SOCIETY FOR NEUROSCIENCE

Michigan Chapter

54th Annual Meeting

May 20, 2024

Rackham Graduate School

University of Michigan

Thank you to our University of Michigan sponsors for supporting this year's meeting!

Department of Molecular, Cellular, and Developmental Biology

Michigan Neuroscience Institute

Department of Anesthesiology, Center for Consciousness Science, and Michigan Psychedelic Center

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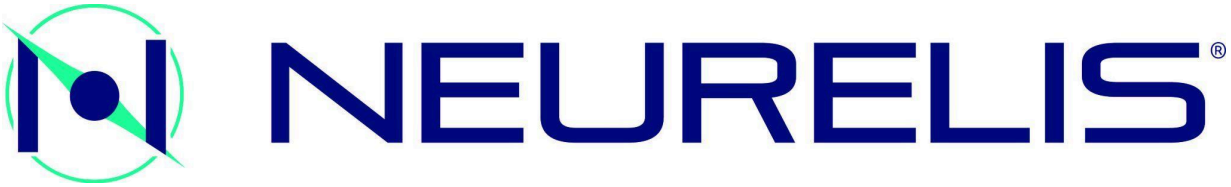

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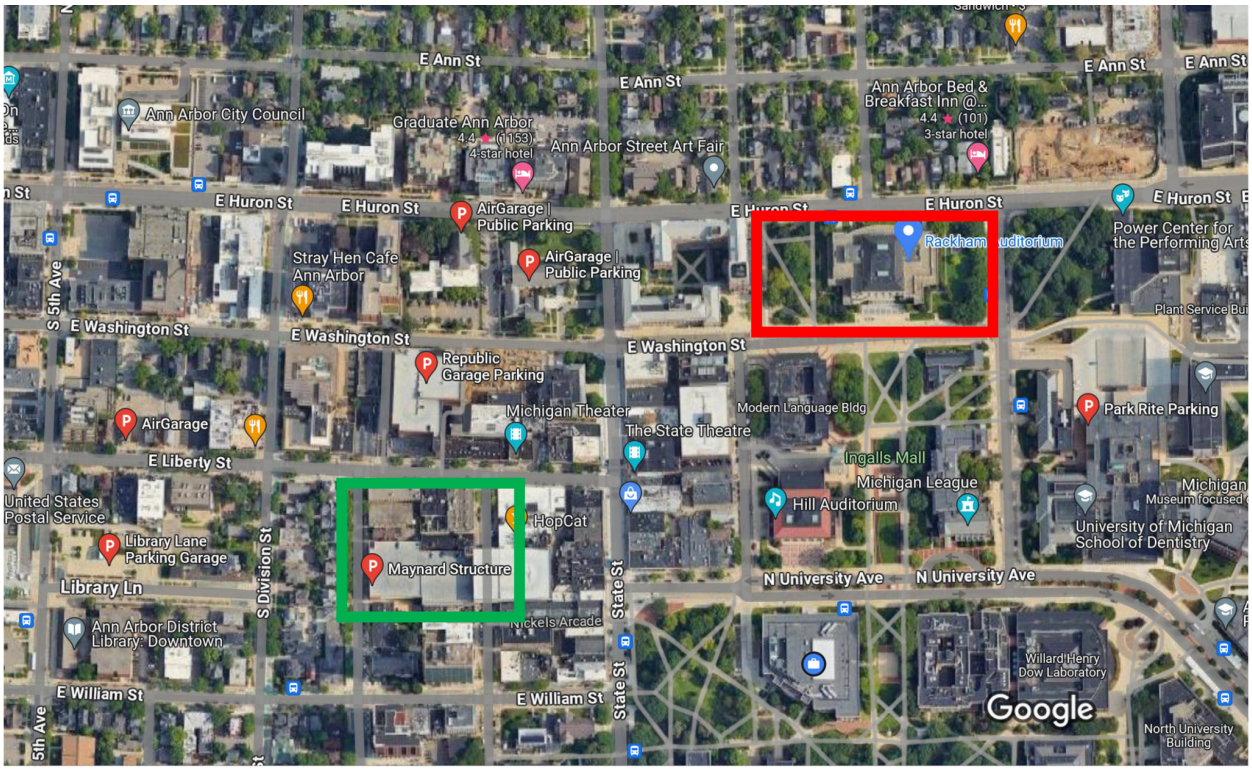
2024 Meeting Schedule

8:30 am-9:00 am	Registration, Continental Breakfast, and Poster Session A setup **(1st Floor Lobby)**
9:00 am-10:15 am	Poster Session A **(1st Floor Lobby, 2nd Floor East Lounge, 2nd Floor West Lounge)**
10:15 am-10:45 am	Break and Poster Session B setup
10:45 am-12:00 pm	Poster Session B **(1st Floor Lobby, 2nd Floor East Lounge, 2nd Floor West Lounge)**
12:00 pm-1:00 pm	Box lunch **(4th Floor Assembly Hall + 4th Floor East and West Conference Rooms)**
1:00 pm-2:00 pm	Business Meeting **(4th Floor Amphitheater)** *Welcome & President's Report; Treasurer's Report; Elections* To be elected during the meeting (self-nominations are welcome): Treasurer (2024 – 2027) MSU Councilor (2024 - 2026) U of M Councilor (2024 - 2026) Awards Chair (2024 - 2026) Councilor at Large (2024 - 2026) Student Councilor (2024 - 2026)
2:00 pm-2:20 pm	Founder's Award Speaker: **Chandi Rana** *"Unraveling the Nigrotegmental Circuit's Role in Dravet Syndrome"* **(4th Floor Amphitheater)**
2:20 pm-2:40 pm	Founder's Award Speaker: **Alixandria T. Mascarin** ***"****Relapse on the brain: Examining the role of sex on cocaine-seeking and the underlying neuronal ensembles****"*** **(4th Floor Amphitheater)**
2:40 pm-3:00 pm	Coffee and Snack Break **(4th Floor Assembly Hall + 4th Floor East and West Conference Rooms)**
3:00 pm-4:00 pm	Keynote Speaker: **Dr. Catherine Kaczorowski:** *"Using Complex Genetics in Mice to Unlock the Secrets of Cognitive Resilience"* **(4th Floor Amphitheater)**
4:00 pm-4:30 pm	**Awards and Adjournment**

VENUE AND PARKING

The **Rackham Building**, is located at **915 E Washington St**, Ann Arbor, MI 48109



Nearby public parking structures include the **Maynard Structure**, at **324 Maynard St, Ann Arbor, MI 48104** - an easy 3-min walk from the Rackham Building (*cost = $2.40 for 2 hours*)

Additional nearby public parking (≤ 10-min walk) is at:

Liberty Square - 510 E Washington St (*cost = $2.40 for 2 hours*)

Library Lane - 319 S 5th Ave (*cost = $2.40 for 2 hours*)

Fourth & Washington - 123 E Washington St (*cost = $2.40 for 2 hours*)

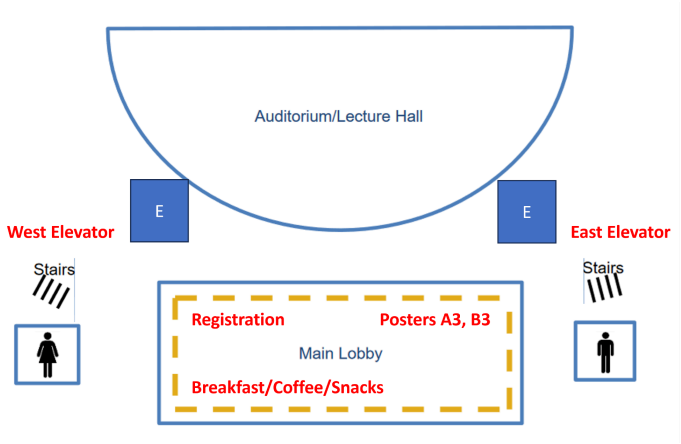
Forest Ave - 650 S Forest Ave (*cost = $2.40 for 2 hours*)

William & Main St - 115 E William St (*cost = $4.40 for 2 hours*)

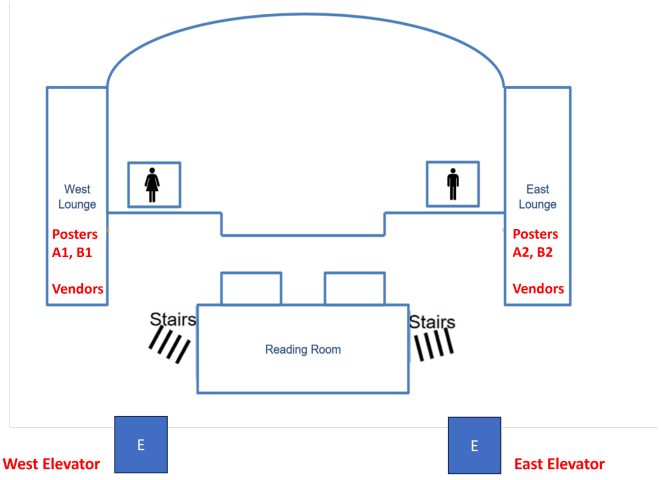
Fifth & William - 351 South Fifth Avenue (*cost = $4.40 for 2 hours*)

Rackham Building Map

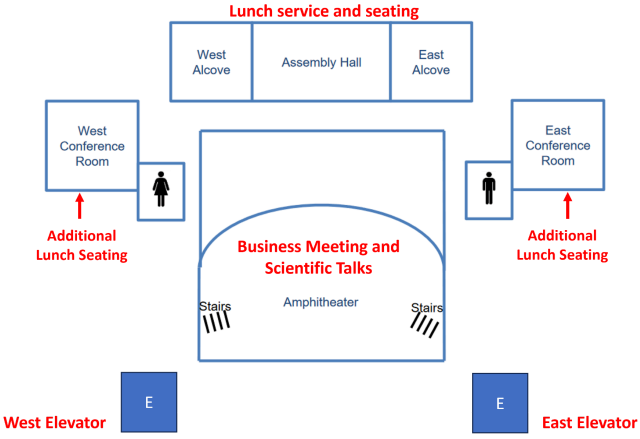
1st floor (Registration, Breakfast/Snacks/Coffee, Posters):



2nd floor (Posters, Vendors):



4th floor (Business Meeting and Scientific Talks [Amphitheater], Lunch):



Michigan Chapter of the Society for Neuroscience Council

	Name:	Institution:	Term Ends:
President	Anna Moszczynska	Wayne State University	2024*
Past President	Jessica Matchynski-Franks	Rochester University	2025
President-Elect	Julien Rossignol	Central Michigan University	2024
Secretary	Harold Greene	University of Detroit-Mercy	2025
Treasurer	Hilary Marusak	Wayne State University	2024
Awards Chair	Eric Ramsson	Grand Valley State University	2024
Website Coordinator	Bhairavi Srinageshwar	Central Michigan University	2025
Councilors:			
CMU	Eric Petersen		2025
MSU	Joseph Patterson		2024
U of M	Sara Aton		2024
WSU	Kelly Bosse		2025
WMU	Lisa Baker		2025
Field Neuroscience Institute	Gary Dunbar		2025
At large 1	Cameron Davidson	Oakland University	2025
At large 2	Kevin Trewartha	Michigan Tech	2024
Graduate student 1	Candace Johnson	Western Michigan University	2024
Graduate student 2	Michael Kubik	Michigan State University	2025

**Past President to retire & President-Elect to start tenure in 2024*

Montford F. Piercey Award

This award is in honor of Dr. Montford F. Piercey, a founding member and past president of the Michigan Chapter Society for Neuroscience.

Awardees are listed in alphabetical order

2024 Winner: Alixandria T. Mascarin

Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine

Relapse on the brain: Examining the role of sex on cocaine-seeking and the underlying neuronal ensembles

Biography: Alixandria received her B.S. in Psychology from Wayne State University in 2019, where her undergraduate research addressed the behavioral effects of chronic cannabidiol administration. She began pursuing her PhD in Translational Neuroscience at the Wayne State University School of Medicine in 2020, and her doctoral research has since focused on cocaine use. In the lab of Dr. Shane Perrine, her dissertation research examines the neuronal ensembles that govern cocaine-seeking in a rat model of relapse to cocaine use. Alixandria has also contributed to several clinical collaborations under the mentorship of Dr. Mark Greenwald, where she examined behavioral phenotypes in samples of recent cocaine users. Alixandria is a passionate science communicator who enjoys sharing her science with scientists and non-scientists alike and hopes to pursue a career as a Medical Science Liaison after completion of her PhD. Outside of the lab, Alixandria volunteers her time towards dog rescue and rehabilitation and enjoys playing soccer.

Summary of work: Alixandria's translational research addresses cocaine use from both clinical and preclinical perspectives. Her dissertation examines sex differences in cocaine-induced behaviors (such as cocaine-induced hyperlocomotion, cocaine-taking, and cocaine-seeking) and the neuronal ensembles that govern them. Alixandria's clinical research uses timeline follow-back interviews to examine what impacts the behavioral health and substance use patterns of recent cocaine users. Through this lens, she has examined the influences of genetic variation in stress signaling and patterns of polysubstance use in characterizing cocaine use phenotypes.

Duncan McCarthy Award

This award is in honor of Dr. Duncan McCarthy, a founding member and past executive board member of the Michigan Chapter Society for Neuroscience.

Awardees are listed in alphabetical order

2024 Winner: Chandni Rana

Neuroscience Graduate Program, University of Michigan

Unraveling the Nigrotegmental Circuit's Role in Dravet Syndrome

Biography: Chandni received her Bachelor's degree in Biomedical Engineering from the University of Florida in 2019. As an undergraduate she trained in a neuroinformatics lab and used machine learning classifiers to decode working memory dynamics from human EEG data. She then completed a summer internship at the RIKEN Center for Brain Science in Japan focused on using EEG decoding to uncover neural dynamics induced by transcranial magnetic stimulation. After graduation, she worked for 2 years as a research associate in the Tech4Health Institute at New York University where she collaborated with an interdisciplinary team of scientists and engineers developing a novel optoacoustic imaging modality for visualizing the rodent brain with enhanced spatiotemporal resolution. In 2021, she began her PhD in Neuroscience at the University of Michigan and subsequently joined Dr. Joanna Mattis's lab to merge her background in engineering and neuroscience to dissect dysfunctional circuits in epilepsy.

Summary of work: Chandni's work centers on the Scn1a haploinsufficient mouse model of Dravet Syndrome, a severe developmental and epileptic encephalopathy. Scn1a+/- mice have recurrent spontaneous seizures in addition to seizures that can be triggered with hyperthermia. Chandni aims to understand how subcortical regions in the brain may be involved in the generation and maintenance of seizures, focusing specifically on the substantia nigra pars reticulata (SNr) and one of its major downstream targets, the pedunculopontine nucleus (PPN). The SNr is considered a seizure "choke point" that can be harnessed to achieve seizure suppression. The PPN is a cholinergic nucleus that densely innervates multiple cortical and subcortical regions involved in seizures. To understand the potential role of the nigro-tegmental pathway in Dravet Syndrome pathology, Chandni's project involves characterizing properties of SNr and PPN neurons in the Scn1a+/- mouse and recording their activity during seizures in vivo.

Keynote Speaker

Using Complex Genetics in Mice to Unlock the Secrets of Cognitive Resilience

Catherine Kaczorowski, Ph.D.

Elinor Levine Professor of Dementia Research

Professor of Neurology

My program moves beyond discovery of genetic risk factors for brain aging and neurodegenerative diseases towards a causal understanding of resilience, a process whereby some individuals maintain normal health and cognitive functions despite age, high-risk mutations, and/or significant pathological or environmental burden. Targeting resilience offers a desperately needed therapeutic approach to mitigate functional decline across the neurodegenerative spectrum -- from before onset to late-stage pathology. However, our ability to capitalize on this is hindered by incomplete understanding of resilience mechanisms. Key questions include: What is the nature of brain and cognitive resilience? How conserved are functional genomic properties of resilience in genetically diverse models? Are resilience mechanisms generalizable across the spectrum of neurodegenerative diseases? What biomarkers of resilience inform about mechanism and predict intervention efficacy? Can resilience mechanisms be modeled in vitro for causal tests and high-throughput screening? To identify molecules, cells and circuits supporting resilience, we implement an integrated, multi-disciplinary approach aligning data from human cases and novel diversity mouse panels my lab developed that exhibit resilience to cognitive decline despite harboring causal Alzheimer's (AD) mutations. To establish the viability of resilience for development of therapeutics, we are evaluating interventions predicted to promote resilience signatures in cell models. Our long-term goal is to generate a complete understanding of resilience to date, encompassing synaptic organization, neuronal circuit function, and inter-region connectivity.

Biography: Dr. Catherine Kaczorowski received her BA *summa cum laude* from University of Wisconsin-Milwaukee in 2000, received her PhD degree from Northwestern University, and received postdoctoral training in Physiology and Biotechnology and Bioengineering at Medical College of Wisconsin as a K99/R00 Pathway to Independence awardee. She started her research group at The Jackson Lab before joining the University of Michigan as a Professor of Neurology and the Elinor Levine Endowed Chair for Dementia Research. Dr. Kaczorowski is both a neurophysiologist and an expert in the systems genetics of 'normal' nonpathological aging and pathogenesis of neurodegenerative diseases including Alzheimer's disease (AD). She has been a driving force in uncovering and describing the phenomenon of cognitive resilience in the context of nonpathological aging, AD, and more recently, Huntington's (HD).

Dr. Kaczorowski's research program - funded by the Simons Foundation, the Cure Alzheimer's Fund, the Alzheimer's Association, the Chan Zuckerberg Initiative, and the National Institutes of Health - entails several collaborative, multi-institutional projects. The Kaczorowski laboratory has pioneered the generation of the first translationally relevant polygenic model of human AD (AD-BXDs; recently published in Neuron). This resource has permitted dissection of aging-specific genetic mechanisms from those controlling the clinical manifestations of disease-specific neuropathologies, which is impossible in human populations. Her research group leverages multi-scale data (genetics, omics, imaging, behavior) from these genetically diverse AD-BXD mouse strains and human patients to identify and validate genetic and cellular mechanisms that promote cognitive resilience to normal brain aging, AD, and other age-related neurodegenerative diseases. Using integrative analyses across multiple scales, data types, environmental factors, and species, the Kaczorowski lab is accelerating the discovery of precise genetic mechanisms of cognitive resilience that could yield the next generation of targets and therapeutic strategies for promoting brain health.

First Author List (Alphabetical)

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Allahyarzadeh Khiabani	B33	B2	ENHANCING THERAPEUTIC EFFICACY: NON-INVASIVE INTRANASAL DELIVERY OF G4 70/30 PAMAM DENDRIMER IN C57 MOUSE MODEL
Allouch	B1	B1	ADIPOSE TISSUE-DERIVED EXTRACELLULAR VESICLES; EFFECTS OF AGING AND INFLAMMATION IN OBESITY AND PREDIABETES
Almaoued	A49	A2	EFFECT OF ADOLESCENT INTERMITTENT CAFFEINE ACCESS ON OPERANT RESPONDING FOR CAFFEINE AND ETHANOL DURING ADULTHOOD IN RATS
Alsharifi	B50	B2	EXAMINING DNA DAMAGE IN OLFACTORY SENSORY NEURONS OF CRAYFISH FOLLOWING COMBINED ATRAZINE AND MICROCYSTIN-LR EXPOSURES
Atasi	A48	A2	ANALYSIS OF THE UBIQUITIN-PROTEIN LIGASE PARKIN IN METHAMPHETAMINE USE DISORDER
Atiratana	A29	A2	EFFECT OF STRAIN AND ARABIAN JASMINE (JASMINUM SAMBAC (L.) AITON) ON ANXIETY-RELATED BEHAVIOR IN ZEBRAFISH
Atwa	B25	B1	BIOID2-BASED INTERACTOME OF FULL-LENGTH AND DOMAINS OF TAU REVEALS NOVEL AND KNOWN PROTEIN INTERACTIONS ASSOCIATED WITH MULTIPLE CELLULAR PATHWAYS

Bartol	A56	A3	CORTICOTROPIN RELEASING FACTOR DISCHARGED BY THE CENTRAL NUCLEUS OF THE AMYGDALA ENCODES POSITIVE VALENCE AND BIASES REWARD PURSUIT
Benovich	B29	B2	UTILIZING THE NOVEL Q111-BXD MOUSE PANEL TO EXPLORE NEUROPSYCHIATRIC TRAITS IN HUNTINGTON'S DISEASE
Benskey	B46	B2	SYNUCLEINOPATHY DIFFERENTIALLY AFFECTS THE EXPRESSION OF FLUID PHASE VERSUS MEMBRANE BOUND COMPLEMENT REGULATORS IN THE SUBSTANTIA NIGRA.
Berchulski	A70	A3	A HERITABLE CHANGE IN ACTION POTENTIAL HALF WIDTH THAT CORRELATED WITH COGNITIVE RESILIENCE
Bermúdez	A40	A2	MODIFYING BEHAVIOR WITH CORTICAL LAYER SPECIFIC NEUROMODULATION
Boas	B43	B3	GERMLINE CAG REPEATS VARY ACROSS GENETICALLY-SEGREGATED BXD BACKGROUNDS AND NEGATIVELY PREDICT MOTOR PERFORMANCE IN A MOUSE MODEL OF HUNTINGTON’S DISEASE
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Boonpraman	B12	B1	DIFFERENTIAL EFFECTS OF CHLORPYRIFOS AND DIAZINON AND THEIR METABOLITES ON MITOCHONDRIAL COMPLEX ACTIVITY AND DOPAMINERGIC NEUROTOXICITY
Bosse	B10	B1	TRAUMATIC BRAIN INJURY ELEVATES EXPRESSION OF THE BIPHASIC SIGNAL TRANSDUCER CARP-1/CCAR1

Brabant	A57	A3	PRO-IMPULSIVE EFFECTS OF 1-(1-BENZOFURAN-5-YL)-2-(METHYLAMINO) PROPAN-1-ONE HYDROCHLORIDE (BK-5-MAPB) ENANTIOMERS IN RATS
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Cao	B41	B2	EXTRACTING BIOLOGICALLY RELEVANT LATENT SPACES FROM ALZHEIMER'S DISEASE MOUSE TRANSCRIPTOMES WITH VARIATIONAL AUTOENCODERS
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Carpenter	A16	A1	SNOOZE OR LOSE: EFFECTS OF SLEEP DURATION ON EXTINCTION RECALL, FEAR EXTINCTION NEURAL CIRCUITRY, AND VULNERABILITY TO ANXIETY IN ADOLESCENTS
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Chatterton	B63	B3	BIOLUMINESCENT KINASE SENSORS FOR DETECTION OF GROWTH FACTOR SIGNALING AND INFLAMMATION SIGNALING
Chen	B24	B1	DATA-INDEPENDENT ACQUISITION PROTEOMIC APPROACH REVEALS CALCIUM-ACTIVATED POTASSIUM CHANNELS AND ADP-RIBOSYLATION FACTORS ARE LINKED TO COGNITIVE RESILIENCE TO ALZHEIMER'S DISEASE.
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Conrad	A74	A3	ORAL CONTRACEPTIVE EXPOSURE MODULATES NEUROIMMUNE ACTIVITY
Crenshaw	A58	A3	BEHAVIORAL TRAINING HISTORY MODULATES AMPHETAMINE-INDUCED IMPULSIVE ACTION IN RATS
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Matsko	A54	A3	BEHIND THE “RUNNER’S HIGH”: EFFECTS OF ACUTE EXERCISE, STRETCHING, AND MEDITATION ON ANXIETY AND ENDOCANNABINOID LEVELS IN YOUTH
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Zhang	B13	B1	AGING DRIVES CEREBROVASCULAR NETWORK REMODELING AND FUNCTIONAL CHANGES IN THE MOUSE BRAIN
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Abstracts

Theme 1 - COGNITION:

CUES AUGMENT WORKING MEMORY PROFICIENCY IN SMOKERS: PRELIMINARY EVIDENCE THAT ATTENTIONAL BIAS ENHANCES EXECUTIVE FUNCTION

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Background. Attentional bias to drug-related cues is a core feature of addiction. Whereas attention bias is often associated with decrements in cognitive function, few studies have investigated the potential for cues to enhance cognition. We investigated the effects of smoking cues on working memory (WM) and neural engagement among cigarette smokers using a novel cued N-back task.

Methods. During multi-echo fMRI acquisition (TE=15,36,56ms; TR=2.83s; 2.9mm isotropic; Siemens Verio 3T), out-of-treatment smokers (N=21; 28.0±3.9yrs old; 17.2±5.9 cigarettes/day) completed a novel cued N-back task: 32s blocks of letter 0- and 2-back (pseudorandom order; interleaved 16s rest periods minimized carry-over effects [discarded]). Blocks contained neutral vs. smoking images (unrelated to performance) positioned behind each N-back letter. After tedana pre-processing, beta weights from first-level contrasts were extracted from a priori brain regions for repeated-measures analyses.

Results. N-back accuracy was greater during 0- than 2-back ($p<.001$) and during cues than neutral images ($p=.01$). Cue-by-Load interactions indicated greater accuracy ($p=.01$; 85.7% vs. 67.9%) and longer latency ($p=.02$; 552.4ms vs. 427.4ms) during cued 2-back compared to neutral 2-back. The Cue effect (cued 2-back>neutral 2-back) was associated with less prefrontal cortex and superior parietal lobule activation ($ps<.01$) and more medial orbitofrontal cortex and temporoparietal junction (TPJ) activation ($ps<.03$), compared to the Load effect (cued 2-back>cued 0-back). Controlling for smoking frequency, TPJ activation correlated with cue-potentiated 2-back accuracy ($r=0.62$, $p=.025$).

Conclusions. Smoking cues enhanced WM proficiency among smokers and shifted attention network engagement from dorsal to ventral structures. Our findings show preliminary evidence that cue-related attentional bias enhances executive function in smokers.

GENDER DIFFRENCES IN AGE-RELATED COGNITIVE FUNCTIONALITY AMONG MICE ADMINISTERED WITH PROGESTERONE

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Progesterone, recognized as a neurosteroid and pivotal sex hormone, has been extensively linked to cognitive function in females, while its impact on males remains relatively unexplored. This study aims to evaluate the influence of progesterone on cognitive ability, considering both sex and age factors. Young (4-month-old) and old (20–23-month-old) male and female mice were subjected to daily subcutaneous injections of progesterone (5 mg/kg) or vehicle (30% 2-hydroxy beta-cyclodextrin). Cognitive assessments were conducted using the water-T-maze and passive avoidance behavioral tasks. Initial analysis involved 17 mice, with ongoing studies involving additional animals. Preliminary findings suggest potential cognitive benefits of progesterone in younger male mice, yet these benefits were not observed in older males. Conversely, progesterone showed detrimental effects on cognitive performance in both young and old female mice, aligning with previous research from our lab. Further investigation is underway to validate these initial observations. This study represents the first examination of cognitive disparities related to age and gender following daily progesterone treatment, prompting further exploration of progesterone's therapeutic potential in cognitive function across sexes and age groups.

SLEEP LOSS DIMINISHES HIPPOCAMPAL REACTIVATION AND REPLAY

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Sleep is vital for consolidation of hippocampal-dependent episodic memories. A growing body of evidence has implicated the reactivation and replay of neuronal patterns from waking experience as strong candidate mechanisms supporting memory consolidation. Reactivation and replay occur primarily during sharp wave ripples (SWRs): transient 150-250 Hz oscillations observed in hippocampal local field potential recordings that occur during quiescence and slow wave sleep. Despite evidence that sleep regulates molecular and cellular level processes important for learning, little is understood about how sleep deprivation impacts circuit-level memory mechanisms in the hippocampus supporting memory, such as SWRs, reactivation, and replay. We utilized high-density silicon probes to perform chronic electrophysiological recordings of large hippocampal ensembles in freely moving rats as the navigated a novel linear track for water reward. Following track running, rats were either sleep deprived for 5 hours by gentle handling followed by 2.5 hours of recovery sleep or allowed to sleep freely for 7.5 hours (natural sleep). In line with previous work from our lab, we found that hippocampal reactivation remained high for a minimum of 5 hours during natural sleep. In contrast, reactivation levels were severely diminished during sleep deprivation and decayed to chance levels much quicker than during natural sleep. Moreover, sleep deprivation was associated with a lower proportion and overall number of sequential replay events. Reduced reactivation and replay were not due to impaired SWRs as SWR rate was elevated during sleep deprivation compared to natural sleep. Sleep deprivation may therefore disrupt memory consolidation by impairing the ability of the hippocampal ensembles to reactivate memory-related information from waking experience following learning.

CROSS-SECTIONAL AND LONGITUDINAL ASSOCIATIONS BETWEEN WEIGHT PERCEPTION AND CARDIOMETABOLIC OUTCOMES AMONG ADOLESCENTS

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Purpose: Cognition can affect cardiometabolic health, as has been elucidated in studies that examine the associations between perceived stress and negative cardiometabolic biomarkers. Weight perception is the way one perceives their own weight regardless of their actual weight status, and it has been shown that perceiving “overweight” is associated with worse psychological and physiological health. However, few studies have been done to examine how cognitive perceptions of weight status, particularly perceiving “overweight”, may affect cardiometabolic health parameters. Thus, we sought to assess the cross-sectional and longitudinal associations between weight perception and markers of cardiometabolic health.

Methods: Data came from waves 4 (Mage =19.20.04) and 7 (three years later) of the NEXT PLUS Generation Health Study, a nationally representative study of United States adolescents (n=454). To assess cross-sectional and longitudinal associations between weight perception (perceived “overweight” vs. did not perceive “overweight”) and cardiometabolic outcomes (fasting blood glucose, HbA1c, high sensitivity C-reactive protein, triglycerides, total cholesterol, HDL, LDL, systolic blood pressure, diastolic blood pressure, and waist circumference), linear regressions adjusting for sociodemographic variables were performed.

Results: Cross-sectionally, when controlled for sex, race/ethnicity, socioeconomic status, and age, those that perceived “overweight” had higher average high sensitivity C-reactive protein (=1.14, p=0.0002), triglycerides (=27.7, p=0.0117), cholesterol (=18.5, p=.0046), LDL (=13.4, p<0.001), diastolic blood pressure (=3.6, p=0.0022), and waist circumference (=18.4, p<0.001). They also had a lower HDL (=-5.4, p=0.0177). When additionally controlling for BMI, cholesterol and LDL remained significantly higher in those that perceived “overweight” ((=12.3, p=0.0449 and =8.3, p=0.0398, respectively). Longitudinally, the perceived “overweight” group had a higher average HbA1c (=0.08, p=0.0495) and a lower LDL (=-9.4, p=0.0098). These associations were lost when additionally controlling for BMI.

Conclusions: At a cross-sectional level, perceiving “overweight” is associated with worse cardiometabolic health biomarkers, some even regardless of actual weight status. Longitudinally, there were fewer significant associations. These findings highlight the influence that cognition may have on cardiometabolic health and can be used to inform policies that aim at correcting weight misperception in adolescents. Future studies are warranted to explore the causal pathway of this relationship, as well as how the effects may differ based on different sociodemographic factors.

AGE-RELATED CHANGES IN BEHAVIOR AND MEMORY FUNCTIONS IN UM-HET3 MICE

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Aging leads to changes in metabolism, immunity, cognition, motor function and mortality risk, at rates that are modulated by biological, genetic, and environmental factors. Several studies have documented age-related changes in behavior and cognitive functions and investigated the molecular changes in aging brain using inbred mouse strains such as C57BL/6, BALB/c etc. In this study using a genetically heterogenous mouse population (UM-HET3) we investigated age-related changes in behavior and memory functions and their association with blood cell measures. We performed a variety of assessments including visual acuity, contextual fear conditioning, grip strength, acoustic startle response and complete blood count (including hemoglobin, white blood cells, neutrophils, monocytes, lymphocytes, platelets). Our results showed significantly lower body temperature, decrease in hemoglobin, changes in blood related parameters (including RBC, hematocrit, platelets, MPV, WBC), reduced visual acuity, reduced grip strength (not normalized by BW) and impaired memory functions in older mice when compared to middle-aged mice. A reduced startle reactivity was also observed in older male and female mice which is likely to be the result of impaired hearing, although these mice were not tested for hearing loss. Our study showed that UM-HET3 mice exhibit age related impairment in sensory and memory functions as compared to middle aged mice. In older mice, reduced memory correlates with increased percentage of neutrophils and decreased lymphocytes in blood, suggesting a possible role of white blood cells in age-related memory decline in UM-HET3 mice. However, further studies are needed to understand the mechanisms driving age-related decline in cognition with focus on changes in immune cells for developing therapies to slow down the rate of cognitive decline in normal aging as well as neurological diseases such as Alzheimer's disease.

FRONTAL AND PARIETAL ERPS MAY MARK THE PLANNING OF UP- VERSUS DOWN-DIRECTED SACCADES

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Saccades (fast eye movements) are controlled by a complex interplay among the cerebellum, brainstem, midbrain, and cerebral cortex. For incoming visual signals, the superior colliculus (SC) in the midbrain serves as a head/eye movement reflex centre that sends saccade commands to brainstem saccade generators. As eye movement is an exclusion problem, the SC must be inhibited from recurrent reflexive responses. If not place under inhibition by the basal nuclei (BN), the SC would chaotically trigger the execution of saccades to attention-grabbing stimuli. Our ability to make saccades at will, comes from activity in frontoparietal regions of the cortex. The balance of control shifts towards frontal regions as visual signals become more endogenous and less exogenous (i.e., peripheral attention-grabbing). Most of our knowledge of saccade operation comes from horizontal saccade studies. The present work was motivated by the following vertical saccade-related observations. First, given the position of the head, there are more attention-grabbing objects in the lower visual field (near the torso) than in the upper visual field. Hence, saccade mechanisms face a vertical asymmetry in saccade selection demands. Second, in the SC, functional discontinuities exist such that response levels are weaker for areas that innervate down-directed saccades. In effect, SC inhibition may be greater in areas responsible for releasing down-directed saccades. Third, within the cerebral cortex, magnetoencephalography (MEG) findings suggest that the left frontal cortex specifically, may be most selective in up vs down saccade planning, such that activity is higher for down- than up-directed saccades. In effect, greater neural effort may be needed to initiate the releasing of down-directed saccades. Saccade-related regularities provide insight on attention and premotor mechanisms. Based on these observations, our aim in this preliminary study was to determine how event-related potentials (ERPs) may be utilized to mark the planning of vertical saccades. We reasonably assumed that pre-saccadic ERPs serve as indices for the planning of saccades. Fourteen neurotypical adult volunteers participated in the experiment. An electroencephalography + electrooculography methodology was utilized to identify ERPs associated with the planning of endogenous, vertically-directed saccades. The planning window was defined as 400ms - 4ms before the execution of a saccade. The reference was the average of 19 electrodes in the 10-20 system. We found polarity differences preceding up- versus down-directed saccades, suggestive of a distinction in saccade planning activity. Also, polarity reversal between frontal and parietal regions suggested a dissociation in function between frontal and parietal saccadic networks. Finally, left frontal activity demonstrated significantly higher planning levels for downward directed saccades. If positive results continue to develop with healthy adults, they may provide a basis for pursuing an ERP research program aimed at determining

saccade-direction-specific deficits in psychiatric disorders characterized by impaired saccade control.

