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Poster Competition
Founder's Award Competition
Online live-interactive event
using gather.town
on October 2nd 2021

Featuring keynote speaker
Dr Brent Funk, PsyD
Post ICU-Clinic for COVID-19 Patients
Department of Behavioral Health,
Henry Ford Health System.

"Clinical Neuropsychology During COVID: What we (think) we know and changes to practice"

51st Annual Meeting
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*Terms are ending this year

Special thanks to the planning committee: Kelly Bosse, Tom Fischer, Hilary Marusak, Jessica Matchynski-Franks, Anna Moszczynska, Eric Ramsson, Julien Rossignol, Bhairavi Srinageshwar, & Kevin Trewartha

Technical Support: Kelly Nack

Conference center programer: Jessica Matchynski-Franks, matchingfranks@gmail.com

Cover Art Designed by: Delphine Rossignol delphine.rossignol@gmail.com
Schedule:

9:00-9:30 AM   Help desk open/ ‘Coffee’
9:30-10:00 AM  Poster Session A
10:00-10:30 AM Poster Session B
10:30-11:00 AM Poster Session C
11:00-11:30 AM Poster Session D
11:30-12:00 PM Vendors/ Institutional booths exclusively open (Also open during Poster Sessions)
12:00-12:30 PM Lunch Break (Meet with colleagues in the lounge or open conference rooms)

Lobby Auditorium:

12:30-12:55 PM Business Meeting:
   Welcome & President’s Report; Treasurer’s Report; Elections
   To be elected during the meeting (self-nominations are welcome)*:
   President-Elect (2021-2022; President 2022-2024; Past-President; 2024-2025)
   Treasurer-Elect (2021-2022; Treasurer 2022-2024)
   Website Coordinator (2021-2023)
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   Western Michigan University Counselor (2021-2023)
   Counselor at Large I: (2021-2023)
   Student Counselor: (2021-2023)
*Due to irregularities related to COVID-19 postponements, note that the 2021 term begins 10/4/2021 and ends 8/31/22. Following years will continue as usual pending any unforeseen circumstances.

12:55-1:30 PM  Data blitz
1:30-2:00 PM  Founder's Award winner talk
2:00-3:00 PM  Keynote address: Dr. Brent Funk, PsyD.
3:00-3:30 PM  Awards & Adjournment
# Posters & Talks

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**Data Blitz**

12:55-1:30 PM, Gather.Town Lobby Stage

*Each presentation will be 8 minutes long and are organized alphabetically by author's last name:*

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Founders Award

This award is in honor of Montford F. Piercey and Duncan McCarthy for their contributions in organizing our chapter.

2021 Winner: Alex Chen, PhD

Bio: Majoring in neuroscience at NYU, Dr. Alex Chen started his research career in Dr. Tom Carew’s laboratory where he studied the time-dependent activation of CREB signaling during the formation of long-term memories in Aplysia californica. He recently attained his PhD at the University of Michigan, probing novel homeostatic metaplasticity mechanisms in hippocampal neural circuits. Dr. Chen will be transitioning from academic neuroscience research to cellular agriculture research, working in the research and development team at New Age Meats to improve cell-based meat systems.

1:30-2:00 PM, Gather.Town Lobby Stage

HOMEOSTATIC METAPLASTICITY IN HIPPOCAMPAL NETWORKS

Chen, A.1, Garay, P. M.1, Iwase, S.1-3, & Sutton, M. A.1,4

1Neuroscience Graduate Program, The University of Michigan Medical School, Ann Arbor, MI, USA, 2Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, Michigan, USA, 3Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, Michigan, USA, 4Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA

Abstract:
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 a network to implement future homeostatic adaptations.
Keynote Speaker

2:00-3:00 PM, Gather.Town Lobby Stage

Brent A. Funk, PsyD, ABPP
One Ford Place
Henry Ford Health System
Senior Staff Neuropsychologist, Neuropsychology
Training Coordinator, Co-Director APPCN
postdoctoral fellowship program & Clinical Assistant
Professor at Wayne State University

Clinical Neuropsychology During COVID: What we
(Think) we Know and Changes to Practice

Dr. Funk specializes in epilepsy, traumatic brain injury and
concussions, and forensic evaluations. He is the neuropsychology
representative on the Comprehensive Epilepsy Program surgical
team and in the Normal Pressure Hydrocephalus clinic. He also
participates in several Athletic Medicine Sports Concussion
Program. Additionally, he trains psychology, psychiatry, and
neurology students in clinical and formal lectures, and has received
multiple awards for his excellence in teaching.

Dr. Funk’s research has focused on epilepsy-related surgery,
temporal lobe seizure prediction, concussion knowledge among
athletes, and cognitive assessments. Recently he was involved in
the team developing Henry Ford's Post ICU-Brain Health Clinic
developed to treat those with neurological issues related to
COVID-19.
Development

ECTOPIC EXPRESSION OF GERMLINE GENES WITH LOSS OF HISTONE DEMETHYLASE KDM5C IN X-LINKED INTELLECTUAL DISABILITY

Bonefas K.M. & Iwase S.
University of Michigan, Ann Arbor, MI

Chromatin regulators are an important component of the epigenetic environment that can influence transcription to ultimately shape cellular identity. Currently, it is unclear why mutations in numerous chromatin regulators cause neurodevelopmental disorders (NDDs). For example, lysine demethylase 5c (KDM5C) erases the histone 3 lysine 4 trimethylation (H3K4me3) and loss-of-function mutations in KDM5C result in intellectual disability, heightened aggression, and comorbidity with Autism Spectrum Disorder. Surprisingly, we found the Kdm5c-KO mouse amygdala and hippocampus — two brain regions important for these behaviors — aberrantly express many germline genes that have no known function in the brain. We found this apparent soma-to-germline transition occurs during early Kdm5c-KO embryogenesis and parallels germ cell development. This failure of Kdm5c-KO embryos to demarcate somatic and germline identity results in aberrant expression of germline genes across multiple tissues. Ectopic transcription of germline genes in the brain suggests Kdm5c-KO neuronal function may be impaired due to a failure to faithfully establish neuronal identity. Our work provides mechanistic insight into how KDM5C helps delineate the somatic and germline transcriptomes. This work also offers early insight into a novel mechanism by which chromatin regulators are essential for neurodevelopment and behavior.
Human Retinal organoids (RO) model human retina development in vivo and can be used as a model of study. The retina is an extension of the central nervous system, therefore neuronal genes and proteins are expressed. The implications of this research allow for further studies of neurological and retinal diseases in humans. Subsequently, research can be done without requiring human retinas. In this study, human embryonic stem cells were grown in culture until maturity, then media supplementation was altered to initiate differentiation. Organoids were cultured on a 35mm plate and in suspension. Data was collected from RO days 30-130 of development. q-RT-PCR was used to identify retina specific genes and neuronal markers and verify development of RO. Genes investigated by this technique were: VSX2 (retinal progenitor cell marker), CRX (photoreceptor precursor), PROX1 (amacrine, bipolar, horizontal cells), RLBP1 (Muller cell marker), RHO (rod photoreceptor marker), PAX6 (early retinal precursor marker), TH (dopamine), OTX2 (neuron marker), Alpha 6 (stem cell marker). In addition, immunostaining showed that organoids at various ages contain the cone-rod homeobox protein (CRX), melanopsin, and tyrosine hydroxylase (TH), at specific ages that were synonymous with q-RT-PCR results. Protein and gene verification prove RO are the end result, due to the neuronal and retina specific markers expressed. In future experiments, specific pathways that contribute to many neurological diseases can be studied in the RO model.
NEUROBIOLOGICAL MECHANISMS THAT INFLUENCE EMOTIONAL REACTIVITY: A DEVELOPMENTAL STUDY OF THE HIPPOCAMPUS

Michigan Neuroscience Institute, Ann Arbor, MI

Debilitating psychiatric disorders such as major depression and chronic anxiety have a major personal and societal impact. Both prevention and treatment strategies require greater understanding of the relationship between genetic predisposition and brain function. For instance, while it is known that there is a genetic component to emotional reactivity, the neurobiological mechanisms through which gene expression patterns shape these behavioral traits remain largely unknown. Using a selective breeding model in rodents, this study aims to elucidate potential cellular mechanisms that link genetic predisposition and the emergence of vulnerable or resilient behavioral traits.

Our laboratory has selectively bred rats for over 60 generations in order to select for locomotor responses to novelty, ultimately generating a robust behavioral model of emotional reactivity and temperament. Bred low responder (bLR) animals and bred high responder (bHR) animals represent extreme ends of emotional reactivity. bLRs represent an internalizing phenotype, are behaviorally inhibited, with a high level of anxiety- and depressive-like behaviors. In contrast, bHRs represent an externalizing phenotype, with low behavioral inhibition and high levels of impulsivity.

Our lab has characterized genetic variants as well as hippocampal gene expression changes in the bLR/bHR model and identified candidate genes that differentiate between the lines. A top candidate gene is the Bone Morphogenetic Protein 4 (Bmp4), which has diverse roles in nervous system development. Preliminary results demonstrate that differences in Bmp4 in the bLR/bHR model arise early in life and are maintained throughout the lifetime of the animals. We perform a comprehensive analysis of cell-type composition and gene expression in the bLR/bHR model using a combination of multiplexed fluorescent in situ hybridization, immunohistochemistry, and confocal imaging. We test the hypothesis that Bmp4 is important for establishing cell-type balance within the developing hippocampus, and that these differences, in turn, play a role in shaping adult behavior.

Support from: NIDA U01DA043098; Office of Naval Research (ONR) 0014-19-1-2149;
The Hope for Depression Research Foundation (HDRF): The Pritzker Neuropsychiatric Research Consortium
DEVELOPMENTAL STIMULATION OF PYRAMIDAL NEURONS DIFFERENTIALLY ALTERS CYTOARCHITECTURE AND EXCITATORY-INHIBITORY BALANCE ACROSS SEPARATE AREAS OF THE NEOCORTEX


1Program in Neuroscience; 2Biochemistry, Cell and Molecular Biology Program; 3College of Medicine, Central Michigan Univ., Mount Pleasant, MI

The malformation of neuronal circuitry in the developing neocortex has been thought to lead to many possible psychiatric disorders later in the organism’s lifespan. By investigating alterations in circuitry through hyperexcitation in the developing brain, adult behavior can be assessed for phenotypic examples of psychiatric disorders. To test the impact of hyperexcitation of neuronal circuitry during early postnatal development, 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 physical light via fiber optics. Mice conditionally expressing LMO3, a fusion of sbGluc luciferase and the blue light sensing opsin VChR1, were crossed with Emx1-Cre transgenic mice, thus limiting expression of LMO3 to cortical pyramidal neurons. Overexcitation of developing pyramidal neurons was achieved chemogenetically by administering the luciferase substrate coelenterazine intraperitoneally during postnatal days 4-14. During adulthood, in vivo extracellular recordings of neocortical circuits revealed functional deficits in excitability. Additionally, alterations in excitatory and inhibitory balance were seen at both the electrophysiological and cytoarchitectural level. While Emx1-positive neurons were hyperexcited during postnatal development across the cortex, behavioral, electrophysiological, and morphological effects manifested differently in distinct areas.
EXAMINING THE IMPACT OF PRENATAL CANNABIS EXPOSURE ON FRONTOLIMBIC WHITE MATTER PATHWAYS

Evanski, J., Borg, B., Morales, A., Faraj, M.M., & Marusak, H.
Wayne State University, Detroit, MI

Recent research has demonstrated that fetal neurodevelopment can be disrupted by cross placental transfer of cannabis constituents, such as delta-9-tetrahydrocannabinol (THC). One of the impacts of this cross placental transfer of THC is the disruption of the fetal endocannabinoid system, which may have adverse effects on the neurodevelopment of the child and adverse effects throughout the lifespan. Previous research has shown that endocannabinoids and cannabinoid receptors are expressed in the fetal brain, as early as five weeks into gestation. We used data from the large-scale NIH Adolescent Brain Cognitive Development Study to examine the impact of prenatal cannabis exposure on integrity of frontolimbic white matter tracts (FWMT) in children. We focused on FWMTs given their critical role in learning, memory, and affect, and given that they are known to be susceptible to cannabis use in adults. Recent studies also indicate that variation in endocannabinoid signaling modulates frontolimbic development in children. This study reports on diffusion tensor imaging data collected from 10,579 children (M ± SD = 9.92 ± 0.62 years; 48% female; 52% White, 12.5% Black, 18.7% Hispanic, 1.8% Asian, 5.3% Other). Prenatal cannabis exposure was measured via a parent retrospective report. Fractional anisotropy (FA) was estimated for five FWMTs in each hemisphere (left (L) and right (R)). In this sample, 3.9% of parents (n = 410) reported using cannabis prior to knowledge of pregnancy, and 1.1% (n = 119) reported using after knowledge. We found that prenatal cannabis exposure before knowledge of pregnancy was associated with lower FA in the L parahippocampal cingulum, L inferior-fronto-occipital fasciculus, and both fornices. After controlling for multiple comparisons (false discovery rate [FDR] < .05) and including potential covariates (i.e., race, gender, ethnicity, prenatal alcohol, and tobacco exposure) only the R fornix remained significant (F [1, 10578] = 15.69, p < .001, $\beta$ = -.038, $r^2 = .001$, p < .001). Prenatal cannabis exposure after knowledge of pregnancy was associated with lower FA in both fornices, R and L cingulate cingulum, R parahippocampal cingulum, and the L uncinate. However, these associations were not significant after controlling for multiple comparisons and including relevant covariates. These data add to evidence linking prenatal cannabis exposure to altered neurodevelopment in offspring. These findings suggest that prenatal cannabis exposure, particularly early in pregnancy, disrupts FWMT. Given the critical role of frontolimbic pathways, these data should be used to encourage women to abstain from THC use during pregnancy or pre-conception.
OVARIAN HORMONES INFLUENCE THE DEVELOPMENT OF SOCIAL DISCRIMINATION IN FEMALE RATS

Henry, M.G., Yoest, K.E., & Veenema, A.H.
Department of Psychology, Michigan State University, East Lansing, MI