NEUROIMMUNE MODULATION CONTRIBUTE TO CHOLINERGIC DYSFUNCTION IN SIGN-TRACKING RATS

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Understanding the intricate interplay between neuroimmune processes and attentional control is crucial in elucidating the underlying mechanism of addiction vulnerability. Certain cues in an environment can gain motivational properties when directly associated with a reward. Sign-tracking (ST) rats are prone to attribute incentive salience toward reward cues, which can manifest as a propensity to approach and contact Pavlovian cues. Compared to their counterparts, goal-trackers (GTs), STs also exhibit poor attentional performance. These attentional control deficits in STs have been linked to intracellular choline transporters marked for dysfunction, ubiquitin. An immune response is a known disrupter of the choline transporter. Activation of the innate immune system by systemic administration of the bacterial endotoxin lipopolysaccharide (LPS) led to a significant increase in choline transporters (CHT) marked with ubiquitin in the cortex and striatum of GTs but not STs. This suggests that in unchallenged STs, CHTs marked with ubiquitin are already at maximum levels and unresponsive to an additional immune challenge, leading to a potential ceiling effect in STs and that differences in neuroimmune state contributes to the cholinergic dysfunction in STs. Here we investigated post-translational modifications responsible for dysfunctional CHTs in STs, with the hypothesis that elevated neuroimmune modulator signaling attenuates cholinergic function causing sign-tracking behavior. Prior to an immune challenge, cytokine levels measured in the frontal cortex and striatum, but not the spleen, were higher in STs than in GTs. Administration of LPS increased levels of cytokines in the spleens of both STs and GTs; however, in the cortex and striatum, LPS increased most all cytokine levels in GTs only. Microglia are immune cells in the brain that, when activated, produce cytokines. Spalt Like Transcription Factor 1 (Sall1) is a microglia gene marker that is important in maintaining the homeostasis of other, more general, microglia. We observed that STs express reduced levels of Sall1, suggesting increased microglia activity, in the frontal cortex compared to that of GTs. Consistent with this finding, STs also showed higher levels of general microglia activation (Iba1, CD45, and CD11b). After LPS, GTs exhibited increased expression of Iba1, CD45, and CD11b and reduced Sall1 expression, consistent with LPS-triggered microglia activation. In contrast, STs showed no increased expression of CD11b and if anything, an increase in Sall1. At baseline, reduced Sall1 expression, elevated levels of microglia, and increased cytokine production, all suggest a more active neuroimmune state in STs. This further supports the interaction between elevated brain immune modulators signaling and choline transporter regulation as an essential component of the neuronal underpinnings of the addiction vulnerability trait, indexed by sign-tracking.

ACTIVATION OF SPECIFIC INTERNEURONAL SUBPOPULATIONS AFFECTS DIFFERENT ASPECTS OF HIPPOCAMPUS-DEPENDENT LEARNING AND MEMORY

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Learning and memory are fundamental cognitive processes that define our ability to remember and respond to past experiences. In particular, spatial memory processing involves the stages of encoding, consolidation, and recall, all of which are thought to rely on the activation of neurons across multiple brain regions. Recent studies have focused on the role of excitatory, or glutamatergic, interneurons during hippocampal memory processing. However, GABAergic interneurons, which act as the primary source of inhibition in the hippocampus and neocortex, likely play a critical part in memory processing as well. To explore how changes in inhibitory interneuron activity can ultimately influence the functional output of the hippocampal circuit, we examined how activation of somatostatin-expressing (SST+) and parvalbumin-expressing (PV+) interneurons affects the different stages of memory processing. Adult male and female SST-IRES-Cre and PV-IRES-Cre mice underwent dorsal hippocampus AAV-mediated transduction to express either the activating DREADD hM3Dq-EGFP, or EGFP alone, in a Cre-dependent manner within these interneuron populations. At the beginning of the light phase, hippocampus-dependent spatial memory processing was assessed using the object-location memory (OLM) paradigm. DREADD agonist Compound 21 (C21) was administered to all mice (i.p.) at different phases of memory processing: 30 min before OLM training, immediately after OLM training, or 30 min prior to OLM testing 24 hrs after training, in order to test for the effects of interneuron activation on memory encoding, consolidation, and retrieval respectively. We find that activation of SST+ interneurons in the dentate gyrus impairs OLM encoding and retrieval in male and female mice, but has no effect on memory consolidation. Furthermore, we find that activation of PV+ interneurons in the dentate gyrus disrupts OLM encoding in male and female mice, while not impacting consolidation or retrieval. Preliminary data from immediate-early gene immunohistochemistry and imaging validates AAV injection sites in C21-injected SST-Cre and PV-Cre. Interneuron populations can be differentially affected by cognitive disruptions like sleep loss, and can differentially regulate activity in brain circuits; thus, examining how these populations contribute to various stages of spatial memory processing will inform our understanding of neurological disorders that may alter their function.

Theme 2 - DEVELOPMENT:

IMPACT OF AN ASD-ASSOCIATED GLUN2B MUTATION ON WT-GLUN2B SUBUNIT TRAFFICKING AND DENDRITE DEVELOPMENT

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De novo mutations within the GRIN2B gene have been associated with autism spectrum disorder (ASD). GRIN2B is the gene that encodes the N-methyl-D-apartate receptor (NMDAR) subunit GluN2B. The first mutation, GluN2B724t, identified in a female patient with severe non-syndromic ASD and intellectual disability (ID), was a splice site mutation that is predicted to produce a premature stop within the extracellular loop (S2). This mutation prevents splicing of the mRNA, and results in the truncation of the 4th transmembrane domain, the cytoplasmic tail and part of the extracellular loop. Previous research in our lab found that the mutant is not trafficked to the neuron surface, instead is restricted to the soma and occasional proximal dendrites. GluN2B724t alters NMDAR function, dendrite development and morphology in cortical neurons. This project aimed to determine if phenotypes associated with GluN2B724t can be replicated through pharmacological inhibition of GluN2B.

EXPLORING THE PREDICTABILITY OF RESTING-STATE CONNECTIVITY PATTERNS ON FRONTOLIMBIC ACTIVATION DURING FEAR EXTINCTION RECALL IN YOUTH

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Background: Anxiety and other fear-based disorders commonly emerge during childhood and adolescence, and are associated with deficits in fear extinction learning and recall. Compared to adults, youth typically exhibit poorer extinction recall and insufficiently engage frontolimbic circuitry, notably regions of the medial temporal lobe and prefrontal cortex. Neuroimaging studies in adults show that patterns of frontolimbic resting-state functional connectivity (rs-FC) following extinction learning can predict brain activation during subsequent recall. This study examined the association between frontolimbic rs-FC patterns and subsequent brain activation during recall in youth.

Methods: Seventy-seven youth (M±SD age=11.5±3.0 years, 54.5% male) completed a two-day Pavlovian fear extinction paradigm. On the first day, participants underwent fear conditioning and extinction learning, followed by a rs-FC paradigm and a test of extinction recall during functional neuroimaging. Regression analyses assessed associations between frontolimbic rs-FC and brain activation during recall.

Results: During extinction recall, youth exhibited activation in the left parahippocampal gyrus (PHG) and right dorsal lateral prefrontal cortex (dlPFC) to a previously extinguished cue. Lower PHG-right supramarginal gyrus rs-FC predicted greater PHG engagement during recall, while higher dlPFC-right fusiform gyrus rs-FC predicted greater dlPFC engagement during recall (p's<0.005, $k>10$).

Conclusion: Similar to adults, rs-FC patterns were linked to subsequent activation of frontolimbic regions during extinction recall in youth. Given the ongoing development of these brain areas during youth and the reliance of current first-line psychotherapies on successful extinction recall, further research elucidating the role of frontolimbic circuitry in these processes is critically needed."

AIR POLLUTION AND MENTAL HEALTH: INVESTIGATING PM2.5 EFFECTS ON SALIENCE NETWORK FUNCTIONAL CONNECTIVITY AND MENTAL HEALTH IN YOUTH

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Exposure to air pollution (PM2.5) is associated with increased risk of mental health disorders in youth, yet the underlying neurodevelopmental mechanisms remain unclear. This study examined the impact of PM2.5 exposure on anxiety and depression symptoms and functional connectivity of the salience network (SN), which plays a pivotal role in orienting attention to emotionally salient stimuli. Fifty youth aged 10-17 (52% female) wore personal air monitors for 7 days and completed surveys assessing both acute (daily) and chronic anxiety and depression symptomatology. A subset of participants (n=35) underwent functional magnetic resonance imaging to examine within-network SN resting-state functional connectivity (rsFC) and between-network rsFC of the SN and the whole brain. Linear regression models tested associations between average PM2.5 and anxiety and depression symptoms using a $p<0.05$ threshold. Linear regressions also linked average PM2.5to rsFC of key SN regions (i.e., anterior cingulate cortex (ACC), insula) using a $p<0.005$, >10 voxel whole-brain threshold. Average PM2.5 varied in the sample from 0.58-31.21 μg/m3 (M =10.14 μg/m3), which exceeded the annual average PM limits set by the Environmental Protection Agency (9.0 μg/m3). PM2.5 was not associated with acute or chronic anxiety or depression symptoms. However, higher PM2.5 was associated with lower rsFC between the ACC and right frontal pole (45 voxels, t = -4.75), and between the ACC and the precuneus (40 voxels, t = 4.56) Further, higher PM2.5 exposure was associated with higher rsFC between left insula and the left and right supramarginal gyrus (Left: 45 voxels, t = 6.93; Right: 13 voxels, t = 4.10), and between the right insula and the left supramarginal gyrus (23 voxels, t = 4.10).

Our findings demonstrate that PM2.5 exposure is associated with altered within- and between-network rsFC of the SN. Lower rsFC between the SN and the frontal pole and precuneus in youth with higher PM2.5 may indicate a shift towards reduced emotion regulation (frontal pole) and default mode network (precuneus) inhibition. Conversely, higher within-network SN rsFC (insula-supramarginal gyrus) may suggest increased network integration. Future longitudinal studies should examine whether the observed SN alterations predict increased risk of mental health disorders.

GLOBAL WARMING AND ENDOCRINE DISRUPTION EXAMINED IN THE FRUIT FLY (DROSOPHILA MELANOGASTER): EFFECTS OF ELEVATED AMBIENT TEMPERATURE AND BISPHENOL A EXPOSURE ON PHYSIOLOGICAL MEASURES OF DEVELOPMENT

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Climate change (i.e. “Global Warming”) is exacerbated by use of carbon-based fossil fuels leading to the emission of greenhouse gases. Various human activities are associated with significant increases in the Earth’s overall environmental temperature in connection with the consumption of fossil fuels. Elevated average environmental temperatures reduce many species’ survival rates due to effects of decreased fertility and physiological stress. Bipsphenol A is a widely used compound in the manufacture of various plastics. Many bisphenol A imbued plastics are used in facets of everyday life. Bisphenol A has been implicated as an endocrine disruptor and hence can exert impact on the endocrine system and the neuroendocrine aspects of the nervous system. Daily exposure to plastics containing bisphenol A occurs through the use of plastic food and beverage containers, receipt papers and in many other consumer goods. A growing facet of endocrine disruptor research is in the examination of potential synergistic effects of multiple environmental stressors related to development. The fruit fly is a translational model organism in research of developmental biology and in this presentation, we examine impacts of embryological, non-lethal, elevated ambient environmental temperatures along with bisphenol A exposure on the shaping of neurobehavioral and physiological responses of fruit flies. We present data on larval travel distances, pupation location measures, and on locomotor behavior of two strains of flies.

HAPLOINSUFFICIENCY OF HISTONE METHYLTRANSFERASE KMT2C IN MICROGLIA CAUSE ASD LIKE BEHAVIORS THROUGH DISRUPTING CORTICAL CONNECTIONS IN MOUSE MODEL

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Autism spectrum disorder (ASD) is marked by atypical social interactions, restricted interests, and repetitive behaviors. From a genetic perspective, mutations in epigenetic-related genes are a significant cause of ASD. Notably, the histone H3 lysine 4 mono-methyltransferase Kmt2C has been identified as a gene associated with ASD. However, the specific contributions of Kmt2C to brain development and ASD remain unclear. Single nuclei RNA sequencing has revealed high expression levels of Kmt2C in microglia, which play a crucial role in neuronal pruning. Consequently, we hypothesize that Kmt2C deficiency may lead to ASD-like behaviors via disrupted brain connectivity. Behavioral analyses indicate that Kmt2C haploinsufficiency in microglia impairs social behaviors, learning, and memory. It has also been established that cortical regions are significantly associated with ASD. Diffusion tensor imaging (DTI) analysis demonstrates that the deletion of Kmt2C in microglia disrupts cortical connections. Additionally, we observed enhanced connections in the medial prefrontal cortex (mPFC) and other cortical areas in both male and female mouse models with Kmt2C-deficient ASD-like traits. These findings suggest that dynamic histone covalent modifications in microglia are a critical factor in the development of ASD.

EFFECTS OF OSMOTIC STRESS AND BISPHENOL – A EXPOSURE ON THE GROWTH, DEVELOPMENT, AND BEHAVIOR OF THE NEMATODE (CANORHABDITIS ELEGANS)

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Environmental osmolarity, the concentration of dissolved solutes in the external environment, is an important neurosensory stimulus for many organisms. Although maintaining proper intracellular osmotic stability through behavioral responses to osmotic changes is important, the mechanisms underlying the development of these behaviors are not fully understood. The nematode Caenorhabditis elegans, which inhabits natural environments where fluctuations in environmental osmolarity occur frequently, provides a useful model for studying these processes. Bisphenol-A (BPA) is a chemical agent found in many plastics used in everyday life, it can and does leech from plastics and is inadvertently ingested by organisms. BPA has been identified as an endocrine disruptor, meaning it impacts the neuroendocrine system and can shape development. A current focus of the endocrine disruptor research examines potential synergistic effects of multiple environmental shapers of development. Data about potential effects of dual stressors of osmotic change and Bisphenol-A exposure are scant and warrant further study. In this research, we examine how environmentally relevant exposures to osmotic stress and bisphenol-A impact the development, growth, and behavior of Caenorhabditis elegans. This nematode is a translational model organism in the study of development due to its translucent body morphology and well-established cell lineage during development. In this work, we also examine new techniques to examine the behavior and neurophysiology of Caenorhabditis elegans.

THE EFFECTS OF GRIN2B HAPLOINSUFFICIENCY ON CORTICAL DEVELOPMENT

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GluN2B, the protein encoded by GRIN2B, is an important subunit of NMDA receptors that plays many instrumental roles throughout cortical development. Disruption of GRIN2B has been linked to a reduced density of spines, reduced dendrite outgrowth, and hindered neuronal differentiation and migration. De novo genetic variation within GRIN2B results in GRIN2B-related neurodevelopmental disorder (NDD), a disorder with a wide array of symptoms including intellectual disability, motor dysfunction and delays, muscle hypotonia, and malformations of cortical development. The majority of GRIN2B-NDD cases are predicted to result from a loss of function mutation in one GRIN2B allele, resulting in haploinsufficiency. To date, investigation into the link between GRIN2B haploinsufficiency and cortical malformation has been limited and unavailing. Therefore, this project aims to address the potential effects of GRIN2B haploinsufficiency on cortical development. To do so, sections of cingulate cortex, primary motor cortex, and primary somatosensory cortex from neonatal heterozygous GRIN2B KO rats have been Nissl stained and analyzed for cortical layering and thickness. Results of this experiment will give insight into the structural consequences of GRIN2B haploinsufficiency alone, which may be a key point for understanding the cortical maldevelopment occurring in patients affected by GRIN2B-NDD.

SNOOZE OR LOSE: EFFECTS OF SLEEP DURATION ON EXTINCTION RECALL, FEAR EXTINCTION NEURAL CIRCUITRY, AND VULNERABILITY TO ANXIETY IN ADOLESCENTS

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Background: Deficits in fear extinction learning and its later recall are associated with increased risk for anxiety disorders. Recent studies in adults indicate that sleep deprivation impairs next-day extinction recall and is associated with lower engagement of extinction-related brain regions, such as the orbitofrontal cortex. This study investigates these effects during late childhood and adolescence, a period marked by decreasing sleep duration and heightened vulnerability to anxiety disorders.

Methods: A total of 101 youth (54.5% female, 10-17 years) completed a two-day Pavlovian fear extinction and recall paradigm. Of these, a subset of youth (n=66) underwent the extinction recall test while undergoing functional magnetic resonance imaging. Youth self-reported their sleep duration on the night preceding the recall phase and anxiety symptoms. Conditioned fear was measured using subjective ratings, and brain activity in a priori extinction recall-related regions was extracted. Regression analyses were conducted to examine associations among sleep, anxiety, fear ratings, and neural responses.

Results: Shorter sleep duration was associated with higher anxiety symptoms, even after accounting for age and sex ($b=-0.040$, $r^2=0.343$, $p=0.002$). Higher anxiety symptoms, in turn, were associated with greater reported fear, even after accounting for age and sex ($b=0.606$, $r^2=0.102$, $p=0.009$). Although there were no main effects of sleep duration on fear ratings, sleep duration was associated with extinction-related brain activity. In particular, shorter sleep duration was associated with lower activity in the lateral orbitofrontal cortex, dorsal anterior prefrontal cortex, and cerebellum ($pFWE<0.05$).

Conclusions: Shorter sleep duration is associated with higher anxiety and lower engagement of brain regions implicated in extinction recall in youth. These results highlight the importance of investigating neural mechanisms to better understand the link between insufficient sleep and anxiety in youth. Such understanding holds promise for informing future research endeavors and intervention strategies aimed at mitigating the impact of insufficient sleep on youth anxiety.

REGULATING RHYTHMS: THE RELATIONSHIP BETWEEN AUTONOMIC NERVOUS SYSTEM MODULATION AND RESTING-STATE CONNECTIVITY PATTERNS DURING ADOLESCENCE

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Background: Heart rate variability (HRV) is an established indicator of parasympathetic activation implicated in emotional regulation and anxiety and depressive disorders. Previous research in adults has demonstrated correlations between HRV and fluctuations in functional connectivity (FC) among key brain regions involved in emotional processing and autonomic control, such as the dorsal anterior cingulate cortex (dACC) and amygdala. However, few studies have explored the neural correlates of HRV during childhood and adolescence—periods associated with heightened risk of anxiety and depressive disorders. The present study investigates correlations between HRV and FC in youth.

Methods: Twenty-one participants (M ± SD age = 13.57 ± 2.29 years, 66.7% male) completed a resting-state functional magnetic resonance imaging scan during which cardiac data was collected using pulse oximetry. We calculated HRV as the root mean square of successive differences between normal heart beats (RMSSD). HRV was then regressed on seed-based FC of the amygdala and dACC.

Results: Preliminary results indicated RMSSD was associated with FC differences in several brain regions. Most notably, RMSSD positively correlated with right amygdala-subgenual cingulate cortex FC and dACC-supplemental motor area FC, and negatively correlated with dACC-parahippocampal gyrus FC ($p < 0.005$, $k > 10$).

Conclusions: The results highlight the link between peripheral and neural mechanisms involved in regulating emotions and autonomic control in youth. A comprehensive understanding of these relationships is essential for preventing and treating anxious and depressive disorders. "

UNVEILING THE LINK: INTOLERANCE OF UNCERTAINTY PREDICTS STARTLE RESPONSE IN TRAUMA-EXPOSED YOUTH

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Background: Intolerance of uncertainty (IU) and trauma exposure each have established links with altered physiological fear responses to unpredictable threat. However, the relationship between these variables is unclear, and research in youth is limited. Here, we explored the interplay between trauma exposure and IU on startle response during a No-Predictable-Unpredictable (NPU) threat task in youth.

Methods: Startle eyeblink response was recorded from 51 Detroit-area youth (M±SD = 14.27±2.03 years, 51% female, 45.1% Black, 39.2% trauma-exposed) during the NPU task. Outcome measures included startle potentiation scores for unpredictable and predictable threat conditions. Linear regression models were used to assess self-reported IU and trauma exposure as predictors of startle response, controlling for NPU task version.

Results: The data revealed unexpected sex effects: females were more likely than males to experience trauma, $X2$ (1, $N = 51$) = 4.76, $p = .029$. Females demonstrated higher startle responses to both predictable ($t42 = 2.09$, $p = .043$) and unpredictable ($t45 = 2.41$, $p = .020$) threat. No trauma-related group differences existed between startle response. For trauma-exposed females only, IU predicted startle response to unpredictable threat ($n = 14$, $\beta = -5.26$, $p = .01$), such that higher IU predicted an attenuated startle response to unpredictable (but not predictable, $p > .05$) threat.

Conclusions: Results suggest that higher IU in trauma-exposed female adolescents predicts an attenuated startle response that is specific to unpredictable threat. Future research should seek to replicate and extend these findings in a larger sample with more trauma-exposed males. "

OUTDOOR AIR POLLUTION EXPOSURE AND ADOLESCENT ANXIETY-RISK: UNRAVELING NEURAL MECHANISMS THROUGH A FEAR EXTINCTION PARADIGM

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Background: Air pollution is a major environmental health threat. Growing evidence indicates particulate matter (PM2.5) air pollution can have adverse effects on brain development and increase the risk of poor mental health outcomes. Recent research has shown that exposure to PM2.5 is associated with a higher prevalence and severity of anxiety and other common mental disorders in youth. However, the neurodevelopmental mechanisms underlying these associations are unknown. Impaired fear extinction recall is a neurodevelopmental marker of anxiety and is tractable to treatment. The current study examines the impact of recent PM2.5 exposure on brain activation and conditioned fear during extinction recall, as well as self-reported anxiety symptoms in youth.

Methods: Sixty-four youth (10-17 yrs, 45% female) completed a two-day fear extinction task during functional magnetic resonance imaging. On day one, participants underwent fear conditioning and extinction. Twenty-four hours later, participants completed an extinction recall task. Conditioned fear was measured using self-reported fear ratings of the conditioned stimuli. Past-month ambient PM2.5 concentrations (mean=8.96 ug/m3) were estimated for each participant using a random forest machine learning model. Additionally, participants completed a validated self-report questionnaire assessing anxiety symptoms experienced during the past month. Linear and logistic regression models, adjusting for age and sex, was used to examine associations between PM2.5 exposure and brain activation and fear ratings during extinction recall, and anxiety symptoms.

Results: Higher PM2.5 exposure was associated with increased odds of meeting or exceeding the cutoff indicating the presence of a clinical anxiety disorder (OR = 1.335 95% CI: 1.061, 1.681). However, no associations were observed between past-month PM2.5 exposure and reported fear during extinction recall. Nevertheless, higher past-month PM2.5 exposure correlated with greater neural response to the previously extinguished cue in the left parahippocampal gyrus (xyz=17, -24, -17, t=3.04, k=5, $p<0.005$) and right insula (xyz= 44, -39, 18, t=3.95, k=6, $p<0.005$). Further, higher insula activation was associated with higher anxiety symptoms ($\beta=10.18$, $p=0.002$).

Conclusion: Youth exposed to higher PM2.5 levels showed heightened neural activity in the insula, a region involved in fear expression, suggesting a return of fear. Further, youth showing higher insula response reported higher anxiety symptoms, indicating a potential neurodevelopmental mechanism linking air pollution exposure to anxiety-risk. Conversely, heightened neural response in the parahippocampal gyrus, associated with context and safety learning, may indicate greater engagement of an extinction-related brain region for recalling safety learning. Future longitudinal studies are needed to track the development of anxiety following air pollution exposure and to develop targeted interventions to stem anxiety symptoms in at-risk pollution exposed youth. "

EFFECTS ON MATERNAL CARE AND OFFSPRING NEURODEVELOPMENT AFTER THE TRANSITION FROM MORPHINE TO BUPRENORPHINE (MEDICATION FOR OPIOID USE DISORDER) DURING PREGNANCY IN A TRANSLATIONAL RODENT MODEL.

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In 2020, 2.7 million people have been diagnosed with Opioid Use Disorder (OUD). Of particular concern, there has been a dramatic increase in opioid misuse among pregnant women with the number of OUD diagnoses during pregnancy increasing by a factor of four from 1999 to 2014. Pregnant women who are diagnosed with OUD are often prescribed medications for opioid use disorder (MOUD) such as buprenorphine (BUP). BUP is a semi-synthetic partial mu-opioid agonist and kappa-opioid antagonist which has since replaced methadone as the gold standard for OUD treatment. Evidence suggests that MOUD treatment (i.e., BUP) results in better outcomes for exposed infants as compared to discontinuation of illicit opioids or the continuous use of illicit opioids during pregnancy.

In the current study, we are utilizing a translational rodent model to investigate the effects on maternal care and offspring neurodevelopment outcomes after the transition from morphine to buprenorphine during pregnancy. Adult female rats were randomly assigned to one of five experimental groups: saline-vehicle (SAL), BUP continuous (BC), morphine continuous (MC), morphine to BUP (MB), or morphine to vehicle (MV). We started to administer drugs 7 days prior to breeding, to ensure drug dependence. The MB group received morphine until gestational day 4 (GD4) and then switched to BUP until postnatal day 2 (PN2). The MV group was switched to saline after GD4. The BC and MC groups received BUP or morphine continuously until PN2. These experimental groups are meant to simulate real-world scenarios in which pregnant women are either already taking BUP (BC), are switched to BUP once they become aware of their pregnancy (MB), try to stop taking the illicit drug (MV), or continue using an illicit opioid (MC). Dams' maternal care behaviors and pup retrieval were observed, and measurements of offspring mortality and neurodevelopment (weight, length, surface righting, and Neonatal Withdrawal Syndrome (NOWS)) were taken. On PN2, dams and pups were sacrificed, and their blood and brains collected for further analysis.

The BUP groups elicited deficits in maternal care behaviors, increased pup mortality, lower pup body weight, and increased pup withdrawal as compared to our control groups.

These findings suggest that BUP exposure during gestation still carries a risk for dam and offspring, warranting further investigation into the underlying mechanisms that contribute to these outcomes."

UNDERSTANDING THE ROLE OF A PATHOGENIC SPLICE SITE VARIANT IN SCN1B GENE IN DRAVET SYNDROME

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

GESTATIONAL BENZENE EXPOSURE LEADS TO SEXUALLY DIMORPHIC NEUROIMMUNE ADAPTATIONS ALONG THE PLACENTA-BRAIN AXIS

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Maternal immune activation (MIA) during pregnancy is highly associated with adverse offspring outcomes, such as neurodevelopmental delays and infectious susceptibility. In the modern era, air pollutants, such as benzene, have become one of the most prominent MIA triggers. The placenta-brain axis provides a unique window into how gestational programing facilitates neuroimmune development and predicts offspring disease risk. Microglia, the primary immune cell of the brain, are especially unique targets of study due to their generation solely in fetal life and shared ontology with placental macrophages. However, the mechanisms of how the placenta modulates MIA and how the fetal neuroimmune system responds to placental signals are poorly understood.

To study this, pregnant C57BL/6J mice were exposed to benzene (5 ppm) for 24 hours/day from embryonic day (E) 0.5-12.5. E12.5 litters were sacrificed for bulk RNA-seq, qPCR, and flow cytometry. Fetal growth measurements showed benzene-exposed litters had significantly decreased crown-rump length, occipito-frontal diameter, and fetal weights. Placenta transcriptomics reveals sex differences in genes associated with neurodevelopment after benzene exposure. Enrichment analysis showed placenta biological pathways were predominately immunological in males and metabolic in females. qPCR confirmed regulation of the inflammatory protein, IFI44, was sexually dimorphic in placenta and fetal brain. Our results suggest benzene-induced MIA causes dysregulation of the developing neuroimmune system in a sex-specific manner. Particularly, the interferon pathway may be a key mechanistic target to understanding sex-specific adaptations and postnatal susceptibility to neurological diseases and infections.

DETERMINING THE FUNCTION OF IL10 RECEPTOR SIGNALING DURING NEURONAL REGENERATION IN ZEBRAFISH

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Zebrafish possess the innate ability to regenerate a variety of damaged tissues, including the heart, fins and central nervous system. In the retina, experimentally induced neuronal cell death ultimately results in neuronal regeneration and restoration of visual function. Regeneration of retinal neurons involves reprogramming and division of Müller glia, a radial glia common to all vertebrate retinas, and proliferation of Müller glia-derived progenitors, which differentiate into neurons. Inflammation, a universal tissue-level response to cellular damage, is required to trigger tissue regeneration in zebrafish.

Interleukin-10 (Il10) is an anti-inflammatory cytokine that downregulates the expression of pro-inflammatory genes. Il10 binds to the tetrameric Il10 receptor complex, which consists of two Il10 receptor alpha and two Il10 receptor beta subunits. In the retina, the alpha subunit is expressed exclusively by microglia, the innate immune cells of the central nervous system, while the beta subunit is ubiquitously expressed. To investigate the mechanisms underlying the microglia-specific function of Il10 receptor signaling, we created loss of function mutants for il10ra and analyzed regeneration following photolytic lesion of photoreceptors.

To mutate il10ra, we utilized genome editing via CRISPR-Cas9. We designed and injected guide RNAs that targeted exons 2, 3, and 4 of il10ra. Genomic DNA of F0 embryos was extracted from both injected and un-injected siblings at 4 days post fertilization, and DNA fragments were amplified by PCR using primers that span the three targeted exons. Genomic DNA from uninjected siblings amplified a single 1.4kb band. Approximately 70% of injected embryos produced bands smaller in size. To validate and characterize the CRISPR-mediated mutations, PCR products were processed for Sanger sequencing, which identified frameshift mutations resulting in premature stop codons and predicted truncations of the Il10ra protein.

Injury-induced proliferation, neurogenesis and the response of microglia were evaluated in wildtype and mutant retinas. Immunohistochemistry for PCNA, proliferating nuclear cell antigen, at 5 dpl (days post lesion) found a notable increase in the number of PCNA-positive cells in the il10ra mutants compared to wildtype. This suggests that Il10ra signaling functions to downregulate proliferation of Muller glia-derived progenitors following a lesion. At 14 dpl, there is no qualitative difference in the number and morphology of regenerated photoreceptors between wildtype and il10ra mutants, whereas the number of newly generated amacrine and ganglion cells was significantly increased. This suggests that Il10ra signaling also plays a role in controlling neurogenesis during regeneration. The microglial response to a lesion was evaluated using the mutant microglia reporter line, il10ra-/-:Tg(mpeg1.1:eGFP). At 5 dpl, the number of microglia in the il10ra mutants was marginally higher than in wildtype retinas. This result indicates that Il10ra signaling also governs the response of microglia to a retinal lesion."

Theme 3 - HISTORY & EDUCATION:

THE NATIONAL HONOR SOCIETY IN NEUROSCIENCE NU RHO PSI

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Nu Rho Psi, The National Honor Society in Neuroscience, is a non-profit, grass-roots organization comprised of neuroscientists, at all stages of their careers. With more than 11,000 members, from 111 chapters in 33 States and the Nation's capital, Nu Rho Psi is a dynamic organization that aims to support the professional growth of its members. Most members are invited to join Nu Rho Psi during their undergraduate training. But qualified graduate students, faculty, and alumni are also welcome to join. Membership in Nu Rho Psi is granted exclusively through local Nu Rho Psi chapters. Nu Rho Psi has become a vibrant contributor to the neuroscience community through: (1) encouragement of professional interest and excellence in neuroscience, (2) recognition of outstanding scholarship, (3) advancement of the discipline of neuroscience, (4) encouragement of intellectual and social interaction between students, faculty, and professionals, (5) promotion of career development in neuroscience and related fields, (6) increased public awareness of neuroscience and its benefits for society, and (7) encouragement of service to the community. Nu Rho Psi goes beyond providing recognition of excellence in neuroscience scholarship and research. We offer our members a variety of grants and awards including competitive research grants to facilitate senior theses or other scholarly projects. Our chapters may apply for Nu Rho Psi Chapter Activity Grants to promote their educational and community outreach initiatives, including those that address our annual theme. The 2023-24 theme is Exercise and The Brain. Members are also eligible for Nu Rho Psi travel grants to present their original research at the annual Society for Neuroscience meeting. Schools wishing to foster a chapter of Nu Rho Psi may contact the National Office located at Washington College. Information regarding the charter application process may be found on our web page: https://nurhopsi.org

U@MNI: AN UNDERGRADUATE WORK-STUDY INITIATIVE TO BRIDGE THE SOCIOECONOMIC GAP IN NEUROSCIENCE RESEARCH OPPORTUNITIES.

Speas, R1,2, Myers, C1,2, Emery, K1,2, Kruskop, J1,2, Varela, M.C1,2, Reeves, J1,2, Walicki, M1,2, Evanski, J1,2, Hoyos Justiniano, R1,2, Meisler, M1,2, Jenkins, P1,2, Seasholtz, A1,2, Duncan, K1,2

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A high percentage of historically marginalized students face financial hardships that prevent them from engaging in research during their undergraduate education. Instead of investing their time in research, they may be required to use this extracurricular time to work for pay. This financial obligation excludes them from participating in labs that typically only support undergraduate volunteers thereby reinforcing socioeconomic barriers to a diverse neuroscience research community. Furthermore, faculty members often do not have funding for undergraduate research assistants, making research experiences exclusive to a for-credit or volunteer basis. To address this disparity in accessibility to research experience, we developed the U@MNI program. We currently fund eight undergraduate work-study students through the Michigan Neuroscience Institute at the University of Michigan. The U@MNI program selectively matches students with faculty who have relevant research interests, strong track records of mentorship, and engagement with diversity, equity, and inclusion efforts. These students work for 8-10 hours per week on a mentored project and attend monthly seminars. The purpose of these seminars is to build community and supplement their research experience with a curriculum designed to introduce the skills and expectations inherent to academia. At the end of the academic year, each student presents a poster on their research as part of a large undergraduate research symposium. U@MNI has opened the door to project-based laboratory research for students who otherwise lack the time or opportunity due to their work commitments. Our program will help to address issues in STEM retention among students of low socioeconomic status and subsequently enhance the diversity of neuroscientists by nurturing individuals with unique perspectives. By making it feasible for students with financial needs to obtain research positions, we target a unique intersection between race, gender, and other underrepresented identities. Our goal is to diversify the makeup of scientists who determine the direction of academic research, science policy, and industrial pursuits.

Theme 4 - INTEGRATIVE PHYSIOLOGY & BEHAVIOR:

Dietary Probiotics Fail to Alter Methamphetamine-Induced Impulsive Action

Kaitlyn A. Steck, Payton D. Brabant, and Lisa E. Baker, Ph.D.

Western Michigan University

Substance Use Disorder (SUD) is a chronic, debilitating condition often comorbid with anxiety and depression. Both SUD and affective disorders are characterized by cognitive dysfunction, including impaired decision-making and impulsivity. A mounting body of research implicates gut microbiome alterations as a contributing factor to the pathophysiology of affective disorders. No published studies have assessed behavioral effects of dietary interventions targeting psychostimulant-induced gut microbiome changes. The present study utilized a differential reinforcement of low rate responding schedule (DRL 18 s) as a behavioral index of drug-induced impulsive action to determine if a dietary probiotic supplement alters the behavioral effects of (+)-methamphetamine in rats. Thirty-two adult male Sprague-Dawley rats were trained to lever press for food reinforcement under a DRL 18 s schedule. Rats were then assigned to two dietary treatment groups, matched on reinforcement rate; the supplement group received continuous access to Bio-Kult Advance® in their drinking water and the control group received standard drinking water. Half the rats in each diet group received intraperitoneal injections of 1 mg/kg (+)-methamphetamine and the remaining rats received saline injections for eight consecutive days. DRL 18 s test sessions were conducted on day 1 and day 8, and subsequently 24 h, 48 h, 96 h, after the last injection. Statistically significant increases in response rate and corresponding decreases in reinforcement rate were observed in (+) methamphetamine-treated animals compared to saline-treated animals. The probiotic-treated rats displayed a higher drug-induced increase in response rate, but reinforcement rates were comparable between diet groups, and the main effect of diet was not statistically significant.

MAST CELL-DERIVED PROTEASES MEDIATE RESOLUTION OF INFLAMMATORY PAIN IN MICE

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Immune cells play a critical role regulating the transition from acute to chronic pain, however, while the initiation of inflammatory pain is relatively understood (Ji et al., 2016), the mechanism behind the resolution is unclear. Mast cells reside in the skin and because of their proximity to sensory nervous, a tight interplay with nociceptors has long been proposed (Hendriksen et al., 2017; Morellini et al., 2018; Mittal et al., 2019). These interactions may be beneficial or detrimental, suggesting their participation in pain modulation. The purpose of this study was to evaluate the contribution of mast cells in inflammatory pain induced by intraplantar injection of Complete Freund Adjuvant (CFA). We compared wild type (WT) and mast cell deficient (Kit^W-sh/W-sh) mice. The lack of mast cells drastically impacted mechanical pain resolution demonstrated by lower paw withdrawal thresholds in response to von Frey filaments compared to the WT. In addition to not recovering from pain, Kit^W-sh/W-sh mice had greater edema and higher levels of nitric oxide. Moreover, mast cells proteases 4 (mMCPT4) and CMA1 (mMCPT5) were significantly upregulated in gene and activity during the resolution phase and the inhibition of these proteases impaired pain resolution. Injection of recombinant mMCPT4 and CMA1 significantly improved resolution of pain. Our findings point toward a protective role of mast cells mediated by the release of proteases.

Innate metabolic profiles are not correlated with exploratory behavior of zebrafish (Danio rerio)

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Examining how physiological factors impact individual differences in behavior can shed light on the mechanisms driving behavioral variability. A potential contributor to behavioral variation is individual differences in metabolic rate. The pace of life syndrome (POLS) hypothesis proposes a correlation between the physiological traits of an individual, such as metabolic rate, and their behavioral traits (i.e., risk-taking, aggressiveness, or exploration). This study aimed to explore if baseline metabolism affects individual differences in the exploratory behavior of Danio rerio (zebrafish). We hypothesized that fish with greater metabolic rates would exhibit more bold and exploratory behaviors in novel environments due to their need to acquire more resources. To test this, we employed the novel tank test (NTT), an assay in which fish were placed in a novel environment and their exploratory behavior was recorded. Two days later, metabolic activity was captured by measuring oxygen consumption of individual fish over 30 minutes. We found a positive correlation between oxygen consumption and body weight, with female fish having higher metabolic profiles than males suggesting our assay is able to capture individual variability in metabolism. However, there were no significant correlations between the metabolic profiles of fish and their behavior in the NTT. Finally, to directly manipulate metabolism, we starved fish for 16 hours prior to the NTT. We found that 16-hour starvation significantly reduced metabolism but had no effect on behavior. This challenges the POLS hypothesis' proposed link between metabolic rate and behavior. The lack of significant correlation suggests metabolism may have less of an influence on behavior than initially thought.

EFFECT OF STRAIN AND ARABIAN JASMINE (JASMINUM SAMBAC (L.) AITON) ON ANXIETY-RELATED BEHAVIOR IN ZEBRAFISH

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Anxiety disorders are prevalent, and treatment options, such as the use of GABAergic or serotonergic drugs, may result in unwanted side effects or withdrawal symptoms. Therefore, medicinal plants are often used as an alternative treatment for anxiety. One such plant Jasminum sambac (L.) Aiton, commonly known as Arabian jasmine, is widely used in Thai traditional medicine for mental health ailments; however, clinical findings are mixed, with some finding jasmine to be anxiolytic and others anxiogenic. Pre-clinical studies can help us understand the conditions in which jasmine may exert these opposing effects. This research aims to determine if J. sambac is anxiolytic or anxiogenic and to determine if sex and/or genetic background influences the effects of Arabian jasmine. To do this, we administered J. sambac to AB and WIK zebrafish prior to their exploration of a novel tank. The flowers of J. sambac were extracted by ultrasonication and high-pressure extraction. The Arabian jasmine crude extract was examined by headspace solid-phase microextraction with gas chromatography-mass spectrometry (HS-SPME-GC-MS), and a total of 33 compounds were identified. The major components were linalool (anxiolytic compound) and benzyl acetate (anxiogenic compound), which could be involved in the opposing effect of anxiety-related behavior. The zebrafish were fed a gelatin pellet with 3 different doses of Arabian jasmine, and a 3-dimensional novel tank test of zebrafish was performed. The results show the anxiogenic effect of Arabian jasmine on the female AB zebrafish but no effect in the WIK strain. These findings suggest that background genetics and sex can play an important role in modulating the anxiety-related effects of Arabian jasmine.

MAPPING THE NEURAL BASIS FOR INDIVIDUAL DIFFERENCES IN THE EXPLORATORY BEHAVIOR OF ADULT ZEBRFISH

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Individual differences in behavior have been observed across a wide range of taxa, including humans, rodents, and fish. One significant axis of behavioral variation is risk-taking, where animals displaying a greater willingness to take risks are classified as bold, while those exhibiting less inclination are characterized as shy. While biological factors are known to contribute to this variation, the underlying mechanisms are not yet fully understood. To investigate the neural mechanisms underlying these behavioral differences, we employ adult zebrafish as a model. We assess behavioral differences in zebrafish by subjecting them to the novel tank test and quantifying their exploration of the new environment. Our findings reveal that bold individuals explore a larger area of the tank and spend most of their time near the top, whereas shy individuals exhibit limited exploration and spend most of their time towards the bottom of the tank.

To gain a better understanding of the neural basis of bold and shy behavior types, we developed tools for whole-brain activity mapping. We used in situ hybridization chain reaction (HCR) to detect the expression of c-fos, an immediate early gene, as a means of labeling active neurons. To visualize brain-wide c-fos expression, we combined tissue clearing technique with light sheet microscopy to generate whole brain images. For automatic detection of c-fos positive cells, we employed CellFinder, a deep learning based cell identification approach integrated into the BrainGlobe computational environment. The images were then registered to our recently created adult zebrafish brain atlas (AZBA) using advanced normalization tools (ANTs). We successfully trained CellFinder to identify c-fos positive cells with an accuracy of 96% and found that c-fos expression peaks at 15-30 minutes following exposure to a novel tank. With this approach, we identified brain regions associated with boldness.

IMPACT OF GLUCOCORTICOID TREATMENT AND WITHDRAWAL ON GLUCOCORTICOID RECEPTOR EXPRESSION IN BRAIN REGIONS ASSOCIATED WITH PAIN AND MOTIVATION

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Synthetic glucocorticoids are prescribed to more than 1% of the population for autoimmune and inflammatory disorders. Despite its efficacy, long-term glucocorticoid therapy is associated with adverse effects, making it imperative for patients to transition to other treatments when possible. Cessation of glucocorticoid treatment is hindered by a profound withdrawal syndrome patients experience manifesting as generalized pain, asthenia, and a loss of interest in normally pleasurable activities. The underlying mechanisms of the glucocorticoid withdrawal syndrome remain poorly understood, and the only treatment is to administer excess glucocorticoids. One potential mechanism of the withdrawal syndrome is alterations to glucocorticoid receptor (GR) expression in the brain, as glucocorticoid receptors can undergo homologous down-regulation. Our study employed a novel mouse model where mice were administered 15 ug/mL of prednisolone for eight weeks followed by a reduced dosage of 2 ug/mL to simulate withdrawal. Control mice received vehicle or continuous prednisolone 15 ug/mL. Male and female mice showed behavioral changes during glucocorticoid withdrawal that resemble key components of the human withdrawal syndrome, including increased mechanical sensitivity. To determine the effect of this prednisolone treatment and withdrawal on GR expression, we utilized immunohistochemistry with 3,3'-diaminobenzadine labeling to label GR and quantified GR+ cells in brain regions implicated in pain and motivated behavior, including the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), nucleus accumbens (NAc), and periaqueductal gray (PAG). Brain slices from each mouse were labeled, imaged, and then analyzed using a custom macro in ImageJ to calculate GR+ cell density. Results were analyzed using a one-way ANOVA, which demonstrated varied responses across the different brain regions. There was a significant effect of treatment on GR+ cell density in both the mPFC ($F(2, 11)=6.332$, $p=0.0148$) and ACC ($F(2, 31)=4.410$, $p=0.0206$). Post-hoc testing showed that prednisolone treatment significantly increased GR+ cell density relative to the control condition (mPFC, $p=0.0117$; ACC, $p=0.0153$), without any significant change in the withdrawal condition. There was no significant effect of glucocorticoid treatment or withdrawal on GR+ cell density in the NAc ($F(2,21)=2.822$, $p=0.0822$), or PAG ($F(2,48)=1.445$, $p=0.2459$). Ultimately, these results suggest that glucocorticoid treatment and withdrawal induce specific alterations in GR expression in the brain in a region-dependent manner. This supports the notion that changes in GR expression could contribute to the behavioral manifestations observed during glucocorticoid withdrawal; however, the role of increased GR+ cell density in the withdrawal phenotype remains to be determined.

EXPLORING THE ORGANIZATIONAL INFLUENCE OF ESTROGEN ON BINGE EATING IN RATS

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Prevalent forms of eating disorders are those characterized by binge eating (BE), which reflects consumption of a large amount of food in a short period of time, with a loss of control over the eating episode. Little is known about the etiology of BE or the biological factors that contribute to its female predominance. Ovarian hormones are key biological candidates to consider as they become elevated during puberty in females and drive physical and neural development during this key phase of development. Moreover, lower levels of estrogen are associated with increased risk for BE in females; however, it is currently unknown whether pubertal exposure to estrogen provides organizational effects that protect against the development of BE. In this study, we used an animal model of individual differences in binge eating to examine the organizational effects of ovarian hormones on risk for BE in female rats and tested the hypothesis that pubertal exposure to estrogen provides protection against the onset of BE. We employed remove and replace hormone paradigms, in which prepubertal female rats (P25) received either sham surgery, ovariectomy with estradiol benzoate replacement, or ovariectomy without hormone replacement. Upon recovery, rats received intermittent access to palatable food (PF) 3x/week for eight weeks to identify binge eating prone (consistently high levels of PF intake) and resistant (consistently low levels of PF intake) rats. Overall, these studies will determine whether the pubertal period is a developmental window of sensitivity to estrogen-dependent protection against BE.

EXAMINING THE NECESSITY OF MESOLIMBIC REWARD CIRCUITRY ON THE DISRUPTION OF COCAINE-SEEKING FOLLOWING MEMORY DEVALUATION

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Cocaine addiction is a major health concern in the US, yet there are no approved therapeutic targets for disrupting cocaine seeking behaviors. In this series of studies, we describe a novel approach in which we attenuate cocaine-seeking by devaluing the memory of cocaine reward via the mesolimbic reward system. In rats, we used a dual-viral strategy to enable projection-specific hM4Di-DREADD inhibition of ventral tegmental area (VTA) cells projecting to the nucleus accumbens (NAc). Following recovery from surgery, rats underwent cocaine self-administration training, in which they responded for cocaine infusions paired with a tone-light cue. Next, rats received presentations of the cocaine-associated tone-light cue alone in a different context. This cue-evoked retrieval of cocaine reward was immediately paired with temporary gastric illness produced by LiCl injection, which served to promote memory devaluation of cocaine reward. This led to a subsequent reduction in cocaine seeking in rats that received the mCherry control virus. Notably, inactivation of the VTA→NAc pathway during memory devaluation prevented the disruption in cocaine seeking in the hM4Di group. Overall, these findings suggest that it is possible to attenuate cocaine-seeking using memory devaluation in a mesolimbic-dependent manner. Moreover, our novel approach can be leveraged to develop therapeutic tools towards the treatment of cocaine use disorder.

NORADRENERGIC CIRCUIT RESPONSE TO HYPERTHERMIA-EVOKED SEIZURES IN THE SCN1A+/- MOUSE MODEL

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Locus coeruleus (LC) neurons are the main source of noradrenaline released to most brain structures, including the seizure-prone neocortical and limbic regions, regulating numerous physiological progresses including arousal, attention, memory, emotional response, and circuit activity. Previous evidence suggests that noradrenergic neurons may be involved in epilepsy and that their activation may be therapeutic for seizures. However, the activity of LC noradrenergic neurons during seizures remains largely unclear.

We directly investigated the response of noradrenergic neurons to seizures by using Scn1a+/- mice, a well-characterized pre-clinical translational model of Dravet Syndrome (DS) in which seizures can be evoked with hyperthermia. We performed EEG recordings of cortical signal concurrently with fiber photometry recordings of a genetically encoded calcium indicator (GCaMP8s) expressed selectively within noradrenergic LC neurons. We quantified the population response of noradrenergic neurons before (pre-ictal period), during (ictal period) and after (post-ictal period) seizures. Age-matched, wild-type littermates were heated as a control for effects of hyperthermia.

We found a profound seizure-induced change in the populational activity of noradrenergic neurons: there was a decrease in the amplitude and frequency of calcium transients. This pattern was not observed in wild-type littermate control mice or heating trials of Scn1a+/- mice that failed to evoke seizures at a similar temperature. Our study showed that the noradrenergic arousal network is acutely inhibited during seizures, which suggests that activation of noradrenergic signaling may be therapeutic in the Scn1a+/- mouse model of DS.

CHARACTERIZING SOCIAL PREFERENCES IN THE MALE NILE GRASS RAT, ARVICANTHIS NILOTICUS

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The Nile grass rat, Arvicanthis Niloticus, is a diurnal rodent that lives in small family structures with offspring reared by both parents in the wild. Their diurnal nature makes A.Niloticus useful for translational studies, as they share similar circadian behavior and physiology as seen in humans. However, it is unclear if this application extends to social behavior, as little is known regarding social behaviors in this species. Here, we aim to understand social behaviors in Nile grass rats by first characterizing social preferences in male Nile grass rats. Utilizing a three-chamber test, male grass rats were tested for sociality (preference for a novel same-sex social stimulus over a novel object) and sociability (preference for a novel over familiar same-sex conspecific) during the light phase. Subjects were tested twice for each of the preference tests over consecutive days to determine consistency in their preferences. We found a significantly higher preference for a novel social stimulus over a novel object stimulus. However, there was no preference for a novel over familiar conspecific. Upon further investigation, there was a two-split variability, with subjects either strongly preferring the novel stimulus or strongly preferring the familiar stimulus. This preference was highly consistent across both testing days. These results suggest that male Nile grass rats prefer to interact with social stimuli over objects, but preferences for novel over familiar social stimuli vary based on the individual. We are currently exploring the option that this could be the result of hierarchical dynamics within a cage. Future investigations could additionally determine how these opposing social preferences (novel versus familiar) are represented by the social decision-making network.

EFFECT OF INACTIVATION OF PREFRONTAL CORTEX ON SLEEP-WAKE STATES IN RAT

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Center for Consciousness Science

Michigan Psychedelic Center

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

EFFECT OF INTRAVENOUS DELIVERY OF N,N DIMETHYLTRYPTAMINE ON SLEEP-WAKE STATES IN RAT

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Psychedelics, including N, N, Dimethyltryptamine (DMT), are being increasingly explored for therapeutic potential to treat psychiatric disorders such as depression, anxiety, and post-traumatic stress disorder. These and other psychiatric conditions have a bidirectional relationship with sleep patterns but the impact of psychedelics, particularly DMT, on sleep architecture is incompletely understood. Therefore, in this study, we determined the effect of intravenous DMT administration on sleep-wake states in male and female rats. Under surgical isoflurane anesthesia, adult Sprague Dawley rats (n=10, 5 male, 5 female) were instrumented with electrodes to record electroencephalogram (EEG) from across the cortex and electromyogram (EMG) from dorsal nuchal muscles. In addition, a chronic catheter was positioned in jugular vein for the infusion of DMT or 0.9% saline (as vehicle control). Each rat received an intravenous bolus (100 uL/min over 5 min) of either one of two doses of DMT (low dose: 3.75 mg/kg, high dose: 7.5 mg/kg) or 0.9% saline. The DMT and saline infusions were performed 30-minutes after the start of the lights-ON period (8:00 am) after which EEG and EMG data were recorded for 24h across the light and dark cycle. EEG and EMG data were manually scored (SleepSign, Kissei Comtec) in 4-second intervals and classified as wakefulness, slow-wave sleep, or rapid eye movement sleep, then averaged in 3h bins across the 24h recording period. A linear mixed model was used to compare the changes in percent time spent in each state, mean duration per episode for each state, and mean number of episodes for each state, between vehicle control and DMT infusion sessions. There was a statistically significant increase in the time spent in wakefulness in the first 3h bin after DMT infusion ($p<0.02$ for low dose, $p<0.03$ for high dose). In the same 3h bin, a significant decrease in slow-wave sleep ($p<0.02$ for low and high dose DMT) was observed. DMT infusion produced a significant increase in the latency to the onset of rapid eye movement sleep ($p<0.01$), but no statistical effect was observed on the time spent in rapid eye movement sleep during the light period. Our results with DMT align with previous reports showing an increase in wakefulness after administration of serotonergic psychedelics. Further analysis of neurophysiologic data is needed to understand the neural dynamics associated with DMT-induced increases in wakefulness.

ASSESSING SYNAPTIC CONNECTIONS WITH INTERLUMINESCENCE USING TRAP2 MICE

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Almost no methods exist for selectively modulating communication between defined cells at the synaptic level, which is key to understanding how functional connectivity creates percepts, engrams and actions. Here, we advance a novel strategy for selectively modulating synaptic transmission, Interluminescence. This approach uses bioluminescent light from a presynaptic axon terminal, generated by a luciferase, to modulate an opsin in its postsynaptic target under experimenter-controlled introduction of a small molecule (luciferin).

We developed two separate methods that target the luciferase to the synaptic cleft, each offering distinct features for experimenter needs. To provide sustained and synapse-specific regulation, the ‘Persist-Int’ strategy places a luciferase in the synaptic cleft tethered to the presynaptic terminal, and an opsin in the opposing postsynaptic membrane. In this configuration, light generation creates sustained and activity-independent modulation. In the complementary ‘Act-Int’ strategy, luciferase is released into the synaptic cleft in response to presynaptic activity, a synapse-specific form of activity-dependent modulation.

We previously demonstrated the effects of Act-Int electrophysiologically at postsynaptic population level in vivo (Prakash et al., 2022). Here, we wanted to test the effects of Persist-Int in vivo, utilizing an approach with higher throughput and requiring no in vivo ephys expertise.

For the presynaptic membrane-tethered light emitter, we fused mNeonGreen-SSLuc to the transmembrane and cytoplasmic domains of neurexin (Nrxn3b) via a spacer consisting of the extracellular domain of the human CD4 (mNeonGreen-SSLuc-linker-CD4-Nrxn3b). For the postsynaptic opsin we selected the highly light-sensitive step function opsin ChR2(C128S). AAV2/9 preparations of these constructs were injected into the lateral hypothalamus and the locus coeruleus, respectively, of double transgenic TRAP2::lox-stop-lox-EYFP mice. Intraperitoneal injection of tamoxifen was followed by injection of the luciferin fluorofurimazine or vehicle. Activation of opsin-expressing neurons by bioluminescence from synaptically connected luciferase-expressing neurons was assessed by Fos2A-Cre-mediated EYFP fluorescence.

Our data demonstrate robust effects of Persist-Int Interluminescence in vivo and show a facile way to assess synaptic connections in different parts of the brain.

INTRAVENOUS PSILOCYBIN ALTERS BRAIN NETWORK DYNAMICS IN RAT

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Tryp Therapeutics

Psilocybin is a naturally occurring psychoactive compound that is being increasingly explored for therapeutic use in psychiatric disorders. Several studies have characterized the neurophysiological effects of psilocybin in human subjects. However, the effect of psilocybin on brain dynamics in animals is not well studied but is imperative for the development of models that can advance mechanistic understanding. Under isoflurane anesthesia, adult male and female Sprague Dawley rats (n=12, 6 female, 6 male, 300-350 g) were implanted with a jugular vein catheter, and electrodes to record electroencephalogram (EEG) across the cortex. After 7-10 days of post-surgical recovery, each rat received 10 mg/kg psilocybin or 0.9% saline (vehicle control group) as a continuous infusion over a period of 60 minutes while the EEG (0.1-300 Hz, sampling rate at 1 kHz) data were simultaneously collected. The EEG data were also collected for 20 minutes before and 120 minutes after psilocybin or saline infusion. The psilocybin and saline infusions were counterbalanced with 5-7 days between each infusion. We computed the absolute power spectrum (PSD), magnitude-squared coherence, and Lempel-Ziv Complexity (LZC) of the EEG time series during 5-minute epochs selected from 1) just before the start of infusion, 2) during psilocybin infusion (beginning, middle, and end), and 3) during the post-psilocybin recovery period. Statistical analyses were conducted using a linear mixed model with treatment condition and epoch as fixed factors, a random effect of rat, and PSD, coherence, or LZC as the outcome variables. Previous human studies have shown the psilocybin-induced psychedelic effect to be associated with increase in 1-30 Hz LZC. In rat, subanesthetic dose of ketamine has been shown to produce sustained increases in 0.5-175 Hz LZC and enhance gamma (65-175 Hz) power and coherence. In contrast, the results from the current study demonstrate the most salient LZC (temporal) changes in 95-150 Hz band, which showed a greater increase relative to pre-infusion baseline during psilocybin vs. saline infusion ($p<0.0001$); there was no significant change in LZC in 1-30 Hz band ($p=0.94$). These complexity changes were accompanied by a decrease in low frequency (4-10 Hz) PSD ($p=0.002$) and coherence ($p=0.008$). No significant changes were found in high frequency gamma (95-150 Hz) PSD ($p=0.37$) or coherence (coherence: $p=0.44$). Further studies and fine-grained analysis of these data are needed to understand the translational validity of psilocybin infusion rat models.

MODIFYING BEHAVIOR WITH CORTICAL LAYER SPECIFIC NEUROMODULATION

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The recent evolution of genetic tools to target neural circuits allows an unprecedented precision in neuromodulation. Genetically targeted circuit manipulation allows to probe nervous system function in the healthy brain, explore pathophysiology of neurodevelopmental and neurodegenerative diseases, and might be used to manipulate neural circuits for therapy. For Parkinson's disease, the goal has been to modulate the basal ganglia circuits in a way that is achieved with deep brain stimulation but with better spatial and temporal control. For Huntington's disease, circuit specific modulation to correct aberrant firing has shown promising rescue of motor deficits. For stroke, excitatory optogenetics has been used to strengthen the function of intact circuits so that they can restore lost motor function. After spinal cord injury, control of spinal neurons distal to the lesion established control of circuits that have been disconnected from brain control. We previously showed that systematically enhancing activity levels of pan-neocortical Emx1-positive pyramidal neurons during postnatal days 4 – 14 using non-invasive BioLuminescent-OptoGenetic (BL-OG)-mediated activation of luminopsin 3 (LMO3) led to decreased social interaction and increased grooming activity in adult animals. In vivo, both prefrontal neural activity and functional markers of cortico-striatal connectivity were impaired in developmentally hyperexcited adult Emx1-LMO3-positive mice. We wanted to further dissect the neural populations and their specific target areas mediating the observed behavioral and electrophysiological changes. Neurons in layer 5 integrate information between cortical areas but also project to subcortical structures involved in the generation of behavior. We carried out developmental hyperexcitation in layer 5-specific Rbp4-LMO3 mice, thus restricting LMO3 expression to L5 projection neurons. The behavioral consequences were compared to those of pan-laminar neocortical developmental hyperexcitation. We hypothesized that narrowing down the population of cells that are hyperexcited in early development will similarly narrow down the set of phenotypes that are altered in adulthood.

Bidirectional manipulation of orexinergic neurons show sexual dimorphism in learning and memory

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Sleep deprivation is thought to affect 30% of the population worldwide. Yet our understanding of how sleep disorders develop, how to treat them, and how to prevent cognitive impairment associated with lack of sleep remains limited. Previous studies from our lab have suggested that orexinergic inputs to the hippocampus are more active when mice are sleep deprived, but whether this plays a role in the mechanism for disruption of hippocampal memories during sleep deprivation is unknown. We have used excitatory and inhibitory chemogenetic manipulations to bidirectionally modulate orexin neurons' activity in the hours following learning, using two hippocampus-dependent, sleep-dependent tasks: contextual fear conditioning and object location memory. Our preliminary data suggest that the effects of these manipulations are both sex- and task-specific. To better understand these differences, we are using Brainbow viral tracing to assess whether there are morphological differences in orexinergic inputs to the hippocampus, and other brain structures, between males and females. These studies will test the hypothesis that sex differences in orexinergic circuitry may lead to sex differences in their contribution to behavior, memory consolidation, and sleep-wake regulation.

TO PLAY OR NOT TO PLAY? EFFECTS OF SOCIAL ISOLATION AND PLAYMATE NOVELTY ON SOCIAL PLAY ENGAGEMENT IN THREE LABORATORY RAT STRAINS

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Social play is a motivating and rewarding behavior displayed by juveniles of many mammalian species, including humans and rats. Social play is vital to the development of emotional, social, and cognitive skills, contributing to appropriate social interactions later in life. Autistic children show less motivation to engage in social play, struggle coordinating play behaviors with others, and find these interactions less rewarding than typically developing children. This reduced engagement in social play can contribute to impairments in social skills throughout life. There is limited knowledge about what external conditions may positively or negatively influence engagement in social play in humans or other animals. Therefore, we determined how two external conditions, social isolation and playmate novelty, modulate social play levels and play styles in juveniles of three common laboratory rat strains: Long-Evans, Sprague-Dawley, and Wistar. Males and females were socially isolated for either 2hr or 48hr prior to social play testing, then exposed to either a familiar or novel playmate, creating four testing conditions: 48hr-Novel, 48hr-Familiar, 2hr-Novel, and 2hr-Familiar. All three strains showed the highest levels of social play in the 48hr-Familiar condition. However, strain differences emerged in conditions yielding the lowest social play levels, which were 48hr-Novel for Long-Evans and 2hr-Familiar for Sprague-Dawley and Wistar. Overall levels of social play were highest in Wistar. No sex differences in social play levels were observed in any strain. Play style analysis is ongoing. Overall, playmate familiarity drives higher play levels in Long-Evans rats whereas time isolated drives higher play levels for Sprague-Dawley and Wistar rats. These strain differences allow for future research into determining the involvement of the brain reward system in condition-induced variations in social play engagement. Our findings are also informative in suggesting that external conditions, like social isolation and playmate novelty, could influence social play engagement in autistic children.

EXPLORING WITHDRAWAL EFFECTS OF A NOVEL BENZOFURAN DERIVATIVE IN RODENTS: A BEHAVIORAL ANALYSIS

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Psychedelics have been of interest to researchers in recent years for treating psychiatric disorders such as posttraumatic stress disorder (PTSD), as the current antidepressant treatments are limited in efficacy. One predominant psychedelic studied for treating PTSD is 3-4 methylenedioxymethamphetamine (MDMA). Although MDMA has been shown to have clinical efficacy in treating PTSD, the adverse withdrawal-like effects of the drug—namely anxiety, lowered mood, and anhedonia—may limit its therapeutic use. The recent development of novel benzofuran compounds, similar to MDMA, may provide more optimal treatments; however, it is unknown if these compounds induce anhedonia and anxiety following their use. This study aimed to compare the withdrawal-related effects of one such compound, 1-(benzofuran-5-yl)-N-methylpropan-2-amine (5-MAPB) (1.2 or 6.0 mg/kg dosage), to that of MDMA (2.5 or 5 mg/kg) and saline treatment (1 ml/kg) in adult female and male rats following binge-pattern administration (3-4 intraperitoneal injections, once every 2 hours). As a measure of anhedonia, rats underwent sucrose preference testing a week before and two days after drug administration. Data collection for the effects of MDMA and derivative administration is still underway, but initial results indicate a baseline sucrose preference in both males and females. We hypothesize that 5-MAPB and saline treatment will result in sucrose preference equivalent to baseline levels, while MDMA administration will lower their sucrose preference, though in a non-sex-dependent manner. Results of this study will aid in understanding the effects of 5-MAPB and the effort to develop newer and safer options for managing PTSD.

COMPARISON OF BLOOD PRESSURE AND SYMPATHETIC NERVE ACTIVITY CHARACTERISTICS IN INTACT VERSUS OVARIECTOMIZED FEMALE RATS

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Studies that examine the neurogenic aspect of cardiovascular disease (CVD) are aimed at improving strategies for reducing the high mortality rates associated with CVD. The incidence of hypertension, a known risk factor of CVD, is reduced in women of reproductive age compared to age-matched males, suggesting a cardioprotective effect of female reproductive hormones. There is a scarcity of research describing whether the presence or absence of cyclic ovarian hormones impact blood pressure regulation. Since blood pressure is in part regulated by the sympathetic nervous system, the purpose of this study was to examine potential differences in resting blood pressure and sympathetic nerve activity (SNA) between ovariectomized and intact female rats.

We hypothesized that blood pressure and SNA would be higher in ovariectomized compared to intact female rats.

Prior to the start of the estrous cycle, six female, Sprague-Dawley rats (4 weeks of age) were anesthetized and received either an ovariectomy [n=3] or sham operation [n=3]. At 16 weeks of age, all rats were anesthetized with Inactin [100 mg/kg, i.v. + supplements] and vaginal lavage samples were collected and analyzed to identify the stage of the estrous cycle. Sham rats were categorized into the high-ovarian-hormone stage if they were in proestrus or estrus, and ovariectomized rats were confirmed to be in the equivalent of diestrus. Arterial blood pressure and SNA were recorded from the femoral artery and splanchnic nerve, respectively. LabChart and the Peak Parameters extension were used to characterize sympathetic bursts by frequency, incidence, height, and width. An unpaired t-test was performed, and effects were considered significant if $p<0.05$.

When compared to intact rats, ovariectomized rats appeared to have higher mean arterial pressure (103±5 vs. 88±13 mmHg, respectively, $p=0.14$). In contrast, burst frequency (2.1±0.6 vs 2.0 ±0.42 Hz, $p=0.85$), burst incidence (46.5±12.5 vs. 46.3±9.9 bursts/100 heartbeats, $p=0.98$), burst width (230 ± 25 vs 248 ±17 ms, $p=0.36$) and burst height (2.87 ± 0.86 vs 1.81 ± 1.00 μV. s, $p= 0.57$) each appeared similar in ovariectomized versus intact rats. These preliminary studies were determined to be statistically under powered (data not shown).

Our preliminary study suggests that blood pressure, but not splanchnic SNA is higher in ovariectomized compared to intact female rats in high-ovarian-hormone stages, but additional experiments are needed, which may then shed light on the cardioprotective effects of ovarian hormones on lowering blood pressure, via mechanisms other than splanchnic sympathetic nerves. Future studies on the contribution of other sympathetic nerves or neurohumoral factors could explored to investigate mechanisms by which ovarian hormones are cardioprotective (R01 HL16123).