Social recognition as defined as the ability to distinguish novel from familiar individuals is crucial for adaptive social interactions. Recent work from our lab found that juvenile female rats do not display social recognition, whereas adult females and adult and juvenile males do, when they are exposed to the social discrimination paradigm. In this paradigm, the experimental animals’ preference to investigate a novel over a familiar 3-week-old same-sex stimulus animal is analyzed. We then tested female rats for social recognition over the course of development. We found that females begin to show this behavior at adolescence. Therefore, we hypothesized that juvenile female rats lacked social recognition because the underlying mechanisms for this process depend on higher circulating levels of estrogen levels as seen during puberty and thereafter. To test this, we injected juvenile female rats with estradiol benzoate (EB) or vehicle 48-hours prior to social discrimination testing. EB increased investigation of familiar animals resulting in a preference for familiar over novel animals. This indicates that EB improved social recognition but not in the typical pattern of social recognition seen in adult females. We then performed ovariectomies on prepubertal female rats to determine whether a lack of estradiol and other ovarian hormones would prevent the maturation of social recognition. Indeed, we found that adult female rats that were prepubertally ovariectomized did not show social recognition. Finally, we found that subsequent administration of EB did not reverse the effects of ovariectomy. This suggests that sustained, rather than acute, increases in ovarian hormone levels during puberty are necessary for the development of social recognition in female rats. In conclusion, our findings provide evidence for sex and age differences in the development of social recognition and its' dependence on the organizational effects of ovarian hormones in females.
A TRANSLATIONAL RODENT MODEL OF GESTATIONAL BUPRENORPHINE OR MORPHINE EXPOSURE: OFFSPRING OUTCOMES

Myers, A., Kulaglic, N., Neole, S., Richardson, L., Wallin, C., Bowen, S., & Brummelte, S.
Department of Psychology, Wayne State University, Detroit, MI

The recent opioid epidemic and escalation of opioid use during pregnancy has resulted in a drastic increase in gestational exposure to opioids. Opioid-dependent pregnant women are typically given opioid maintenance therapies (i.e., buprenorphine or methadone) to mitigate harmful effects on the fetus induced by in utero exposure to opioids. However, the consequences of exposure to synthetic opioids such as buprenorphine during gestation on fetal outcome and brain development are poorly understood. Our translational rodent model aims to characterize neonatal mortality and development after continued or discontinued gestational opioid exposure. Female rats received either morphine (2-6mg/kg twice daily, s.c.; to mimic opioid use disorder) or buprenorphine (1mg/kg s.c.; to mimic opioid maintenance therapy) from preconception until late gestation (‘discontinued’ on gestational day (GD) 19, mimicking withdrawal before parturition) or until postnatal day 2 (‘continued’). Pups were either sacrificed on postnatal day 2 or fostered to a naïve foster dam and allowed to reach young adulthood for behavioral testing. Buprenorphine resulted in higher pup mortality, reduced body weight, and increased neonatal withdrawal symptoms compared to controls and morphine-exposed pups. Somewhat surprisingly, discontinuation of buprenorphine on GD 19 did not appear to rescue these effects. Preliminary behavioral data from young adult offspring suggest that gestational opioid exposure did not result in changes to anxiety-like behavior (elevated zero maze) or pain sensitivity (hot-plate test). This suggests that fostering opioid-exposed offspring may potentially rescue or protect them from the negative effects of gestational opioid exposure or opioid-induced deficits in maternal care. Offspring brains collected at postnatal day 2 will be analyzed to determine the effects of gestational opioid exposure on brain-derived neurotrophic factor (BDNF) concentration in the hippocampus and prefrontal cortex as a measure of neurodevelopment. More research is needed to understand how buprenorphine affects pregnancy outcomes and subsequent offspring well-being in order to avoid negative consequences in human mothers undergoing opioid maintenance treatment.
HIPPOCAMPAL VOLUME PREDICTS REDUCTIONS IN POSTTRAUMATIC STRESS SYMPTOMS IN CHILDREN WITH CANCER: EFFECTS OF A NOVEL MARTIAL ARTS-BASED INTERVENTION

Wayne State University, Detroit, MI

Youth with cancer experience an immeasurable amount of stress and trauma prior, during and after their cancer treatments. The treatment phase can take an emotional and physiological toll on youth and many pediatric cancer patients report posttraumatic stress symptoms (PTSS). Our prior research in pediatric cancer populations has demonstrated correlations between PTSS and variation in stress-sensitive brain regions, such as the hippocampus. In tandem, prior studies in adults with breast cancer consistently link smaller hippocampal volumes to cancer-related PTSS, particularly re-experiencing PTSS (e.g., flashbacks, nightmares). The current study examines the impact of a novel martial-arts based intervention on hippocampal volume and PTSS in pediatric cancer patients, and whether baseline (i.e., pre-intervention) hippocampal volumes predict changes in PTSS over time. Over a 4-week period, eighteen pediatric cancer patients or survivors (ages 5-17 years, 8 female) participated in this prospective study. Before and after the 4-week program, participants self-reported on cancer-related PTSS. Participants underwent structural magnetic resonance imaging (MRI) scans at baseline and then completed 4 weekly Kids Kicking Cancer classes. Kids Kicking Cancer is a martial arts-based therapy that integrates meditation, breathing, and physical movements, and has been shown to lower PTSS in pediatric cancer and other populations (e.g., sickle cell, schoolchildren, and adults with opioid use disorder). Further analyses examined whether PTSS and hippocampal volumes changed over time, and whether hippocampal volume at baseline predicted change in PTSS. In conclusion, PTSS symptoms and hippocampal volumes did not change significantly over the 4-week period. However, youth with smaller left (but not right) hippocampus at baseline demonstrated greater reductions in PTSS over time. Exploratory analyses suggest that the association between hippocampal volumes and change in PTSS was driven by re-experiencing PTSS. Exploratory analyses suggest that the association between hippocampal volumes and change in PTSS was driven by re-experiencing PTSS. Results from this pilot study suggest that smaller hippocampal volumes may predict reductions in cancer-related PTSS following a martial arts-based intervention. They also extend prior studies suggesting that a martial arts-based approach may be effective for reducing pain in pediatric cancer populations.
A TRANSLATIONAL RODENT MODEL OF GESTATIONAL MORPHINE OR BUPRENORPHINE EXPOSURE: EFFECTS ON PREGNANCY OUTCOMES, MATERNAL CARE, AND MATERNAL BEHAVIOR

Richardson, L., Wallin, C., Myers, A., Kulaglic, N., Neole, S., Bowen, S., & Brummehte, S.
Department of Psychology, Wayne State University, Detroit, MI

In recent years, the opioid epidemic has continued to worsen, with a rise in prescribed and illicit opioid usage. As a result, many opioid-dependent pregnant women are receiving opioid maintenance therapies, specifically that of buprenorphine or methadone to combat the negative effects of misused opioids on both the mother and her developing fetus. Clinically, buprenorphine produces preferable outcomes for exposed infants as compared to methadone or the abuse of opioids. However, there is dearth of knowledge on the behavioral and neurological effects of buprenorphine on the maternal brain and maternal caregiving behaviors. Through the use of a translational rodent model, we were able to mimic chronic opioid (mis)use (morphine exposure) or maintenance opioid drug (buprenorphine exposure) to investigate the behavioral and neurochemical consequences of gestational opioid exposure on dams and their offspring. Opioid administration to female rats began prior to pregnancy and was either continued until postpartum day 2 or discontinued shortly before birth on gestational day 19. Dams were evaluated for appropriate maternal behaviors through careful maternal care observations, as well as the behavioral pup retrieval test. Our preliminary findings indicate that buprenorphine exposure resulted in more maternal care deficits, increased postpartum pup mortality, and maternal deficiencies in the pup retrieval task as compared to our control group. On the other hand, morphine exposure seemed to result in fewer successful pregnancies compared to other groups, but contrarily, fewer deficits in maternal care as compared to buprenorphine dams. Further research is imperative to understanding the mechanism in which buprenorphine interacts with the maternal brain network during the transition to motherhood in order to help with diminishing the possible negative consequences of maternal care deficits on the offspring.

Funding: ReBUILDetroit Bridge Grant
HUMAN DIET'S INFLUENCE ON DENDRITOGENESIS REGULATING COGNITION

Roychoudhury, R.1, Dubey, H.2, White, A.2, Liu, S.2, & Knickmeyer, R.C.3
1Col. of Osteo. Med., 3Pediatrics, 2Michigan State Univ., East Lansing, MI

The ability of the gut microbiota to regulate brain development and behavior is increasingly recognized. For example, germ-free mice exhibit altered anxiety-like and social behaviors in a variety of paradigms as well as altered dendritic morphology in relevant brain regions such as the amygdala. However, the clinical relevance of these germ-free studies has been challenged as the majority of human development occurs in the presence of complex microbial communities. To address this limitation and determine whether variation in human microbiomes influences development of the neural circuits regulating anxiety and social behavior, we introduced microbiomes from typically developing infants into pregnant germ-free mice and evaluated dendritic morphology in the prefrontal cortex and amygdala of their adult offspring. We chose to focus on infant microbiomes because infancy is a critical period in the establishment of the gut microbiome and in brain development. Furthermore, our team, led by Dr. Knickmeyer from the MSU College of Human Medicine Department of Pediatrics, recently reported associations between features of the human infant gut microbiome and amygdala and medial prefrontal cortex volumes. One infant inoculum was characterized by high levels of Bacteroides, which is often considered anxiogenic, while the other was characterized by high levels of Bifidobacterium, which is often considered anxiolytic. Dendritic length and volume of pyramidal neurons were significantly greater in the prefrontal cortex, amygdala, and hippocampus of humanized Bif mice compared to humanized Bac mice, p < 0.05, suggesting that microbiomes with a high relative abundance of Bifidobacterium may promote dendritogenesis of pyramidal neurons in this region. Specific-pathogen free mice (N=4) appeared more similar to the Bac mice, but did not differ significantly from either group. No differences in dendritic length and volume were observed in aspiny inhibitory neurons in either the nucleus accumbens. These findings suggest that differences in the composition of the human gut microbiome influence development of the prefrontal cortex, hippocampus, and amygdala which may influence social information processing and emotional regulation.
Preterm births account for over 10% of all U.S. live births and the rate is rising. Preterm children often display impairments in neurodevelopment associated with exposure to neonatal stressors, such as painful procedures in the Neonatal Intensive Care Unit (NICU). Maternal care can alleviate stress during these procedures but infants in NICUs receive reduced maternal care compared to peers, and this may further impair neurodevelopment. This translational study aimed to investigate how the interaction of neonatal repeated procedural pain and maternal separation influences neuroplasticity in neonatal and adult rodents.

Male and female rat pups were exposed to stressors in 4 sessions per day on postnatal days 1 through 4 (PD 1 – 4). During each session subjects received either repeated needle pokes, maternal isolation, needle pokes followed by isolation, or neither (touch control group). Animals were sacrificed at PD 8 or at adulthood and brain tissue sections were processed via immunohistochemistry for Ki67, a marker of cell proliferation, and reelin, a protein that modulates neurodevelopment and synaptic activity.

Reelin levels were increased in adult female rats exposed to either pain or maternal separation, but not both, and there was no effect in male rats. However, hippocampal cell proliferation in neonates and adults was unaffected by either stressor. These results suggest that neonatal stressors that mimic NICU experiences can have long-lasting effects on brain plasticity, but these outcomes are not necessarily exacerbated by exposure to more than one type of stressor. More research is needed to better understand the contribution of the various neonatal stressors to long-term neurodevelopment.
Neural Excitability, Synapses, and Glia

D3

ALTERATIONS IN GLIAL PROTEIN EXPRESSION IN OBESITY-PRONE RATS LEAD TO CHANGES IN GLUTAMATE METABOLISM AND NUCLEUS ACCUMBENS NEUROCHEMISTRY

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Obesity rates continue to rise despite obesity being declared a global epidemic by the WHO. Although the risk of obesity is well known, many individuals continue to struggle with weight loss and maintenance. Recent studies have shown that alterations within regions of the brain involved in motivation, such as the nucleus accumbens (NAc), likely make it more difficult to lose excess weight and keep it off. Glucose metabolism and neurotransmitter production in the brain is a complex interaction involving both astrocytes and neurons. Most of the work examining the roots and effects of obesity in the brain have focused on neurons. Recent work has illustrated alterations in the neurochemistry of the NAc that is consistent with changes in astrocytes. Here we examined key proteins in glutamate and GABA metabolism and synthesis to examine the possibility that astrocytes may be altered in response to obesity.

Four selectively bred obesity-prone and five obesity-resistant adult rats monitored for weight gain across five weeks before being anesthetized using isoflurane. Microdissection of the brain was performed to isolate the NAc and tissue was flash frozen. Western blot analysis was performed using antibodies to glutamine synthetase (GS), GABA Decarboxylase (GAD-67), microglial marker IBA-1, and glial glutamate transporter (GLT-1) in duplicate. Images were developed and analyzed using a C-DiGit Blot Scanner and ImageStudio.

GS was significantly lower in OP rats when compared to OR rats (Unpaired t-test: p=0.0313). The functional GLT-1 monomer was significantly lower (2 tailed T Test: p=0.0247) in OP rats while a large smear indicative of polyubiquitination was increased (2 tailed T Test: p=0.0437). GAD-67 did not show any significant difference in expression between OP and OR rats (2 tailed T test: p=0.2588). IBA-1 appeared to be decreased in OP rats (1 tailed T Test: p=0.0518).