LOW- VERSUS HIGH-OVARIAN-HORMONE STAGES OF THE ESTROUS CYCLE ARE POTENTIALLY ASSOCIATED WITH HIGHER RESTING BLOOD PRESSURE AND SYMPATHETIC NERVE ACTIVITY IN PHYSICALLY ACTIVE FEMALE RATS.

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Cardiovascular disease (CVD) is the leading cause of death in the USA. Two of the most common risk factors for CVD are a sedentary lifestyle and high blood pressure. Additionally, females of reproductive age have a low incidence of hypertension compared to age-matched males, suggesting cyclical variations in ovarian hormones are cardioprotective. Although blood pressure is determined in part by the magnitude and frequency of bursts of action potential discharge from sympathetic nerves, little is known of the effects of the normal, cyclical fluctuations in ovarian hormones on resting blood pressure and sympathetic nerve activity (SNA). The purpose of this study was to determine if low versus high levels of endogenous ovarian hormones affect resting blood pressure and SNA in physically active female rats.

We hypothesized that physically active females in low-ovarian-hormone stages of the estrous cycle will have higher resting blood pressures and SNA than their high-ovarian-hormone stage counterparts.

Eight female Sprague Dawley rats were single-housed with 24-hour access to running wheels. At 16 weeks of age, the acute effects of exercise were minimized by removing wheels 24 hours prior to experiments. Rats were anesthetized with Inactin and instrumented to record arterial pressure and splanchnic SNA. The Peak Analysis extension of LabChart 7 was used to characterize sympathetic burst characteristics. Vaginal lavage samples were obtained to determine the stage of the estrous cycle. Rats were assigned to high-ovarian-hormone (proestrus or estrus, n=4) or low-ovarian-hormone (metestrus or diestrus, n=4) groups.

Mean arterial pressure (101±7 vs 90±3 mmHg; p=0.2018), burst width (258.3 ±17.6 vs 217.0 ± 19.4 ms; p=0.1670) and burst height (2.20±0.60 vs 1.25±0.47 μV.s; p=0.2541) appeared to be higher in the low- ovarian-hormone females as compared to their high-ovarian-hormone counterparts. In contrast, burst frequency (2.0±0.2 vs 2.6±0.3 Hz; p=0.20304) and incidence (47.4±5.9 vs 59.6±8.8 bursts/100 heartbeats; p=0.2911) appeared to be lower in the low hormone groups. Our studies were underpowered statistically (data not shown), suggesting more experiments are needed to more firmly support or refute our hypotheses.

In conclusion, our preliminary studies may suggest that low-ovarian-hormone stages of the reproductive cycle may be associated with increased resting blood pressure, due to more prolonged and higher amplitude bursts from sympathetic nerves. In contrast, high-ovarian-hormone stages of the estrous cycle may produce acute, short-term reductions in blood pressure and SNA, which may lead to long-term cardioprotective benefits. (R01 HL161233).

EXPLORING THE IMPACT OF OXYTOCIN DOSE AND ADMINISTRATION ROUTE ON ENHANCING MATERNAL-INFANT INTERACTIONS AND OFFSPRING OUTCOMES FOLLOWING GESTATIONAL BUPRENORPHINE EXPOSURE: A PILOT STUDY

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Over the past two decades, the United States has experienced an ongoing crisis of opioid abuse, which has reached epidemic proportions. Notably, from 2010 to 2017 opioid-related diagnoses have increased by 131% among pregnant women. Opioid dependent women are commonly prescribed Medication for Opioid Use Disorder (MOUD), such as buprenorphine (BUP), given that more favorable outcomes for infants have been demonstrated as compared to methadone or continuation of illicit opioids. Despite this, the neurological effects of BUP on mothers and the implications for maternal behavior remain poorly understood, but our lab has previously demonstrated that gestational BUP exposure can impair maternal care behaviors and offspring survival in a translational rodent model. This pilot study used the same translational rat model to test whether oxytocin administration could improve maternal behavior in BUP-exposed dams. For this, we investigated postpartum oxytocin administration (I.P and Intranasal) at varying doses following prenatal BUP exposure (1.0 mg/kg), beginning on gestational day 5 throughout postnatal day (PN) 2. Adult female rodents (N=14 total, n=2-3/group) were assigned to one of 5 experimental groups; BUP (BUP) + mock handling, BUP + I.P oxytocin low dose (BUP-IPL, 60 IU/kg), BUP + I.P oxytocin high dose (BUP-IPH, 180 IU/kg), BUP + intranasal oxytocin low dose (BUP-INL, 0.8 IU/kg) and BUP + intranasal oxytocin high dose (BUP-INH, 4 IU/kg). Oxytocin was administered once following parturition on PN0. Dams were assessed for nesting quality, maternal caregiving behaviors (nursing, licking/grooming, time spent on/off nest), and maternal motivation (pup-retrieval task). Offspring were assessed for presence of milk bands, and developmental milestones. Results from this pilot study indicate that postpartum oxytocin administration, specifically intranasal oxytocin at the higher dose, may increase pup-directed maternal caregiving behaviors, nesting quality, and maternal motivation. As well, results indicated oxytocin administration improved offspring outcomes, with a higher percent of milk bands present within the litter, as well as improved developmental milestones. This pilot data suggest that a single oxytocin administration shortly after birth may partly rescue the maternal brain network from the adverse impact of BUP. However, more research is needed to illuminate the effects of BUP and oxytocin on the maternal brain and subsequent offspring outcomes.

LOW- VERSUS HIGH-OVARIAN-HORMONE STAGES OF THE ESTROUS CYCLE ARE POTENTIALLY ASSOCIATED WITH HIGHER RESTING BLOOD PRESSURE AND SYMPATHETIC NERVE ACTIVITY IN PHYSICALLY ACTIVE FEMALE RATS

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ANALYSIS OF THE UBIQUITIN-PROTEIN LIGASE PARKIN IN METHAMPHETAMINE USE DISORDER

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The use of methamphetamine (METH) has become a significant public health concern, with over 2.7 million users in 2022, representing a staggering 107% increase over the past decade. This growing problem is compounded by the fact that mortality rates due to METH overdose are also on the rise. Adding to the challenge, there is a lack of any FDA-approved medication to treat METH use disorder (MUD). Our group has demonstrated that ubiquitin-ligase protein Parkin has a pharmacotherapeutic potential as a novel drug target to treat heavy use of METH, which is often associated with overdoses. Specifically, using an extended-access METH self-administration (SA) paradigm we showed that overexpression of Parkin in the nucleus accumbens (NAc) of male Long Evans rats led to a significant decrease in METH intake and relapse to METH seeking, while Parkin-deficient rats displayed higher METH consumption and were more vulnerable to relapse than the wild-type controls. To investigate the molecular pathways underlying Parkin's anti-addictive properties, we analyzed accumbal proteomes from Parkin-overexpressing (PO) and wild-type (WT) rats across different experimental conditions (saline-yoked rats, METH SA withdrawn rats, and rats that relapsed to METH-seeking behavior), employing the Ingenuity Pathway Analysis (IPA). Based on the z score, biological functions activated the most in PO NAc (regardless of an experimental condition) included those related to cell viability and survival. The most inhibited process was that of cell death The magnitude of change was higher in METH-exposed PO rats as compared to PO saline controls. Based on the p-value, the pathway statistically changed in PO rats (regardless of an experimental condition) was the EIF2 pathway, a pathway that regulates RNA responses under stresses. Other significantly changed pathways were those involved in neural adaptations, neuronal repair, responses to stress, and degradation systems. Filtering the enriched pathways to that were changed by 1.5-fold or more revealed statistical differences in responses to stresses in METH relapsed rats whereas in METH withdrawn rats, synaptosomal synaptogenesis and ERK/MAPK pathway (related to reinforcement of drug-related behaviors) were the most statistically different from WT rats. Studies have revealed that stress-related pathways can significantly impact drug reinforcement, particularly through ERK/MAPK signaling. This suggests that Parkin may play a crucial role in the specificity of this pathway during METH withdrawal. Together, these findings suggest that Parkin anti-addictive properties include stress response pathways and indicate complex interplay between parkin mediated molecular pathways and MUD.

Theme 5: MOTIVATION & EMOTION

EFFECT OF ADOLESCENT INTERMITTENT CAFFEINE ACCESS ON OPERANT RESPONDING FOR CAFFEINE AND ETHANOL DURING ADULTHOOD IN RATS

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Caffeine is a widely consumed substance found in a variety of common beverages. Early exposure to substances that alter behavior, like caffeine, could potentially influence later substance use patterns during adolescence and affect activity levels. The purpose of this study was to determine if adolescent intermittent access to caffeine in rats will escalate caffeine consumption during intermittent access, enhance sensitivity to motor-increasing effects of caffeine or elevate operant responding for caffeine. Another purpose was to determine if the caffeine exposure elevates operant responding for ethanol or enhances sensitivity to motor effects of oral ethanol. Male and female adolescent rats received six weeks of intermittent caffeine. The control groups received two water bottles, while the experimental group chose between 300 g/L caffeine and water three days a week. After the 6-week caffeine access, motor activity was assessed following injections of saline or caffeine (10 mg/kg and 30 mg/kg). Reinforcing effects of caffeine were tested via operant responding during 20-min sessions on fixed ratio 2 (20'FR2) and a progressive ratio (PR). Motor activity was tested again following orally self-administered caffeine on 10'FR1 schedule. To test ethanol reinforcement, rats responded for 6% ethanol during 20-min sessions on FR2 and PR. Motor activity was also tested following self-administered oral ethanol. Results indicate that adolescent intermittent caffeine access failed to escalate caffeine consumption during bottle drinking. Females consumed more caffeine early in adolescence as compared to late adolescence/early adulthood, with higher intake in females (15.2 mg/kg) than males (10.1 mg/kg) during early adolescence. Motor activity was unaltered by either injected caffeine or oral caffeine. Responding for caffeine was higher for females vs. males on FR2 but not on the PR. Responding for ethanol was higher for caffeine females vs. control females. However, male responses were similar across groups. Although responding was not significantly different for females vs. males, ethanol intake (g/kg) was higher in females than males, attributed to their lower body weight. Lastly, the motor activity was unaffected by orally self-administered ethanol. In conclusion, early-life oral consumption of caffeine may increase future motivation for caffeine and ethanol but only in females. Additional research can potentially investigate the relationship between caffeine consumption and ethanol as a gateway drug. Examining how intermittent access to caffeine may alter neurobiological and behavioral mechanisms could serve as the framework for future research.

REGULATION OF STRESS AND MOOD IN A MOUSE MODEL OF ORAL CONTRACEPTIVES

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Hormonal contraceptives, including oral contraceptives (OCs) are a critical part of healthcare, with broad health and economic benefits. For many users, OCs have beneficial mood effects, with decreased premenstrual mood changes and overall improved mood. Yet for 4-10% of people, OCs trigger adverse mood effects and increased risk for depression. We used a mouse model of OC exposure, newly developed in our laboratory, to identify how commonly used OC formulations regulate stress responses and contribute to the vulnerability to stress-induced behavioral changes. In this project, we aimed to understand the mechanisms by which OCs modify hypothalamic-pituitary-adrenal (HPA) axis. Female C57Bl6/N mice were given ethinyl estradiol (EE, 0.02μg) and levonorgestrel (LVNG, 0.75μg)– daily in 0.25mL 10% sucrose; control animals received 0.25mL of 10% sucrose. All treatments were given for at least 2 weeks prior to, and throughout, behavioral testing. At these doses, EE+LVNG suppresses the estrous cycle, has no gross effects on locomotor activity, and does not increase anxiety-like behavior. However, EE+LVNG does decrease sucrose preference suggesting a specific anhedonia-like effect and increases risk-assessment behavior suggesting more subtle changes in anxiety-related processes. Consistent with reliable findings from people using OCs, mice treated with EE+LVNG, show a significantly blunted acute stress response, as measured by corticosterone levels. Here, we hypothesize that OCs increase levels and expression of FKBP5, which in turn blunts the peripheral stress response. We determined OC-induced changes in glucocorticoid receptors, mineralocorticoid receptors, and FKBP5 levels/expression in the paraventricular nucleus, amygdala, and hypothalamus to identify molecular mechanisms by which OCs interact with stress. Together these findings demonstrate that the modulation of the HPA axis are key mechanisms for vulnerability and resilience to OC-triggered depression. Identifying individual differences in stress responsivity may help predict which individuals will benefit from which OC formulations, improving precision medicine.

SEX-DEPENDENT ASSOCIATION OF FOS-EXPRESSING NEURONAL ENSEMBLES IN THE NUCLEUS ACCUMBENS WITH COCAINE-PRIMED SEEKING IN RATS.

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Background: Individuals with Cocaine Use Disorder experience high rates of relapse that contribute to increased morbidity and mortality. Evidence from rat models of relapse to cocaine use suggests that sparse groups of neurons (i.e., neuronal ensembles) in the nucleus accumbens (NAc) encode learned associations that drive cue-induced cocaine-seeking, a relapse-like behavior. However, the role of neuronal ensembles in drug-primed cocaine-seeking has not been explored. Additionally, the role of sex in neuronal ensemble activation associated with drug-primed cocaine-seeking is poorly understood. Since females reportedly exhibit greater cocaine-primed seeking than males, determining if neuronal ensemble activation drives this behavioral sex effect is essential. Methods: To address these knowledge gaps, the present study investigated the role of sex on volitional cocaine-taking, cocaine-seeking following forced abstinence, and Fos-based neuronal ensemble activation in the rat NAc. Results: Consistent with previous literature, females self-administered more infusions of cocaine and exhibited greater cocaine-primed seeking than males. Neuronal ensemble activation was observed, but contrary to our hypothesis, females and males did not differ in NAc Fos activation after cocaine-seeking. Lastly, NAc Fos activation was significantly correlated with cocaine-seeking in males but not females. Conclusions: These results suggest that, while necessary for cocaine-primed seeking behavior, NAc ensemble activation did not drive the observed behavioral sex-effects. Circulating sex hormones and sexually dimorphic reward circuitry are implicated in cocaine-seeking, and future investigations should be performed to examine their interaction with relevant ensemble populations.

MELANIN CONCENTRATING HORMONE NEURONS DIFFERENTIALLY REGULATE FEEDING AND AROUSAL AS A FUNCTION OF DOWNSTREAM PROJECTION AREA

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Animals must make informed decisions about what to eat to maintain a proper balance of nutrients. Yet homeostatic need is not the sole factor in the decision to eat. Non-homeostatic motivators to eat, such as craving of sugary or fatty foods even when sated, are a contributor to human eating disorders. Melanin-concentrating hormone (MCH) neurons of the lateral hypothalamus and zona incerta are a relevant neural target for both homeostatic and non-homeostatic motivators to eat. MCH neurons project to many brain areas including the arcuate nucleus, nucleus accumbens, and cerebral cortex, and have a role in numerous behaviors including feeding, sleep, learning, and reward. We hypothesize that MCH projections to the nucleus accumbens (NAc) promote hedonic motivations to consume food but do not have a role in sleep-wake regulation. To address this hypothesis, we instrumented MCH-ChR2 mice with EEG/EMG headcaps and optic fibers either over the MCH neurons in the LH or their terminals in NAc and investigated how optogenetic stimulation affected feeding and sleep behavior in different behavioral contexts. When stimulation was delivered continuously, we observed that mice with stimulation of MCH neurons in the LH spent more time in REM sleep as well as transitioned into REM sleep bouts more frequently, while mice with terminal stimulation in the NAc did not show a sleep effect. When given the opportunity to choose between a port which delivers both food and acute optogenetic stimulation or a port which delivers stimulation alone, mice with terminal stimulation in the NAc show a preference for food paired with stimulation, while mice with cell body stimulation of all MCH neurons in the LH do not. These results begin to elucidate a mechanism by which MCH neurons differentially regulate feeding and arousal as a function of downstream projection area.

THE ROLE OF NUCLEUS ACCUMBENS CRF NEURONS IN INCENTIVE MOTIVATION

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Corticotropin-releasing factor (CRF) neurons are traditionally assumed to generate aversive stress states, particularly during withdrawal from drugs of abuse (George et al., 2012). However, other evidence shows that CRF signaling in nucleus accumbens (NAc) can generate positively valenced incentive motivation to pursue and consume rewards (Lemos et al., 2012; Peciña et al., 2006). Additionally, optogenetic laser stimulation of CRF neurons in the NAc of crh-Cre rats intensifies and focuses pursuit of a laser-paired sucrose or cocaine reward over an equal reward without laser stimulation. Optogenetic excitation of NAc CRF neurons also supports laser self-stimulation indicating positive valence of CRF neuronal excitation in NAc (Baumgartner et al., 2021, 2022). However, CRF neurons co-release other neurotransmitters such as GABA which may be more likely to account for these incentive effects. Thus, it is unknown whether CRF itself versus other neurotransmitters mediate this positively-valenced motivation. To specifically test the role of CRF receptor activation in NAc CRF neuronal incentive motivation, we administered i.c.v. microinjections of a global CRF antagonist or vehicle prior to laser self-stimulation by crh-Cre rats, or prior to 2-choice tasks in which rats could choose to earn either laser-paired sucrose reward or identical sucrose reward without laser. We found that CRF receptor blockade reduces laser self-stimulation of NAc CRF neurons and eliminates focused and intensified incentive motivation for laser-paired sucrose rewards in the two-choice sucrose task. This work builds on recent evidence that CRF neurons are capable of generating incentive motivation without distress and support an alternative role for CRF in salience without necessitating stress.

Behind the “Runner’s High”: Effects of Acute Exercise, Stretching, and Meditation on Anxiety and Endocannabinoid Levels in Youth

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Background:Exercise and meditation are known to positively impact mental health. Studies in adults and animals suggest that acute exercise raises circulating endocannabinoid (eCB) levels, potentially reducing anxiety. Yet, the effects of exercise and meditation on eCBs in youth remain unexplored. This gap is critical given the onset of mental health issues during childhood and adolescence, coinciding with changes in eCB signaling. This randomized controlled trial examined the effects of a 30-minute session of moderate-intensity treadmill exercise, light-intensity stretching, or seated meditation on state anxiety and eCB concentrations in youth.

Methods:This study reports on a community sample of 55 youth (50.9% male, 9-17 years) from Metro Detroit. Participants were randomly assigned to one of three conditions: treadmill exercise (n=19), stretching (n=20) or meditation (n=16). During a single 3-4 hour study visit, participants completed informed parent/guardian consent and youth assent, baseline anxiety and mental health questionnaires, a baseline blood draw, a 30 minute exercise or meditation session, post-session anxiety and mental health questionnaires, and a post-session blood draw. Liquid chromatography with tandem mass spectrometry was utilized to quantify eCB concentrations in plasma. While awaiting full eCB data, preliminary findings from a subset are reported here.

Results:Anxiety scores significantly decreased from pre to post the 30-minute session ($p<0.001$) across all three conditions. However, there were no significant main effects of condition or time by condition interaction on anxiety scores ($p=0.708$). While anandamide, an eCB, showed higher concentrations after treadmill exercise, these differences did not reach statistical significance ($p>0.05$).

Discussion:Our results underscore the effectiveness of 30-minute sessions of treadmill exercise, stretching, and meditation in reducing state anxiety among youth. These activities may serve as valuable, low-cost, and low-risk behavioral interventions, potentially complementing pharmacotherapy or psychotherapy in addressing youth mental health issues. While not statistically significant, preliminary analyses indicate a potential association between moderate-intensity exercise and increased circulating eCBs in youth.

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

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Corticotropin-releasing factor (CRF) from neurons in the central amygdala (CeA) have traditionally been posited to generate distress that motivates reward seeking and relapse in addiction. However, new evidence suggests that CRF is alternatively involved in promoting incentive motivation to increase reward pursuit without requiring an aversive stress state. For example, optogenetic laser stimulation of CRF-containing neurons in the CeA can intensify reward pursuit and be positively valenced, demonstrated by rat laser self-stimulation. It is unclear what signaling mechanisms and circuitry underlie these effects as CeA CRF neurons corelease several neurotransmitters at multiple projection targets. To determine whether CRF is necessary for appetitive motivation, we optogenetically stimulated CRF neurons in the CeA after administering an intraventricular CRF antagonist or vehicle prior to two-choice tasks and self-stimulation sessions. Furthermore, we optogenetically activated CeA CRF projections to the lateral hypothalamus (LH) and the dorsomedial striatum (DMS) to characterize their role in CeA CRF-driven incentive motivation. We found that optogenetic stimulation of CeA CRF neurons drives incentive motivation that is eliminated by global CRF antagonism, indicating that CRF is necessary for this motivational effect. Furthermore, we show preliminary data that suggest the projection of CeA CRF neurons to the LH generates aversive motivation and pilot evidence suggesting that activation of the CRF CeA-DMS projection may also be aversive. Together, this work adds complexity to CRF's role in driving incentive and aversive motivation that may contribute to drug seeking and relapse.

Corticotropin Releasing Factor Discharged by the Central Nucleus of the Amygdala Encodes Positive Valence and Biases Reward Pursuit

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Corticotropin releasing factor (CRF) is stress neuropeptide that is traditionally associated with anxiety and distress. CRF signaling in the extended amygdala, including central amygdala (CeA), increases during withdrawal and is posited to generate distress and drive drug consumption as a mechanism of hedonic self-medication. In contrast, recent work from our lab has demonstrated that activation of CeA CRF neurons can bias and intensify reward seeking without distress and that Crh-cre rats will optogenetically self-stimulate CeA CRF neurons. This presents a novel role for CRF in mediating reward salience. First, since addiction is characterized by intense drug pursuit at the expense of other rewards, we sought to determine whether CeA CRF neuronal activation can bias reward decision making in a sucrose vs cocaine two-choice task. Previous work in our lab has shown that intense attraction can be created by pairing CeA laser stimulation to either sucrose or cocaine can make rats pursue either sucrose or cocaine exclusively. We used this two-choice task where one group Crh-cre rats could press distinct levers to choose between either Sucrose + Laser and Cocaine Alone and another group could choose between Cocaine + Laser or Sucrose Alone to determine whether CeA CRF neuronal activation could create ‘sucrose addicts’ or ‘cocaine addicts’ at the whim of the experimenter. We determined that CeA CRF may play a role in intensifying pursuit of existing preferences for sucrose or cocaine but may not be sufficient at generating exclusive reward pursuit for a non-preferred reward. We were additionally curious to see whether a positive experience paired with CeA CRF neuronal activation can enhance incentive motivation in a laser self-stimulation task. We assessed self-stimulation of CeA CRF neurons before and after lever training with sucrose rewards to determine if self-stimulation increased following training. We demonstrate that self-stimulation of CeA CRF neurons can be intensified following pairing CeA CRF activation with a sucrose reward.

PRO-IMPULSIVE EFFECTS OF 1-(1-BENZOFURAN-5-YL)-2-(METHYLAMINO) PROPAN-1-ONE HYDROCHLORIDE (BK-5-MAPB) ENANTIOMERS IN RATS

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Recreational use of novel psychoactive substances (NPS) is a global public health concern. Benzofurans are a popular group of NPS on the illicit drug market with comparable psychoactive effects to the entactogen, methylenedioxymethamphetamine (MDMA) and other phenethylamines. In rodent drug discrimination studies, the S- and R- enantiomers of 1-(1-benzofuran-5-yl)-2-(methylamino)propan-1-one (BK-5-MAPB) display differential effects that are likely related to differing potencies at dopamine (DA) and serotonin (5-HT) transporters. Psychostimulant drugs have pro-impulsive effects in rodent operant conditioning procedures utilizing differential reinforcement of low rate responding (DRL) schedules. Previous studies utilizing a DRL 36 s schedule showed differential effects of DA and 5-HT releasers. This study examined the acute effects of S- and R-BK-5-MAPB on responding maintained by a DRL18 s schedule of food reinforcement. Sixteen adult male Sprague-Dawley rats with an extensive training history were assessed following acute injections of saline (N=8) or S- and R- BK-5-MAPB (N=8). Tests were conducted once per week with ascending doses (0.675 mg/kg, 1.35 mg/kg, 2.7 mg/kg). S-BK-5-MAPB produced dose-dependent increases in response rate and decreases in reinforcement rate, as well as a leftward shift in the inter-response time (IRT) distribution. Similar effects were observed only following the highest dose of R-K-5-MAPB. These results are consistent with drug discrimination findings that S-BK-5-MAPB produces behavioral effects comparable to MDMA, though the observed enantiomeric differences in IRT distribution changes opposed predicted outcomes based on differential potencies as DA releasers.

BEHAVIORAL TRAINING HISTORY MODULATES AMPHETAMINE-INDUCED IMPULSIVE ACTION IN RATS

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Substance use disorders (SUD) present a complex interaction of neurobiological, psychological, and social factors affecting millions of individuals globally. SUDs are characterized by escalated, frequent use and compulsive drug-seeking behaviors despite adverse consequences. Understanding the role of behavior inhibition in SUDs is critical to their prevention and treatment. Behavior inhibition can be assessed experimentally in rodents by utilizing an operant conditioning schedule known as differential reinforcement of low rate responding (DRL). Previous studies utilizing DRL schedules have shown psychostimulant drugs reduce behavior inhibition, as indicated by more rapid responding and reinforcement loss. The influence of differential training history on drug-induced disruption of impulse control has not been evaluated. This study utilized short and long DRL schedules to assess the influence of prior learning history on amphetamine-induced impulsivity. Sixteen adult male Sprague-Dawley rats were trained to lever press for food reinforcement under either a DRL 18 s (N=8) or DRL 72 s (N=8) schedule until responding was relatively stable under each training condition. Both groups were subsequently tested following 2.0 mg/kg d-amphetamine or saline injections with a DRL 18 s schedule in effect. In both training groups, amphetamine produced the expected outcome of increased responding, reduced reinforcers earned, and a leftward shift in the inter-response time distribution. Of interest, animals with the DRL 72 s schedule training history exhibited higher impulsive action compared to those with the DRL 18 s schedule history, and these differences were evident following both amphetamine and saline injections. These findings suggest the importance of considering prior learning history when evaluating drug effects on behavior inhibition. Future studies are planned to determine if a longer training history will differentially influence pro-impulsive responses to various drugs of abuse.

ROLE OF LATERAL ENTORHINAL CORTEX PROJECTIONS TO THE NUCLEUS ACCUMBENS IN ENCODING A CONTEXTUAL FEAR MEMORY

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The lateral entorhinal cortex (LEC) is a brain region important in associative memory. The LEC contains a subpopulation of neurons that project to the nucleus accumbens (NAc; LEC-NAc), a region important in reward, avoidance, and motivated behavior. Glutamatergic projections from other regions (e.g. cortex, amygdala) to NAc become hijacked in neuropsychiatric diseases like depression and addiction, however, the role of LEC-NAc neurons are unknown. Because LEC mediates associative memory and NAc mediates motivated behavior, we hypothesized LEC-NAc neurons may mediate associative memories underlying motivated behavior via activation during encoding and retrieval of a contextual memory. To investigate this, we inactivated LEC-NAc neurons using DREADDs during contextual fear conditioning (CFC), specifically during the learning (encoding) and test (recall) period. Additionally, we performed CFC in a separate cohort and collected LEC tissue 1 hour post learning to test for c-fos immunohistochemistry, which is a protein marker of high neuronal activity. Inactivation of LEC-NAc neurons impaired contextual fear learning, but didn't affect the recall, suggesting LEC-NAc neurons are necessary for the encoding, but not the recall, of a contextual fear memory. Quantification of c-fos expression in LEC-NAc neurons suggested these neurons are activated by a novel context, rather than specifically during encoding or recall. These findings elucidated a previously unknown role of this understudied neuronal population; demonstrating the necessity of LEC-NAc projections in encoding of contextual fear memory, which has interesting implications for memories that underlie neuropsychiatric disease.

CHRONIC INTERMITTENT GESTATIONAL MORPHINE USE IMPAIRS MOUSE MATERNAL BEHAVIOR DEVELOPMENT

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Background: Although incidence of opioid use disorder (OUD) during pregnancy has quadrupled from 1999-2014 (CDC, 2018), the behavioral and biological effects of opiates on pregnant women, and their infants, remain poorly understood. The shared neural circuitry that modulates drug processing and maternal behavior development may explain mechanistically the risk of reduced maternal care in the presence of opioids. For instance, several clinical studies have suggested that chronic morphine use leads to opioid receptor sensitization and reduced responses to infant cues (Wallin et al. 2021; Miranda-Paiva et al. 2001). Furthermore, in rodents, morphine use in late gestation has been shown to decrease preference for pup odors, prolong the latency of last pup retrieval, and increase non-maternal activities, such as self-grooming and digging (Wallin, et al. 2021; Slamberová & Szilágyi, 2001). The purpose of this study is to evaluate whether intermittent gestational morphine exposure started prior to conception - modeling human patterns of drug taking - differentially alters maternal behavior. We hypothesize that active consumption of morphine by female mice prior to breeding will interfere with their maternal behavior development. This study expands upon previous studies in the field, in that morphine consumption in female mice was begun much earlier with hopes of reproducing previous rodent study results and modeling human observations in a translationally relevant approach.

Methods: Female mice (9-10 weeks old) orally consumed morphine (0.2 mg/ml) (0.2% morphine in sucrose) starting 2 weeks prior to breeding until pups were weaned. Dams were longitudinally assessed for anxiety with open field and elevated plus maze tests, as well as maternal behaviors, including pup retrieval, olfactory discrimination, nesting behavior, carrying pups, licking, and grooming. Results: Pup retrieval time was decreased when measured at mid (4) compared to early (1-2) postnatal days (PD) in control dams, reflective of maternal learning, which was absent in morphine exposed dams. Morphine dams also displayed reduced licking and nesting behavior, spent less time seeking pup olfaction cues, and showed greater anxiety-like behavior dependent on the test compared to their control counterparts.

Conclusion: These data support the hypothesis that chronic intermittent morphine intake will negatively impact maternal behavior development, and that this outcome may involve mood dysregulation. While this study assesses behavior, future work will examine neurobiological mechanisms to pinpoint pathology on a cellular level with hopes of identifying interventions for targeted therapeutic development.

DIFFERENTIAL EFFECTS OF REPEATED TOLUENE EXPOSURE ON LOCOMOTOR ACTIVITY IN FEMALE AND MALE MICE

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Inhalant abuse is a worldwide public health concern, particularly among adolescents. Recent research shows, that 2.3 million people aged 12 or older have used inhalants in the past year in the US. Limited studies have examined the behavioral effects of repeated toluene exposure in adolescence and even less including females. We aimed to address the gap in the literature by examining the dose-dependent effects of toluene inhalation between males and females, as well as the differential effects of acute and repeated toluene exposure on rodent behavior. Adolescent male and female Swiss Webster mice (PN 27-38) were exposed to toluene concentrations of 0, 2000, and 4000 parts per million (ppm) for 30 minutes daily for 10 days. Locomotor activity was observed during each session. Acutely, toluene produced a concentration-dependent increase in locomotor activity. With repeated exposure, locomotor sensitization was observed with increased locomotor activity over 10 days at 4000 ppm that differed significantly from the controls. Additionally, the 4000 ppm concentration of toluene led to a significant greater locomotor activity for males as compared to female mice. Ongoing experiments are assessing the effects of acute exposure in adolescent mice at the same concentrations of toluene to compare differences between acute and chronic exposure.

IDENTIFICATION OF A NOVEL HEDONIC HOTSPOT IN ANTERIOR CINGULATE CONTROL THAT CONTROL LIKING AND WANTING FOR SWEET REWARD

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Brain hedonic hotspots are small regions of mesocorticolimbic systems that amplify the hedonic impact of palatable tastes. They have been identified in the nucleus accumbens medial shell, caudal ventral pallidum, rostromedial orbitofrontal cortex, and insula cortex by pairing certain brain manipulations like drug microi jections that activate opioid and orexin receptors with the taste reactivity test. This test measures and categorizes affective expressions emitted to taste into positive 'liking' and aversive 'disgust' hedonic components, and these orofacial reactions are shared between rodents, nonhuman primates, human infants, and other mammals. Recently, an anatomically restricted site in the anterior cingulate cortex of humans was discovered where brain stimulations in patients undergoing treatment for epilepsy produced intense happiness and joyful laughter. Here we use optogenetic stimulations in rats to probe whether a homologous site capable of control affective reactions may exist in the rat. We report that channelrhodopsin stimulations in a mid-to-caudal region of cingulate cortex doubled the number of affective 'liking' reactions elicited by intra-oral sucrose infusions. Further, optogenetic manipulations at this same site that increase hedonic impact further promoted incentive motivation, here measured as focused and directed instrumental responding for a laser-paired sucrose reward over an alternative yet identical sucrose reward that was available but never paired with ACC neuron stimulation. Finally, we assessed whether ACC neurons would promote self-stimulation in the absence of sucrose reward using two laser self-stimulation tests. We note that ACC hotspot ChR2 rats self-stimulated for laser alone in our two tests. Our results suggest a never previously identified site of hedonic enhancement exists in the anterior cingulate cortex of the rat brain capable of causally amplifying the hedonic impact of pleasant tastes, and may have important implications for the development of treatments to various affective disorders.

DIETARY PROBIOTIC SUPPLEMENT ATTENUATES METHAMPHETAMINE-INDUCED CONDITIONED PLACE PREFERENCE IN FEMALE SPRAGUE-DAWLEY RATS

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Substance use disorders (SUD) represent a global public health crisis with limited effective treatment options. Recent findings implicate the gut microbiome and gut/brain axis in the pathophysiology of SUDs. Several preclinical studies have established that psychostimulants alter gut microbiome composition, but no published studies have assessed behavioral effects of dietary probiotic supplements on drug-induced behavioral changes. This study utilized rodent conditioned place preference (CPP) as a behavioral index of conditioned drug reward to determine if a dietary probiotic supplement alters the behavioral effects of (+)methamphetamine (METH). Fifty-four adult female Sprague-Dawley rats were randomly assigned to receive either a standard rodent diet or daily supplements of Bio-Kult®, a multispecies probiotic supplement in their daily food rations. After four weeks, CPP commenced while dietary treatments continued. Each diet group was randomly assigned to one of three treatment groups: saline (N=9), 0.5 mg/kg METH (N=9), or 2.0 mg/kg METH (N=9). Following a 15-min habituation session to assess time spent on each chamber side, a biased CPP procedure was implemented. Conditioning trials were conducted once per day, with alternating drug and saline conditions over 10 days, while locomotor activity was monitored. On day 12, animals were allowed to explore both chamber sides for 15 min to determine CPP scores. Fecal samples were collected weekly throughout the study for analysis of gut microbiome composition and brains were harvested 72 hours after the last injection. Behavioral results showed that METH increased locomotor activity in both diet groups. CPP was established by METH in the control diet animals, but the probiotic diet animals did not show strong evidence for CPP. These results demonstrate that a commercially available probiotic supplement attenuates the conditioned rewarding effects of METH in female SD rats. Although profiling the microbial communities obtained from fecal samples collected throughout the study will be essential to interpreting these findings, the behavioral results alone support continued exploration of dietary probiotics as a potential complementary treatment for psychostimulant-induced gut dysbiosis. Other future directions include replication of this study with male rats and evaluation of cytokine levels in selected brain regions related to drug reward."

Individual Differences in Emotional Memory Consolidation in Male Rats

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Post-traumatic stress disorder (PTSD) is a psychiatric disorder resulting from exposure to trauma. Most Americans will experience a traumatic event in their lifetime, and develop acute fear reactions following the incident. However, about 10% will go on to develop PTSD, experiencing recurring recollection of the emotional event, psychological distress towards trauma-related cues, difficulty concentrating, and nightmares. This suggests 1) vulnerability to PTSD varies among individuals, and 2) PTSD pathology influences emotional memory processes. Identifying individual differences in memory offers a potential solution for why only some individuals develop PTSD. Pavlovian conditioned approach (PCA) training can be used to assess individual variation in emotional memory consolidation. Sign-tracking animals (ST) who approach a predictive cue exhibit less fear in contextual fear memory (CFM) paradigms than GTs, who approach the reward site. Administration of the histone deacetylase inhibitor, sodium butyrate (NaB), enhances fear memory in ST. These results suggest histone modifications underlie individual differences in emotional memory consolidation. Importantly, memory consolidation is a sleep - dependent process well known to modify histone acetylation. We thus hypothesized increasing acetylation using NaB to improve emotional memory consolidation in sleep deprived STs and GTs, and rested STs. STs and GTs rats underwent CFM training, were immediately administered NaB or saline, and subsequently allowed sleep or sleep deprived. Animals were placed in a novel context one day prior to returning to the CFM context. Our results show that sleep deprivation impairs memory in STs and GTs, though NaB does not improve memory performance under any sleep condition. Both sleep deprived STs and GTs express increased fear towards the novel context, but NaB only improves context discrimination in GTs. These results suggest that histone modifications underlie CFM memory in STs and GTs, and sleep deprivation may impair context discrimination.

CHEMOGENETIC INHIBITION OF LOCUS COERULEUS DURING FEAR CONDITIONING IN WISTAR RATS DOES NOT AFFECT EARLY FEAR EXPRESSION, BUT DAMPENS FEAR EXPRESSION AT THE START OF EXTINCTION.

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RATIONALE: Posttraumatic stress disorder (PTSD) is known to have a lifetime prevalence of ~8% in US adults, that results in significant functional burden to patients and poses financial burden to the US healthcare system. Major symptoms in those with PTSD include intrusive vivid memories of their traumatic event and intense psychological and physiological distress in response to reminders of their traumatic event. Pavlovian fear conditioning is thought to model intrusive PTSD symptoms as those with PTSD show greater fear expression during Pavlovian fear acquisition and recent research has shown that PTSD treatment leads to improved extinction learning. The memory processes involved with fear conditioning (FC) are dependent on brain regions known as the fear circuit: the amygdala, hippocampus and prefrontal cortex. The fear circuit is densely populated with noradrenergic receptors and noradrenergic neurons stemming from the locus coeruleus (LC). The activity of the fear circuit, and formation of fear memories, is modulated by central norepinephrine (NE). We hypothesized that inhibition of NE during fear conditioning would dampen expression of fear-like behaviors.

METHODS: Female rats underwent intracranial surgery to receive injection of viral vectors containing inhibitory DREADDs (AAV9-HM4Di-PRSx8) into the LC or received a sham surgery (cranial openings, no virus). After 6 weeks of incubation, all rats underwent a fear conditioning protocol including tests of fear expression, extinction and extinction retention. Footshocks (0.6 mA, 0.5 s) were used as the unconditioned stimulus, paired with a chamber light as the conditioned stimulus. Fear-potentiated startle was used as the measured behavior during FC tests. Clozapine-N-oxide (CNO, 5 mg/kg i.p.) was injected in all rats on fear acquisition days and extinction training days to activate the DREADD receptors, inhibiting NE release from the LC.

RESULTS: Rats in both the DREADD and control groups displayed successful fear expression after fear acquisition training. Both groups displayed successful with- and between-session fear extinction. At the start of extinction training, rats in the DREADD group displayed significantly less startle potentiation than controls, indicating a less intense expression of fear-like behavior.

DISCUSSION: Lesser fear-like behavior at the start of extinction training in the DREADD group may indicate that, due to inhibition of NE in the brain, rats formed a weaker fear memory during acquisition training, that is more susceptible to extinction training. It may also indicate that inhibition of NE release may dampen the expression of fear-like behaviors acutely. Future studies will be employing this DREADD system in combination with a model of PTSD, single prolonged stress, to investigate whether NE inhibition can dampen fear expression in rats that experienced extreme stress. Results of this and future studies may help develop targeted, neurological treatments for PTSD.

THEME 6: NEURAL EXCITABILITY, SYNAPSES, & GLIA

SEX DIFFERENCES IN THE VSUB-ABNST-PVN CIRCUIT ENGAGED DURING AN ANXIOGENIC TASK

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The ventral subiculum (vSub) is a major output structure from the ventral hippocampus, a central controller of neuroendocrine and behavioral stress responses. The vSub, like other limbic brain regions, projects to the bed nucleus of the stria terminalis (BNST), which integrates top-down inputs to shape downstream physiological and behavioral output. Sex differences in this vSub-BNST circuit supporting stress responses are practically unexplored. We previously showed that in both male and female mice, ventral subiculum (vSub) neurons projecting to the anterior BNST (aBNST) control both anxiety-like behavior and the associated neuroendocrine response in an anxiogenic environment. Chemogenetic activation of vSub-aBNST neurons expressing a Gq-DREADD using CNO injection decreased anxiety-like behavior in the elevated zero maze (EZM) in both sexes. The present study used c-fos immunolabeling to explore the effects of this vsub-aBNST activation on overall neuronal activity levels in response to the EZM, measured as c-fos protein immunolabeling, in the vSub-aBNST-PVN circuit. We developed a novel ImageJ script which automated quantification of c-fos+ cells with high sensitivity and few false positives, and used it to quantify c-fos+ cell densities along the entire dorsal-ventral axis of the vSub and in the anteroventral and anterodorsal VNST. While vSub-aBNST neuron activation had a similar effect on anxiety-like behavior in male and female mice, it had sex-specific effects on c-fos expression. In the vSub, there was a trend toward an interaction between CNO treatment and sex in a 2-way ANOVA ($F(1,23) = 0.4.235$, $p = 0.0511$); CNO appeared to increase c-fos expression in males, but not females. In the BNST, there was also an interaction between CNO treatment and c-fos+ cell density in the BNST (anteriodorsal: $F(1,37) = 3.735$, $p = 0.0610$; anteroventral: $F(1,37) = 8.948$, $p = 0.0049$). Post-hoc testing showed that CNO increased c-fos+ cell density in the BNST in females, but not males. There were no significant differences in PVN c-fos as a function of CNO or sex, and no significant interactions. These observed sex differences in c-fos expression suggest that sex differentiated neural circuitry can underly the same behavioral response. In particular, activation of vSub-aBNST neurons prior to the EZM increased overall aBNST activity in females, but not males, suggesting stronger overall engagement of the BNST by this circuit in females.

INVESTIGATING THE KYNURENINE PATHWAY IN TRAUMATIC BRAIN INJURY (TBI)

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TBI affects approximately 2.87 million Americans annually and can result in long-term inflammation, which may underlie debilitating post-injury outcomes such as chronic pain. Numerous studies have implicated the kynurenic pathway (KP) in neuroinflammatory responses with KP metabolites kynurenic acid (KA, anti-inflammatory) and quinolinic acid (QA, pro-inflammatory) demonstrating divergent inflammatory effects. While the KP has been investigated in the context of inflammation, the role of the KP and its metabolites in TBI-induced pain remains understudied. Initial rodent studies report improvements in pain sensitivity with KP inhibitors after surgical brain injury, highlighting the unrealized potential for using KP inhibitors to mitigate chronic neuroinflammation post-TBI. As a preliminary study to examine KP modulation after immune challenge, we quantified a comprehensive panel of KP metabolites from E. Coli lipopolysaccharide (LPS) infected and saline-control animals (10-12 week old, C57Bl/6 male mice). Mice were administered ether two saline or two LPS injections (0.83mg/kg i.p. each), separated by 16 hrs. Brains and serum were harvested 24 hrs. after the last injection and analyzed using Liquid-Chromatography-Tandem-Mass-Spectrometry. LPS increased the Kynurenine-to-Tryptophan ratio in both serum and brain hemisections compared to respective control animals, indicative of robust KP activation. Also, brain levels of KA, and precursors of QA production, were increased and QA was enhanced in serum, after LPS, compared to respective saline controls. Our findings indicate that KP metabolite levels are elevated systemically and in the brain 24 hrs after peripheral administration of two LPS doses. Together, these findings suggest that an acute neuroinflammatory challenge upregulated KP metabolism with divergent effects on KA (anti-inflammatory) in the brain, suggestive of an astrocyte response, vs. QA in the periphery (pro-inflammatory), suggestive of a macrophage response.

EXOSOMES SECRETED FROM EPILEPTOGENIC TUBEROUS SCLEROSIS COMPLEX (TSC) TUBERS ARE ENRICHED FOR PROTEINS ASSOCIATED WITH VESICLE-MEDIATED TRANSPORT AND ALTERED ELECTROPHYSIOLOGY.

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Rationale: Tuberous Sclerosis Complex (TSC) is a genetic disorder caused by mutations in the TSC1 and/or TSC2 genes that lead to the formation of benign tumors in the brain known as cortical tubers. A subset of tubers are capable of inducing seizures; however, the molecular mechanism responsible for this process has yet to be fully elucidated. Recent evidence suggests exosomes may play a role in this process. Exosomes are small extracellular vesicles (EV) that transport molecular cargo between cells. Recent studies have demonstrated the importance of exosomes in intercellular communication and disease pathology. Exosome cargo includes RNA, DNA, protein, and lipids, which can be selectively packaged into the vesicles by the source cell and provoke a functional response in recipient cells. The protein cargo in exosomes secreted from human epileptogenic brain tissue has not been characterized. We used quantitative proteomics to compare EV cargo proteins in epileptogenic and non-epileptogenic TSC tubers.
Methods: We isolated exosomes from archived, frozen cortical tissue that was surgically resected during the treatment of drug-resistant epilepsy in TSC patients. Specimens included 4 epileptogenic tubers (ET), 3 non-epileptogenic tubers (NT), and 3 non-tuber controls (NC). Protein cargo was extracted from exosomes and analyzed using quantitative LC-MS/MS proteomics. Statistical analysis with control of the false discovery rate was used to identify proteins differentially expressed among the groups. Differently expressed proteins were analyzed to identify those potentially involved in altered electrophysiology and seizures utilizing Enkefalos, a bioinformatics software developed in our laboratory. This research was approved by the Wayne State University Human Subject Institutional Review Board.
Results: Isolated vesicles were comprised primarily of exosomes (<150 nm), and 1866 proteins were quantified. Principal component analysis demonstrates that exosomal protein cargo is distinct in ET compared to NT and NC controls. Statistical analysis identified 140 proteins differentially expressed in epileptogenic exosomes compared to exosomes secreted from non-epileptogenic tubers and non-tuber cortical tissue. Pathway analysis, utilizing Enkefalos, revealed enrichment for proteins involved in vesicle-mediated transport, including Syntenin-1 and YKT6. Syntenin-1 was previously reported to have increased expression in TSC neurons and caused a reduction in spine synapse density. We also identified elevated proteins in exosomes secreted from ET that were associated with alterations in electrophysiological properties and seizures.
Conclusion: Epileptogenic tubers secrete exosomes with altered protein cargo. Differentially expressed proteins suggest increased vesicle-mediated transport. Elevated proteins are also associated with altered electrophysiology, synapse morphology, and seizures. Our results warrant further investigation of the role of the altered exosomal proteins in intercellular signaling and epilepsy.
Funding: This research was supported by the TSC Alliance (A19-0151-001), National Institutes of Health (R56 NS079429), and the Department of Defense (W81XWH-22-1-0798).

MEDIAL SEPTUM DYSFUNCTION IN A MOUSE MODEL OF DRAVET SYNDROME

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Dravet syndrome (DS) is a severe neurodevelopmental disorder caused by heterozygous loss-of-function mutations in the SCN1A gene, which encodes the voltage-gated sodium channel Nav1.1. DS manifests with infant-onset epilepsy, developmental delay and intellectual disability. The medial septum (MS), pivotal for hippocampal theta rhythm generation, plays a crucial role in cognitive function. Both cholinergic (ChAT+) neurons and parvalbumin-expressing (PV+) neurons within the MS express SCN1A. Yet, whether these neurons are functionally impaired in DS remains uncertain.

In this study, we employed patch clamp techniques to investigate the electrophysiological characteristics of ChAT+ and PV+ neurons in acute brain slices from Scn1a haploinsufficient mice (Scn1a+/-) and age-matched wild-type littermate controls. Our findings revealed no discernible genotype-related differences in MS ChAT+ neurons; however, we observed a pronounced impairment in the intrinsic properties of PV+ neurons in Scn1a+/- mice. Further analysis revealed heterogeneity among MS PV+ neurons: we categorized them into continuous-firing and burst-firing subtypes based on their firing patterns. Both subtypes exhibited impaired firing.

Our results highlight a significant dysfunction of MS PV+ neurons, consistent with similar findings in PV+ neurons within neocortex and hippocampus. Future studies will elucidate potential dysfunctions in synaptic transmission from the MS to the hippocampus, as well as the possibility of a restoring the firing ability of PV+ neurons by enhancing Scn1a expression. Even though Scn1a is reportedly expressed in nearly all MS ChAT+ neurons, we did not observe genotype differences in somatic action potential initiation. Future studies will assess whether cholinergic transmission is nevertheless impaired in Scn1a+/- mice, either due to deficits in action potential propagation, or reorganization of the septohippocampal circuit.

A HERITABLE CHANGE IN ACTION POTENTIAL HALF WIDTH THAT CORRELATED WITH COGNITIVE RESILIENCE

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

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

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

ACH-MEDIATED BURSTS INHIBIT DOPAMINERGIC AXONAL EXCITABILITY

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Dopamine release from terminal axons of dopaminergic neurons in the striatum is crucial for movement, motivation, and proper function of the basal ganglia network. Loss of dopamine release is linked to neurodegenerative disorders like Parkinson's Disease, and dysregulation in the ventral striatum is linked to the development of substance use disorders. Dopamine release is regulated by somatodendritic and axonal mechanisms. While much is known about somatodendritic mechanisms that regulate dopamine release, much less is understood about mechanisms intrinsic to the axon. Here we use direct axonal recordings together with calcium imaging in dopaminergic axons to show that bursts of action potentials in the dopaminergic axon initiate a refractory period that inhibits excitability for ~200 milliseconds. The bursts of action potentials occur in experimental situations by electrical stimulation that coordinates the timing of an action potentials in the dopaminergic axon with one or more action potentials mediated by acetylcholine activation of axonal nicotinic receptors. Of note, these bursts can also be triggered by cholinergic activation of axonal nicotinic receptors, even in the absence of electrical stimulation. Burst pause firing patterns are important for generating meaningful dopamine signals, and have long been observed during in vivo recordings of dopaminergic neuronal firing or dopamine release. Our data reveal a novel mechanism for generating burst-pause firing that is intrinsic to the axonal compartment of dopaminergic neurons.

RECENT ADVANCE IN SEIZURE PREDICTION IN AN IN VITRO AND AN IN VIVO MODEL OF EPILEPSY

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Epilepsy is a common brain disease characterized by spontaneous and recurrent seizures. Seizures happen suddenly and greatly devastate patients' lives; thus, seizure prediction is of research interests when seizures are not managed by conventional strategies. Genesis of seizures is not associated with manifesting changes, like structural alterations in the epileptic neuronal networks, making predicting seizures difficult. Electrographic recordings like EEGs, as the last approach revealing instantaneous neuronal network behaviors, provide unsatisfying insights into the neuronal dynamics leading to seizures. Therefore, a quantifiable and more accurate measure is still desired for ideal seizure prediction. In this study, we investigated the genesis of seizures in two models respectively: A) in mouse hippocampal slices induced by higher concentration of potassium ([K+]o = 6-11 mM) in the artificial cerebrospinal fluid, and B) in intrahippocampal tetanus toxin (20 ng) induced epilepsy in rats. In the A model, although epileptiform discharges were provoked by moderate increase of [K+]o, further addition of [K+]o disturbed epileptiform discharges. Interestingly, CA3, the common drive of oscillations in the hippocampus, disturbed the neuronal synchrony in this in vitro model. In the B model, simple to complex seizures spontaneously presented in the experimental rats. We found a rising trend of the variances and the coastline lengths, as two measures extracted from the 30-minute pre-seizure recordings. Albeit preliminary, the two undergraduate projects refined the models and advised the directions for our future advance in seizure predictions.

THE EFFECT OF POST-LEARNING SLEEP DEPRIVATION ON NEURONAL ACTIVATION, ENGRAM REACTIVATION, AND TRANSCRIPT EXPRESSION IN THE MOUSE DENTATE GYRUS

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Post-learning sleep deprivation (SD) disrupts hippocampal memory consolidation, including consolidation of contextual fear memory (CFM) in mice following contextual fear conditioning (CFC). This observed SD-induced memory deficit may result from disrupted neuronal engram reactivation. Areas of the hippocampus are heterogenous in structure and function and may respond differently to post-learning sleep or SD. Here we investigate the overall impact of post-learning sleep and SD on neuronal activity, neuronal engram reactivation, and transcript expression in the subregions of the mouse hippocampus. Analysis of neuronal activity markers in the hippocampi of male C57BL6 mice show that following a 6-h period of SD, neuronal activity is higher in the superior blade compared to the inferior blade of the dentate gyrus (DG). This effect is driven by significant reductions in neuronal activity in the inferior blade, but not the superior blade, following 3- or 6-h periods of SD. To understand how memory encoding-activated engrams are reactivated in the hours following learning, we used targeted recombination of activated populations (TRAP) in male reporter mice to label context-specific neurons (engram cells) in the DG. We then quantified engram cells' reactivation after a period of sleep or SD. The inferior blade showed greater engram reactivation compared to the superior blade. Animals allowed to sleep freely following learning exhibited a higher level of engram reactivation compared to animals that underwent 6 hours of post-learning SD in the inferior blade. In the 3-hour post learning mice after either sleep or SD, we did not observe significant ensemble reactivation, and thus no change in reactivation across subregions. Transcript expression analysis demonstrated that 6-h SD led to differential expression of select transcripts in the DG inferior vs. superior blades, and in other hippocampal subregions, suggesting a significant and region-specific impact of sleep on gene expression. Transcripts associated with neuronal activity, protein synthesis regulation, synaptic signaling, and cytoskeletal remodeling were differentially affected in the DG blades (compared with other hippocampal subregions) following SD. Taken together, our results suggest that sleep plays an important role in regulating hippocampal DG activity, engram cell reactivation, and transcription in the hours following learning. These effects are subregion-specific, and likely contribute to the beneficial effects of sleep on memory consolidation.

ORAL CONTRACEPTIVE EXPOSURE MODULATES NEUROIMMUNE ACTIVITY

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Hormonal contraceptives including oral contraceptives (OCs) are the most widely prescribed class of drugs in the world. OCs are typically a combination of ethinyl estradiol and a synthetic progestin. At the dosage used, OCs induce negative feedback to suppress endogenous ovarian hormones and prevent pregnancy. Because ovarian hormones modulate function beyond the reproductive system, OCs have a variety of beneficial and adverse side effects. For example, the ovarian hormone estrogen has modulatory capabilities on the peripheral and neuroimmune systems. By changing ovarian hormone levels, it is likely that OCs change immune processes as well. In this project, we aimed examined how cytokine levels in the peripheral and neuroimmune systems were altered after an acute immune challenge and OC treatment. Female C57Bl/6N mice readily drank ethinyl estradiol and levonorgestrel in sucrose (EE, 0.02μg + LVNG, 0.75μg in 0.25mL 10% sucrose) given daily in their home cage. Control animals received 0.25mL of 10% sucrose. After treatment of EE+LVNG for one month, mice from each group were injected with either a single strand RNA immune challenge (R848 in DMSO and PBS; 1000 μg/kg) or a vehicle injection (DMSO+PBS; 1000 μg/kg) before brain tissue and blood collection. Brain regions collected include the prefrontal cortex, dorsal hippocampus, and hypothalamus. We used bead based multiplex cytokine assays (Magpix/Luminex) to assess 15 different cytokines. We found that mice treated with EE+LVNG showed significantly higher levels of CXCL10 after R848 treatment as compared to OC untreated mice. Cytokines IL-1 and IL-10 showed no differences between treatment groups. This change in response differs across brain regions suggesting a distinct effect of EE+LVNG and R848 treatment on specific areas of the brain. These individual changes suggest altered cytokine network dynamics (Finnell et al., 2023). As neuroimmune processes can influence future cognitive functioning, further research will expand on cytokine networks across brain regions to determine how OC mediated neuroimmune changes can modify the development of Alzheimer’s disease.

SLEEP LOSS ALTERS THE STRUCTURE OF SOMATOSTATIN-POSITIVE INTERNEURONS IN BRAIN REGION SPECIFIC FASHION

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Even a single night of sleep loss impairs memory processing. In addition, previous work shows that only a few hours of sleep deprivation affects spine densities in specific regions of the hippocampus–a brain structure crucial for memory processing. Interestingly, state-dependent activity of somatostatin-positive (SST+) interneurons in the hippocampus increases after a few hours of sleep deprivation, increasing their inhibitory effect on neighboring excitatory neurons, impairing memory consolidation. To better understand the underlying mechanism of how SST+ interneurons cause memory impairments associated with sleep deprivation, we investigated morphological changes in SST+ interneurons in various brain regions that are important for memory function. SST-Cre mice were injected with a cre-dependent Brainbow virus in the primary visual area (V1), medial prefrontal cortex (mPFC), and various subregions of the hippocampus, including the CA1, CA3, and dentate gyrus. Mice either underwent a 6 hour-period of sleep deprivation or were allowed ad-lib sleep. Brain sections were visualized using immunohistochemistry and the structure of SST-interneurons were traced using Neurolucida 360 software . We found that six hours of sleep deprivation changes overall spine density in a region and type-specific fashion.. In the CA1, we observed an overall increase in spine density, and more specifically an increase in thin and mushroom spine density. Contrastingly, in the CA3, a change in overall spine density was not observed but there was a significant decrease in thin and mushroom spine densities, whereas SST+ dendrites were significantly longer and had a higher volume. In the V1, we observed a decrease in overall density, including a decrease in thin, stubby, mushroom, and filopodia spine densities. While no differences in spine density was observed in the mPFC, dendritic length was reduced. Dendritic spines are the major sites of synaptic transmission in the central nervous system, and alterations in spine numbers and morphology can directly change the functional output of a specific brain structure. The increase in spine density in the CA1 affirms our theory that increased synaptic density of SST+ interneurons results in increased inhibition onto surrounding excitatory cells in the hippocampus, leading to reductions in spine density of excitatory principal cells, driving memory impairments. Altogether, this research is fundamental to understanding the contribution of SST interneurons to the memory impairments associated with sleep loss. Eventually, this knowledge could be utilized to treat patients with disturbances in sleep or excitation/inhibition balance due to neurodegenerative disorders, such as Alzheimer’s disease.

ADIPOSE TISSUE-DERIVED EXTRACELLULAR VESICLES; EFFECTS OF AGING AND INFLAMMATION IN OBESITY AND PREDIABETES

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Inflammation, particularly adipose tissue inflammation, is commonly associated with multiple diseases and disorders, including obesity, prediabetes, type 2 diabetes, and aging. This inflammation is thought to promote the neurological complications that are common in these disorders. Adipose tissue-derived extracellular vesicles (EVs) are key mediators of cell-cell communication. EVs can cross the blood brain barrier and may promote central nervous system (CNS) inflammation, potentially via activation of the primary immune cells of the CNS, the microglia. Therefore, our goal was to assess potential inflammatory effects of age and obesity/prediabetes on microglia-adipose crosstalk via EVs. To do this, we induced obesity/prediabetes by feeding young adult (5 weeks of age) or middle aged (1 year of age) male C57BL/6 mice either standard diet (SD) or high fat diet (HFD) for 13 weeks. Metabolic and cognitive phenotyping was performed at terminal and fresh epididymal white adipose tissue from these animals (n=3/group) was used to isolate EVs. HFD-fed animals developed cognitive impairment, which was aggravated by age. We also showed adipose tissue hypertrophy in HFD animals, which was similar in both age groups. Cognitive deficits were accompanied by changes in CNS inflammatory profiles, which varied dependent upon age. Complimentary in vitro work in a human microglial cell line (SV40; applied biological materials, British Columbia, Canada) was used to characterize adipose-microglial crosstalk by treating microglia for 24 hours with adipose tissue-derived EVs. Western blots of microglial NFκB protein expression showed increased expression in cells treated with adipose tissue-derived EVs from adult HFD mice, as well as EVs from aged SD fed animals relative to adult SD controls. These data indicate that HFD and age promote cognitive impairment and inflammatory changes. HFD and age may also disrupt adipose-microglia crosstalk via EVs, contributing to an inflammatory environment in the CNS.

SINGLE-NUCLEUS RNA-SEQ REVEALS ALTERED TRANSCRIPTION IN THE NUCLEUS ACCUMBENS AFTER AD LIBITUM SUCROSE CONSUMPTION

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It's estimated that up to 75% of all foods and beverages in the US contain high amounts of added sugar and this is thought to contribute to a number of poor health outcomes. For example, human subjects studies have found that an increase of one sugar-sweetened beverage serving per day was associated with in an increased risk for Type 2 Diabetes Mellitus, cardiovascular disease, and coronary heart disease. Recent studies suggest that ingestion of excessive sugar may alter reward and motivational processes in ways that promote over-eating. This is likely due to the effects of enhanced sugar consumption on the function of brain regions that mediate reward and motivation, including the nucleus accumbens (NAc). For example, consumption of foods high in sugars and fats alters the function of medium spiny output neurons (MSNs) in the NAc, even in the absence of increased adiposity. However, relatively little is known about how sugar alone affects NAc MSN, and few studies have examined effects on other cell types within the NAc (e.g., interneurons, astrocytes etc). Therefore here, we used single-nucleus RNA sequencing (snRNA seq) to compare the transcriptomes of NAc cells in male rats that were given ad libitum access to 1M sucrose for 28 days vs controls that were never exposed to sucrose. Bioinformatic analysis of snRNA-Seq revealed 30 transcriptionally-distinct cell types within the NAc, including 10 MSN and 8 interneuron types, and three types of astrocytes. A total of 5,492 differentially expressed genes (DEGs) were found across all cell types. 88.2% of DEGs were up-regulated in the sucrose group compared to controls, while 11.8% of DEGs were down-regulated. Our analysis here focused on genes related to dopamine, glutamate, GABA, and acetylcholine transmission, ion channels (potassium, sodium, and calcium), peptide hormone receptors (insulin, leptin, and ghrelin), and opioid receptors. The Drd2 gene was up-regulated in just one type of MSN and was the only DEG related to dopamine transmission. Genes encoding some AMPA receptor subunits increased in several cell types, while genes encoding NMDA receptor subunits were down-regulated. Genes encoding GABAA receptor subunits were up-regulated mainly in astrocytes and some D1-MSN types, while genes encoding acetylcholine receptors were upregulated in in GABA-1 cells. Genes encoding potassium channel subunits were both upregulated and downregulated across astrocytes, MSNs, and GABAergic interneurons but genes encoding sodium and calcium channels were upregulated in all cell types but two. Additional analysis is ongoing, and includes examination of nuclear genes encoding mitochondrial proteins involved in cellular respiration. Data will be discussed in light of the role of the NAc in the pursuit of food, and current understanding of local NAc circuits.

THEME 7: NEURODEGENERATIVE DISORDERS & INJURY

IRONING OUT TRAUMATIC BRAIN INJURY

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Background: Annually, an estimated 1.5 million people sustain traumatic brain injury (TBI), resulting in approximately $40 billion in healthcare costs. Mechanisms underlying the onset and progression of debilitating symptoms after injury must be further understood to treat TBI safely and effectively. Brain iron accumulation has been observed after TBI and is associated with excess lipid peroxidation that can lead to ferroptosis, an iron-mediated form of cell death. Furthermore, iron accumulation can be accompanied by reactive oxygen species and long-term inflammatory mediator production. Although there have been advancements examining iron accumulation after TBI, there are divergent data on putative underlying mechanisms and chronology of iron dysregulation following injury, and no studies have utilized a closed-skull TBI model to investigate the role of iron post-injury. Therefore, the objective of this study is to evaluate temporal and regional patterns of iron accumulation and expression of iron transporter proteins (ITPs) in the brain following closed-skull, non-contusive TBI.

Methods: Male 10-week old C57Bl/6 mice (n=8-11/group) were anesthetized and subjected to a single closed-skull impact (5 mm diameter rounded tip; 2 mm impact, 5 m/s velocity) or sham (control) surgery. At 6 hrs, 7 days and 30 days post-injury, mice were terminally anesthetized and transcardially perfused with phosphate buffered saline. Whole brains were collected and frozen at -80° until use. Anterior cingulate cortex (ACC) and dorsal hippocampus (DHC) were harvested using a derm punch (1.5 mm diameter) and homogenized in whole cell lysis buffer. The levels of 4 ITPs, transferrin (TF), transferrin receptor (TFR1), ferroportin (FPN), and divalent metal transporter (DMT1) were measured using immunoblotting. Band densities were obtained using ImageJ and analyzed in Prism by GraphPad using unpaired t-tests.

A second set of identically prepared mice was transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde at 30-day post-TBI or sham surgery. Whole brains were collected, post-fixed in 10% formalin for 24 hrs, cryoprotected in 30% sucrose for 48 hrs, then frozen using 2-methylbutane, and stored in –80°C until use. Coronal sections (30 μm) were stained using Perl's solution and iron accumulation was evaluated using ImageJ.

Results: TBI increased FPN levels in the ACC, but not the DHC, at 6 hrs with no significant effects of injury in TF, TRF1, DMT1 levels in either region examined. There were no significant differences in ITP levels in either region at 7 and 30-days post-TBI. Iron accumulation was evident 30 days after TBI, notably localized to the corpus collosum.

Discussion: Here we demonstrate acute injury-induced changes in FPN, a critical mediator of iron entry as the sole iron exporter in the brain via endothelial cells of the blood-brain barrier. Evidence of long-term accumulation of iron after TBI may be implicated in neuroinflammation commonly observed chronically post-injury. In future studies, cell-specific changes in ITP levels will be examined using immunofluorescence.

WHERE DO PATHOLOGY AND COGNITIVE DECLINE MEET IN ALZHEIMER'S DISEASE?

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6.5 million nationwide today are suffering from Alzheimer's disease (AD), the most common type of dementia, in addition to their caregivers. The disease is characterized clinically by severe cognitive problems and memory impairment and pathologically by two major protein aggregates, amyloid plaques and neurofibrillary tangles (NFTs) formed by aggregation of tau protein. Studies have shown that physiological tau goes through several posttranslational modifications, leading its aggregation into pathological oligomeric forms that are toxic and potent to spread the pathology further in the brain. Therefore, the goal of this study is to examine the spatiotemporal accrual of early pathological tau moieties in samples of the frontal cortex (FC), posterior cingulate cortex (PCC), and precuneus (PreC) obtained postmortem from cognitively intact control subjects and those who died with early-stage or advanced AD. Notably, these three cortical regions comprise a large-scale brain network called the "Default mode network (DMN)". Communication among these DMN hubs is altered very early in AD patients. Hence, our study aims to help understand the relationship between the early pathological tau in the DMN and changes in cognitive functions mediated by these connected brain regions. Using immunohistochemical analysis and custom-made ELISA assays, our findings so far indicate that early pathological tau elevates in the DMN as early as Braak stage 4 (n=36, p=0.002), which is earlier in the disease process than predicted by Braak NFT staging; and it inversely correlates with the Mini-Mental State Exam (MMSE) global cognitive score (n=36, p=0.004). We found in a different cohort that the pathological tau levels rise in Braak stage 5 cases and reach significance by stage 6. Intriguingly, these pathology scores are correlated with patients' episodic and semantic memory scores, which are largely attributed to the PCC and PreC function, while working memory (which remains fairly intact in AD patients) scores are not. With this study, we hope to better understand when and where tau starts to accumulate within the DMN and how that relates to cognitive impairment. These findings may inform new strategies for early therapeutic interventions.

OPTIMIZING MOTOR FUNCTION RECOVERY IN SPINAL CORD INJURY: A PHOTOBIMODULATION THERAPY PERSPECTIVE FROM PRECLINICAL STUDIES

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Spinal cord injury (SCI) is the leading cause of disabilities worldwide. It can result in temporary or permanent impairment of function, presenting a significant challenge in medical treatment (Noonan et al., 2013). Despite various therapeutic strategies, including systemic medication (McKinley et al., 2002), surgical intervention, and rehabilitation therapy (Elbasiouny et al., 2010), enhancing neurological function following SCI remains limited and inconsistent. Photobiomodulation therapy (PBMT) has emerged as a promising avenue due to its multifaceted benefits, including suppressing inflammation, repairing damaged tissue, and providing analgesia (Janzadeh et al., 2020 ; Silva et al., 2019 ; Keshri et al., 2020). While initial preclinical studies have shown potential for PBMT in axonal regeneration and inflammation reduction, there is currently no standardized protocol or timeline for its application in SCI treatment.