These data, in combination with previous studies, suggest that differences exist in key glial cell proteins between OP and OR rats. First, decreased IBA-1, a microglial marker, suggests that obesity may lead to microglial dysfunction within the nucleus accumbens. Second, astrocyte protein expression in the NAc is significantly different in OP rats compared to OR rats with the observed differences suggesting decreased uptake and metabolism of glutamate. These changes would lead to elevated glutamate levels in the NAc, which have been observed through microdialysis studies. Interestingly, elevated glutamate levels can lead to excitotoxicity including loss of dendritic spines, which has been observed in other brain regions of obese rats. Finally, there were no significant changes in GAD-67 levels, indicating that obesity does not appear to impact the GABAergic neurons. Taken together, these results indicate that astrocytes play a role in obesity-related changes in glutamate metabolism in the NAc.
 Precisely tuned regulation of pre and post-synaptic communication depends on the ability to adjust synaptic protein levels via coordinated protein synthesis and degradation mechanisms. Previous work has demonstrated activity-dependent proteasome recruitment into dendritic spines in response to synaptic stimulation, indicating that remodeling the synaptic landscape via active degradation is likely an important aspect of postsynaptic functional plasticity. In axons, where the abundance of proteasomes is dramatically lower than in dendrites, the redistribution of the proteasome to appropriate synaptic terminals could be a critical mechanism governing protein degradation in the presynaptic compartment. Indeed, we report here that intrinsic firing governs activity-dependent proteasome trafficking to and from synaptic terminals in axons of cultured hippocampal neurons, and this dynamic proteasome localization is critical for trans-synaptic signaling to homeostatically adjust presynaptic neurotransmitter release. Using epitope- and fluorescently-tagged subunits of the 19S proteasome, we find that increasing neuronal firing rates enriches proteasome accumulation at synaptic terminals, whereas inhibiting neuronal firing results in a dramatic redistribution away from synaptic terminals to non-synaptic areas. This altered localization is due, at least in part, to an activity-dependent active sequestration mechanism at presynaptic terminals, as revealed by live monitoring of fluorescence persistence after synaptic photoactivation of GFP-tagged proteasomal subunits. Moreover, we find that activity dependent phosphorylation of the Rpt6 subunit of the 19S proteasome is necessary and sufficient for axonal proteasome redistribution, and that this altered localization plays a critical role in establishing retrograde homeostatic changes in presynaptic function after loss of postsynaptic drive. Together, our data reveal that dynamic redistribution of the proteasome is a novel mechanism whereby the activity-dependent “state” of synaptic compartments determines the specific forms of plasticity they can exhibit.
DETERMINING THE MOLECULAR, CELLULAR, AND CIRCUIT EFFECTS OF CHRONIC CHEMogenetic NeurAl STimulation

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In the last decade, different chemogenetic tools have been developed that are allowing neural circuits to be controlled and investigated non-invasively, which in turn has many clinical applications. Chemogenetics uses synthetic or orthogonal proteins that respond to otherwise inert ligands to control neural activity. These genetically encoded proteins act as actuators in cellular pathways and can be targeted to specific cell populations ultimately leading to changes in neuronal membrane potential. DREADDs, or Designer Receptors Exclusively Activated by Designer Drugs, are engineered G-protein coupled receptors while Pharmacologically Selective Actuator Modules, or PSAMs, are ligand-gated ion channels. Luminopsins, LMOs, a third chemogenetic technology, change the neuronal membrane potential by conducting ions through a channel, like PSAMs. Unique to LMOs is that it does this in a light-dependent manner through bioluminescent activation of light-sensing ion channels in the presence of a luciferin. Extensive studies report the causal effects of chemogenetic activation of circuits driving animal behavior, yet we have a limited understanding of the neurophysiological changes arising from a direct consequence of their implementation. To address this knowledge gap, cultured cortical neurons were transduced with viral constructs each containing an excitatory chemogenetic actuator (1) DREADDs, (2) PSAMs, or (3) LMOs. On DIV 17-19, the expressing cortical neurons were chronically treated with the appropriate chemical ligand once a day for five days to selectively activate each respective chemogenetic actuator. Microelectrode array recordings of spontaneous baseline activity revealed an increase in firing rate when neurons were chronically excited using DREADDs. However, all three groups of stimulated neurons were less responsive to electrical stimulation than the vehicle-treated culture, suggesting that the stimulated neurons have decreased excitability after treatment. To determine if chronic chemogenetic stimulation alters neuronal morphology, Sholl analysis revealed more complex dendrite arborization in chronically stimulated neurons as compared to the control. Chronic stimulation of cultured cortical neurons with three distinct chemogenetic technologies impacts neural physiology in unique ways. To bridge these chemogenetic technologies to the clinic, it is pertinent to characterize any electrophysiological and morphological alterations that arise due to implementing chemogenetic technology—warranting further investigations in vivo.
GENETICALLY ENCODED RED SHIFTED LIGHT SOURCES FOR NON-INVASIVE NEURONAL OPTOGENETICS.

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BioLuminescent-OptoGenetics (BL-OG) is a minimally invasive approach in which genetically expressed luciferases are tethered to opsins. In the presence of the luciferase substrate coelenterazine (CTZ), the generated light activates the opsins and alters ion flow. Thus, neuronal activity can be manipulated without the need for invasive surgeries and LEDs. Here, we developed three excitatory luciferase-opsin constructs (luminopsins; LMO) using a red-shifted version of Renilla Luciferase (RLuc8.6) tethered to red-shifted opsins. In each red-shifted LMO, RLuc8.6 was fused to a different fluorescent protein, generating different peak emission wavelengths (592nm, 589nm, 560nm). The cation channel ChrimsonR was utilized in the two red-most LMOs while Volvox carteri channelrhodopsin-1 (VChR1) was utilized in the more yellow shifted LMO. Upon IVIS imaging, the bioluminescent emission recordings confirmed LMO expression in cortical neurons. After CTZ stimulation, current clamp recordings showed that LMOs with ChrimsonR were more robust, exhibiting more consistent and higher frequency neuronal spiking, than the LMO with VchR1. Additionally, CTZ stimulation during voltage clamp recordings showed that LMOs with ChrimsonR had consistently more pronounced inwardly directed current when compared to the LMO with VchR1. These findings suggest the potential application of our red shifted LMOs as optogenetic tools for manipulation of independent neural populations when coupled with previously described blue shifted optogenetic tools, thus allowing for multi-modal control of neural circuitry.
DIVISION OF LABOR AMONG HISTONE H3 LYSINE 4 METHYLTRANSFERASES REGULATE DISTINCT FACETS OF HOMEOSTATIC SYNAPTIC PLASTICITY

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Methylation at lysine 4 of Histone 3 (H3K4me) is an evolutionarily conserved post-translational modification that is associated with transcriptionally active areas in the genome. In mammals, the majority of this mark is placed by six writer enzymes, namely KMT2A-G. Interestingly, all six KMT2 genes are monogenetically associated with neurodevelopmental disorders characterized by autism and intellectual disability, revealing a non-redundant role for these H3K4me enzymes in the development and function of the central nervous system. The roles of KMT2A-G have been intensely studied in embryonic development, but the role of these enzymes in postmitotic neurons remains largely unknown. Using genetics, electrophysiology, neuronal imaging, and gene expression profiling, we have found that all six H3K4me writer enzymes are readily expressed in excitatory neurons and engaged in an enduring form of synaptic plasticity to homeostatically maintain the stability of neuronal activity. Intriguingly, rather than playing overlapping roles, each KMT2 writer appears to play a specific and unique role in distinct facets of homeostatic plasticity. Finally, H3K4me is dynamically controlled by the balance of writer and eraser enzyme activity, but how H3K4 writers and erasers functionally interact in neural plasticity is unknown. We revealed a clear interaction between the H3K4me writer KMT2A and the eraser KDM5C in synaptic downscaling, suggesting that targeting specific H3K4me enzymes may be a future therapeutic strategy for neurodevelopmental disorders characterized by H3K4me dysregulation.
DEVELOPING A METHOD TO IDENTIFY THE PROTEIN INTERACTOME OF TAU USING THE BIOID2 APPROACH

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Aberrant aggregation of tau protein is a hallmark of neurodegenerative diseases collectively known as Tauopathies, of which the most common is Alzheimer’s Disease. Although the tau protein is most commonly known as a microtubule-associated protein involved in regulating microtubule dynamics, accumulating evidence suggests tau is likely involved in many other biological functions. For instance, several studies implicate a scaffolding protein role for tau where it can act to regulate cellular pathways through direct or indirect interactions with several other proteins. Deciphering the tau protein-protein interactome is a critical step towards better understanding the physiological and pathological roles of tau. The goal of this work is to identify tau interacting partners using a method that allows in situ identification of protein-protein interactions. We are adapting a technique based on biotin ligase (BioID2) that allows in situ protein labelling with biotin followed by biotin-targeted pulldown and mass spectrometry. Here, we describe the methodological approach used to adapt this technology for studying tau interactions. Full length human tau (ht40) consists of the N-terminus, microtubule binding region (MTBR), and the C-terminus. To map the tau protein interactome, we began by creating fusion proteins between tau and BioID2, with BioID2 on the N-terminus (BioID2-ht40) or C-terminus (ht40-BioID2) of full-length human tau. To further dissect the role of each domain in protein interactions, we generated BioID2 fusion proteins with the following tau domains: a) 1-224 amino acids (Nterm-BioID2), b) 225-380 amino acids (MTBR- BioID2), c) 381-441 amino acids (Cterm-BioID2), d) 1-380 amino acids (Nterm-MTBR-BioID2), and e) 225- 441 amino acids (MTBR-Cterm-BioID2). For each constructs plasmids are cloned and validated at the DNA level using Sanger sequencing and restriction digestions. Then protein expression of fusion proteins is validated in HEK293T cells using western blotting. The constructs are then produced in lentiviruses, which are titrated using digital droplet PCR and protein expression is confirmed in HEK293T cells. Ongoing studies will use validated lentiviruses to stably express the tau-BioID2 proteins in primary hippocampal neurons from tau knockout mice. Studies will isolate biotinylated proteins from neurons expressing the various tau-BioID2 proteins through affinity-capture using streptavidin-coated magnetic beads and identified by mass spectrometry. We expect this approach will provide a method to identify novel tau interacting partners that could help shed light on tau’s growing functional roles in neurons.

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EARLY SYNUCLEINOPATHY TRIGGERS CONVERSION OF ASTROCYTES TO THE TOXIC A1 PHENOTYPE AND MICROGLIAL COMPLEMENT COMPONENT 3 UPREGULATION PRIOR TO NEURODEGENERATION

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Parkinson’s Disease (PD) is a complex and progressive neurodegenerative disorder that culminates in the deposition of alpha synuclein (a-syn) containing protein aggregates, named Lewy bodies, and the degeneration of nigrostriatal dopamine neurons. Evidence suggests that neuroinflammation plays a causative role in the pathogenesis of PD. Post-mortem- and longitudinal PET imaging studies of the PD brain reveal an increase in the number of activated microglia that occur early in the disease process and remain elevated throughout the course of the disease. Activated microglia can convert astrocytes to a subtype of toxic “A1-astrocyte”. Preliminary evidence suggests that these reactive astrocytes are present in the PD brain. A1 astrocytes coordinate selective neurodegeneration by releasing components of the complement system and as-of-yet unidentified neurotoxins. As such, A1 astrocytes may coordinate selective degeneration of dopamine neurons in the context of PD, and therefore represent a potential therapeutic target. To date, A1 astrocytes have only been identified in PD human tissue and animal models of PD after significant neurodegeneration has occurred. Accordingly, it is impossible to determine if A1 astrocytes appear as a response to cell death, or if they are present prior to cell death, in which case they may actively contribute to neurodegeneration. Here we aimed to test the hypothesis that A1 astrocytes are present in the substantia nigra pars compacta (SNc) prior to over neurodegeneration in a model of synucleinopathy. Intra-striatal injection of recombinant alpha-syn pre-formed fibrils (PFFs) results in progressive aggregation of endogenous a-syn which peaks at 2 months post injection, followed by significant nigral degeneration (~48% loss of tyrosine hydroxylase positive neurons) by 6 months post injection. During peak a-syn aggregation we observe peak microglial activation as well as robust astrogliosis. To determine if synucleinopathy triggers the conversion of astrocytes to the A1 phenotype prior to cell loss, we performed droplet digital PCR (ddPCR) to quantify A1-associated transcripts in the SNc 2 months following unilateral, 2 site intra-striatal injections of mouse a-syn PFFs (total 16 μg, 2 x 2 μl, 4μg/μl) or PBS vehicle control (2 x 2μl). We observed a significant increase in many A1-associated transcripts, including complement component 3 (C3), guanylate-binding protein 2 (GBP2), and Serping 1. Expression of transcripts associated with the neuroprotective A2 astrocyte phenotype were either not increased or showed a small but significant increase. We next performed HiPlex RNAscope dual in situ hybridization combined with immunofluorescence to determine the cellular source of A1-associated transcripts. As predicted, Serping 1 colocalized to GFAP positive astrocytes. However, C3 overwhelmingly colocalized to IBA1 positive microglia, which is surprising as most published reports show that astrocytes are the primary cellular source of C3 in the central nervous system. Taken together these data lend support to the idea that the aggregation of a-syn triggers a multifaceted response of the innate immune system involving neuroinflammatory gene expression by both astrocytes and microglia prior to, and potentially participating in, neurodegeneration.
TAU MUTANTS IMPAIR AXONAL TRANSPORT VIA FUNCTIONAL INTERACTION WITH PROTEIN PHOSPHATASE 1

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Alzheimer's disease and related dementias are characterized by neuronal and axonal degeneration that may be caused by disruption of microtubule-based axonal transport. Several pathological tau modifications disrupt fast axonal transport in a squid axoplasm model by activating a protein phosphatase 1 (PP1)-mediated signaling pathway. However, several of the mechanistic details surrounding this effect remained unknown. We sought to characterize this interaction and elucidate its role in tau toxicity in mammalian neurons by using wild-type and two mutant forms of tau (P301L and R5L) that cause inherited frontotemporal dementias (FTLD). We found that tau interacts with the PP1α and PP1γ isoforms, but not PP1β, and the interaction is enhanced by the P301L mutation. Additionally, tau expression increased levels of active PP1 as measured by reductions to phosphorylation at an inhibitory site on PP1. Finally, we used primary rat hippocampal neurons to examine mutant tau's effect on axonal transport of fluorescent-tagged synaptophysin. P301L tau disrupted normal fast axonal transport in primary neurons as shown by increased rates of cargo pausing. Using isoform-specific shRNA-mediated knockdown we determined that mutant tau's effects on axonal transport effects were mediated by PP1γ but not the other PP1 isoforms. Deletion of tau's N-terminal phosphatase activating domain (PAD) rescued mutant tau-induced increases to active PP1 levels and axonal transport pausing. These results demonstrate that tau and PP1 have a functional relationship with important implications for tau toxicity in disease. This aligns with a pathogenic tau-induced disruption to normal axonal transport in mammalian primary neurons that is dependent upon PP1γ and supports a model whereby tau toxicity is mediated by increased interaction with PP1 that leads to aberrant signaling pathway activation and axonal transport deficits. We hypothesize that this mechanism ultimately contributes to axonal and neuronal degeneration in Alzheimer's disease and other neurodegenerative tauopathies.
Diabetes mellitus affects more than 30 million Americans, roughly half of which are expected to develop peripheral diabetic neuropathy (PDN), which greatly impacts patient quality of life. PDN usually begins as hypersensitivity to touch (hyperesthesia) and pain, which often progresses to a loss of touch sensitivity (hypoesthesia) due to irreversible neurodegeneration. There are treatments that address neuropathic pain, however, preventative therapeutics for neurodegeneration are lacking. Thus, there is an unmet medical need to identify novel therapeutic targets for PDN. Recent studies demonstrate that inhibition of soluble epoxide hydrolase (sEH), an enzyme that hydrolyzes epoxy fatty acids, is an effective strategy in attenuating hyperesthesia and pain in rodent models of PDN. Furthermore, treatment with sEH inhibitors prevents neurodegeneration in other disease models, as well as in the hippocampus in rodent diabetes models. However, whether sEH inhibition can prevent neurodegeneration in touch sensory neurons under hyperglycemic diabetes conditions is unknown.

Studying high glucose-induced neurodegeneration in mammal models is difficult (necessitating surgical interventions), time consuming, and costly. To overcome these challenges, we will use *Caenorhabditis elegans* (*C. elegans*) as a model to determine whether pharmacological sEH inhibitors can prevent high glucose-induced neurodegeneration in touch sensory neurons. The results from this study will build a strong foundation for future studies in complex mammalian models.