This paper presents three of my studies aimed at optimizing PBMT as a therapy for SCI. Firstly, we compared the effects of one-week PBMT, and two-week PBMT with treatments using methylprednisolone sodium succinate (MPSS) on motor function and inflammation in male rats with moderate compression SCI (Mojarad et al., 2018). We found that two weeks of PBMT treatment was as efficacious as MPSS in motor recovery and inflammation reduction, without causing weight loss or mortality, suggesting long-term PBMT as a preferable option due to fewer side effects.

Building on these findings, our second experiment investigated the efficacy of two PBMT protocols administered over two and four weeks in male rats with moderate compression SCI (Janzadeh et al., 2023). The four-week PBMT showed greater effectiveness in alleviating SCI complications, such as neuropathic pain and motor dysfunction, compared to the shorter duration protocols, emphasizing the importance of extended PBMT durations for optimal outcomes in motor function recovery.

Our third study extended the PBMT timeline to seven weeks in both male and female rats with severe compression SCI (Mojarad et al., 2024). Daily PBMT administration throughout the experiment demonstrated enhanced motor recovery compared to untreated groups, highlighting the potential of extended PBMT durations in improving outcomes post-SCI.

In conclusion, our studies underscored that prolonged PBMT therapy can significantly enhance motor recovery following SCI. Our ongoing studies are aimed at utilizing PBMT in combination with both neural stem cell transplantation and functional electrical stimulation to promote axonal regeneration, reduce inflammation, and restore functional recovery following SCI. Because our results indicate that PBMT is an effective therapy, even for severe SCI, we are optimistic that when used in combination with other therapies it may optimize treatments for SCI."

THE NIGROTEGMENTAL CIRCUIT IN AN SCN1A+/- MOUSE MODEL OF DRAVET SYNDROME

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Rationale: Dravet Syndrome (DS) is a severe developmental and epileptic encephalopathy (DEE) characterized by prominent seizures, with non-seizure aspects of the phenotype progressively becoming more significant over time. DS primarily arises due to pathogenic variants in SCN1A, which lead to impaired expression of Nav 1.1, a sodium channel crucial for neuronal signal generation and propagation. Nav 1.1 is expressed throughout the brain, including canonical epilepsy regions such as the neocortex and hippocampus, but also within subcortical regions like the substantia nigra pars reticulata (SNr) and pedunculopontine nucleus (PPN). Despite the extensive expression of Nav 1.1, the specific contributions of subcortical networks, particularly the nigro-tegmental circuit, to the DS phenotype remain poorly understood.

Methods: We investigated the nigro-tegmental circuit in an Scn1a+/- mouse model of DS using whole-cell patch clamp recordings and fiber photometry. We compared the intrinsic cell properties of parvalbumin-expressing (PV+) neurons in the SNr and cholinergic (ChAT+) neurons in the PPN between Scn1a+/- and wild-type mice. We also monitored SNr and PPN neuron population activity during seizures induced by hyperthermia using simultaneous EEG and fiber photometry recordings.

Results: PV+ SNr neuronal firing in response to depolarizing current steps was significantly impaired in Scn1a+/- mice although Scn1a+/- SNr neurons had slightly lower rheobase. Individual spike width was also wider in Scn1a+/- SNr neurons. Scn1a+/- ChAT+ PPN neurons however were indistinguishable from wild-type. Preliminary imaging of population level fluorescence activity revealed that SNr neurons had a profound decrease in activity starting at seizure onset. In contrast, PPN neurons had a significant increase in calcium transients prior to seizure onset and large increase in baseline fluorescence during the seizure, followed by post ictal attenuation.

Conclusions: The dysfunction observed in PV+ SNr neuron spike generation suggests a potential explanation for the motor deficits observed in DS, underscoring DS as a whole-brain disorder. Notably, our study also revealed normal spike generation in ChAT+ PPN neurons despite Nav1.1 expression. This suggests Nav1.1 may not be crucial for somatic spike initiation in cholinergic neurons. Future investigations will explore potential impairments in spike propagation and cholinergic transmission. Additionally, while previous studies show exogenous SNr inhibition suppresses seizures, our finding of endogenous SNr inhibition during seizures suggests the SNr may contribute to intrinsic seizure termination mechanisms. We hypothesize that PPN excitation observed during pre-ictal and ictal periods may result from a combination of excitatory cortical input and SNr disinhibition, serving as an endogenous seizure termination mechanism. Future experiments manipulating cortico-pontine and nigro-pontine projections during seizures may identify this circuit as a therapeutic target. "

EVALUATING THE LONG-TERM CAPACITY AND SURVIVAL OF OPTOGENETIC DOPAMINERGIC NEURONAL-LIKE STEM CELL TRANSPLANTATION WITH VARYING LEVELS OF ENCOURAGED COMPLEX LIMB-USE IN A 6-OHDA RAT MODEL OF PARKINSON'S DISEASE (PILOT)

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Parkinson's disease (PD) is a progressive, idiopathic neurodegenerative disorder that predominantly affects motor function through the loss of dopaminergic neurons in the nigrostriatal pathway. This pathway's deterioration leads to the hallmark symptoms of PD such as tremor, rigidity, and bradykinesia. The current cornerstone of treatment, L-DOPA, while initially effective, gradually loses efficacy and leads to dose-related complications over time. Thus, alternative therapies are urgently needed. One promising avenue is stem cell therapy, aimed at replacing the lost neurons. Our laboratory explores an innovative approach by combining stem cell therapy with the stimulation of transplanted cells that have been genetically engineered to respond to light via a luminopsin construct, which is activated by coelenterazine (CTZ). This method is complemented by a swimming task, serving as an exercise regimen, to investigate the integration of these cells and their impact on behavioral recovery. Initial short-term studies show promising results, leading to further investigation into the long-term presence and functionality of these cells. Histological analyses have confirmed variable levels of cell integration, which appear to be influenced by the exercise regimen, suggesting a potential interaction between exercise and transplantation endurance. This finding could be pivotal for enhancing the treatment of neurodegenerative diseases. Given that these transplanted cells can persist within the host circuitry for extended periods, our ongoing research now focuses on the controlled release of dopamine from these cells over time, using microdialysis for real-time assessment. Our objective is to ensure that these early stimulated cells integrate robustly within the host brain, thereby enabling timely dopamine release to support appropriate behavioral responses, potentially offering a long-term therapeutic strategy for managing Parkinson's disease.

DEVELOPMENT OF A NOVEL TRANSLATIONAL RAT MODEL OF DEMENTIA WITH LEWY BODIES

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Dementia with Lewy Bodies (DLB) is the second most common neurodegenerative cause of dementia after Alzheimer's disease. Clinical features of DLB include variable attention and alertness, spontaneous parkinsonism, rapid eye movement sleep disorder, and recurrent visual hallucinations. DLB is characterized by: 1) aggregates of, alpha-synuclein (α-syn) Lewy bodies (LBs), amyloid-beta (Aβ) peptides as plaques and, to a variable extent, hyperphosphorylated tau protein as neurofibrillary tangles (NFTs); 2) Nigrostriatal degeneration; and 3) DLB-relevant behavioral symptomatology. Aggregated co-occurring proteinopathies in DLB are particularly prominent in cortical (temporo-occipital) and limbic (entorhinal cortex, cingulate, hippocampus CA1) regions. Some characteristics of DLB such as LB and plaque co-pathologies have been replicated in rodent models. However, no rodent model has recapitulated the full spectrum of DLB. Thus, we are developing a novel DLB rat model by combining the transgenic (Tg) F344 rat model of Alzheimer's Disease (AD) with specifically targeted intracerebral injections of mouse α-syn preformed fibrils (PFFs). The TgF344-AD rat model expresses mutant human amyloid precursor protein and presenilin 1 genes resulting in age-dependent accumulation of plaques and NFTs, cortical and hippocampal neurodegeneration, and cognitive disturbances. Nigrostriatal α-syn PFF injections result in accumulation of pathological α-syn in cortical, limbic and nigrostriatal regions, followed by nigrostriatal degeneration and motor deficits. Total of 14 male F344 wildtype (WT) and AD Tg rats have received bilateral intranigral injections of α-syn PFFs or α-syn monomer (control) at 6 months of age, which were followed by additional bilateral intrastriatal PFF and monomer injections at 10 months of age. We hypothesize that PFF injected TgF344-AD rats will exhibit: 1) abundant LBs, plaques, and NFT co-pathologies with upregulation of neuroinflammatory markers in cortical and limbic brain areas; 2) significant nigrostriatal degeneration; and 3) behavioral impairments. Postmortem assessments will focus on the amygdala, entorhinal cortex, cingulate cortex, hippocampus, striatum and substantia nigra to determine the impact of plaques + NFTs with and without LB co-pathology; glial reactivity; and neurodegeneration. A rodent model that integrates the entire repertoire of DLB co-pathologies and related behaviors will increase understanding of the proteinopathy in DLB and facilitate preclinical assessment of novel disease modifying therapies.

DENDRITIC SPINE MORPHOLOGICAL SIGNATURES OF COGNITIVE RESILIENCE TO ALZHEIMER'S DISEASE

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

TRAUMATIC BRAIN INJURY ELEVATES EXPRESSION OF THE BIPHASIC SIGNAL TRANSDUCER CARP-1/CCAR1

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Apoptosis and inflammatory activation are essential to induce tissue repair and restore homeostatic mechanisms following insults, such as traumatic brain injury (TBI). However, prolongation or exacerbation of these responses is directly tied to TBI pathophysiology. CARP-1/CCAR1 (Cell division Cycle and Apoptosis Regulatory Protein1) is a perinuclear protein that subserves biphasic pathways regulating cell growth and apoptosis, in part through its immunomodulatory signaling. As such, CARP-1 is hypothesized to act as a critical mediator to differentiate the induction of neurodegenerative vs repair processes that may occur after TBI. Therefore, the present study assessed CARP-1 levels and cellular expression patterns in brain tissue at subchronic and chronic timepoints after experimental TBI in the mouse. TBI increased CARP-1 levels relative to shams, as quantified by immunoblotting, in the dorsal hippocampus, a region that is highly susceptible to pathology post-injury. The relative increase in CARP-1 levels in TBI mice was greater in tissue harvested at chronic (30 days post-TBI), compared to subchronic (7 days post-TBI) timepoints. An increase in hippocampal CARP-1 levels was also evident by immunofluorescence in tissue harvested at 30 days post-injury, and this elevation was localized to the cytosol of dorsal hippocampal neurons in the CA2/3 subregions. CARP-1 was also co-localized with GFAP (glial fibrillary acidic protein)-expressing astrocytes within the dorsal hippocampus of chronically injured mice (30 days post-injury), suggesting CARP-1 may be involved in regulating tissue repair and synaptic remodeling. Together, these findings indicate that CARP-1 is modulated by TBI even at chronic timepoints. Current expansion of this project with transgenic models will delineate if CARP-1 represents a critical response factor to distinguish promotion of beneficial or detrimental pathological events based on concurrent signaling. This work was supported by the resources and facilities of the John D. Dingell VA Medical Center.

USING COMPLEX GENETICS IN MICE TO UNLOCK THE SECRETS OF RESILIENCE TO DEMENTIA

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Aims: Cognitive resilience to Alzheimer's disease (AD) is a phenomenon whereby an individual presents with normal cognitive function despite harboring a familial Alzheimer's disease (FAD) mutation and has corresponding brain neuropathology. Determination of the underlying mechanisms of cognitive resilience to AD will likely offer novel disease modifying therapeutics for individuals at-risk for AD.

Methods: We used the contextual fear memory paradigm to assess short-term memory function in the AD-BXD mouse reference panel, which incorporates the 5xFAD human mutations on a genetically diverse background that better mimics human AD. The degree resilience or susceptibility was based on the age-related change in cognitive function relative to that of the entire AD-BXD population; strains showing no or lower than average decline were considered resilient, while those showing more decline were considered susceptible. To determine transcriptional changes associated with resilience, we profiled the hippocampal transcriptome at the single cell level in top resilient and susceptible strains.

Results: We show that cognitive resilience in 5xFAD mice is characterized by a transcriptional signature that aligns with that of non-transgenic littermates in excitatory neurons of CA1, dentate gyrus and intratelencephalic neurons in layer 3 and 6 of the entorhinal cortex. We found that the transcriptional profile of resilient strains is enriched for regulation of transmembrane transport in presymptomatic stages that included a notable upregulation of Reln and Ntng2, whereas translation at the CA1 synapse corresponded to an upregulation of ribosomal genes in neurons from resilient AD mutation carrier mice.

Conclusions: Our findings suggest that resilience is conferred in memory-relevant regions through unique transcriptional changes in a cell-specific manner and provide a foundation for mechanistic studies required for resilience-based drug development.

DIFFERENTIAL EFFECTS OF CHLORPYRIFOS AND DIAZINON AND THEIR METABOLITES ON MITOCHONDRIAL COMPLEX ACTIVITY AND DOPAMINERGIC NEUROTOXICITY

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Organophosphate (OP) pesticides and their oxon derivatives are known for their neurotoxic effects. While extensively studied for their acute cholinergic toxicity, the impacts on other non-target neurons remain obscure. This study compares two major OP pesticides, Chlorpyrifos (CPF) and Diazinon (DZN), and their metabolites on dopaminergic neurons and mitochondrial complex activity. Previous studies demonstrated that CPF and its active metabolite, CPF-oxon, exert different levels of neurotoxicity and mitochondrial enzyme inhibition. CPF being banned in the year 2021; DZN is one of the most commonly used OP pesticides. Dopaminergic cell loss was assessed in C. elegans strain BZ555 (dat-1p::GFP), and the effect on mitochondrial enzyme activity was assessed by acutely treating mitochondria isolated from rat liver. Previous studies showed that CPF leads to dose-dependent dopaminergic cell loss, with CPF-oxon exhibiting relatively higher DA toxicity than CPF at equimolar concentrations. Paradoxically, while CPF exhibited significant inhibition of mitochondrial complex II, CPF-oxon showed no inhibition. Furthermore, posterior deirid (PDE) neurons were found to be most susceptible, partly due to developmental delay and neurotoxicity. In comparison to CPF and CPF-oxon, DZN and its metabolite, Diazoxon (DZO), exhibited little and a little neurotoxicity, respectively. In DZO-treated worms, PDE neurons remained mostly spared the neurotoxic effects of DZO, while ADE neurons, followed by CEP neurons, were mostly affected. DZN appears to decrease complex I activity, while its metabolite, DZO, does not show any effect. These findings implied the differential impacts of OP pesticides and their metabolites on dopaminergic insults and mitochondrial function, highlighting the complexity of their neurotoxic mechanisms. Overall, our study suggests that these OP and metabolites are potential neurotoxicants and inhibitors of mitochondrial enzymes. Future studies will entail elaborated investigations of DZN and DZO for effect on mitochondrial enzymes and whether inhibition ameliorated upon co-exposure with Coenzyme Q.

AGING DRIVES CEREBROVASCULAR NETWORK REMODELING AND FUNCTIONAL CHANGES IN THE MOUSE BRAIN

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Introduction: Cerebrovascular dysfunction has been implicated in age-related cognitive decline and dementia, but the underlying vascular mechanisms are not well understood. An improved understanding of the nature of normal cerebrovascular aging is needed to help to establish the role that vascular dysfunction might play in cognitive decline and dementia.

Methods: Here, we asked how normal aging differentially impacts the vascular structure and function in different brain areas in mice. We investigated structural changes in aged cerebrovascular networks and pericytes utilizing serial two-photon tomography (STPT). To further investigate potential remodeling of different vascular compartments and pericyte subtypes, we utilized tissue clearing, 3D immunolabeling, and light sheet fluorescence microscopy (LSFM) imaging. We also assessed how healthy aging impacts brain hemodynamics in response to voluntary locomotion and whisker stimulation in awake, head-fixed mice using wide field optical signal imaging and two-photon imaging. We tested mice of both sexes at 2-month, 18-month (early aging), and 24-month (late aging) of age.

Results: Whole-brain vascular tracing using STPT showed an overall ~10% decrease in vascular length and branching density, and LSFM imaging with 3D immunolabeling further revealed increased arteriole tortuosity in aged brains. We also uncovered a selective vascular and pericyte loss in deep cortical layers, basal forebrain regions, and the hippocampal network. This may contribute to their regional vulnerabilities in neurodegenerative disorders. Moreover, our in vivo imaging in awake, head-fixed mice identified delayed neurovascular coupling response and inefficient oxygen delivery in aged brains.

Conclusions: Our study reveals aging-related brain-wide changes in vascular and mural cell types that can explain vulnerability and resilience of different brain areas in normal aging. Moreover, we identified an age-related decrease in brain oxygenation and delayed neurovascular coupling responses which can be linked with cognitive impairment in aged brains. These aging-related changes will serve as a common factor to understand many neurodegenerative disorders and cognition decline in the elderly population."

AGE AND GENETIC BACKGROUND MODULATE THE EFFECT OF ALZHEIMER'S DISEASE ON SLEEP

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BACKGROUND: Alzheimer’s Disease (AD) is known to contribute towards changes to sleep, including decreased sleep duration and increased sleep fragmentation.1 While significant advances have been made in characterizing these changes,2,3 the underlying genetics are yet unknown. As such, we decided to use a forward genetic approach to identify genes controlling sleep in AD in a panel of genetically diverse mice (AD-BXDs).4

METHODS: Female mice from 47 strains in the AD-BXD panel4 carrying the 5xFAD transgene (n =214) and non-transgenic littermate controls (n = 216) completed sleep testing in the PiezoSleep Tracking System5 at 6 and 14 months of age. The percent of time spent sleeping was calculated over 4 testing days by automated sleep/wake scoring.

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

CONCLUSION: Genetic background modulates the effect of AD and age on sleep in the AD-BXD panel. Since sleep is vital for memory consolidation,6 future work will aim to examine cognitive performance of these strains and map the genes causing changes to sleep in AD. "

EXAMINING THE IMPACT OF TAU TOXICITY ON CLIMBING ABILITY IN DROSOPHILA

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Alzheimer's disease is a significant medical and societal challenge marked by memory loss, cognitive decline, and behavioral changes. It belongs to a disease class named tauopathies, characterized by tau protein toxicity in the human brain. Tau, a microtubule-associated protein, typically stabilizes microtubules but can aggregate due to post-translational modifications, leading to cognitive impairment. Drosophila melanogaster, the fruit fly, is an ideal model for studying the role of tau in disease due to the genetic conservation of molecular pathways. To assess neurodegenerative fitness in multiple groups of flies simultaneously, many labs utilize the rapid iterative negative geotaxis (RING) assay, commonly called a climbing assay. My project uses this assay to assess how expression of wild-type and mutant forms of human tauopathy-associated genes affect negative geotaxis. To identify potential therapeutic intervention strategies, I employed the climbing assay with tau-expressing flies that were fed either kinase or calpain inhibitors to measure whether these drugs could significantly improve the tau-associated decline in locomotion. Not only does this assay provide a statistically relevant assessment of how our genetic and pharmaceutical manipulations of flies influence a disease-associated behavioral phenotype, but it also provides an alternative method to characterize neuronal toxicity in circumstances when the modification of tau toxicity may be difficult to quantify (such as modification of the tau-induced rough eye phenotype). This research represents a crucial step toward understanding the role of tau in neurodegeneration and its implications for Alzheimer's disease treatments.

THE IMPACT OF PHARMACOLOGICAL INHIBITION IN SUPRESSING TAU TOXICITY

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Alzheimer's disease (AD) is a neurodegenerative disease that affects more than 6 million Americans. It is characterized by a multitude of symptoms, including memory loss, seizures, and mood swings. There are treatments on the market that are aimed at targeting these symptoms; however, there is no known cure. AD is characterized anatomically by the presence of neurofibrillary, tau tangles and beta-amyloid plaques. Because of this, AD belongs to a family of diseases called tauopathies, which are characterized by abnormal phosphorylation of tau proteins by kinases in the brain, leading to the characteristic neurofibrillary tangles and ultimately, miscommunication and death of hippocampal neurons. Our laboratory uses the model system Drosophila melanogaster to investigate the molecular basis of tau toxicity. Cleavage of full-length tau (65kD) by the protease calpain results in the production of a 17kD fragment that our lab and others have shown is intrinsically toxic to flies and cultured human neurons. Using deficiency lines, we showed previously that genetic reduction of fly calpain suppresses tau-induced toxicity. For this project, we tested the efficacy of pharmacological calpain and kinase inhibitors to suppress tau-induced neurodegeneration. The aim of this study is to determine whether pharmacologically inhibiting two main drivers of tau-induced neuronal toxicity, phosphorylation and proteolytic cleavage, are viable treatment strategies to alleviate the neurodegeneration associated with AD.

HISTOPATHOLOGICAL ANALYSIS OF DENDRIMER PROGESTERONE IN STROKE RAT BRAIN ABSTRACT

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Stroke is the top cause for disability and second most cause of death in the world. Ischemic stroke occurs when blood clot or narrowed artery blocks the blood flow into the brain resulting in neuroinflammation and hypoxic brain damage, which results in brain function loss. Progesterone may be a possible treatment for stroke, due to its neuroprotective and anti-inflammatory characteristics. However, progesterone does not cross the blood brain barrier (BBB) making it impossible to deliver to brain. Polyamidoamine dendrimers are commonly used to deliver treatment into the brain. The purpose for this study was to treat MCAo rats with progesterone and dendrimer-progesterone complex via intraperitoneal injection. The rats underwent stroke or sham surgery, treatment injection, and euthanization. The brains then were extracted, stored in the freezer, then sliced into 30 μm thick tissue slices. We performed hematoxylin and eosin staining to quantify the stroke volume in different treatment groups. We performed immunohistochemistry (IHC) staining to quantify the expression of GFAP and IBA-1 in different treatment groups. After the IHC brains were mounted, and cover slipped. We saw a decrease in stroke volume in MCAo rats treated with progesterone-dendrimer complex and dendrimer alone. We also saw a reduction in GFAP and IBA-1 expression in progesterone dendrimer and dendrimer alone treated MCAo rats. Our result showed that dendrimer was able to cross BBB and deliver progesterone into the brain. Moreover, our results showed dendrimer itself has anti-inflammatory properties, confirming our previous findings.

GENERATION OF SELF-ORGANIZING SINGLE-ROSETTE CORTICAL ORGANOIDS (SOSR-COS) LACKING ATXN3, THE DISEASE GENE IN SPINOCEREBELLAR ATAXIA TYPE 3

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

IDENTIFICATION OF THE EARLY MICROGLIAL TRANSCRIPTOMIC RESPONSE TO ALPHA-SYNUCLEIN INCLUSION FORMATION IN THE SUBSTANTIA NIGRA FOLLOWING PREFORMED FIBRIL INJECTION

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Increasing evidence suggests that chronic inflammation may contribute to disease progression in Parkinson's disease (PD). Lewy body deposition in PD tissue is correlated with microglial reactivity, specifically upregulation of major histocompatibility complex class II (MHC-II). In the rat alpha-synuclein preformed fibril (PFF) model we similarly observe a positive association between phosphorylated alpha-synuclein (pSyn) inclusion accumulation and MHC-II expression in the substantia nigra (SN). RNA-sequencing in the SN of PFF model rats at the peak of pSyn accumulation and MHC-II expression (2-months) revealed immune effector processes to be the most enriched pathway, including increased expression of Cd74, which mediates assembly and subcellular trafficking of MHC-II. However, studies examining the microglial transcriptome support the concept that microglial phenotypes are disease stage-specific, with at least one report in an amyloid-beta model suggesting microglial MHC-II expression defines a late response of microglia to disease pathology. The purpose of the present study is to evaluate whether the microglial transcriptomic response to early pSyn formation in the SN (1-month post PFF) is distinctive from that we have previously observed during the later, established peak of pSyn accumulation (2-months post PFF). Male TH-EGFP rats received two intrastriatal injections of either mouse α-syn PFFs, mouse α-syn monomer or vehicle and were euthanized via saline perfusion 1-month after surgery. Ipsilateral SN tissue was punched from frozen brains and nuclei suspensions prepared for single nuclei RNA-sequencing. Transcripts will be clustered together using Seurat, allowing for direct comparisons of the microglial-specific transcriptome between PFF, monomer and PBS control rats. Results will be compared to microglial-associated transcripts upregulated in our existing 2-month RNA-Seq dataset. Future studies will include validation of subsets of identified transcripts within microglia following PFF injection using in-situ hybridization. Results from this study will determine whether the microglial transcriptomic response to early vs. late pSyn inclusions is temporally distinctive.

POOR GLUCOSE METABOLISM EXPLAINS COGNITIVE DECLINE IN NORMAL AGING

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Background: High Health Burden of Diabetes and Dementia. Diabetes and dementia each have high prevalence rates in the U.S. and an unexpectedly high rate of comorbidity. Individuals diagnosed with diabetes have a 1.4–2.2-fold higher risk of dementia than those without diabetes. However, the healthcare burden of these diseases differs by race/ethnicity backgrounds (higher in non-Hispanic Black individuals) and by biological sex (higher in men for diabetes but women for dementia). Given the differences in these prevalence rates, we aimed to determine how glucose metabolism modifies normal aging-related cognitive decline in a genetically diverse mouse panel: AD-BXD (Fig. 1). During the breeding process of the AD-BXD, there is a 50/50 chance that the F1 progeny will be non-transgenic for AD mutations. We utilized the non-transgenic mice to study the normal aging process under different diets, one of which was standard chow.

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

Conclusions: In summary, we found that in the normal aging process poorer glucose metabolism is associated with cognitive decline in males on a chow diet. These same findings were not exhibited in AD males/females or non-transgenic females. The use of the AD-BXD mouse panel illuminated the fact that this decline differs across strains of diverse genetic backgrounds independent of AD mutations. It also illustrates the point that strain results may differ by sex. These findings and the use of a mouse panel emulating human diversity are proving to be invaluable in developing precision medicine practices tailored to individuals.

Methods: Glucose Metabolism and Contextual Fear Memory - The data were collected longitudinally from 516 non-transgenic AD-BXD mice (39 unique strains). Glucose metabolism was assessed through an intraperitoneal glucose tolerance test (IPGTT). Contextual fear memory (CFM) was the measure of cognitive function. We used multivariable linear regression to model the relationship between cognitive decline from 6 to 14 months and glucose metabolism at 14 months.

USE OF CRISPR/CAS9 GENE EDITING TOOL MEDIATED GENES KNOCKOUT IN PRIMARY ADULT RAT ASTROCYTES FOR NEURONAL REPROGRAMMING AND CONVERSIONS

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In the event of a stroke, the blood flow in the circle of Willis becomes disrupted, leading to a lack of oxygen in the brain tissue and causing neuronal death which triggers an immune response called neuroinflammation that activates the protective functions of astrocytes, also known as reactive astrocytes. Previous research has shown that it is possible to convert these astrocytes into functional neurons by inhibiting specific pathways, including the Notch, GSK-3 β, and BMP pathways. In this study, we used the CRISPR/Cas9 gene-editing tool to knock out the target genes that of these pathways Hes5, NF-kB1, and Blc2 for Notch; Nlrp3 for GSK-3β and Smad1, and Smad5 for BMP) and receptor associated genes Psen1 for Notch pathways, Bmpr1a for BMP pathway, and Gsk3b for GSK-3β in adult rat brain-derived astrocytes. Sanger sequencing showed knockout of above-mentioned genes. The Western blot analysis showed a reduction in the expression of the Hes5, Nfkb1, Smad1, Smad5 proteins, suggesting the successful knockout of these target genes in the Notch and BMP pathways. The aim of this in vitro study was to investigate the potential of using dendrimers to deliver CRISPR/Cas9 to astrocytes in primary rat cell culture to edit the genes involved in the pathways described above and convert the astrocytes into neuroblasts in vitro. In a previous experiment, we have shown that dendrimers can cross BBB of adult rat. This preliminary study suggests that using CRISPR/Cas9 plasmid-dendrimer complexes to edit genes and pathways to convert astrocytes into neurons in stroke could be a promising strategy of these therapeutics into the brain.

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

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

QUANTIFICATION OF CHOLINERGIC CELL NUMBER IN THE PEDUNCULOPONTINE NUCLEUS AND MEDIAL SEPTUM OF AN SCN1A+/- MOUSE MODEL OF DRAVET SYNDROME

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Epilepsy is a common neurological disorder characterized by recurrent seizures. Patients with epilepsy also frequently suffer from quality of life-limiting comorbidities including depression, anxiety, sleep disturbances, and inattention. The mechanistic link between seizures - which are paroxysmal and typically brief periods of uncontrolled electrical activity in the brain - and the development of chronic comorbidities - which persist on a much longer timescale - is not well understood. Converging evidence has associated epilepsy with dysregulation in arousal-promoting neuromodulatory networks, including cholinergic circuits known to be crucial for mood, attention, and sleep regulation.

We hypothesize that seizures reduce cholinergic expression within and neurotransmission from cholinergic nuclei including the pedunculopontine nucleus (PPN) in the brainstem and the medial septum (MS) in the basal forebrain. We are testing this hypothesis in the well-validated preclinical Scn1a+/- mouse model of Dravet Syndrome, a developmental and epileptic encephalopathy. Mice were subjected to three heat-induced seizures at postnatal day (P)19, P21, and P23. We then quantified cholinergic cell density in the caudal PPN and the MS with gold-standard stereology and additionally measured single-cell features such as cell size.

Our findings revealed that Scn1a+/- mice have a significant ~20% reduction in cholinergic cell numbers within the PPN and a preliminary similar finding in the MS relative to wild-type littermate controls (which also were exposed to hyperthermia but did not have behavioral seizures). To ascertain whether this reduction is attributable to the induced seizures, we are additionally testing a parallel cohort of mice that were not subjected to hyperthermia. Additional future directions of the project will be to elucidate the mechanism and the circuit- and behavioral-level consequences of these findings, with the ultimate goal of identifying a novel therapeutic strategy for comorbidities in epilepsy."

DATA-INDEPENDENT ACQUISITION PROTEOMIC APPROACH REVEALS CALCIUM-ACTIVATED POTASSIUM CHANNELS AND ADP-RIBOSYLATION FACTORS ARE LINKED TO COGNITIVE RESILIENCE TO ALZHEIMER'S DISEASE.

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

BIOID2-BASED INTERACTOME OF FULL-LENGTH AND DOMAINS OF TAU REVEALS NOVEL AND KNOWN PROTEIN INTERACTIONS ASSOCIATED WITH MULTIPLE CELLULAR PATHWAYS

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Tau protein aggregation is central to neuropathological alterations in Alzheimer's Disease (AD) and other neurodegenerative diseases collectively known as tauopathies. Tau was initially reported as a microtubule-associated protein involved in regulating microtubule dynamics. Multiple lines of evidence suggest that Tau plays diverse microtubule-independent functional roles regulated, in part, by direct and/or transient protein interactions. Deciphering the Tau interactome is a critical step toward better understating the physiological and pathological Tau-mediated cellular processes. In this work, we sought to map potential members of the Tau interactome using the BioID2 approach that allows for in situ protein labeling in living neurons. Biotin-labeled proteins are identified by biotin-targeted pulldown and mass spectrometry. We generated lentiviruses expressing fusion proteins between full-length human Tau (2N4R isoform) with BioID2 on either the N-terminus (BioID2-Tau) or C-terminus (Tau-BioID2). Tau was further subdivided into three domains: the N-terminus domain (N-term), the microtubule binding region (MTBR), and the C-terminus domain (C-term). To further dissect the Tau interactome, we created fusion proteins between Tau domains and BioID2 (Nterm-BioID2, MTBR-BioID2 and Cterm-BioID2). A control lentiviral construct was created to express only the BioID2 protein. Embryonic day 18 Tau knockout (TKO) primary cortical neurons were transduced on the 4th day in vitro (DIV4), and lysates were collected on DIV12 for biotin-targeted pulldown and mass spectrometry identification (n=3 biological replicates). Utilizing this approach, we identified 323 proteins as candidates of the Tau interactome among which 161 proteins interacted with the N-term, 166 proteins with the MTBR, 179 proteins with the C-term. Tau protein interactions with synapsin-1, FUS, and prune1 were further validated by co-immunoprecipitation from adult human Tau knockin (hTKI) mouse cortical tissue (n=3) and proximity ligation assay in hTKI cortical neurons (n=3). Gene Ontology enrichment analysis mapped protein interactors associated with the somatodendritic compartment, mitochondria, cytoskeleton, synapses, ribosomes, ubiquitin-proteasome system, and the ribonucleoprotein complex. KEGG pathway analysis identified proteins associated with neurodegenerative diseases, including AD, Parkinson's disease, and Huntington's disease. Thus, this approach can identify potential members of the Tau interactome via in situ labeling. This work helps expand the growing list of Tau's potential functional roles and may advance our understanding of its biological and neurodegenerative functions.

NEURON SPECIFIC REGULATION OF REPEAT ASSOCIATED NON-AUG TRANSLATION IN AGE RELATED DEMENTIAS

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Expansion of short tandem repeats (STRs) causes over 60 neurological diseases including C9orf72 associated frontotemporal dementia/amyotrophic lateral sclerosis (C9 FTD/ALS) and Fragile X Associated Tremor/Ataxia Syndrome (FXTAS). These expanded repeats form GC-rich regions which create stable secondary structures such as G-quadruplexes, alter RNA dynamics, and support a non-canonical initation process known as repeat-associated non-AUG (RAN) translation. While canonical translation initiation requires various factors to bind to the 5’ cap of mRNA and then scan the mRNA until the start codon (AUG) is reached, RAN translation initiation can occur in two distinct ways: cap-dependent, in which initiation factors bind to the cap but initiate translation at a near cognate codon due to stalling at the repeat, and cap-independent, in which translation does not require a cap and utilizes poorly characterized internal ribosome entry sites (IRES). Here, we examined the influences of cell-type on cap-independent translation. While RAN translation is largely a cap-dependent process in cell lines and in vitro, cap-independent translation of RAN translation specific reporters is upregulated in primary rat hippocampal neurons and in iPSC-derived neurons. In primary rat hippocampal neurons, cap-independence is not specifically influenced by G-quadruplex destabilization and responds differently to ER stress compared to findings in HEK293 cell lines. Taken together, these data indicate an effect of cell type on the cap-dependence of RAN translation, with implications for how repeat expansion disorders repeats might elicit toxicity primarily within the nervous system.