*C. elegans* are microscopic, transparent nematodes with a simple nervous system that includes mechanoreceptive and nociceptive neurons. These worms are particularly advantageous for assessing neurodegeneration because transgenic strains are available with fluorescently labeled neurons. Furthermore, the worms have short lifespans, which allows for aging experiments to be conducted over the entire lifespan, thereby increasing the throughput. When grown under high glucose conditions, *C. elegans* experience diabetes-like phenotypes, including elevated levels of glucose and shortened lifespans with short-term increased touch sensitivity and long-term losses in touch sensitivity (similar to what is observed in PDN). Additionally, multiple studies have shown that worms on high glucose conditions develop neurodegeneration in several neuron types. However, these studies did not assess morphological changes in specific touch sensitive neurons. In this presentation, we will show that *C. elegans* grown under high glucose conditions may have morphological changes in touch sensitive amphid sensory neurons compared to control worms grown under normal conditions. In addition, the sEH inhibitor 12-[(tricyclo[3.3.1.13,7]dec-1ylamino)carbonyl]amino]-dodecanoic acid (AUDA) attenuates high glucose-induced shortened lifespan. Future experiments in our lab will include phenotypic assays to determine whether morphological changes in these specific neurons correspond with reductions in touch sensitivity. By establishing high glucose-induced changes in these neurons, we may be able to use *C. elegans* to test whether sEH inhibitors can prevent high glucose-induced PDN.
EXAMINING THE INFLUENCE OF COGNITION ON THE RELATION BETWEEN BACKWARD WALKING AND FALLS IN PERSONS WITH MULTIPLE SCLEROSIS

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Background: Backward walking (BW) is a complex motor task that requires increased cognitive demands. In persons with multiple sclerosis (MS), our laboratory has demonstrated BW velocity as a marker of fall risk and cognitive correlate of information processing speed (IPS) and visuospatial memory. However, the extent to which the relation between BW and falls is dependent upon core cognitive function is unknown.

Objectives: The objective of our study was to examine the discrete influences of processing speed and visuospatial memory on the relation between BW and falls in persons with MS. Based on published preliminary data, we hypothesized that the relation between BW and number of falls is conditional upon processing speed and visuospatial memory.

Methods: In a single session, spatiotemporal measures of forward and backward walking, processing speed as measured by the symbol digit modalities test (SDMT), visuospatial memory as measured by the BVMT-R and self-reported retrospective fall history were collected. Using hierarchical regression modeling, moderation was tested in a second step including an interaction term predicting number of falls. Co-variates for all analyses included age and disease severity (Patient Determined Disease Steps [PDDS]).

Results: Thirty-eight individuals with relapsing-remitting MS were included in the moderation analyses. BW, processing speed and co-variates significantly predicted the number of falls ($R^2 = 0.301$, $p = 0.016$), but processing speed did not change the relation to BW ($\Delta R^2 = 0.013$, $p > 0.1$). In a separate model, BW, visuospatial memory and co-variates also significantly predicted the number of falls ($R^2 = 0.332$, $p = 0.008$), but visuospatial memory did not change the relation to BW ($\Delta R^2 = 0.001$, $p > 0.1$). The FW models generated comparable results.

Conclusions: There was no statistical evidence to suggest processing speed or visuospatial memory as moderators of the relation between BW and falls in our small sample of individuals with MS. Larger scale studies examining additional cognitive domains impacted by MS (i.e., executive function and attention) are needed to establish a cognitive framework aimed at characterizing neurobiological processes relevant to BW and its clinical application in the assessment of fall risk.
DNA DAMAGE IN THE CELLS OF LATERAL ANTENNULES OF CRAYFISH (FAXONIUS VIRILIS) IS INCREASED FOLLOWING EXPOSURE TO ENVIRONMENTALLY RELEVANT CONCENTRATIONS OF ATRAZINE

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Atrazine (ATR) is an herbicide commonly applied in agricultural regions in the Midwestern United States. Excess ATR can enter nearby aquatic environments through run-off and seepage, causing ATR concentrations to increase and placing non-target aquatic organisms, like crayfish, at risk of ATR exposure. It has been shown that acute exposure to 80 ppb (μg/L) ATR can cause chemosensory deficits in crayfish. Knowing that ATR causes impairments on olfactory-mediated behaviors, our aim for this study was to determine the effects of ATR in olfactory sensory neurons, located in the lateral antennules of crayfish. We exposed crayfish to environmentally relevant concentrations of ATR (0, 10, 40, 80, 100 and 300 ppb) for 10 days. Following exposures, the distal portion of the lateral antennule was cryosectioned, and a TdT mediated dUTP nick-end labelling (TUNEL) assay was done in order to determine if cells in the lateral antennules had DNA damage. We found a significant increase of TUNEL-positive cells as atrazine increased above 10 ppb. The data that we obtained showed that DNA damage is caused in the cells of lateral antennules, including olfactory sensory neurons, which ultimately compromises the chemosensory abilities of crayfish. This is concerning as crayfish rely heavily on chemosensory abilities for many aspects of their lives.
ASSESSING DOPAMINE RELEASE DURING ENCOURAGED MOVEMENT IN A UNILATERAL 6-OHDA LESIONED THEN DIFFERENTIATED STEM CELL TRANSPLANTED PARKINSONIAN RAT MODEL

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Our laboratory has been interested in ways to encourage and document more efficient incorporation of dopamine (DA) producing transplantable cells into behaviorally functional circuits in our preclinical Parkinson's Disease (PD) research. Major unilateral nigrostriatal DA depletion (> 90%) using the neurotoxin 6-OHDA results in very apparent limb use deficits that are expressed during different forms of locomotion. It is our goal to document the establishment of both behavioral recovery from these limb-use deficits as well as DA release control obtained from direct in vivo measurements as a sign that our regimens of support for training transplants and encouraging their integration into host tissue are working. This poster represents a history of what we have found so far, and an introduction of our current research plan that can serve as a springboard for discussion. Both historical data will be placed in context regarding our demonstrations that our mesenchymal stem cell derived dopaminergic neuronal-like cells are adequate for our plan as well as signs that correlated exposure to swimming with simultaneous targeted stimulation appear to improve the efficiency of these cells once transplanted. Though our laboratory has yet to demonstrate the establishment of DA release control in correlation with improved behavioral recovery, we expect to do so with the current plans for in vivo microdialysis in the context of swimming. Our goal is to establish a confirmation that the regimen of transplant targeted stimulation in the context of behavioral drive designed to activate the basal ganglia both improves limb use symmetry and DA release control derived from the transplants.
REPETITIVE BLAST TRAUMATIC BRAIN INJURY INDUCES CHRONIC ANXIETY, FOREPAW THERMAL SENSITIVITY AND NEUROINFLAMMATION IN RATS.

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Repetitive blast traumatic brain injuries (rbTBI) are a common cause of chronic pain as well as anxiety and cognitive deficits in many U.S. Veterans. Persistent neuroinflammation has been postulated to be a key player; however, much of the current knowledge on long-term rbTBI changes are primarily related to behavioral changes with a limited focus on underlying inflammation. To address this knowledge gap, this study assessed neuroinflammation in conjunction with changes in pain and anxiety at chronic post-rbTBI timepoints. Anesthetized Sprague Dawley rats were subjected to rbTBI (one blast exposure/day for 3 consecutive days using a shock tube) or sham procedures. Following rbTBI or sham procedures, surface righting duration was immediately measured, and 1 or 3 months after rbTBI or sham procedure, anxiety-like behavior was measured by elevated plus maze (EPM) and pain behavior was tested by forepaw thermal withdrawal latency to radiant heat exposure. Perfused, fixed brain sections were collected following behavioral testing and assessed for neuroinflammation using immunofluorescence for microglial and astrocytic proliferation in the prefrontal cortex. Ionized calcium-binding adapter molecule 1 and glial fibrillary acidic protein were measured in astrocytes and microglia. Acutely, rbTBI animals showed prolonged surface righting duration compared to sham animals. At both 1- and 3-month post-blast, rbTBI rats displayed anxiety-like behavior, indicated by decreased open arm time/entries, and they had higher pain sensitivity as indicated by shorter thermal withdrawal latency compared to sham-controls. A high number of microglia and astrocytes were observed in rbTBI animals compared to sham. Overall, these data suggest the development of anxiety-like and thermal sensitivity changes long after rbTBI which may be mediated by the underlying chronic inflammatory state. Future studies will be directed at understanding the effects of rbTBI on opioid-taking behaviors and the ensuing neuropathology.

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A7 Featured Abstract

COMPARISON OF OVINE- AND BOVINE- SOURCES OF GM1 GANGLIOSIDE FOR TREATING BEHAVIORAL DEFICITS, NEUROPATHOLOGY AND EXTENDING LONGEVITY IN THE R6/2 MOUSE MODEL OF HUNTINGTON’S DISEASE

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Huntington’s disease (HD) is an autosomal dominant neurodegenerative disease caused by mutations in the Huntington (HTT) gene containing a long polyglutamine (CAG) stretch. This mutation leads to excessive production and accumulation of mutant huntingtin protein (mHTT) in the brain. This excessive mHTT protein accumulation causes motor, psychiatric, cognitive impairments and, ultimately, neuronal death. Currently, no effective treatment has been approved for HD. However, a promising line of research in therapeutics for HD, involves the use of GM1 ganglioside, as levels of GM1 are reduced in HD patients, which is thought to contribute to the disease progression. GM1 ganglioside is a sialic acid-containing glycosphingolipid that is found abundantly in the outer leaflet of the neuronal membrane in the brain. The initial source for GM1 came from bovine brains, but new sources, including synthetic as well as those obtained from the ovine brain, have been developed subsequently. Treatments with synthetic- and bovine- sourced gangliosides have been shown to reduce motor dysfunction in HD rodent models. However, the efficacy of treatment using ovine-sourced GM1, which is much more economical, has yet to be tested. The goal of this study was to compare the efficacy of ovine-sourced GM1 with that obtained from bovine sources on the motor and cognitive dysfunctions in the R6/2 mouse model of HD. In the current study, we used intraventricular osmotic pumps to deliver the different-sourced GM1 gangliosides for four weeks in five-weeks-old R6/2 mice. Rotarod-, open field-, Barnes-maze- and novel-object-recognition- tasks were used to measure behavioral abilities in the treated and non-treated mice. Results indicated that ovine-sourced GM1 gangliosides significantly reduced behavioral deficits in R6/2 mice on the rotarod- and open-field-, but not on Barnes-maze- or novel-object-recognition- tasks, compared to vehicle-treated and bovine-sourced-GM1-treated R6/2 mice. In addition, HD mice treated with ovine-sourced GM1 ganglioside showed a significant increase in body weight over their lifespan compared to vehicle-treated and bovine-sourced-GM1-treated R6/2 mice. Ovine-sourced GM1 treatment significantly increased the BDNF levels in the cortical and striatal regions and reduced the neurodegeneration of medium spiny neurons. Ovine-sourced and bovine-sourced GM1 ganglioside-treated HD mice increased longevity by nine and twelve days, respectively, compared to vehicle-treated HD mice. Our results suggest that ovine-sourced GM1 is at least as effective as bovine-sourced GM1 for reducing deficits in the R6/2 mouse model of HD.

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REDUCTION OF MEMORY DEFICITS FOLLOWING PROGESTERONE TREATMENT IN THE 3XTG MOUSE MODEL OF ALZHEIMER’S DISEASE

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Alzheimer’s Disease (AD) is a neurodegenerative disease that is characterized by severe memory deficits, aggregation of amyloid-beta (Aβ), and hyperphosphorylation of tau. Current treatments fail to alter the progression of AD, but progesterone, a gonadal and neurosteroid hormone with pleiotropic effects, exerted neuroprotective effects in several studies of brain dysfunction, including AD. However, our study is the first to test the efficacy of progesterone in reducing cognitive deficits in an animal model (3xTg mice) of AD. We utilized 48, 12-month-old male and female 3xTg and wild-type (WT) mice and treated them with daily, oral-gavage administration of progesterone (5 mg/kg) or vehicle (2,hydroxypropyl-beta- cytodectrine) for 40 days. Ten days after initiation of treatments, the mice were given 10 daily trials for 21 days in a water-T-maze, in which the mouse swam to find a hidden platform at the end of one of the two arms. Once the animal located the platform 80% of the time, the platform was re-located to the other arm and the number of successful reversals was used as the primary dependent variable. Our results indicated that progesterone-treated C57 wild-type males made more reversals than all other groups and the progesterone-treated 3xTg male mice performed at the level of vehicle-treated WT controls, indicating an ameliorative effect of progesterone on AD-like deficits in male 3xTg mice. Although further data analysis is required for the additional behavioral, histological, and biochemical assays, the current project clearly indicates that progesterone can enhance cognitive functioning in both AD and WT male mice.

![Figure 1. Number of Reversals in Water T-Maze Test:](image)

![Figure 2. Serum Progesterone levels.](image)

Blood serum levels were Daily oral-gavage injections of progesterone (5 mg/kg) or equivalent collected at time of behavior testing and tested via Luminex assay. volumes of the vehicle was administered for 40 days to C57 and 3xTg Progesterone-treated C57 wild-type mice had higher progesterone levels. For 21 of those days, the mice were tested in the Water-T-Maze levels than all other groups. One-way ANOVA was performed with a Eight ‘correct’ platform findings per day per mouse indicated a reversal Tukey’s HSD post-hoc. *** p < 0.0001, Progesterone treated- versus of platform side for the next day. From day 8 onwards C57 male mice vehicle-treated C57 groups. # p < 0.001, Progesterone treated C57 treated with progesterone had the most reversals. 3xTg vehicle treated mice versus Progesterone treated 3xTg mice. male mice had more reversals (#) and 3xTg vehicle treated male mice had significantly less reversals (*) compared to all other groups, using repeated measures ANOVA and Tukey’s HSD post-hoc analyses.
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ASSESSING PATHOGENIC CONFORMATIONS OF TAU IN PRIMARY NEURONS SEEDED WITH HUMAN-DERIVED PATHOLOGICAL TAU

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One of the pathological hallmarks of Alzheimer’s disease (AD) is the accumulation of pathological tau inclusions, and the multimerization of tau is likely a critical part of tau’s role in disease pathogenesis. Tau is capable of seeding aggregation in cells; however, the extent to which specific disease-associated pathogenic conformations can be effectively seeded remains an area of active investigation. To help address this gap, we seeded aggregation in primary neurons and assessed known pathogenic tau conformations that are linked to mechanisms of tau-induced toxicity. We used hippocampal primary neurons derived from tau knock-in mice. We seeded aggregation by treating neurons with human AD brain-derived tau (AD-tau). The AD-tau seed material was characterized by transmission electron microscopy (TEM) and biochemical assays that measure total tau and pathological modifications, such as phosphorylation (e.g. PHF-1+ tau) and two conformation specific antibodies (TNT1 and TOC1). TNT1 labels conformation-dependent display of an N-terminal domain known as the phosphatase activating domain (PAD) and TOC1 labels oligomeric tau, both of which are linked to toxicity. The AD-derived material showed morphologies consistent with small pieces of the characteristic paired helical filaments of AD on TEM, phosphorylation at the PHF-1 site on western blot, and the presence of oligomeric tau and PAD-exposed tau species in sandwich ELISAs. Following treatment of neurons with AD-tau, we used immunostaining to label TNT1 and TOC1 conformations at multiple time points (4-28 days days post-treatment). We observed a robust increase in TNT1 labeling over time in AD-tau-treated primary neurons, but little to no evidence of TOC1+ oligomeric tau species. To begin assessing consequences of tau seeding in this model, we determined whether overt cell loss occurred up to 28 days after seeding. There were no significant effects on the number of neurons or astrocytes in seeded cultures out to 28 days post-treatment suggesting that robust degeneration did not occur. Ongoing studies will assess additional functional consequences (e.g. axonal transport impairment) and potentially earlier measures of toxicity (e.g. synaptic and axonal toxicity) in seeded neurons. Together, our results suggest that seeding primary neurons with AD-tau drives some of the pathogenic conformations observed in human disease, but overt degeneration does not occur within 28 days of seeding. The presence of pathological forms of tau but lack of overt degeneration could indicate that more time is needed and/or that the functional consequences of these tau species are more subtle leading to synaptic loss or axonal degeneration.

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Widespread accumulation of cellular inclusions (Lewy Bodies) comprised of pathological alpha-synuclein (a-syn) and degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) are the defining pathological hallmarks of Parkinson’s disease (PD). A-syn preformed fibril (PFF) injections into rodents recapitulate both pathological features. In the rat PFF model, phosphorylated a-syn (pSyn) inclusion-containing neurons and reactive microglia (major histocompatibility complex II immunoreactive) peak in the SNpc at 2 months. This is followed by loss of dopaminergic phenotype and ultimately neurodegeneration at 5-6 months post-injection. In the present study, we leverage the early 2-month peak of synucleinopathy and neuroinflammation in the SNpc to detect transcriptomic changes relevant to early PD with the ultimate goal of identifying potential therapeutic targets for neuroprotection.

Three-month-old male and female hTH-EGFP rats received unilateral, intrastratal injections of sonicated mouse a-syn PFFs (total =16 μg) or an equal volume of vehicle as a control for surgical injection. At 2 months post-injection, GFP fluorescence was used to visualize the SNpc, allowing for precise laser capture microdissection (LCM) to enrich each sample for SNpc dopaminergic neurons and immediately adjacent cell subtypes. Total RNA isolation, followed by ribosomal RNA depletion, and library preparation were performed for RNA sequencing (RNASeq)

We identified 2,534 and 1,491 differentially expressed transcripts associated with PFF- inclusions in males and females, respectively (FDR < 0.2). Differentially expressed transcripts from both males and females were compared to determine transcripts impacted by early synucleinopathy across sex. In both males and females, 326 transcripts were differentially expressed; of those, 172 were similarly upregulated and 137 similarly downregulated in both sexes, with the remaining 17 transcripts uniquely regulated in each sex (FDR < 0.2). After Weighted Gene Correlation Network Analysis (WGCNA), we identified 22 differentially expressed genes that were also hub genes in correlated expression networks. Upregulated hub genes showed enrichment for immune related pathways, where downregulated hub genes showed enrichment for dopamine signaling pathways. Our results are the first comprehensive analysis of transcriptomic changes specifically associated with early synucleinopathy. In future studies, pharmacological or genetic manipulation of identified synucleinopathy-associated pathways will design neuroprotective strategies.

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NEW ROD PHOTOTRANSDUCTION-SENSITIVE OCT-BASED BIOMARKERS

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Purpose: To test the sensitivity of novel imaging biomarkers to phototransduction.

Methods: Light vs. dark-adapted C57BL/6J mice, and mice null for transducin (i.e., Gα; Gnat1−/−) that can not undergo phototransduction, were studied. We compared the extent of dark-induced thinning of the external limiting membrane-retinal pigment epithelium (ELM-RPE) thickness, a known proxy for phototransduction / cGMP / mitochondrial-driven / pH-triggered / RPE-water removal from the subretinal space to the magnitude of a hyporelective band (HB) at the rod tips, a biomarker that is correlated with ELM-RPE thickness in wildtype mice, but shows a pH-independent response. An additional comparison was performed against a new parameter, the shape of the light-scattering, mitochondria-rich IS measured in an unbiased manner with a quantitative ellipse descriptor to generate the minor: major aspect ratio (AR) from its AC profile.

Results: Dark-adapted C57BL/6 mice showed the expected thinning of the ELM-RPE, as well as reduction in HB magnitude compared to that in light-adapted conditions. In addition, we also found that the IS AC AR was significantly greater in the dark than in the light. In contrast, Gnat1−/− mice did not show light-dark differences in ELM-RPE, HB magnitude, or IS AC AR.

Conclusions: Our first-in-kind data unequivocally demonstrate that the HB and shape of the IS AC profile are functionally regulated by phototransduction, a major regulator of rod mitochondria activity, as has been shown earlier for the ELM-RPE thickness. This suite of OCT-based biomarkers is expected to address a so-far unmet gap in the diagnosis and treatment of rod energy-landscape abnormalities in patients with neurodegenerative diseases.
REVERSAL OF COGNITIVE DEFICITS FOLLOWING SILENCING OF THE MUTANT HUNTINGTIN GENE THROUGH CRISPR-CAS9 THE YAC128 MOUSE MODEL OF HUNTINGTON’S DISEASE

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Huntington’s disease (HD) is an inherited neurodegenerative disorder that is characterized by progressive cognitive and motor behavioral abnormalities. HD is caused by trinucleotide repeat expansions of cytosine adenine and guanine (CAG), on chromosome 4 which results in the production of the mutant huntingtin (mHTT) protein/aggregates. This excessively produced and accumulated mHTT is detrimental to the medium spiny neurons, the major cell population on the caudate and putamen regions of the brain affected in HD. The wild type HTT protein is a nucleic and cytoplasmic protein that is involved in chemical signaling, but not mHTT protein. These mHTT protein aggregates impair the transport of brain-derived neurotrophic factor (BDNF) by cortical neuron axons which further worsen the availability of BDNF in the striatum and prevent its binding on TrkB receptors on striatal neurons. This in turn results in the triad symptoms (motor, cognitive, and psychiatric) seen in HD patients. To date there is no cure available for HD. Recent advancements with the gene editing tool, CRISPR-Cas9, to knock down or block the transcription of mHTT. In our study, we have constructed two CRISPR-Cas9 plasmids with gRNAs that target open reading frame (uORF) of 5’ UTR. We injected the adeno-associated virus encapsulated CRISPR-Cas9 intracranially, in the striatum of YAC128 mice to investigate the gene-silencing ability. This mouse model is genetically modified to mimic the human HD conditions having the full length human HTT gene in the mice. Behavioral assessments using rotarod performance testing indicated that gene silencing with CRISPR-Cas9 did not reduce motor behavioral deficits in YAC128 mice but showed significant improvements in cognitive deficits in learning and memory during water-T-maze testing. Although further data analysis is required for the additional behavioral, histological, and biochemical assays, the current project clearly indicates that gene editing with CRISPR-Cas9 can enhance cognitive functioning in HD mice.

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Integrative Physiology and Behavior; Motivation and Emotion

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LOST IN TRANSLATION? COMPARING THE PROPERTIES OF SYMPATHETIC SPLANCHNIC NERVE ACTIVITY OF RATS UTILIZING ANIMAL AND HUMAN DATA ANALYSIS METHODS

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INTRO Blood pressure is regulated by the brain via multiple mechanisms, with significant contributions from the sympathetic nervous system. Electrical recordings of sympathetic nerve activity (SNA) can be measured directly in humans and animals, using the technique of microneurography. SNA occurs in the form of bursts when individual nerve fibers fire together as groups in response to specific stimuli. The splanchnic nerve for instance, contains sets of sympathetic efferent fibers which tonically fire to increase or decrease blood vessel constriction in response to small changes in blood pressure. Differences in signal processing and physiology between human and rat models could lead to differences in how SNA bursts are quantified – consequently, influencing the interpretation of how SNA modulates blood pressure. PURPOSE The purpose of this study was to investigate whether analysis methods used for human SNA (but tailored for rats) leads to different results and interpretation compared with analysis typically conducted in rats. HYPOTHESIS We hypothesized that burst frequency (the number of SNA bursts per second) will be significantly lower when using human versus rat analysis methods. METHODS To test this hypothesis, we analyzed splanchnic nerve recordings (90 seconds) from 4-week-old, anesthetized, Sprague-Dawley rats (n=6). To resemble the analysis of human data, we used a longer duration integration time constant (100 msec), but maintained an appropriately short latency and time window to detect one burst for each cardiac cycle. To conduct the analysis of rat data, we used a shorter integration time constant (28 msec), while enabling more than one burst to be detected for each cardiac cycle. We used ShinyApp, a customized software on the R Studio platform written for human data and LabChart7, often used in rat studies. RESULTS After conducting a paired samples t-test, our preliminary results were unable to detect a difference in burst frequency when comparing ShinyApp (3.1352 (1.1350 Hz; STD)) compared to LabChart7 (4.1533 (1.1173 Hz)); p = 0.2663. The statistical power to detect a difference was calculated to be low (0.34), indicating the necessity for further research. CONCLUSION Preliminary results suggest that data analyses for human and rat data lead to comparable results. However, we speculate that SNA bursts are being integrated as fewer larger and individual bursts per heartbeat for human data and numerous, smaller bursts per heartbeat for rat data. It would be important to understand how human and animal nerve activity is being differently analyzed in research on blood pressure regulation because this may affect the development of interventions targeting neural pathways in managing blood pressure.

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BIG 5 PERSONALITY TRAITS AS THEY RELATE TO RISK BEHAVIORS

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Prior research has suggested that different neurological components have significant implications regarding an individual’s personality, and thus the likelihood of engaging in risk behaviors. Extraversion has been found to be linked to increased cortical folding within the reticular formation as well as increased cortisol levels, which suggest the need for more external stimuli, while Neuroticism significantly impacts sleep patterns in individuals. The impact of neurological structures and processes on personality traits and individual behaviors suggests a relationship between personality traits and risk behaviors. In order to test the relationship between personality traits and risk behaviors, a survey was conducted in which participants aged 18-59 were assessed for Big-5 personality traits, as well as questions regarding their engagement in and consideration of engaging in risky behaviors such as self-harm, drug use, and sexual recklessness. It was hypothesized that extraversion and neuroticism would have significant relationships with the total amount of risk behavior committed, as well as with the motive for engaging in risk behaviors. Significant relationships regarding neuroticism, conscientiousness, and risk-behaviors were discovered. Because of the relationship between personality traits and the chemical/structural make-up of the brain, and because of the relationship between personality and risk-behavior engagement, there is a potential for a relationship between an individual’s neurological make-up and their likelihood of engaging in risk behaviors. Using the data found here, there is potential for identifying individuals who may benefit from professional intervention prior to their participation in behaviors that put themselves or others at risk of harm. Future research should explore neurological variables more in-depth, such as looking at chemical/structural components in individuals who engage in risky behaviors.
INFLUENCE OF ENDOGENOUS OVARIAN HORMONES ON SYMPATHOEXCITATION PRODUCED BY ACTIVATION OF THE ROSTRAL VENTROLATERAL MEDULLA IN ACTIVE AND SEDENTARY FEMALE RATS

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Cardiovascular disease (CVD) is the leading cause of mortality globally. One of the major modifiable risk factors for CVD is a lack of regular exercise. A non-modifiable risk factor for CVD is male sex, where risks for CVD are lower in young women compared to age-matched men, possibly due to protection afforded by female sex hormones. However, following menopause, women are at greater risk for high blood pressure. Blood pressure is controlled by a region of the brainstem known as the rostral ventrolateral medulla (RVLM). The RVLM directly regulates vasoconstriction produced by the sympathetic nervous system. Interestingly, in previous studies, four-week-old female rats, which lack an overt estrous cycle, exhibited greater sympathoexcitation following activation of the RVLM when compared to age-matched males. Although these data suggest inherent sex differences independent of the estrous cycle, the contribution of endogenous ovarian hormones to sympathoexcitation produced by direct activation of the RVLM in the context of sedentary versus active conditions is currently unknown. Therefore, the purpose of our study was to examine the impact of sedentary versus active conditions in the presence and absence of ovarian hormones. We hypothesized that removal of the ovaries (OVX) in female rats would yield low estradiol levels and enhance centrally-mediated sympathoexcitation when compared to intact (sham-operated), sedentary female rats. We also predicted that physically active conditions offset enhanced centrally-mediated sympathoexcitation by the RVLM in ovariectomized (OVX) female rats. In preliminary studies, female Sprague-Dawley rats underwent OVX or sham surgeries at 4 weeks of age. Following recovery, active rats were placed in cages with 24-hr access to running wheels, while sedentary rats were placed in cages without running wheels. At 16 weeks of age, splanchnic sympathetic nerve activity (SSNA) and blood pressure were recorded from Inactinanesthetized, sham-operated or OVX, active or inactive females (n=2 ea). Microinjections of glutamate [30 nl] at 100 mM were performed into the RVLM. Lavages and blood samples were performed to determine the estrus cycle and confirm OVX. Our preliminary data indicate that glutamate microinjected into the RVLM increased SNA in all groups but appeared to produce greater increases in SNA in OVX sedentary versus sham-operated, sedentary rats (2.2±0.9 mV vs. 0.9±0.3 mV, respectively). In contrast, glutamate produced greater increases in SNA in active sham-operated versus OVX rats (1.9±0.1 mV vs. 1.0±0.1 mV, respectively). Our results suggest that ovarian hormones appear critical to RVLM neuroplasticity and attenuate glutamatergic excitation in sedentary but not active females. They also demonstrate the complexity of the interaction between a sedentary lifestyle and a loss of female reproductive hormones in contributing to increased risks of CVD and hypertension. [R01-HL096787; Wayne State University Barber Program].
Neuronal Ensemble in the Medial Prefrontal Cortex is Involved in Anxiety-like Behavior in Rats with a History of Binge Cocaine Administration and Drug-abstinence