DISEASE-MODIFYING POTENTIAL OF THIRD GENERATION ROCK INHIBITOR KL-00974 IN SYNUCLEINOPATHY MODELS OF PARKINSON'S DISEASE

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Microglial activation is associated with Lewy Body deposition in Parkinson's disease (PD) and rodent models of synucleinopathy which leads to a chronic proinflammatory environment that may contribute to neurodegeneration. Microglial migration, phagocytosis and release of proinflammatory cytokines are mediated by Rho-associated protein kinase (ROCK) activity. The third generation ROCK inhibitor KL-00974 is more selective, potent and brain penetrant than classical ROCK inhibitors (e.g. fasudil). In the present study we evaluated the anti-inflammatory and neuroprotective potential of KL-00974 in both the alpha-synuclein adenoassociated viral (AAV) vector and alpha-synuclein (a-syn) preformed fibril (PFF) model in rats. Daily oral administration of KL-00974 significantly decreased microglial immunofluorescence and significantly prevented degeneration in tyrosine hydroxylase immunoreactive (THir) nigral neurons in the AAV a-syn overexpression model. In the a-syn PFF model, no impact of daily oral KL-00974 on accumulation of phosphorylated a-syn (pSyn) or number of major histocompatibility complex class II immunoreactive (MHC-IIir) microglia in the substantia nigra (SN) are observed at 2 months. Ongoing studies will determine whether KL-00974 can impact nigrostriatal degeneration in the PFF model. Future studies will examine transcriptomic changes in the SN associated with KL-00974 administration in the context of PFF-induced pSyn inclusions. Systematic vetting of the neuroprotective potential of KL-00974 in multiple synucleinopathy models will provide critical data to support continued development of KL-00974 as a therapeutic strategy for PD.

EVALUATING THE THERAPEUTIC EFFECTS OF DELIVERING CRISPR/CAS9 AND SMALL INTERFERING RNA MOLECULES VIA G4-70/30 PAMAM DENDRIMER NANOMOLECULES ON HUMAN GLIOBLASTOMA CELLS IN VITRO

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Glioblastoma (GB) is the most common and aggressive central nervous system tumor, with a 15-month median survival time after diagnosis. There has never been a cured case reported, thus stressing the need for new GB treatments. One promising novel GB treatment involves targeting the AVIL gene, the overexpression of which has been found to be essential for the survival, migration, and invasion of GB cells. In this study, we evaluated the efficacy of AVIL CRISPR/Cas9 and AVIL siRNA therapy in vitro, which target the AVIL gene and AVIL mRNA, respectively. The efficacy of these gene therapies is limited by poor cellular uptake and low stability. Therefore, we used G4-70/30 PAMAM dendrimers, which improve the stability and bioavailability of encapsulated treatments. Encapsulating siRNA molecules in these dendrimers yielded a stable complex. RT-PCR data showed that siRNA treatment using the RNAiMAX transfection reagent conferred AVIL knockdown in both HEK and U87 cells. Treatment with the dendrimer-siRNA complex produced AVIL knockdown in U87 cells to a lesser degree. Hence, we are currently working to further optimize this treatment to confirm these results. CRISPR/Cas9 transfected U87 cells were analyzed for gene knockout via Sanger sequencing and for protein expression via Western blotting. Sanger sequencing of transfected U87 cells showed 55% AVIL gene knockout after 3 days of transfection. Western blot analysis of CRISPR/Cas9-treated U87 cells revealed reductions in the proteins p92, which is encoded by the AVIL gene, and FOXM1, which is a downstream target of the AVIL gene. However, Western blot analysis of siRNA-treated U87 cells revealed reductions in p92 but not FOXM1, suggesting that CRISPR-Cas9 may be more effective in treating the U87 model of GB than siRNA treatment. The preliminary data gathered in this study suggests that AVIL gene inhibition is a potential treatment option for GB.

Support for this study was provided by the 2023 Student Endeavors Grant, the Neuroscience Program, the College of Medicine, the Department of Chemistry and Biochemistry, the John G. Kulhavi Professorship in Neuroscience, and the E. Malcolm Field and Gary Leo Dunbar Endowed Chair in Neuroscience at CMU.

UTILIZING THE NOVEL Q111-BXD MOUSE PANEL TO EXPLORE NEUROPSYCHIATRIC TRAITS IN HUNTINGTON'S DISEASE

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

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

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

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

VERSATILE GENETIC PERTURBATION APPROACH TO UNDERSTAND TUMOR-IMMUNE INTERACTIONS IN HUMAN GLIOBLASTOMA

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The mechanism underlying how macrophages induce a transition of glioblastoma (GBM) cells into a mesenchymal (MES)-like state is unclear. Macrophages produce a cytokine known as Oncostatin M (OSM), which is proven to cause this transition in GBM cells. Although the involvement of OSM is recognized, the precise mechanism and potential additional factors remain uncertain. In this study, previous work used single-cell RNA sequencing (scRNA-seq) to analyze GBM subtype expression in various cellular states. GBM cells (MGG23) were treated with macrophage-derived ligands for 24 hours and assessed for glioblastoma states via CD24 and CD44 markers with flow cytometry. Additionally, we employed the Neon electroporation system that used CRISPR/Cas9 to knock out the OSM gene in U937 macrophages, achieving high transfection efficiency. Single-cell cloning by limiting dilution produced putative OSM knockout (KO) clones, which were assessed by PCR and gel electrophoresis. Given the central roles of the MES-like program in GBM, this research endeavors to contribute valuable insights into the relatively unknown microenvironment of GBM cells, with the ultimate goal of helping to create targeted therapeutic interventions to combat this disease.

SCN2A DISEASE-ASSOCIATED VARIANTS DISRUPT THE INTERACTION WITH ANKYRIN AND ALTER THE SODIUM CHANNEL FUNCTION, IMPACTING NEURONAL EXCITABILITY

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SCN2A and ANK2 are strong risk genes for autism spectrum disorders (ASD). The voltage-gated sodium channel NaV1.2 (product of SCN2A) is located to the dendrites in mature neurons, where it promotes action potential backpropagation necessary for normal synaptic function. Ankyrin-B, (product of the gene ANK2) is an intracellular scaffolding protein that is highly enriched in the soma and distal dendrites. We recently discovered that ankyrin-B is the dendritic scaffold for NaV1.2, and that deleting ankyrin-B significantly reduced NaV1.2 dendritic localization. While we have identified disease-associated variants within the binding interface between the two proteins, not all the variants in the interface influenced the binding affinity of NaV1.2 to ankyrin-B, suggesting that they may influence channel function in other ways. Our research project involves performing whole-cell electrophysiological voltage-clamp recordings in mammalian cells to compare the biophysical properties of wild-type NaV1.2 channels to those of NaV1.2 channels with autism-associated polymorphisms that do not disrupt the binding to ankyrin-B. This is critical because changes in ion channel electrophysiological properties can influence neuronal excitability and synaptic function, and thus can contribute to the etiology of autism.

CAUSAL ROLE OF KIRREL3 IN SLEEP DISRUPTION OF PRESYMPTOMATIC ALZHEIMER'S DISEASE MICE

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Sleep disruptions are early risk factors of Alzheimer’s Disease (AD), appearing decades prior to the accumulation of Aβ and phosphorylated tau in the brain, and worsening throughout the course of the disease. Though sleep is highly regulated by genetics (heritable ~17-69%), causal genes and gene variants regulating AD-related sleep disruptions remain unknown. Here, we provide the first evidence that genetic factors interact with AD mutations to influence sleep behavior. To identify potential gene candidates involved in AD related sleep disturbances, we first characterized sleep in 25 strains from the AD-BXD genetic reference mouse panel, which better models the complex genetics and etiology of human AD. Sleep phenotyping was done using PiezoSleep system (n = 179, AD-BXD mice). We found that at 7-8 months of age, both male and female AD mice demonstrated significant sleep loss in the dark cycle and hourly sleep even before the onset of cognitive symptoms. Quantitative trait loci (QTL) mapping revealed a significant QTL peak associated with variation in sleep in zeitgeber time 14 (ZT14) that was mapped to intron 1 of the Kirrel3 gene on chromosome 9, suggesting involvement of Kirrel3 in sleep/wake activity. To validate the effect of Kirrel3 on sleep, we developed a Kirrel3-knockout mouse carrying familial AD mutations (5XFAD) (Kirrel3-HET-5xFAD). Sleep phenotyping in these mice (n = 153) revealed a significant reduction in dark cycle and hourly sleep only in Kirrel3-HET-5XFAD mice. These results suggest that Kirrel3 plays a causal role in sleep susceptibility that is dependent on the presence of 5XFAD transgene.

Enhancing Therapeutic Efficacy: Non-Invasive Intranasal Delivery of G4 70/30 PAMAM Dendrimer in C57 Mouse Model

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Various routes of drug administration to the brain have been explored, yet the blood-brain barrier (BBB) poses a significant challenge in achieving effective drug delivery for neurological diseases. Over the years, nanocarriers have appeared as a promising approach to overcome this barrier. Notably, intranasal administration offers a direct pathway for drug delivery to the brain, circumventing the BBB. The olfactory nerve route, which includes the olfactory bulbs, can provide an entrance point for NPs into the human brain. Dendrimers show potential as carriers for central nervous system (CNS) drugs via intranasal administration, offering reduced systemic exposure and limited side effects after in vivo administration. highlighting their potential as a safe and effective strategy for CNS drug delivery.

Our study was designed with male (n=3) and female (n=3) C57 mice. The treatment group received daily intranasal delivery of CY5.5-labeled G4 70/30 PAMAM dendrimers, while the control group received HBSS. In Vivo Imaging System (IVIS) measures were conducted weekly for three weeks after intranasal treatment initiation. After three weeks, fresh organs were extracted from all mice. The extracted organs (brain, lung, liver, kidney) were frozen and sectioned. The sections were imaged with fluorescence microscope. Results from fluorescence evaluation of intranasal brain inoculated mice showed greater accumulation of G4 70/30 PAMAM dendrimer in the brain, liver and kidney of CY5.5-labeled G4 70/30 PAMAM dendrimers. In conclusion, our study revealed that G4 70/30 PAMAM dendrimer can be delivered intranasally in the C57 mice brain, which shows the efficiency of intranasal delivery for neurological diseases, and can be used for delivering gene and drugs such as curcumin to treat brain tumors.

Effects of Coelenterazine Stimulation of Modified Bone Marrow Derived Mesenchymal Stem Cells in the Context of Exercise on Motor Restoration in a 6-OHDA Rat Model of Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder resulting from the loss of dopaminergic neurons within the nigrostriatal pathway. Popular PD treatments (L-DOPA) increase dopamine (DA) without task-integrated host control. Our lab aims to enhance host-controlled DA release by enhancing transplant integration. We stimulated transplanted cells optogenetically during active swimming to address these shortcomings and promote improved amelioration of motor deficits. Mesenchymal stem cells derived from bone marrow (BM-MSCs) were rendered dopaminergic-like and altered to allow direct activation by the catalyst coelenterazine (CTZ) via the luminopsin construct LMO3. Male Sprague Dawley rats underwent unilateral 6-OHDA lesioning before receiving striatal transplantation of modified BM-MSCs. Following transplantation, directly stimulating these transplants in concert with encouraged exercise (swimming) appears to increase supportive integration of transplants, eliciting improved movement control at the Post testing time point. Results from the first instance of stimulation (Day 7) for Groups 3 and 4 appear to show shifts in symmetry of forelimb use at the interval level, with a general trend towards more symmetrical forelimb use. Groups 1 and 2 displayed sustained asymmetry at the Day 7 time point. At the Post testing time point, subjects that received direct transplant stimulation at the time of exercise (Group 3, Group 4) showed greater motor control when compared to subjects not receiving stem cell stimulation (Group 1, Group 2). After two stimulation events, Group 4 subjects exhibited improved motor control compared with only one stimulation event (Group 3). These results indicate the more efforts to correlate transplant stimulation with swimming, the more motor control is restored per treated animal. Non-transplanted PD rats were also stimulated, yielding no motoric benefits, and confirming behavioral improvements likely derived from transplants being "trained."

DELIVERY OF PROGESTERONE INTRAPERITONEALLY IN MCAO RAT USING PAMAM DENDRIMERS

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A stroke occurs when the blood supply to a specific area of the brain is interrupted, resulting in brain tissue damage leading to loss of brain function, and neuroinflammation. Ischemic stroke, the most common type, happens when blood supply to brain is blocked due to a blot clot or narrowed artery supplying to brain region. Previous studies have shown progesterone has anti-inflammatory properties. To confirm these properties, we used G4 PAMAM dendrimers to systemically deliver progesterone the in rat models with middle cerebral artery occlusion (MCAo). The rats received intraperitoneal (IP) injections of dendrimer-progesterone complex, progesterone, dendrimer only, and HBSS every other day for 10 days, starting on the sixth day after surgery. Behavioral assessments, including the ladder test, cylinder test, and modified Garcia scale for neuro-scoring, were conducted weekly to evaluate motor function. Results of the ladder test and cylinder tests showed improved function of left paw in dendrimer-progesterone and dendrimer-only treated MCAo rats compared to untreated rats. The neuro-scoring test showed improvement in score in dendrimer progesterone-treated MCAo rats. These findings confirmed that dendrimers could cross the blood-brain barrier when injected intraperitoneally. These results further suggest that dendrimers have potential as a promising delivery method for drugs. Currently, we are performing IHC imaging to analyze GFAP and IBA-1 expression in brain sections.

COVID-19-INDUCED NEUROIMMUNE CHALLENEGES AS A LONG-TERM RISK OF AGE-RELATED DEMENTIA

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COVID-19 has affected more than 770 million individuals worldwide, and up to 40% of survivors experience post-acute COVID sequelae (PASC, “long COVID”). The symptoms of long COVID include “brain fog”, cognitive impairments, and mood-related symptoms such as depression or anxiety. SARS-COV-2 virus only rarely infects the brain, suggesting that other effects of COVID-19 cause changes including memory impairments. In this project, we examined the hypothesis that a COVID-like inflammatory event causes lasting changes in neuroimmune function that contribute to risk for age-related cognitive decline and dementias including AD. We have previously demonstrated the effects of a peripheral immune challenge on memory can last months after resolution of the inflammatory response (Tchessalova & Tronson 2019, 2020). Specialized immune cells in the brain, including microglia, and immune molecules including cytokines play a variety of regulatory roles in the normal and pathological brain including neuroplasticity, memory, and neurodegeneration. Single-stranded RNA (ssRNA) viruses like SARS-COV2 trigger innate immune activation via TLR7 and TLR8. TLR7 has previously been linked with cognitive impairments and neurodegenerative disorders including Alzheimer’s Disease (AD). We used our two-week subchronic immune challenge protocol with TLR7 agonist R848 (400-1000μg/kg) to identify the time course of cytokine responses to R848 through this period, and the alterations in microglia number and morphology after subchronic immune challenge. Although there were no elevations in overt immune or neuroimmune activation (e.g., circulating or hippocampal cytokines) during the period in which memory deficits are evident, we observed sex specific elevations in microglia number and reactivity that persists at least 8 weeks after subchronic immune challenge. These data suggest that a mild-moderate transient illness, such as COVID-19, can trigger sex-specific and long-lasting changes in neuroimmune processes that change neuroplasticity and other neural functions, thereby contributing to memory impairments that emerge and persist long after the illness itself. Importantly, if transient illness or subchronic immune challenge modifies microglia function, this has implications for the progression and exacerbation of Alzheimer’s disease and other age-related dementias in the years following the illness.

Thalamic Gene Expression Changes in a Rodent Model of Parkinson Disease

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Parkinson Disease, the most prevalent neurodegenerative movement disorder, arises from a depletion of striatal dopamine levels, leading to disruption of the cortico-striato-thalamic-cortical circuit. The most common treatments work by attempting to replace the decrease in dopamine (levodopa) or stimulating specific structures within the circuit (deep brain stimulation), resulting in an immediate improvement in symptoms known as the short-duration response. However, longer-lasting changes are also observed in the long-duration response, which may be explained by gene expression changes within the circuit. Using whole transcriptome RNA Sequencing, we identified differentially expressed genes within the central structure of this circuit, the thalamus, in a 6-hydroxydopamine rat model of Parkinson's Disease. We identified 167 differentially expressed genes between the thalamus of rats with (n=2) and without (n=2) striatal dopamine ($q<0.05$). To provide context to the differentially expressed genes, gene enrichment analysis was performed on both the upregulated genes (positive log2foldchange, n=77) and downregulated genes (negative log2foldchange, n=90). Importantly, the ‘glutamatergic synapse’ KEGG pathway was enriched in the upregulated group, while the ‘cation channel complex’ cellular component was enriched in the downregulated group. The results of this study revealed several differentially expressed genes that were previously unknown to be affected by dopamine depletion within the thalamus. Interestingly, some of these genes are implicated in processes relevant to the modulation of the reticular thalamus and its afferents, suggesting a potential decrease in thalamic nuclei excitability. This observation emphasizes the necessity for in-depth exploration of the thalamic reticular nucleus's role within the framework of Parkinson Disease.

A COGNITIVE RESILIENCE GENE EXPRESSION SIGNATURE IN EXCITATORY INTRATELENCEPHALIC CORTICAL NEURONS

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

Dietary *Hericium erinaceus* Effects on Radial Arm Maze Acquisition and Reversal in Elder Rats

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Hericium erinaceus, commonly known as lion's mane mushroom, is a fungal fruiting body historically consumed for its medicinal properties. Scientific research has identified several bioactive compounds within this and other medicinal fungi purportedly responsible for their positive health promoting effects. Two classes of active compounds within the fruiting body and mycelium of H. erinaceus, hericenones and erinacines, increase brain derived neurotrophic factor (BDNF), a protein marker associated with neurogenesis and neural plasticity. Given that reduced BDNF is associated with neurodegenerative diseases such as Parkinson's and Alzheimer's disease as well as age-related cognitive decline, dietary supplements with H. erinaceus may be a viable complementary treatment to improve age-related cognitive decline. Few published empirical studies have utilized objective behavioral measures to evaluate the putative pro-cognitive effects of H. erinaceus. The present study investigated the effects of a dietary supplement containing H. erinaceus dried powder on the acquisition and subsequent reversal of a spatial learning task in elder rats. Thirty eight-month-old male Sprague-Dawley rats were randomly assigned to three treatment groups and received powdered H. erinaceus (0, 100, 1000 mg) mixed with their daily food rations for six weeks prior to the onset and for the duration of radial arm maze training sessions. Eight animals in each group were assessed for maze acquisition, with one trial per day for 23 days in which four of eight arms were baited. Following maze acquisition, a reversal task was implemented for 14 days in which the opposite four arms were baited. Working memory errors (repeat arm entries) and reference memory (non-baited arm entries) were scored. No statistically significant differences were observed among the three treatment groups in task acquisition or reversal. Although the present results failed to support pro-cognitive effects of dietary H. erinaceus treatment, additional analyses are ongoing to determine if this treatment altered BNDF expression in the brain.

THE EFFECTS OF LEVODOPA ON SKILLED REACHING IN THE RAT 6-OHDA MODEL OF PARKINSONS DISEASE

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Parkinson's Disease (PD) is characterized by degeneration of nigrostriatal neurons responsible for dopamine production, leading to the motor symptoms of bradykinesia, rigidity, and rest tremor. While dopamine replacement therapy with levodopa effectively mitigates bradykinesia, its impact on coordination is limited. Levodopa increases tonic brain dopamine levels, but likely does not restore normal dynamic dopamine signaling. Hence, we propose that disrupted dopamine dynamics underlie the persistence of coordination deficits despite increased dopamine levels with levodopa treatment. In this study, our goal was to establish a 6-OHDA rat model that recapitulates the disrupted dopa-responsiveness of dexterity in human PD., We assessed skilled-reaching task performance across varying degrees of nigrostriatal degeneration and evaluated the effect of levodopa on forelimb-digit coordination during skilled reaching. Rats with partial 6-OHDA lesions and sham-lesioned controls maintained skilled reaching performance. However, rats with complete hemispheric lesions were impaired in skilled reaching, which was not rescued by levodopa. This preliminary investigation suggests that appropriate 6-OHDA lesions can model the impaired dexterity of PD, setting the stage to study the pathologic basis of impaired dexterity in PD.

EXTRACTING BIOLOGICALLY RELEVANT LATENT SPACES FROM ALZHEIMER'S DISEASE MOUSE TRANSCRIPTOMES WITH VARIATIONAL AUTOENCODERS

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Cognitive resilience is a complex trait influenced by genetic, molecular and environmental factors. The identification of cellular-level mechanisms underlying cognitive resilience to Alzheimer's disease (AD) pathology is essential for resilience-based drug discovery. Latent variable modeling (LVM) has been pivotal in enabling researchers to detect underlying features that cannot be directly identified from transcriptomes. This study aims to apply LVM on single-nucleus RNA sequencing (snRNA-seq) datasets acquired from prefrontal cortex tissue of a genetically and phenotypically diverse mouse model of familial AD at 6 and 14 months of age. Linear LVM techniques such as PCA may fail to preserve the non-linear relationships that exist among genes which are essential for capturing the complexity, significance, and variability of biological processes. Common non-linear methods like t-SNE and UMAP focus more intensely on capturing the local relationships such as which points with the same cell type are similar or different to each other but are less effective on preserving global structure which might reveal how different genotypes are related to each other on a higher level. Variational autoencoders (VAEs) surpass standard LVM techniques with the regularization which helps shaping the latent space to ensure that the features extracted are robust and generalizable. To unravel the difference in latent spaces between resilient and susceptible cells, we built a Conditional-Gaussian Mixture Variational Autoencoder framework (C-GMVAE) where AD resilient and susceptible labels were used to train the model with a conditional learning process and shaped the resultant latent space of genetic features to follow a mixture of gaussians. This allowed us to obtain a heterogenous mixture of resilient and susceptible features in the latent space. For effective computational efficiency and model tuning, the model was trained with random samples taken from the dataset to generate 10 latent variables and evaluated on the whole original dataset. Statistical significance via Mann-Whitney U test revealed excitatory neurons contained most of the latent variables that were significantly different between resilient and susceptible features. This preliminary work demonstrates an efficient approach for building a transcriptomic spectrum of AD cognitive resilience to further explore potential genomic drivers that relate to resilience at a cellular level.

GENETIC BACKGROUND MODIFIES NEURONAL ELECTROPHYSIOLOGICAL RESPONSES TO Aβ1-42 IN VITRO

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Alzheimer's disease (AD) is the most common form of dementia and is characterized by cognitive impairments (particularly in learning and memory). These impairments are thought to be driven by neuropathological features including beta-amyloid (Aβ) plaques and changes in neuronal excitability. However, little is known how genetic background influences the effects of Aβ on neuronal activity. Here, we addressed this gap by measuring neuronal activity after Aβ1-42 treatment using Microelectrode Array (MEA) plates with cortical and hippocampal mixed cultures isolated from a genetically diverse mouse panel. Interestingly, treatment with 2.5μM Aβ1-42 initially reduced neuronal activity in all conditions. However, B6-BXD39 males tended to recover neuronal activity earlier with Aβ1-42 treatment while males from the strain B6-BXD124 maintained reduced activity. These data demonstrate that genetic background is a critical determinant of the reduced neuronal excitability in the presence of Aβ1-42. We plan to extend our investigation in several important ways including examining region-specific (cortical vs. hippocampal) and sex-specific differences in Abeta's effect on neuronal excitability, as well as investigating molecular pathways and signals that may further modulate this effect. For example, treatment with β-estradiol (E2) has been shown to reduce Aβ accumulation in both preclinical and clinical studies. Furthermore, E2 can be produced by neurons and astrocytes in both males and females, and modulates many cellular functions including neurogenesis, synaptic plasticity, mitochondrial functioning, and cellular transcription. Additional investigation into the influences of E2 on neuronal activity in a genetically diverse model system can help elucidate the role E2 plays in AD and help identify novel therapeutic targets.

GERMLINE CAG REPEATS VARY ACROSS GENETICALLY-SEGREGATED BXD BACKGROUNDS AND NEGATIVELY PREDICT MOTOR PERFORMANCE IN A MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a dominantly-inherited neurodegenerative disease caused by pathogenic CAG repeat expansion within the huntingtin gene. CAG repeat length is generally predictive of disease severity, with earlier onset and worsened symptomology associated with longer repeats. Additionally, CAG length and disease severity tend to progress with successive generations; however, some families present with later than predicted onset and stable intergenerational CAG transmission. To model variation observed between human HD families, we utilized a Q111 knock-in HD mouse model crossed to a panel of genetically-segregated (BXD) backgrounds.

In Q111 females from 31 BXD strains, CAG repeat length evaluated from tail-DNA collected at weaning was calculated to be highly heritable ($H2R\bar{I}_{\bar{x}} = 0.84$). On average, all strains showed expansion beyond initial CAG knock-in length, with median strain lengths ranging from 112 to 118; however, individual animals across several strains showed repeat constriction. Interestingly, several strains showed multimodal distribution, suggesting that inherited CAG length is differentially stable between genetic backgrounds. Quantitative trait loci (QTL) mapping of median CAG length revealed a suggestive QTL peak between chromosomes 8 and 9. In order to map CAG 'stability', QTL mapping was run on the bimodality coefficient of each strain. This analysis showed a significant QTL peak in chromosome 3. We will next investigate variants within this QTL as potential modifiers of CAG stability.

We are also currently testing Q111 carriers and non-transgenic (Ntg) littermates on a battery of motor and nonmotor assays to assess variation and heritability of HD-relevant traits across strains. Preliminary assessment across several strains showed that CAG repeat length was negatively correlated with Q111-BXD performance on accelerating rotarod (rho = -0.41, p-value = 0.015) and wire-hang (rho = -0.41, p-value = 0.016) at 6-months of age. Overall, these data demonstrate our panel as a promising translational model of genetic variation in HD. By leveraging the genetic mapping power of the BXD panel, we will identify putative genetic modifiers to illuminate new and desperately needed therapeutic targets for the clinical population.

VERSATILE GENETIC PERTURBATION APPROACH TO UNDERSTAND TUMOR-IMMUNE INTERACTIONS IN HUMAN GLIOBLASTOMA

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The mechanism by which macrophages induce a transition of glioblastoma (GBM) cells into a mesenchymal (MES)-like state remains unclear. Macrophages produce a cytokine known as Oncostatin M (OSM), which has been shown to facilitate this transition in GBM cells. Although the involvement of OSM is recognized, the precise mechanisms and potential additional factors are still uncertain. In this study, previous work utilized single-cell RNA sequencing (scRNA-seq) to analyze GBM subtype expression in various cellular states. Human GBM cells (MGG23) were treated with macrophage-derived ligands for 24 hours and assessed for glioblastoma states via CD24 and CD44 markers with flow cytometry. Additionally, we employed the Neon electroporation system using CRISPR/Cas9 to knock out the OSM gene in human macrophages (U937), achieving high transfection efficiency. Single-cell cloning by limiting dilution produced putative OSM knockout (KO) clones, which were assessed using PCR and gel electrophoresis. We plan to perform Sanger sequencing on clones suspected of being knocked out to ascertain the precise DNA sequence at the OSM locus. Upon confirming a successful knockout, the modified clones will be co-cultured with glioblastoma cells to investigate their interactions in vitro. Future studies will further elucidate how OSM contributes to a heterogeneous microenvironment in mesenchymal-like GBM.

THE ROLE OF UBQLN2 MUTATIONS IN NEURODEGENERATIVE DISEASES

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Protein misfolding and subsequent aggregation disrupts normal protein function, and in the nervous system can lead to neurodegenerative disorders such as Alzheimer's disease, Frontotemporal Dementia, and Huntington's disease. Within cells, various mechanisms prevent misfolding and facilitate the clearance of misfolded proteins, collectively termed as protein quality control (PQC). Ubiquilins (UBQLN) are a family of conserved ubiquitin adaptor proteins that participate in ubiquitin-dependent PQC. Among them, UBQLN2 is most abundantly expressed in the brain and body and is implicated in several neurodegenerative diseases, including as a direct cause of fatal inherited neurodegenerative disease on the Frontotemporal Dementia/Amyotrophic Lateral Sclerosis (FTD/ALS) spectrum through a mutation. However, the mechanisms underlying its function in health and disease and the interacting proteins that regulate its function remain largely unexplored. Previous studies have shown the interaction of UBQLN2 with a pathogenic huntingtin (HTT) polyglutamine protein in a mouse model of Huntington's disease. The goal of this study is to determine the effect of mutant UBQLN2 on subcellular localization and function, illuminating the way normal and mutant UBQLN2 behavior differ. Various experiments using brain tissue of this pathogenic HTT mouse model with mutant and wild type UBQLN2 will be conducted to determine the difference in ability of each UBQLN2 to rescue the Huntington pathology and localize in condensates through phase separation within individual neurons in the hippocampus and cerebellum. We hope this research will build our knowledge of UBQLN2 in neurodegenerative pathology and demonstrate potential therapeutic strategies.

SYNUCLEINOPATHY DIFFERENTIALLY AFFECTS THE EXPRESSION OF FLUID PHASE VERSUS MEMBRANE BOUND COMPLEMENT REGULATORS IN THE SUBSTANTIA NIGRA.

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Parkinson's disease (PD) is characterized by loss of midbrain dopamine neurons, accumulation of pathological alpha synuclein (a-syn) in Lewy bodies and neuroinflammation. Neuroinflammation occurs in early-stage PD patients and remains elevated through the course of the disease, suggesting it may contribute to neurodegeneration. In the CNS, activated glia coordinate neuroinflammation by releasing complement proteins. Normally, “self” cells are protected from complement attack by inhibitory complement regulators. In the PD brain, levels of activated complement proteins are increased, where complement opsonins and components of the membrane attack complex (MAC) deposit on Lewy body containing DA neurons. Complement regulators normally prevent deposition of complement opsonins and the MAC. Thus, the presence of these proteins on nigral DA neurons in the PD brain implicates complement regulator dysfunction. Here we hypothesized that aggregation of a-syn may affect the expression and/or localization of complement regulators. To test this hypothesis, we analyzed the expression of both fluid phase and membrane bound complement regulatory proteins in the substantia nigra of rats injected with recombinant a-syn preformed fibrils (PFFs). Intra-striatal injection of a-syn PFFs results in progressive aggregation of endogenous a-syn and gliosis that peaks 2 months post injection, followed by significant nigral degeneration at 6 months post injection. Two-months post PFF injection, levels of total and activated complement component 3 protein were increased in both the striatum and the SNc of PFF injected rats. Interestingly, levels of activated C3 correlated with levels of pSer129 a-syn (pSyn; marker of pathological a-syn), indicating that complement activation is due to synucleinopathy. We next quantified gene expression of select fluid phase and membrane bound complement regulators in the SNc using droplet digital PCR. The expression of most fluid phase complement regulators was significantly increased, while expression of two membrane bound complement regulators, CD55 and CD59, were significantly decreased. Immunofluorescence (IF) analysis of the SNc revealed a decrease in CD55 immunoreactivity on nigral neurons of PFF injected rats, where neurons containing phosphorylated a-syn (pSyn) were almost completely devoid of CD55 immunoreactivity. Finally, to determine if changes observed in the rat PFF model are relevant to human PD, we performed IF intensity analysis of CD55 on neuromelanin+ neurons in the SNc of PD and control brains. Here, CD55 IF intensity was significantly decreased on neuromelanin+ neurons in the PD brain. Taken together, these data demonstrate the synucleinopathy causes a robust activation of the complement system and a decrease in the expression of membrane bound complement regulators in nigral neurons. Importantly, these changes occur months prior to neurodegeneration in our PFF model, indicating that complement activation and regulator dysfunction may contribute to neurodegeneration in PD.

BEHAVIORAL EFFECTS OF STZ ADMINISTRATION IN LIRAGLUTIDE-TREATED AGED C57 MICE

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Recent studies have shown that the anti-diabetic drug, liraglutide (LIR), can reduce age-related deficits in rodent models of Alzheimer's disease (AD) and age-related cognitive decline. The aim of the present study was to see if the addition of a potent toxin, streptozotocin (STZ), could exacerbate behavioral performance in aged C57 mice treated with LIR. STZ, which has been used to model sporadic AD, is a toxin derived from the bacteria Streptomyces achromogenes that can produce vascular dysfunction within mammalian models, likely through inducing endothelial dysfunction. In our study, ten, 19-month-old C57 mice (6 males and 4 females) received either bilateral intracerebroventricular injections of STZ toxin or citrate buffer solution (CBS) vehicle and were subsequently treated with LIR, intraperitoneally, once a day for 37 days. Mice were evaluated on three behavioral tests: passive avoidance, open-field, and novel object recognition tasks. In all three behavioral tests, no statistically significant results were observed. These results partially support the findings of Gaspar and colleagues (Scientific Reports, 2022) who observed that the addition of STZ to aged rats did not exacerbate their performance on the passive-avoidance task. However, those researchers observed a worsening of performance on the novel object recognition task in aged rats, which was not observed in our study. Further work with STZ-treated aged C57 mice that do not receive LIR treatments is needed to discern whether the effects of STZ were attenuated by LIR and to determine whether STZ alone can differentially affect behavioral performance in aged C57 mice.