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Cocaine abuse remains a public health problem with no FDA-approved medications to treat cocaine use disorder. Previous results show that rats given chronic binge-pattern cocaine administration display a disinhibited hyper-exploratory phenotype in novel environments, indicative of anxiety-like behavior. A growing body of evidence also suggests that targeted neuronal ensemble ablation in the medial prefrontal cortex (mPFC) can affect cocaine seeking and taking behaviors. Therefore, we assessed the contribution of mPFC neuronal ensembles in the disinhibited hyper-exploratory phenotype observed in a rat model of chronic binge cocaine administration. Female and male heterozygote Fos-LacZ Wistar rats were administered either cocaine (15 mg/kg/injection) or saline (1 ml/kg/injection) for 14 days using a binge-pattern administration paradigm (3 times daily at 1-hour intervals) followed by 14 days of forced abstinence (in the home cage). Injections took place in locomotor activity chambers (LMA) on days 0, 1 and 14 and their respective home cages on other days. Rats were then assessed for novelty induced exploration in a black plexiglass open field box. After 90 minutes, the time point of maximum Fos expression and hence -galactosidase expression in Fos-LacZ rats, Daun02 or vehicle was intracranially-injected bilaterally into the mPFC. After 3 days of rest in the home cage, all rats were tested for cocaine-induced hyper-exploratory behavior on a raised elevated plus maze consisting of closed/open arms. Our results show that a sexually dimorphic LMA response to cocaine occurred over the 14 days of cocaine administration, with females showing greater behavioral sensitization. Additionally, rats treated with cocaine displayed higher anxiety-like behavior by spending less time in the center of open field following 14 days of cocaine withdrawal. In the elevated plus maze, cocaine-treated rats who received vehicle injections continued to show anxiety-like behavior, but Daun02 chemoablation of mPFC neuronal ensemble attenuated cocaine-induced anxiety-like behavior by increasing time/entries in open arms. The finding that ensemble ablation of one context carries over to a new context is an interesting finding and suggests a shared neuronal ensemble in anxiety provoking stimuli of the mPFC. Furthermore, neuronal ensembles that underlie anxiety-like behavior during cocaine withdrawal can contribute to dysfunction of the mPFC that plays a role in drug-relapse.
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ONE BODY AND MIND: TRAUMA-RELATED PSYCHOPATHOLOGY IS STRONGLY ASSOCIATED WITH SOMATIC SYMPTOMS AND HEALTH PERCEPTIONS IN PERSONS RESETTLED AS REFUGEES OF SYRIA AND IRAQ

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Objective and Rationale: Somatic symptoms are commonly reported in persons with posttraumatic stress disorder (PTSD) and may contribute to worsened objective and perceived health. Elevated proinflammatory cytokines and acute phase proteins have also been associated with severity of PTSD (as well as anxiety and depression) and may mediate the relation between severity of psychological and somatic symptoms. Persons resettled as refugees experience higher rates of trauma-related psychopathology compared to the general population due, in part, to civilian war trauma exposure, torture, forced migration, and chronic stress of post-migration living difficulties. Therefore, the objective of this research was to determine the relation between severity of trauma-related psychopathology (PTSD, anxiety, and depression) and somatic symptoms, and to investigate inflammation as a biological mechanism linking psychopathology and somatic symptoms. We leveraged a relatively homogenous cohort of Middle Eastern/North African (MENA) refugees who had been exposed to civilian war trauma and forced migration within the same timeframe, resettled between 2016 and 2017, and were living in Southeastern Michigan.

Methods: n=81 participants (43F, Mage=38.11 +/- 11.18 years) were assessed one to three years following resettlement in Southeastern Michigan from Syria. Trauma histories were obtained using the Life Events Checklist (LEC), and severity of self-reported posttraumatic stress, anxiety, depression, and somatic symptoms were measured using the PTSD Checklist, Hopkins Symptoms Checklist, and Somatic Symptoms Scale 8. Capillary blood samples were obtained for measurement of interleukin 18 (IL-18) and C-reactive protein (CRP), that were then quantified using the Perkin Elmer AlphaLISA platform.

Results: Somatic symptoms were significantly, positively correlated with total PTSD symptoms (r=.61, p<.001), reexperiencing (r=.45, p<.001), avoidance (r=.39, p<.01), and arousal symptoms (r=.49, p<.001); anxiety (r=.61, p<.001); and depressive symptoms (r=.61, p<.001). Cumulative trauma was not significantly associated with severity of somatic symptoms, r=.14, p>.05. Trauma-related psychopathology (PTSD, anxiety, and depression) predicted significant variance in somatic symptoms above and beyond that of age, sex, medical conditions, medication use, and trauma exposure. However, levels of pro-inflammatory IL-18 and CRP did not explain any additional variance in somatic symptoms. We did not observe any significant differences between the biological sexes, nor did we find sex to be a moderator of the relations described herein.

Conclusions: Somatic symptoms could be a major contributor to the dysfunction and distress that patients may experience in the aftermath of trauma. Somatization is not always addressed in traditional psychotherapy and pharmacotherapy, highlighting the need for integrated care. In our sample, unlike previous studies, inflammation was not associated with trauma-related psychopathology. These findings highlight the importance of future biomarkers studies examining the role of inflammation to also look at variables such as time from exposure, levels of glucocorticoids, sympathetic arousal, and genetic variability. These findings also highlight the need to prioritize inclusion of currently underrepresented populations, as biological processes may vary based on ancestral variation and environmental exposures, requiring personalized medicine practices.
BDNF-RELATED NEUROPLASTIC MECHANISMS IN THE ROSTRAL VENTROLATERAL MEDULLA OF ACTIVE VERSUS SEDENTARY RATS

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The rostral ventrolateral medulla (RVLM) is an integrative region in the brainstem containing sympathoexcitatory neurons that control blood pressure by regulating sympathetic nerve activity. The regulation of sympathetic activity is crucial in maintaining long-term cardiovascular health, but sympathetic overactivity likely contributes to cardiovascular disease, the leading cause of death in the United States and globally. RVLM neurons undergo structural and functional neuroplasticity in response to chronic sedentary conditions compared to physically active conditions. For example, previous studies from our laboratory show that RVLM neurons of sedentary animals have increased dendritic branching compared to physically active animals, suggestive of greater excitability in inactive conditions. Dendritic branching is regulated by brain-derived neurotrophic factor (BDNF). BDNF is initially synthesized in its pro-form, proBDNF, before undergoing proteolytic cleavage to release its mature form, mBDNF. Unpublished studies from our laboratory indicate that chronic physical activity increases proBDNF in the RVLM; whereas, mBDNF is higher in the RVLM following sedentary conditions. The conversion of proBDNF to mBDNF is facilitated by the serine protease tissue plasminogen activator (tPA). It is possible that differences in the expression of tPA in the RVLM contributes to physical activity- and inactivity-induced neuroplasticity. The purpose of this ongoing study is to determine whether physically active or sedentary conditions affect the synthesis of mBDNF through tPA in the RVLM. We hypothesized that tPA levels will show increases in the RVLM of sedentary animals compared to active animals. To test this hypothesis, we will divide 4-week-old male Sprague-Dawley rats into two groups (n=4 each): physically active (24-hour access to in-cage running wheel) and sedentary (no running wheel), housed for 12 weeks. Rats will be sacrificed for fresh tissue removal, and brainstems will be cryosectioned at 80 μm and serial sections will be collected on uncoated slides. Bilateral tissue punches will be retrieved from cryostat sections and placed in centrifuge tubes containing lysis buffer and protease inhibitors. Post-punched sections will be stained with cresyl violet to determine the location of the RVLM relative to the caudal pole of the facial nucleus. Punches will be pooled for western blotting to examine the expression levels of tPA using validated antibodies. Preliminary data shows that expression of tPA occurs from the caudal to rostral aspect of the RVLM. Ongoing experiments are analyzing tPA expression following sedentary versus physically active conditions. Since our previous studies indicate that physically active rats have decreased mBDNF levels in the RVLM compared to sedentary rats, we expect that tPA levels will also be lower in active versus sedentary rats. Decreases in mBDNF could lead to decreased dendritic branching and lowered sympathetic nerve activity. Alternatively, increased tPA levels in sedentary individuals could stimulate increases in mBDNF, dendritic branching, and sympathetic nerve activity. High blood pressure is one of the main contributors to cardiovascular disease. Exercise or a sedentary lifestyle each could cause changes in the brain, leading to changes in sympathetic nerve activity and blood pressure. Understanding how exercise and inactivity alter the brain could provide a means to decrease the prevalence of cardiovascular disease. (HL096787, WSU Physiology SURF).
DESIGNING AN OPEN-SOURCE DIGITAL HOMECAGE FOR CONTINUOUS PHENOTYPING OF DEPRESSION-LIKE BEHAVIOR IN MICE

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Acquisition of behavioral and physiological data in the mouse is integral to the success of many neurobiological experiments. We aim to develop an automated method of reliably tracking mouse behavior in the home cage, particularly for the longitudinal study of depression-like behavior in a more naturalistic environment than brief assays such as the forced swim test. The core system consists of an infrared camera combined with the automated Cleversys mouse behavior analysis system. In addition to video data, food pellet consumption, water consumption, and running wheel revolutions are me-stamped and recorded. A more comprehensive analysis of behavioral patterns may reveal phenotypes characteristic of depression-like behavior, and provide further insight into the consequences of psychological stress.
ROLE OF THE VENTRAL PALLIDUM IN THE REGULATION OF SOCIAL PLAY BEHAVIOR IN JUVENILE RATS,

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Social play is a rewarding behavior that is displayed by juveniles of many mammalian species. Engagement in social play behavior is important for the development of social competencies throughout life. Children diagnosed with neurodevelopmental disorders such as autism spectrum disorder (ASD) show deficits in social play, which may contribute to life-long impairments in social communication. It is theorized that children diagnosed with ASD experience social interactions as less rewarding, which may be due to impairments in the mesolimbic reward pathway, the brain’s reward pathway. Therefore, it is essential to understand how the mesolimbic reward pathway modulates the expression of typical and impaired social play behavior. In the present study, we aimed to determine the role of the ventral pallidum (VP) in modulating social play behavior in juvenile male and female rats. The VP is a brain region in the mesolimbic reward pathway that regulates adult social behaviors such as maternal behavior, pair-bonding, and sociosexual motivation in rodents. However, the role of the VP in regulating juvenile social behaviors, such as social play behavior, is unknown. We first determined whether activation of the VP is required for the expression of social play behavior by temporarily inactivating the VP via local infusions of the GABA\(_A\) receptor agonist muscimol. We found that pharmacological inactivation of the VP decreased social play behaviors in juvenile male and female rats compared to their vehicle-treated counterparts. Next, we determined whether exposure to social play altered neuronal activation of the VP by using \(fos\) as a marker of cellular activity. We observed that exposure to social play increased the number of \(fos^+\) cells in the VP of males while no changes in \(fos^+\) cells were observed in females. We found a potential sex-specific correlation between the time spent engaging in social play and number of \(fos^+\) cells in the VP, with males trending in a negative and females trending in a positive correlation. Together, these findings provide the first evidence that activation of the VP is required for the typical expression of social play in both sexes but that exposure to social play recruits VP cells in a sex-specific manner.
BENZOFURAN DERIVATIVES ARE POTENT SEROTONIN RELEASERS AND SUBSTITUTE FOR THE DISCRIMINATIVE STIMULUS EFFECTS OF MDMA IN RATS.


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3, 4-Methylenedioxymethamphetamine (MDMA) is currently under evaluation in phase III clinical trials for the treatment of post-traumatic stress disorder (PTSD) and is expected to be approved by the FDA for clinical use in the near future. MDMA is also a popular abused substance with risks for cardiovascular toxicity and neurotoxicity, particularly when misused at higher doses. Characterization of the behavioral and neurochemical effects of novel psychoactive substances is an essential step in the development of safer alternative therapeutic agents. Drug discrimination is a preclinical behavioral assay with pharmacological specificity for characterizing in vivo drug actions in the central nervous system. This project implemented rodent drug discrimination studies and serotonin transporter assays to characterize the enantiomers of 5-(2-methyaminopropyl) benzofuran (5-MAPB) and 5-(2-methylaminobutyl) benzofuran (5-MBPB), two benzofuran derivatives with potential MDMA-like effects.

Eight male Sprague-Dawley rats were trained in a standard two-lever operant drug discrimination procedure under a fixed ratio 20 schedule of food reinforcement to discriminate MDMA (1.5 mg/kg) from saline. Once criteria for stimulus control were established, stimulus substitution tests were conducted with (RS) 5-MAPB, (R)-5-MAPB, (S)-5-MAPB, (R)-5-MBPB, and (S)-5-MBPB. All substances produced dose- dependent increases in MDMA-lever responding and full substitution at the highest dose assessed with minimal effects on response rate. The 5-MAPB and 5-MBPB enantiomers differed slightly in potency. Serotonin release assays using rat synaptosomes and [3H] serotonin indicated all substances are substrate-type releasers of serotonin with nanomolar potency. S-enantiomers appeared to have a somewhat higher potency than their corresponding R-enantiomers. The benzofuran scaffold may allow the development of substances that retain MDMA-like therapeutic effects while reducing toxicities associated with MDMA.
Investigations on complementary and alternative treatments for anxiety and depression have contributed to a recent re-emergence of psychedelic research. Alongside the success of psychedelic-assisted psychotherapy, microdosing of psychedelics, the practice of consuming a sub-perceptual dose of a psychedelic substance, has become increasingly popular among recreational users. User reports from blogs and self-report studies suggest that microdosing with psychedelics such as Lysergic Acid Diethylamide (LSD), has positive effects on mood, anxiety, and cognitive flexibility. Despite the increasing popularity of microdosing, few preclinical studies have systematically evaluated the effects of chronic, intermittent sub-perceptual doses of LSD that approximate dosing patterns similar to those reported by users who have microdosed. The current study investigated the effects of chronic, intermittent low-dose Lysergic Acid Diethylamide (LSD) in two rodent screening models predictive of the anxiolytic or anxiogenic properties of drugs. Sixty-four adult, male Sprague-Dawley rats were injected with LSD (0.02, 0.04, 0.08 mg/kg) or vehicle (N=16 per group), once every three days over 43 days (15 injections in total). Eight rats from each treatment group were assessed in the light-dark box (L/D) test, and the remaining animals were assessed in an open field test (OFT) 48 hours after the eighth injection and again 24 hours after the 15th injection. L/D test dependent variables were latency to enter, number of entries and time spent in the brightly lit compartment. OFT dependent variables were distance traveled and time spent in the center. Statistical analyses indicated no significant treatment effects on any of the dependent variables assessed in the L/D test or OFT after either eight injections or after 15 injections. A two-way ANOVA with repeated measures on treatment duration (test day) indicated a statistically significant main effect of test day for distance traveled, but not for time in center in the OFT. A similar analysis of the L/D test results also revealed a statistically significant effect of test day on latency to enter and time spent in the lit side, but not entries into the lit side. These findings suggest chronic, intermittent low-dose (CILD) LSD does not appear to produce significant anxiolytic or anxiogenic-like behaviors in rats as indexed by the light/dark or open field tests. More research involving additional behavioral tests is warranted to help elucidate the behavioral effects of CILD LSD in rodents, to provide a better understanding of the phenomenon reported by humans.
CHEMOGENIC EXCITATION OF HYPOTHALAMIC OXYTOCIN NEURONS MODULATES SOCIAL PLAY IN JUVENILE FEMALE RATS

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One social behavior that is commonly displayed by juveniles of many mammalian species is that of social play. This highly rewarding behavior is critical in the development of important social skills throughout life. Deficits in social play behavior are observed in children diagnosed with autism spectrum disorder (ASD), and it has been hypothesized that these deficits contribute to their lifelong impairments in social skills. Although the number of ASD diagnoses is rising, there are no leading therapeutic options that can help alleviate the social deficits associated with ASD. The neuropeptide oxytocin (OXT) has an important role in regulating social behaviors, including social play behavior, and is currently being tested for its potential to alleviate social deficits in ASD. However, the underlying neural mechanisms of OXT signaling in juvenile social play are unknown. OXT is mainly synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. Therefore, we aimed to investigate the role of PVNOXT and SONOXT systems in social play in juvenile female rats. To accomplish this we used an excitatory chemogenic approach referred to as Designer Receptor Exclusively Activated by Designer Drugs (DREADDs). These DREADDs are under the control of the OXT promoter, which allows for selective stimulation of OXT producing neurons in the PVN or SON when given the inert ligand Clozapine-N-oxide(CNO). We demonstrate that stimulation of PVNOXT, but not SONOXT, neurons increases the duration of social play. However, stimulation of SONOXT neurons increases social investigation in female juvenile rats. Our preliminary studies demonstrate the selective involvement of PVNOXT neurons in social play of female juvenile rats which could be helpful in a more targeted approach when using OXT as a potential therapeutic option for children with ASD.
COMPARISON OF SYMPATHETIC BURST CHARACTERISTICS IN PREPUBERTAL MALE AND FEMALE RATS

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Cardiovascular disease leads to an overall reduction in the quality of life. In the United States, cardiovascular disease is also the leading cause of mortality, costing over $300 billion annually. Interestingly, the risk of cardiovascular disease differs among men and women. Women of reproductive age have a lower prevalence of cardiovascular diseases; however, after the onset of menopause, the risk is higher in females compared to males. The cardiovascular system is controlled by the sympathetic nervous system in part via its innervation of the heart and blood vessels. One way to analyze sympathetic nerve activity is by quantifying the characteristics of sympathetic bursts. Increases in the frequency, amplitude, and size of sympathetic bursts may contribute to cardiovascular diseases including hypertension. The purpose of this study was to analyze sympathetic bursts for potential differences between four-week-old male and female rats, an age at which the estrous cycle has not begun and the cardioprotective effects of reproductive hormones are presumed to be minimal. We hypothesized that resting splanchnic sympathetic burst characteristics would be lower in female rats compared to male rats as the risk to develop cardiovascular diseases is lower in premenopausal females compared to age-matched males. Splanchnic sympathetic nerve activity (SSNA) was recorded from 5 male and 5 female Inactin-anesthetized rats at 4 weeks of age using LabChart. Sympathetic bursts were obtained by rectifying and integrating the raw SSNA signal. Burst frequency was calculated in one-second intervals by using the cyclic measurements tool in LabChart. Burst height and width were obtained by using the Peak Parameters extension. Burst frequency, height and width were calculated using the Peak Parameters extension in LabChart. Our results showed that there was no significant difference in burst frequencies between male and female rats (4.18 ± 0.46 vs 5.56 ± 1.25 Hz, respectively, p= .32). In addition, there was no significant difference between males and females in burst height (1.6 ± 0.4 vs 1.3 ± 0.5 mV.s, p = .62, respectively) or width (135.3 ± 10.8 ms vs 123.8 ± 18.0 ms, p= .59, respectively). Our preliminary data suggest prepubertal male and female rats display similar SSNA burst frequency, height, and width; however, additional studies are necessary to achieve appropriate statistical power before these conclusions can be confirmed. Future experiments may be conducted to explore the differences in burst characteristics in females once the estrous cycle begins. To explore this possibility, we plan to study SNA burst characteristics in sexually mature rats. We expect to further understand the effect of hormones and sex-dependent development of cardiovascular diseases.

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EFFECTS OF BULLYING ON ANXIETY SYMPTOMS IN ADOLESCENTS: PARENTAL WARMTH AS A POTENTIAL BUFFER

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Anxiety disorders are the most common psychiatric disorder, affecting nearly one in three, and commonly begin during adolescence. Peer victimization, or bullying, is common (10-30%) among adolescents and has been shown to increase risk of anxiety disorders. Prior research suggests that a healthy parent-child relationship may protect adolescents from anxiety. Here, we examine whether peer victimization is associated with anxiety symptoms in early adolescents, and whether parental warmth can buffer the effects of peer victimization on anxiety. One-hundred and fifty early adolescents (M±SD = 12±1.5, 45% female) and one parent/guardian completed online surveys. Adolescents self-reported on peer victimization, anxiety symptoms, and parental warmth using validated questionnaires, including the Revised Personal Experiences Checklist (De Los Reyes & Prinstein, 2004), Screen for Child Anxiety Related Disorders (Birmaher et al., 1997; Monga et al., 2000), and the Parent Child Relationship Scale (Pianta, 1992), respectively. Correlation analyses were used to examine bivariate associations among peer victimization, anxiety, and parental warmth, and hierarchical regression was used to test for interactive effects of peer victimization and parental warmth on anxiety. Peer victimization was positively associated with anxiety symptoms, and negatively associated with parental warmth. Parental warmth was also negatively associated with anxiety. There was no significant interaction between peer victimization and parental warmth on child anxiety symptoms. All results remained consistent when controlling for age and gender. These results suggest that peer victimization may increase risk of adolescent anxiety. Although parental warmth was associated with lower anxiety, we did not find evidence that parental warmth can buffer the negative effects of peer victimization on anxiety. These findings highlight the need to improve mental health outcomes in victims of bullying. They also suggest that parents, in general, can play an important role in supporting adolescents' mental health.
SIZE COMPARISONS BETWEEN RAT BRAINSTEM BASED ON SEX, AGE, AND ACTIVITY LEVELS

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Background:
Organ function, including that of the heart, will often differ based on size. For example, a bigger heart will produce more cardiac output. Similarly, bigger lungs contain more ventilating capacity. Recent studies from our laboratory have found that female rats have bigger responses in peripheral nerves that control blood pressure in response to brain activation. This led to a question: Does brain size affect brain function? And if so, does brain size correlate with any statistic about the rat itself? Therefore, the purpose of this study was to examine the brainstem using measurements of area, height, and hemi-section width, and determine whether a relationship between brainstem size varies with exercise versus sedentary conditions, between males and females, or if the size develops with age. This led us to our hypothesis: If a rat is an active male rat who is older, then it will have the largest brain stem area.

Methods:
Brainstem sections containing the RVLM were stained with Neutral Red on slides so brain regions could be observed microscopically. To ensure that the same cross-section of brainstem was measured for all of the slides, the caudal pole of the facial nucleus (FN0) was identified in every animal. Sections containing FN0 were measured for all brainstems. Data collection started with measurement of brainstem slides containing rostral ventrolateral medulla (RVLM) cross-sections from different rats. The features that were measured from these brainstems were: area (mm^2), height, and hemi-section width (mm). The brainstems belonged to rats of different size, weight, sex (male vs female), activity level (wheel runner vs sedentary), and age (4-wks old, 8-wks old, and 16-wks old). Sufficient data was not available for all groups. After finding the area, height, and hemi-section width, the data was statistically analyzed in the context of age, sex, and activity level.

Results:
In four-week-old rats, we were unable to detect a difference in brain stem area in males and females (18.7+0.5 vs. 18.0+0.3 mm^2, respectively, SEM; n=5). However, we did find sex differences in the 16-week-old active rats, with males having a significantly greater brainstem area compared to females (21.8 + 0.36 vs. 20.4+0.42 mm^2 ; respectively; p=0.03). We also found that 16-week-old males had significantly more area compared to 4-week-old males (21.8+ 0.4 vs. 18.7+ 0.6 mm 2 ; p=0.00). Brain stem areas of 16-week-old active vs. sedentary female rats were not significantly different.

Conclusion:
We conclude that at 16 weeks of age, male rats have a larger brain stem area than do age-matched females. We also found that 16-week-old male rats had larger brain stems than 4-week-old male rats. Lastly, exercise versus sedentary conditions did not change brain stem size. Thus, our hypothesis that, “If a rat is an active male rat who is older, then it will have the largest brain stem area.”, was not validated by the current study.
PROFILING INDIVIDUAL BEHAVIORAL DIFFERENCES IN ZEBRAFISH ACROSS TIME USING THE NOVEL TANK TEST

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Every individual behaves differently. For example, some people like to stay home and enjoy their own company, but others enjoy an outing and social gathering. Such individuality in behavior is common, but how these differences arise is poorly understood. Behavior can vary among individuals as a function of biological factors such as sex and genetics. To model individual differences, we have analyzed zebrafish swim behavior during the exploration of a novel tank. When exposed to a novel environment, zebrafish exhibit a variety of exploratory behaviors. To assess these behaviors, we automatically captured postures and three-dimensional swim patterns using depth-sensing cameras. We recorded behavior from 400 animals across four commonly used inbred strains (AB, Tu, TL, and WIK) and both sexes to study the influence of sex and genetics on behavioral variability. Markerless tracking of the fish was done using a deep learning approach (DeepLabCut) to identify the relative positions of different body parts (Head, Trunk, and Tail). We found that fish posture stratifies into approximately 11 different clusters. Additionally, our analysis of behavioral parameters found that fish behavior categorizes into 5 distinct clusters. Finally, we found that clustering membership is consistent over time and is influenced by strain and sex.
PRECLINICAL BEHAVIORAL ASSESSMENT OF THE ANXIOLYTIC AND ANTIDEPRESSANT-LIKE EFFECTS OF CHRONIC, INTERMITTENT LOW-DOSE PSILOCYBIN IN MALE SPRAGUE-DAWLEY RATS

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Recent studies have demonstrated the clinical efficacy of psychedelic-assisted psychotherapy for treatment-resistant depression and anxiety. Amidst the overall success of recent clinical trials using a single high dose of psilocybin, anecdotal reports indicate anxiolytic and antidepressant effects following a repeated low dose regimen. As therapeutic outcomes are often tightly intertwined with the individual’s subjective experience, animal models are used as objective measures to investigate the underlying mechanisms responsible for the putative antidepressant/anxiolytic effects of psychedelics. Three rodent models predictive of anxiolytic or antidepressant effects were used to evaluate effects of chronic intermittent low dose (CILD) psilocybin treatment; the Light/Dark conflict test (L/D), an open field test (OFT), and the forced swim test (FST). Rats were treated with vehicle or psilocybin (0.025, 0.05, 0.1 mg/kg) every 72 hours over a 48-day period. Tests were conducted 48 hours after the eighth injection (day 24) (L/D, OFT), 48 hours after the 16th injection (day 48) (L/D, OFT, FST), and 12 days after the last injection (day 58) (OFT, FST). Results from the current study indicate that CILD psilocybin does not produce significant differences in exploratory, locomotor, or swimming behavior of rats in the L/D, OFT, or FST paradigms. Rats administered 0.1 mg/kg psilocybin entered the light compartment significantly sooner in the L/D test on day 48 than was observed on day 24. Additionally, rats administered 0.05 or 0.1 mg/kg spent significantly more time in the center of the open field apparatus on day 48 and/or 58 compared to time spent in center on day 24. Measures of stereotypy from the OFT also varied within group, trending in the same direction (reduced stereotypy counts) as saline control animals on day 48 and 58 suggesting that repeated administration of low dose psilocybin does not appear to cause anxiogenic behavior. Overall, the apparent lack of anxiolytic and antidepressant-like effects of CILD psilocybin in the present study suggest that there may be distinct mechanistic and behavioral differences between sub-threshold, “microdosing” of psychedelics and hallucinogenic high doses. Future studies with more complex behavioral models, such as operant tasks, or ecologically representative rat populations are warranted to further investigate the behavioral effects of CILD in rodents in order to determine if the cognitive/behavioral effects of “microdosing” reported in humans is exclusively a human phenomenon.
C12

MANGANESE-ENHANCED MRI DETECTS SUBREGIONAL DIFFERENCES IN NEURONAL ACTIVITY IN THE PARAVENTRICULAR NUCLEUS WITH POTENTIAL DIFFERENCES IN SEDENTARY VERSUS PHYSICALLY ACTIVE RATS

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Cardiovascular disease (CVD) is the leading cause of death globally. Factors including physical inactivity, sleep quality, stress levels, and body weight affect the development of CVD. The paraventricular nucleus of the hypothalamus (PVN) is a critical part of the brain and plays a role regulating blood pressure. The neuronal activity in the PVN stimulates sympathetic nerve activity (SNA) and constricts blood vessels resulting in higher blood pressure. However, it is currently unknown whether sedentary conditions produce differences in neuronal activity in subregions of the PVN. The purpose of the study was to test the hypothesis that the sedentary rats have greater neuronal activity within the PVN when compared to physically active rats. A group of four-week-old male, Sprague-Dawley rats were divided into 11 active (access to a running wheel) and 12 sedentary (no access to a running wheel) rats and were observed over a 12-week period. Manganese (Mn2+)-enhanced MRI (MeMRI) was used to assess neuronal activity longitudinally. Prior to each MRI scan, rats were injected with MnCl2 (66 mg/kg, i.p). Eight MRI slices of the PVN were obtained at 260-μm intervals (range 0-1820 μm). Prior to designation into active and sedentary groups, rats demonstrated significantly lower neuronal activity in the -780 μm slice, p<0.001) with no significant differences between groups at baseline. Following 12 weeks of sedentary or active conditions, higher neuronal activity in subregions of the PVN appeared to occur in sedentary versus active rats, but this difference did not reach significance whether expressed as an absolute (p=0.078) or percent change in intensity (p=0.115). Our data suggest that sedentary conditions may alter neuronal activity in the PVN in ways not previously detected in other studies. Since CVD is more common in sedentary humans, alterations in the PVN of inactive rats may relate to mechanisms by which CVD is increased in sedentary humans. (HL096787; AHA258).
Techniques

D12

PRODUCTION OF SUMOYLATED RECOMBINANT TAU IN ESCHERICHIA COLI

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Aggregation of tau is a pathological hallmark in tauopathies, and phosphorylation is thought to play a major role in regulating tau aggregation and dysfunction in disease. However, tau is subject to a myriad of other post-translational modifications, such as SUMOylation, whose contribution to the pathophysiology of tau is not fully understood. Herein, we describe a method for producing and purifying recombinant SUMO1-modified tau protein in bacteria (E. coli) to study the impact of SUMOylation on tau biology and pathobiology. BL21 (DE3) competent cells were co-transformed with 2 plasmids: a plasmid encoding for the full-length isoform of tau (hT40) and the SUMO plasmid encoding for SUMOylation machinery (SUMO-activating enzyme (SAE1 and SAE2), SUMO-conjugating enzyme (Ubc9), and His-tagged SUMO1). Bacteria were then grown in Luria Broth, and protein expression was induced using IPTG. SUMO1-modified hT40 was purified using a 3-step procedure that started with boiling followed by his-tag purification to capture SUMOylated proteins. The third step was to further purify SUMO1-modified tau using size exclusion chromatography. The modification of tau by SUMO1 was verified by western blot (WB) and mass spectrometry (MS). His-tag purification showed enrichment of hT40 from E. coli co-transformed with tau and SUMO plasmid compared to competent cells transformed with tau only. Moreover, WB data show that tau is modified by SUMO1, giving a unique banding pattern. Results from MS indicated that tau is SUMOylated at K150, K174/K180, K254/K257, K280/281, and K340. Our data demonstrate that recombinant SUMO1-modified tau can be produced in bacterial cells using a co-transformation approach with two plasmids. The identified modification sites agree with prior studies assessing SUMOylated tau in HEK293T cell line (K340) and include novel sites at (K150, K174/180, K254/K257, and K280/K281). Ongoing studies will utilize the recombinant SUMO1-modified tau protein to characterize the impact of SUMOylation on tau behavior (e.g., conformation, multimerization, etc.) using a series of biochemical and in-cell assays.