THE DISTRIBUTION AND INTERACTION BETWEEN TAU AND PROTEIN PHOSPHATASE 1 IN PRIMARY HIPPOCAMPAL NEURONS

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Tau is a microtubule-associated protein that is traditionally thought of as a microtubule stabilizing protein primarily found in the axon of neurons, but evidence shows that tau is distributed throughout other neuronal compartments (i.e. dendrites, synapses, nuclei, somata, and axons) and in glial cells. Recent studies show that tau is more likely involved in regulating microtubule dynamics at the labile domain rather than truly stabilizing them. Additionally, several studies support the idea that tau acts as a scaffolding protein involved in regulating various biological pathways under physiological conditions. Tau appears to play such a role in regulating protein phosphatase 1 (PP1), a member of the serine/threonine phosphatase family with three isoforms expressed in the mammalian brain: PP1α, β, and γ1. Like tau, PP1 is found throughout multiple compartments of neurons where it mediates protein dephosphorylation to regulate several biological processes including protein synthesis, axonal transport, synaptic activity, and nuclear functions. The functional diversity of PP1 is driven by its binding to over 200 confirmed regulatory partners. Physiological and pathogenic tau species are known to interact with and alter PP1 activity and PP1-mediated pathways. While data suggest physiological tau can act as a PP1 scaffolding protein that directly modulates PP1 function, several gaps in our knowledge exist. Using primary hippocampal neuron cultures from E16 human tau knock-in (hTau-KI) mice, we first established the distribution of tau and PP1 in this model. Confocal microscopy analysis using total tau and PP1 isoform-specific antibodies in DIV 14 hTau-KI neurons confirmed that tau and PP1 are present within many of the same subcellular compartments. Proximity ligation assays further support a close association between tau and all PP1 isoforms in neurons, with potential isoform-dependent differences. Prior work implicates the MTBR domain of tau as a primary mediator of the interaction with PP1. Within this domain there are two endogenous cysteines, C291 and C322, which may contribute to tau conformation and interaction with other proteins via disulfide bridging. To determine the role of these endogenous cysteines in mediating the tau-PP1 interaction, we generated tau point mutants C291A, C322A, and C291A/C322A. WT tau or cysteine mutant tau proteins (all C-terminally NanoLuciferase tagged) were co-expressed in HEK 293T cells with N-HaloTag PP1 or a HaloTag only control. Preliminary HaloTag pulldown and in-cell NanoBRET protein interaction assays suggest removal of the endogenous cysteines reduces the interaction with PP1, indicating that they may serve a critical role in mediating the tau-PP1 interaction. Together, this work will provide additional insight into the complexity of the tau-PP1 interaction and the physiological role of tau in modulating PP1-dependent functions in multiple neuronal compartments.

This work was supported by NIA R01AG067762 and NINDS R01NS082730.

THEME 8: SENSORY & MOTOR SYSTEMS

USING A REVERSE VISUALLY GUIDED REACHING TASK TO DISTINGUISH BETWEEN HEALTHY AGING AND COGNITIVE IMPAIRMENT

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While Alzheimer's disease (AD) is associated with impairments in learning and memory, recent studies suggest that subtle changes in motor task performance may reflect early cognitive changes. The current study identified the impact of aging and cognitive impairment on performance of the reverse visually guided reaching task (rVGR) which rotates visual feedback of participant's hand position 180^{0} relative to the actual hand position. We also examined differences in initial learning and overall learning curves in the rVGR task and probed the cognitive correlates of rVGR performance with a neuropsychological battery. We recruited young adults, and older adults (55 – 85 years old) with and without cognitive impairment to complete a VGR task with veridical mapping, and then the rVGR task. Age differences were observed for nearly all overall measures of performance. Overall, cognitively impaired adults exhibited longer reaction times and performed more corrective movements than healthy older adults. They also exhibited slower learning across the task and larger angular errors in the earliest trials of the rVGR task. Both overall- and early- measures of performance were correlated with measures of cognitive control. These findings add to the growing literature suggesting that sensorimotor adaptation tasks may be sensitive to early cognitive changes in AD.

EXAMINING DNA DAMAGE IN OLFACTORY SENSORY NEURONS OF CRAYFISH FOLLOWING COMBINED ATRAZINE AND MICROCYSTIN-LR EXPOSURES

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Anthropogenic activities can negatively impact freshwater ecosystems. Freshwater aquatic systems contain a variety of toxins such as heavy metals, pharmaceuticals, pesticides, plastics, and algal toxins. Crayfish are an abundant aquatic species that are differentially exposed to aquatic pollutants in their environment. They serve as a bioindicator species for pollution exposure and are a keystone species, meaning they play a pivotal role in the aquatic food web, transferring energy from the benthic to pelagic zones of the aquatic environment. Both the herbicide atrazine and the algal toxic microcystin-LR are known to be neurotoxins and are both found in the aquatic environment in areas where crayfish are found. In this experiment, we exposed crayfish to atrazine (10 ppb), microcystin-LR (10 ppb), or a combination of both (10 ppb of both) and examined olfactory sensory neurons using a terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick-end labeling (TUNEL) assay to determine if they contained any DNA damage. Preliminary data suggests that both atrazine and microcystin-LR cause DNA damage to olfactory sensory neurons. Understanding the impacts of exposure to herbicides and algal toxins on neurons and potential neuron death is important for determining if exposure impacts chemoreception of important odors like food, mate, predators, and alarm cues. Further, understanding combinatorial effects is important for determining if multiple subacute exposures can have additive, negative neurological impacts. Future quantification of DNA damage will determine if exposures to both atrazine and microcystin-LR have additive effects.

A MORPHOLOGICALLY DISTINCT SUBCLASS OF SOMATOSTATIN-EXPRESSING INTERNEURONS IN THE SOMATOSENSORY CORTEX ARE STRONGLY RECRUITED BY INPUT FROM THE MOTOR CORTEX

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The motor cortex (M1) and somatosensory cortex (S1) are strongly interconnected regions, and their interactions are essential for sensory perception and motor execution. However, the mechanisms by which M1 activity modulates sensory processing within S1 are poorly understood. In particular, M1 recruitment of distinct GABAergic inhibitory cells could impact S1 responsiveness. Preliminary work in our lab identified a subpopulation of somatostatin (SOM) expressing inhibitory interneurons in layer 6 (L6) of S1 that are strongly recruited by M1 input. We hypothesize these M1-responding L6 SOM cells are an electrophysiological and morphologically distinct subclass that express the enzyme neuronal nitric oxide synthase (nNOS). To stimulate the M1 to S1 pathway and test this hypothesis, we injected adeno-associated virus (AAV) encoding the light-sensitive cation channel, channelrhodopsin-2 (ChR2), in M1 of postnatal day 21 (+/-1) mice in vivo. After three weeks of expression, we prepared acute coronal brain slices for targeted loose-patch recordings and selective optical stimulation of M1 terminals. We found two groups of L6 SOM cells based on their spiking behavior during photostimulation: responsive (15%) and non-responsive (85%). Whole-cell recordings and neurobiotin injections into responsive and non-responsive SOM cells revealed robust electrophysiological and morphological differences. Initial anatomical reconstructions indicate that the non-responsive SOM cells (n=12) exhibit both Martinotti (with an L1 axonal projection) and non-Martinotti (no L1 axonal projection) morphologies, whereas the responsive SOM cells had axonal arborizations projecting toward the underlying white matter (n=10). The responsive SOM cells had quasi-fast-spiking electrophysiological properties (n=10) and were negative for nNOS (n=9), whereas the non-responsive SOM cells exhibited non-adaptive spiking behavior. Importantly, responsive SOM cells were negative for parvalbumin (PV), a marker for a distinct class of fast-spiking interneurons in the cortex, indicating these cells were not mislabeled PV cells due to the known off-target recombination in the SOM-IRES-Cre mouse line (n=4). In summary, our data show that input from M1 strongly recruits a previously unknown SOM-expressing interneuron in lower L6 with distinct morphological and electrophysiological features that could influence sensory responsiveness in S1. Future studies will focus on the mechanisms of this selective activation and identifying the postsynaptic targets of these cells to further our understanding of this sensorimotor circuit.

Funding: NIH R01NS117636 (SRC), APS SURF (MKS)

Androgen hormones regulate the production on the pain-inducing inflammatory molecule IL-1β in a mouse model of inflammatory pain

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Chronic pain prevalence varies between sexes with a higher incidence and duration reported in women compared to men. This disparity suggests that biological factors, such as sexual hormones, may influence pain perception and development. Among the key players in pain mechanisms, interleukin-1 beta (IL-1β), an inflammatory molecule, has been identified to activate neurons involved in pain sensation. This study explores the hypothesis that sexual hormones, particularly androgens, regulate IL-1β production in inflamed tissues, thereby influencing pain responses. To investigate this, we induced inflammatory pain in male and female mice using Complete Freund's Adjuvant (CFA), injected into the hind paw. To assess the effect of sex hormones on the production of IL-1β and pain, we modulated the levels of sex hormones by surgical and pharmacological approaches: ovariectomy, orchidectomy, and administration of flutamide, an androgen receptor antagonist. The analysis of IL-1β levels was conducted through quantitative Polymerase Chain Reaction (QPCR). We measured mechanical pain sensitivity thresholds using the von-Frey method. Our results indicate that injection of CFA drastically increased the levels of IL-1β in the inflamed skin. Blocking IL-1β significantly reduced pain sensitivity. We found decreasing systemic androgen levels, by orchidectomy or flutamide, significantly increase IL-1β expression and pain recovery times. Overall, we found that androgens reduce the levels of IL-1β and facilitate the resolution of pain. These findings underscore the intricate relationship between sexual hormones and inflammatory mediators in the context of chronic pain, suggesting potential avenues for developing sex-specific pain management strategies that target the hormonal regulation of inflammatory pathways.

INFERIOR COLLICULUS NEURONS RESPOND SELECTIVELY TO SPECIFIC AUDITORY MOTIFS WITHIN FREQUENCY MODULATED SWEEPS

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Rapid changes in sound frequency known as frequency-modulated (FM) sweeps are an important component of both human and animal vocalizations, however, how FMsweeps are detected and encoded in the brain remains poorly understood. The inferior colliculus (IC) is a hub of auditory processing that receives inputs from the cochlear nucleus and other auditory brainstem nuclei and sends projections to the auditory thalamus. Some IC neurons are selective for the direction of FM sweeps, with strong firing preference for upward or downward sweep direction. However, whether and how direction selectivity is shaped by different properties of the FM stimuli, such as the intensity, sweep frequency range, and speed of the sweep, is poorly understood. To investigate how these factors shape FM direction selectivity in IC neurons, we performed in vivo juxtacellular recordings from IC neurons in awake, head-fixed mice while playing FM sweeps of different speeds (10 – 200 octaves/second), directions (up, down), frequency ranges (4 octave, 2 octave, and 1 octave at intervals between 4 – 64 kHz), and intensities (10 – 70 dB SPL). We also assessed the frequency tuning of each neuron using 4 – 64 kHz tone bursts played at intensities ranging from 0 – 70 dB SPL. In response to 4 –64 kHz FM sweeps played at 70 dB SPL, we found that some IC neurons display selective firing for upward or downward sweeps. However, we found that the selectivity of an individual cell for FM sweep direction was strongly dependent on the frequency range, intensity, and speed of the sweep, with some neurons only exhibiting selectivity at particular intensities, speeds, or sweep ranges. This indicates that rather than simply computing sweep direction, IC neurons detect specific combinations of sound features, highlighting a mechanism IC neurons may use to build representations of complex auditory inputs.

STATE-DEPENDENT COMPLEXITY OF STIMULUS-INDUCED NEURONAL FIRING PATTERNS IN RAT VISUAL CORTEX

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Complexity of neuronal firing patterns is an important index of sensory information processing that may be directly affected in altered states of consciousness. Recent investigations found that general anesthesia disrupts visual cortex neuronal responses to flash stimuli up to hundreds of milliseconds post-stimulus and decreases the complexity of the early post-stimulus response. How anesthesia alters complexity of the late response component remains unclear. The latter is important as it likely reflects recurrent processing necessary for conscious vision. Additionally, there is growing evidence for spontaneous shifts in large-scale brain states at constant anesthetic agent concentration, suggesting a partial dissociation between neuronal state and anesthetic level. Whether spontaneous state transitions at fixed anesthetic concentration occur also during post-stimulus activity and complexity is unclear. Here we aimed to investigate these questions in rats at different levels of anesthesia produced by the inhalational agent desflurane. Extracellular unit activity was measured with chronically implanted 64-site silicon microelectrode arrays in cortical layers 5/6 of primary visual cortex of six freely moving male rats during stepwise decrease of desflurane concentration at 6, 4, 2, and 0%. Discrete light flashes of 10 ms duration were delivered to the retina at random interstimulus intervals (2-4 s; 100 trials per concentration level) by transcranial illumination. Single-unit activity was identified using the clustering software SpyKING CIRCUS. The first two principal components of neuron population spiking distinguished four neuronal states, each characterized with distinct visual-evoked spiking patterns. State 1 was associated with the awake condition (0% desflurane), whereas states 2-4 reflected, but not consistently, the concentration-dependent effect of desflurane. In state 1, visual stimulation induced an early increase and a subsequent suppression in population firing rate, followed by a late response up to 500 ms. General anesthesia delayed the peak time of the early spike response in a state-dependent manner and suppressed the amplitude of late response ($p=0.008$, state 3 vs. state 1). The complexity of stimulus-evoked population firing patterns depended on the studied time windows. While complexity of the late (200-500 ms) response was reduced in states 2, 3 and 4 ($p<0.001$), complexity of the early (1-200 ms) response decreased in state 4 only ($p=0.003$). The complexity of post-stimulus responses within the time window of 1-500 ms demonstrated a gradual decrease from state 1 to state 2 and 3 at 58.2 [51.6, 64.7] % and 44.3 [35.7, 52.9] %, respectively ($p<0.001$). The results suggest the presence of multiple neuronal states that represent distinct visual stimulus-evoked spiking patterns on the time scale of tens of seconds at constant anesthetic concentrations. The complexity of post-stimulus responses (1-500 ms) decreases in neuronal states at increasing depth of anesthesia, which may reflect the disruption of sensory information processing.

EXPLORING STRUCTURAL BIOMARKERS: UNDERSTANDING THE ROLE OF WHITE MATTER IN PREDICTING CHRONIC PAIN AFTER TRAUMA

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

THEME 9: TECHNIQUES

CYTOFLARE: A GENETICALLY ENCODED TOOL FOR REPORTING AND MANIPULATING NEURONS ACTIVATED DURING COGNITION AND BEHAVIOR

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

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IMPROVING BIOLUMINESCENT GAMMA-AMINOBUTYRIC ACID SENSOR RESPONSE TO LIGAND

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Many neurological diseases such as Alzheimer's Disease, Parkinson's Disease, and autism spectrum disorder have been shown to be caused, in part, by neurotransmitter disfunction. Expanding on the types of neurotransmitters that can be detected is important to study the causes and treatments of these diseases. In this study, we focus on the amino acid gamma-aminobutyric acid (GABA), which is an inhibitory neurotransmitter found throughout the brain and is involved in many neurological disorders. We developed a variety of genetically encoded bioluminescent GABA sensors that are an attractive alternative to using fluorescent sensors because they do not require an excitation light source, allowing deeper areas of the brain to be recorded without damaging tissue and improving signal-to-noise ratio due to the lack of autofluorescence. We created a library of bioluminescent GABA sensor variants and tested them for improved responses to GABA. Taking bioluminescence readings on a plate reader, we found that the sensors with a mutated GABA binding domain and optimized linkers have higher responses to saturating amounts of GABA than the ones with the native GABA binding protein. To further improve the response of the sensors to GABA with the goal of using them to image brain activity in rodents, we will use rational design and further linker optimization to mutate amino acids in different areas of these GABA sensors with the goal of improving response amplitude and signal-to-noise ratio.

QUANTIFYING SOCIAL ROLES IN MULTI-ANIMAL VIDEOS USING SUBJECT-AWARE DEEP-LEARNING

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Analyzing social behaviors is critical for many fields, including neuroscience, psychology, and ecology. While computational tools have been developed to analyze videos containing animals engaging in limited social interactions under specific experimental conditions, automated identification of the social roles of freely moving individuals in a multi-animal group remains unresolved. Here we describe a deep-learning-based system – named LabGym2 – for identifying and quantifying social roles in multi-animal groups. This system uses a subject-aware approach: it evaluates the behavioral state of every individual in a group of two or more animals while factoring in its social and environmental surroundings. We demonstrate the performance of subject-aware deep-learning in different species and assays, from partner preference in freely-moving insects to primate social interactions in the field. Our subject-aware deep learning approach provides a controllable, interpretable, and efficient framework to enable new experimental paradigms and systematic evaluation of interactive behavior in individuals identified within a group.

DEVELOPING AND IMPROVING A BIOLUMINESCENT GABA SENSOR

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Many neurological diseases such as Alzheimer's Disease, Parkinson's Disease, and autism spectrum disorder have been shown to be caused, in part, by neurotransmitter disfunction. Expanding on the types of neurotransmitters that can be detected is important to study the causes and treatments of these diseases. In this study, we focus on the amino acid gamma-aminobutyric acid (GABA), which is an inhibitory neurotransmitter found throughout the brain and is involved in many neurological disorders. We developed a variety of genetically encoded bioluminescent GABA sensors that are an attractive alternative to using fluorescent sensors because they do not require an excitation light source, allowing deeper areas of the brain to be recorded without damaging tissue and improving signal-to-noise ratio due to the lack of autofluorescence. We created a library of bioluminescent GABA sensor variants and tested them for improved responses to GABA. Taking bioluminescence readings on a plate reader, we found that the sensors with a mutated GABA binding domain and optimized linkers have higher responses to saturating amounts of GABA than the ones with the native GABA binding protein. To further improve the response of the sensors to GABA with the goal of using them to image brain activity in rodents, we will use rational design and further linker optimization to mutate amino acids in different areas of these GABA sensors with the goal of improving response amplitude and signal-to-noise ratio.

OPTIMIZATION OF G4 70/30 PAMAM DENDRIMERS FOR ENHANCED DELIVERY OF YWHAB SIRNA IN GLIOBLASTOMA TREATMENT

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Glioblastoma (GB) is a primary tumor of the brain that arises from central nervous system malignancies. Typically, the treatment for glioblastoma includes a combination of therapy options like neurosurgery, chemotherapy (temozolomide), and radiotherapy. Despite these treatment options, the survival rate is incredibly low because of the reoccurrence of tumor and absence of precision treatments. siRNA (small interfering RNA) is highly target specific and efficient at low doses with a simple design. This study investigates the potential of YWHAB siRNA delivered using a novel carrier system to combat glioblastoma. YWHAB siRNA encodes for 14-3-3β protein which is specifically upregulated in glioblastoma and causes an increase in malignancy. Previous research from our lab has shown that loss of 14-3-3β significantly reduces cellular proliferation and spheroid formation of U87MG cells. However, a significant challenge with most therapeutics is their inability to cross the blood-brain barrier (BBB). Poly-amido(amine) (PAMAM) dendrimers can offer a promising alternative for siRNA delivery as they offer several advantages such as crossing the BBB due to their small size, increased cellular uptake of siRNA by protection against enzymatic degradation, stable dendrimer-siRNA complex (dendriplex) across a wider pH range, and high solubility. In this study, we used a generation 4 (G4) 70/30 PAMAM dendrimers with a modified cystamine core with YWHAB siRNA in HEK293 cells for optimization of dendriplex. G4 70/30 PAMAM dendrimers with 70% hydroxyl and 30% surface amine groups have been used as delivery agents in many studies with high transfection efficiency and low cellular toxicity. In our preliminary investigations using HEK293 cells, we achieved a significant knockdown efficiency of 70% for the YWHAB gene with the dendriplex formulation. Furthermore, we determined that a dendriplex concentration of 1x exhibited maximal knockdown efficacy compared to higher (2x) and lower (0.5x) concentrations. Additionally, our initial investigations revealed that the dendriplex formulation achieves peak knockdown efficiency 120 hours post-transfection.

PEDIATRIC FMRI DATA QUALITY ENHANCEMENT WITH MULTI-ECHO DATA ACQUISITION AND PREPROCESSING

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Functional magnetic resonance imaging (fMRI) has emerged as an important tool for understanding brain development and the neural bases of psychiatric disorders. However, head motion and signal dropout are common challenges, particularly for neuroimaging in children and adolescents. Here, we describe a multi-echo fMRI data acquisition and preprocessing pipeline for more robust artifact reduction and quality assurance in pediatric fMRI data. We examine the effects of this pipeline on head motion and signal dropout, as compared to the raw data (prior to preprocessing). A custom preprocessing pipeline using tedana was applied to multi-echo multi-band fMRI scans (51 slices, TR=1.5s, TE=15.0, 30.7, 46.4ms) from an ongoing study in 99 youth (ages 6-17 years). Participants completed resting-state (eyes closed) and fear extinction recall paradigms. Primary outcomes were head motion and temporal signal to noise ratio (tSNR). Head motion was measured by framewise displacement (FD), and tSNR was measured in the ventromedial prefrontal cortex (vmPFC), an area particularly vulnerable to signal dropout due to its proximity to the orbital sinus. Repeated measures ANOVA were conducted to examine the effects of time (raw, preprocessed) and paradigm (resting-state, fear extinction recall) on FD and tSNR. Visual inspection showed substantial head motion (average FD>0.5 mm) in 13% of participants. In addition, the majority of participants showed signal dropout in the vmPFC in raw data. ANOVA showed a significant main effect of time for both FD and tSNR, such that there was a blunting of head motion ($F(1,98)=30.02$, $p<0.001$,) and recovery of signal following preprocessing ($F(1,98)=63.14$, $p<0.001$,) with large-sized effects ($\eta p2=0.234$ and $\eta p2=0.392$, respectively). For head motion, there was also a significant main effect of paradigm ($F(1,98)=6.22$, $p=0.014$, $\eta p2=0.06$) and paradigm x time interaction ($F(1,98)=8.21$, $p=0.005$, $\eta p2=0.08$), which was driven by higher head motion during the recall paradigm compared to resting-state only in the raw (not preprocessed) data. Our multi-echo preprocessing pipeline reduced head motion across the brain and recovered signal in an area of high susceptibility as compared to raw (and/or single-echo) fMRI data. Importantly, the benefits were large in size for both task- and resting-state fMRI paradigms. Our approach minimized artifact in children and adolescents, thus enhancing fMRI data quality and accuracy.

BIOSENSOR OPTIMIZATION THROUGH OPTIMIZED CELLULAR TRAFFICKING PROTEINS

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Optical imaging is an evolving method and promising technology to enhance indications of biological processes and study biological function and disease conditions. Through the use of discrete membrane trafficking proteins, protein-based biosensors can be improved for their ability to report more specific cellular processes and utilized to efficiently observe biological mechanisms and the movement of neurotransmitters. Neurotransmitters are biologically significant because they are the chemical communicators between nerve cells. These neurotransmitters can be prevalent and causative of bioluminescence, which is the production of light by a living organism. The common problem within the imaging of these in targeted cells is that there is not enough necessary research and utilization of possible membrane trafficking peptides to observe the neurotransmitter movement. The purpose of this experiment is to enhance the existing bioluminescence of the glutamate sensor. The imaging of the engineered sensors can be adapted and improved through including new enzymatic and genetic combinations in response to different wavelengths of light. Identifying these pathways and optimal signaling is important because analyzing the abundance of neurotransmitters and their locations can be useful in identifying current or potential future conditions such as Alzheimer's or neurodegenerative diseases/conditions. To address this problem, the BLING (BioLuminescent Indicator of the Neurotransmitter Glutamate) construct was augmented with the addition of a GPI insert, COBL9. Preliminary results suggest that the addition of COBL9 can improve bioluminescent response to glutamate when compared to the BLING 8.10 base. Additionally, COBL9 shows the capability of integrating with other neurotransmitter sensors, such as GABA, to increase cellular bioluminescence response levels to a variety of neurotransmitters. The introduction of some GPIs to current bioluminescent sensors has proven to be beneficial in neurotransmitter identification and enhanced cellular luminescence and shows promise for additional testing and clinical application.

BIOLUMINESCENT KINASE SENSORS FOR DETECTION OF GROWTH FACTOR SIGNALING AND INFLAMMATION SIGNALING

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Growth factor signaling is an important component of a large variety of cellular processes including metabolism, differentiation, proliferation, and migration. When growth factor signaling is altered it can lead to pathologies like cancer cells forming and proliferating within the body such as glioblastoma multiforme (GB). In this study, we focus on investigating and proposing novel therapeutic approaches utilizing genetically encoded Bioluminescent Kinase Sensors (BlinKS) to respond to growth factor signaling via kinases in the epidermal growth factor receptor (EGFR) signaling pathway. Specifically, this study is targeting the kinases within the MEK, RAS, RAF signaling pathway. We are developing a rational library of BlinKS variants with altered phospho-amino acid binding domains (PAABD) as well as varying kinase substrate peptides and permutations of the linker regions, either flexible or rigid at the interfaces of the protein fusion sites. To test our BlinKS construct in association with U87 glioblastoma cells expressing our candidate sensor variants, treated the cells with epidermal growth factor (EGF) and measured the response of the BlinKS sensors allowing for light emitted by the sensor and by measuring an optogenetic transcriptional readout via a fluorescent reporter protein. Bioluminescence readings were conducted on a plate reader, and it was found that the cells treated with EGF produced more luminescence than those not treated with EGF and those treated with chlorambucil (a chemotherapy drug). We also found our sensors targeting this signaling cascade to be able to control an optogenetic transcription system, reporting EGFR activation with GFP expression. To further improve the BlinKS sensor, we will be testing different substrate peptide variants as well as RAF mimic substrates to determine the effect that they have on improving the sensors. We ultimately aim to test these in rodents to reign in uncontrolled growth in cancer cells when using BlinKS to drive expression of a therapeutic protein to halt cell division in response to EGF signaling. BI

DELIVERY OF PAMAM DENDRIMERS AND DENDRIPLEXES ACROSS NATURAL BARRIERS (BLOOD-BRAIN BARRIER AND PLACENTAL BARRIERS) IN HEALTHY PREGNANT MICE

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For treating diseases that affect the fetus, gene therapy is an important tool that can deliver and integrate therapeutic genes into the genome of cells carrying mutations. Nanomolecules, especially PAMAM dendrimers have recently come into wider use as cargo, carrying vectors that have several advantages over viral vectors due to their 1) surface chemistry; 2) uniform size; 3)ability to target specific tissues; and 4) carry large biomolecules and drugs. Recently, we demonstrated that 4th generation (G4) PAMAM dendrimer nanomolecules (D) with a cystamine core; cys (S=S) with a non-toxic 90: 10 OH:NH2 (known as D-Cys) surface modification can cross the blood brain barrier following injection into the bloodstream. In the current study, as a proof of concept, we are focusing on delivering the dendrimers alone (D-Cys) tagged with Cy5.5 (D-Cys-Cy5.5) as well as dendrimers conjugated 10kb plasmid (known as D-Cys-Cy5.5-EF1a-Luc2-dTom complexes) to healthy pregnant C57BL/6J mice to determine the fate of these dendrimers and complexes in the pregnant mice as well as in the fetus. Systematic diffusion of the D-Cys-Cy5.5 and D-Cys-Cy5.5-EF1a-Luc2-dTom and was evaluated 3 days after intraperitoneal injection using in vivo imaging (IVIS), demonstrating that the dendrimer was taken up into the circulation and away from the injection site. Analysis of sections by fluorescence microscopy showed that D-Cys-cy5.5 and complexes were able to successfully cross the maternal blood brain barrier. They were taken up by the brain cells such as neurons and astrocytes as evidenced by NeuN and GFAP staining, However, analysis of the fetal brains failed to detect dendrimers or complexes in the brain tissue, although the dendrimers appeared to be retained in the placenta. This is one of the first studies to analyze the distribution of surface modified PAMAM dendrimer and dendrimer plasmid complexes in the pregnant mouse and fetus following systemic injection.

Support for this study was provided by the Neuroscience Program, the College of Medicine, the Department of Chemistry and Biochemistry, the E. Malcolm Field and Gary Leo Dunbar Endowed Chair in Neuroscience, and the John G. Kulhavi Professorship in Neuroscience at CMU.

A BIOELECTRONIC SENSING PLATFORM COMBINING FLEXIBLE DUAL-SIDED MICROELECTRODE ARRAY AND INSECT OLFACTORY NEURONS FOR LUNG CANCER BIOMARKER DETECTION.

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Early detection of lung cancer significantly enhances treatment outcomes, yet current screening methods are limited by accessibility, sensitivity, and cost. Detecting volatile organic compounds (VOCs) related to lung cancer offers the possibility for noninvasive, early, and accessible diagnosis of lung cancer in humans. This study introduces a bioelectronic sensing platform that integrates the highly sensitive locust olfactory system with a flexible dual-sided microelectrode array (MEA), for robust, efficient and label-free detection of volatile lung cancer biomarkers. By leveraging a unique folding-bonding fabrication method and poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) surface functionalization, we developed and optimized flexible dual-sided MEAs in 4-, 6-, or 8-channel configurations with a high spatial resolution of 55-75 μm and a low electrochemical impedance of electrodes in a range of 1.03 ×104-2.85×104 Ohm. These MEAs retain mechanical flexibility and stability while being robust enough to penetrate the insect brain tissues without any mechanical reinforcement. Lung cancer VOC-evoked in vivo electrophysiological recordings showed the MEAs were capable of capturing distinct neural activities in the locust's antennal lobe, producing high-quality recordings across all channels. By combining neural responses across electrophysiological recordings and employing dimensionality reduction techniques, we obtained unique trajectories and clusters associated with distinct population responses for each lung cancer VOC biomarker. Utilizing a high dimensional population neuronal response analysis via leave-one-trial-out methodology, we obtained a high classification success rate of unknown VOCs. Moreover, the ability of this integrative bioelectronic sensing platform to classify each lung cancer VOC biomarker from a single electrophysiological recording was shown. Combining all neurons obtained from a single electrophysiological recording showed 88% classification success of each VOC indicating the potential of this platform for one-shot and real-time lung cancer detection. This study presents a novel platform for lung cancer biomarker detection and opens new avenues for biological brain-based, non-invasive, and efficient cancer screening methods.

SPARSE RECORDING: A WINDOW ON BRAIN-WIDE COMMUNICATION DYNAMICS?

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It has become clearer over the past decade that many interesting cognitive functions depend on circuits linking distinct subsets of cells distributed over several brain regions. Therefore, neuroscientists increasingly want to record activity of individual neurons simultaneously across many connected brain regions. However, it seems impractical to record from very many individual cells over many brain regions. The trade-off seems to be detail versus breadth of coverage.

One recent approach to resolve this dilemma, at least for purposes of recording cortical activity, is to label a sparse subset of L2/3 cortical neurons which can be individually detected through one photon epifluorescence imaging over a wide cortical area. The question that arises for such recordings is: how well can the sparse recording represent population activity over a cortical region? This poster addresses that question through both empirical data analysis and simulation.

Semedo (2019) and other studies suggest that most information about population activity participating in cross-region communication is contained within the first few principal components (PCs) of population activity in each communicating region. Thus if the information in the first few PCs of the full network can be well represented in the dominant PCs of the sparse subset, then a sparse recording may suffice to represent full population activity.

I studied the relationship of full PCs and sparse PCs in several public data sets of recordings from very many neurons. Most of the scientifically useful information about the activity of these large neural populations could be represented by PCs of a sparse random subset of neurons: over 2/3 of the population information used for most analyses could be captured adequately by working with PCs of only 5%-7% of the neurons.

I conclude that recordings from sparse subsets of neurons in several regions of cortex, may be sufficient to study large-scale cortical dynamics and inter-regional communication. To illustrate this, I show a few examples of relationships between population activity in several distal regions of a mouse brain involving subsets of neurons in each region acting independently of the bulk level of activity in those regions.

OPTIMIZATION METHODS FOR NEUROSCIENCE DATA ANALYSIS WITH APPLICATIONS TO CELL-LEVEL WIDEFIELD IMAGING AND TO NETWORK ANALYSIS IN MULTI-MODE OPTICAL-IMAGING.

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For many brain imaging technologies one of the key upstream issues is separating faint brightness fluctuations due to neural activity from a fluctuating background. An increasingly prominent downstream analysis issue is how to infer from activity measures the dominant modes of communication within a neural network. Both of these questions are best addressed through modern optimization methods, but these methods are still poorly known in the neuroscience community.

Optimization can be used for both fitting model parameters from data, and for machine learning approaches to finding features from data, such as neurons in fluorescence images. Optimization generally proceeds by defining a cost-function which is to be minimized, which includes terms representing both fidelity (how well the model fits to data) and regularization (penalization of over-fitting). Further constraints on model parameters come in the form of equality or inequality conditions that must be enforced. The Alternating-Direction-of-Multipliers Method (ADMM) is a general framework for optimization which allows us to solve arbitrary combinations of L1 or L2 fidelity and regularization terms, combined with various constraints. We apply ADMM here to extract single-neuron activity traces from very large imaging videos, and to connectivity network inference over many cortical and subcortical brain regions.

We have recently developed the WISLaC system for Widefield Imaging of Sparsely Labeled Cortex. The imaging optics allow recordings of the whole surface of a mouse cortex at single-neuron resolution, through an implanted glass full-cranial window. When combined with sparse expression of GCaMP in ~5% of L2/3 cells, activity traces of individual neurons can be extracted simultaneously across all exposed cortical areas. In order to automatically identify 10s of thousands of neurons, extract their GCaMP activity, and remove background fluorescence, we have developed an ADMM version of Non-Negative Matrix Factorization, which incorporates L1 sparseness constraints, and an L2 penalty term that extracts the background fluorescence level. The validity of cell-identification by this method can be assessed by subsequent 2P imaging and/or histology.

In a second project, we are applying optimization methods to fit a feed-forward network regression-model to data simultaneously recorded with widefield imaging of cortex and multi-fiber imaging of subcortical thalamic regions, in order to characterize thalamocortical network circuitry. Again this model is fit using non-negativity constraints and L1 regularization for sparseness, yielding a network structure that is significantly different from that obtained by correlation-based methods. Network connectivity inferred by this approach is compared to anatomical connectivity datasets.