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METHOD FOR HIGH THROUGHPUT ANALYSIS OF IMMUNOFLOUORESCENCE IMAGES

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With the increasing availability of high-resolution microscopy, it is critical to develop and improve methods for accurate and automated assessment of immunofluorescence images to provide more reliable information about the relationships between protein expression and localization and behavioral observations in a high throughput manner.

The present study was designed to evaluate the relationship between fear conditioning responding and protein expression/localization of Fos, β-galactosidase and the neuronal marker, NeuN, with the hypothesis that fear conditioning would lead to greater expression of Fos and β-galactosidase in the medial prefrontal cortex (mPFC). A rigorous method to determine appropriate imaging thresholds and parameters was developed to establish an unbiased, automated method to quantify neuronal activation in subregions of the medial prefrontal cortex which were then statistically compared between groups and with behavioral outcomes.

We used 84 40-micron thick brain slices (perfusion fixed) obtained from control and footshock- exposed Wistar rats homozygous for the Fos/LacZ gene (W1-Tg(cFOS-LacZ)1ottc) containing medial prefrontal cortex regions (cingulate area (Cg1), the infralimbic cortex (IL), and the prelimbic cortex (PrL)) that had been stained with standard immunofluorescence methods. Images were collected using epifluorescence wide field microscopy and the Leica THUNDER imaging system to create the best focused image with technology available. Mosaic merging of 200x scanned images was performed and merged, which was done to make images visible at the scale necessary for accurate evaluation as the microscope takes images at the scale of 200 images to make a single slice. In Image J, the images were then processed by uniformly-defining cortical regions of interest (ROIs) which were digitally circumscribed on each image in accordance with “The Rat Brain In Stereotaxic Coordinates” (Paxinos,Watson) at 2.0-3.0 mm Bregma. Cell count, average cell size, area covered in cells in the region by percent, and the average threshold of the region being measured, were all recorded. Adjustments were made to the circularity requirements of cells counted, aspects of disqualifiers that were quantified, and cell size counted were adjusted until the average number from the cell analysis best represented the naturalistic observations. The data were then analyzed using ANOVA to establish freezing:FOS correlation using Prism software.

Fear conditioning elicited a greater expression of Fos, and β-Gal in the mPFC compared to the sham conditioned and the naïve groups, upholding the present hypothesis and confirming prior data indicating that the medial prefrontal cortex plays a role in the process of consolidation or recall of fear or extinction memory, which can be detected using this novel method of quantification.
SELECTIVE BEHAVIORAL DEFICITS IN ADULTHOOD FOLLOWING POSTNATAL EXCITATION OF LAYER V NEOCORTICAL PYRAMIDAL CELLS

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Patterns of neuronal activity during early development are believed to guide the assembly of neural circuits. Thus, alterations of neuronal activity in this time window may cause structural and functional changes that persist into adulthood. Specific alterations may lead to unique patterns of aberrant circuit formation, a mechanism which has been implicated in some psychiatric conditions such as autism. We took advantage of a bimodal experimental approach, bioluminescence-driven optogenetics, to assess the impact of hyperexciting pyramidal cells in layer V of the neocortex during early development. To enable highly specified manipulation of pyramidal cell activity in layer V, mice expressing the excitatory luciferase-opsin fusion LMO3 (sbGluc fused to VChR1) under a Lox-Stop-Lox sequence were crossed with Rbp4-Cre mice. In presence of the luciferase substrate coelenterazine (CTZ), light emission from sbGluc drives activation of VChR1 to depolarize the cell and evoke action potentials. CTZ was delivered intraperitoneally once per day during post-natal days 4-14 in the developing mouse pups; a control group received a vehicle treatment instead of CTZ. Starting at postnatal day 50 (P50), we examined the behavior of all mice across several testing paradigms. To standardize our quantification of behavioral data we implemented DeepLabCut, an open-source machine learning tool that leverages recent advances in computer vision to allow accurate, markerless tracking of animal limbs across behavior testing videos. Three deep residual neural networks were each trained on 100-300 human-labeled frames from open field, elevated plus maze, or novel object videos, respectively. The networks were used to analyze full videos of tests corresponding to their training data: an analysis of one video outputs a dataframe of X/Y coordinates and labeling confidence values (0 < p < 1) for each user-specified “body part”, indexed frame-by-frame in temporal order. This positional data can be used to measure many different behavioral phenotypes downstream. Here, we quantified the total amount of time (number of frames divided by the video’s frame rate) that each mouse’s snout or centroid was located within user-specified regions of interest. Regions of the open field arena were defined manually per video; our elevated plus maze algorithm was trained to label each corner of the arena, so rectangular ROIs corresponding to each ‘arm’ could be defined automatically during analysis (utilizing code from Sturman et al., 2020). The latter approach was less labor-intensive and more robust to variability in camera position. CTZ-treated Rbp4-Cre/LSL-LMO3 mice displayed adult behavioral phenotypes similar to those observed in our previous study, where pyramidal neurons in all layers of the neocortex were hyperexcited in the Emx-Cre/LSL-LMO3 mouse line during the same time window (P4-14). In conclusion, we leveraged the versatility of a dual chemogenetic and optogenetic method to study the impact of neuronal hyperexcitation during early development on adult neurophysiology and behavior. Exploratory analysis demonstrates the viability of a DeepLabCut data pipeline and shows ASD-like behavioral deficits in adult mice following developmental hyperexcitation of layer V neocortical pyramidal neurons.
DIRECT MEASUREMENT OF NEURONAL ENSEMBLE ACTIVITY USING PHOTOACOUSTIC IMAGING IN THE STIMULATED FOS-LACZ TRANSGENIC RAT BRAIN: A PROOF-OF-PRINCIPLE STUDY

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Measuring neuroactivity underlying complex behaviors facilitates understanding the microcircuitry that supports these behaviors. We have developed a functional and molecular photoacoustic tomography (F/M-PAT) system which allows direct imaging of Fos-expressing neuronal ensembles in Fos-LacZ transgenic rats with a large field-of-view and high spatial resolution. F/M-PAT measures the beta-galactosidase catalyzed enzymatic product of exogenous chromophore X-gal within ensemble neurons. We used an \textit{ex vivo} imaging method in the Wistar Fos-LacZ transgenic rat, to detect neuronal ensembles in medial prefrontal cortex (mPFC) following cocaine administration or a shock-tone paired stimulus. Robust and selective F/M-PAT signal was detected in mPFC neurons after both conditions (compare to naive controls) demonstrating successful and direct detection of Fos-expressing neuronal ensembles using this approach. The results of this study indicate that F/M-PAT can be used in conjunction with Fos-LacZ rats to monitor neuronal ensembles that underlie a range of behavioral processes, such as fear learning or addiction.
TROUBLESHOOTING THE USE OF ESTRADIOL ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) TESTS FOR RODENT SERUM

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Estradiol is the most common type of the female sex hormone estrogen. Fluctuations in estradiol levels across the female’s estrous cycle have been associated with alterations in behavior and physiology as well as brain anatomy. Thus, we aimed to measure estradiol levels in our current study that examines sex differences with regards to the effects of exercise on cardiovascular disease. In order to do this, we ordered ELISA estradiol kits from two different companies (Abcam and Calbiotech) that claimed to be able to measure estradiol levels in rodent serum in the range we expected them to be. We included serum samples from ovariectomized (OVX) and sham female rats, as well as male and intact female rats that were either sedentary or had access to a running wheel in their cages to explore a spectrum of estradiol variation. We ran the first ELISA on an Abcam Estradiol plate (range of 20-2000pg/ml and sensitivity of 8.68 pg/ml), which showed a low sensitivity for estradiol. OVX and sham rats had similar levels of estradiol, which was unexpected. We then ran another Abcam plate on which we spiked some of the samples with a known amount of estradiol to bring them into the range of better sensitivity of the standard curve of the assay. Interestingly, spiking the samples also failed to produce consistent results, despite both plates having valid standard curves and accurately measuring the value of the control sample provided by the kit. We then tried an ELISA kit from a different company (Calbiotech) that advertised a lower sensitivity, with a range of 10-1000 pg/ml. This plate also produced unreliable results (i.e. OVX and sham females having similar levels.). We additionally analyzed cytology from vaginal lavage slides to determine the estrous cycle of each female rat, however this is only an indirect measure of estradiol levels at the time of testing and collection. The difficulty in obtaining reliable estradiol measures indicates that there is a need for a more reliable way to accurately measure estradiol levels in rodents, especially given the NIH mandate to include females in all basic research studies and the need to take the estrous cycle variation in sex hormone levels into account. The ability to obtain reliable estradiol results in future studies will help continuing research into sex differences, including our current study examining sex differences in cardiovascular disease.
DEVELOPMENT OF A FEED-BASED DRUG DELIVERY SYSTEM FOR ZEBRAFISH

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Zebrafish (Danio rerio) have recently become a popular animal model in the study of behavioral pharmacology. The popularity of zebrafish is due to the presence of orthologues for over 70% of protein-coding genes found in humans. In addition, their high fecundity, external fertilization of eggs, abundant genetic techniques, and high-density housing make zebrafish an excellent model organism.

Current methods to administer drugs to zebrafish are stressful, such as placing the fish into a dosed beaker, anesthetizing the fish to administer an intraperitoneal injection, or, less commonly, administer an oral gavage. Although these methods have been successful in administering compounds to zebrafish, there are several drawbacks, especially when studying fear and anxiety due to excess handling and stress which may cause injury or unduly influence experimental results.

To circumvent these issues, we have developed a feed-based drug delivery system which allows for minimal handling of zebrafish, while allowing for accurate drug administration according to individual body weight. Utilizing a leaching assay, we found that the feed remains stable with minimal loss of compound for up to five minutes. To test the effectiveness of the feed-based system, we tested the behavioral effects of an anxiolytic compound, fluoxetine (a selective serotonin reuptake inhibitor), using the novel tank test for anxiety-related behavior. Consistent with prior work, we found that fish who were treated with fluoxetine (10 mg/kg) had an increase in locomotor activity. We also found that fluoxetine reduced anxiety-like behavior in female, but not male, fish.

In conclusion, we developed a novel gelatin-based feed drug delivery system developed for zebrafish which allows for minimally invasive delivery of pharmaceuticals based off individual body weight.
DELIVERY OF PAMAM DENDRIMER NANOMOLECULES ACROSS THE NATURAL BIOLOGICAL BARRIERS AND ANALYSIS OF DENDRIPLEX-CLEARANCE FROM THE MOUSE BRAIN

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Dendrimers are 3-dimensional nanomolecules with promising biomedical applications. They can carry biomolecules to the central nervous system as they can cross the blood-brain-barrier (BBB) following systemic injections. The conventional G4 100% amine surface dendrimers (G4-NH₂) are highly toxic to cells due to their dense positively charged amine groups on the surface. Therefore, to reduce the toxicity, we have modified them to have only 10% surface covered with -NH₂ and remaining 90% surface covered with hydroxyl groups (-OH; G4-90/10). Our work indicates that these surface-modified dendrimers are taken up by brain cells in vitro and in vivo. Moreover, the G4-90/10 dendrimers are capable of forming complex with plasmid DNA (known as dendriplex) of various sizes and can deliver them to different cells types in vitro and in vivo. Further, we also focused on delivering them to the rodent fetal brain following i.p injections and analyze their ability to cross the BBB and the placental barrier in pregnant mice. The future application lies in delivering therapeutic biomolecules using dendrimers to treat fetal diseases affecting the nervous system that can result in numerous neurological deficits. Finally, we also injected the dendriplexes into the rodent brain and tracked them using in vivo imaging system (IVIS) to analyze the longevity of the dendriplexes in mice. Our results show that the PAMAM dendrimers can successfully form complex with large plasmids and were able to deliver them into the cells in vitro and in vivo. Moreover, the PAMAM dendrimers were able to cross the maternal BBB and reach the brain, however, very negligible amounts of dendrimers were found in the fetal tissue, and majority of the dendrimers were found in the placental tissue. In conclusion, we were able to track the PAMAM dendrimers and the complexes in the rodent brain using IVIS showing steady clearance of these PAMAM nanomolecules over time.

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IMAGING BRIGHT AND HIGH-CONTRAST BIOLUMINESCENT CALCIUM INDICATORS IN CULTURED CORTICAL NEURONS

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Calcium (Ca2+) ions serve as a proxy for neuronal activity as they appear as second messengers in the cell. In neurons, electrical activity is accompanied by an influx of intracellular Ca2+. Previously established fluorescent Ca2+ sensors have problems with bleaching and tissue death from the need of external illumination. To date, published bioluminescent Ca2+ indicators have not been the preferred mode of Ca2+ sensing because they were not as bright and the signal change in Ca2+ ion concentration responses were harder to detect. Here we report on, “Calcium BioLuminescence-Activity Meters” (CaBLAMs). This is a novel class of bioluminescent Ca2+ indicators made from a luciferase and Ca2+ sensor optimized through directed evolution. Each component of the indicator was optimized from the luciferase domain to the linker length and composition, in order to create a sensor which exhibits extremely low levels of resting light emission. Bioluminescent Ca2+ imaging of cultured cortical neurons revealed physiological responses to chemical stimulation in a dose dependent manner upon perfusion of glutamate. Next generation CaBLAM sensors will pave the way for deep brain calcium imaging in behaving mice addressing a major limitation when imaging with fluorescent indicators.
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