SOCIETY FOR NEUROSCIENCE

Michigan Chapter

52nd Annual Meeting

August 20, 2022

Health Professions Building

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-Mitochondrial copy number kits: Cat # MCN1 (human), MCN2 (mouse), MCN3 (rat)

PLEASE MAKE SURE TO VISIT OUR VENDORS!

Map and Directions

Conference Location:

Health Professions Building, 1280 E. Campus Drive, Mount Pleasant, MI, 48859

Parking on Lot 5 and 12 in front of the Health Professions/College of Medicine Building

Lunch and Business meeting will be at the Bovee University Center Rotunda

Schedule

8:30am to 9:00am: Registration and Continental Breakfast

9:00am to 10:15am: Poster session A*

10:15am to 10:30am: Coffee break and visit vendors and Institutional booths exclusively open (Also open during Poster Sessions)

10:30am to 11:45am: Poster session B*

11:45am to 1:45pm: Lunch and Business meeting

Welcome & President's Report; Treasurer's Report; Elections

To be elected during the meeting (self-nominations are welcome) *

Awards Chair (2022-2024)

Central Michigan University Counselor (2022-2024)

Michigan State University Counselor (2022-2024)

University of Michigan Counselor (2022-2024)

Field Neuroscience Institute Counselor: (2022-2024)

Counselor at Large: (2022-2024)

Student Counselor: (2022-2024)

1:45pm to 2:35pm: Founders awards

2:35pm to 3:15pm: Coffee break/Ice cream social and Vendor Visits

3:15pm to 4:15pm: Keynote speech by Dr. Deborah Shear, PhD

"Gaps, Challenges, and solutions to diagnosing and treating traumatic brain injury"

4:15pm to 4:30pm: Awards and Adjournment

**Posters from session A and B will be displayed throughout both the poster sessions*


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Michigan Chapter of SfN Council

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President	Jessica Matchynski-Franks	Rochester University	2023*
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Counselors			
CMU	Julien Rossignol		2022
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U of M	Paul Jenkins		2022
WSU	Kelly Bosse		2023
WMU	Lisa Baker		2023
Field Neurosciences Institute	Gary Dunbar		2022
At large I	Jessica Matyas	Rochester University	2023
At large II	Kevin Trewartha	Michigan Tech. University	2022
Grad student	Lana Grasser	Wayne State University	2023

**President to become Past-President & President-Elect to become President in 2022*

Founders Awards

(1:45-2:35 PM)

These awards are in honor of Montford F. Piercey and Duncan McCarthy for their contributions in organizing our chapter

Awardees are listed in alphabetical order.

Daniel Doyle

Michigan Neuroscience Institute, University of Michigan

CHROMATIN REMODELER ARID1A ORCHESTRATES SUBPLATE-DEPENDENT WIRING OF CORTICAL CONNECTIVITY

doyledan@umich.edu

Bio: Dan Doyle is currently a 6th year doctoral candidate in Dr. Ken Kwan's lab in the Michigan Neuroscience Institute and Neuroscience Graduate Program (NGP) at the University of Michigan. In the Kwan lab, his dissertation work focuses on the genetic and molecular mechanisms that orchestrate developmental assembly of neural circuits and how disruption of these mechanisms can contribute to neurodevelopmental disorders. Prior to joining the NGP, he completed his undergraduate degree in Cell & Molecular Biology and Biomedical Sciences at Grand Valley State University (GVSU). While at GVSU, Dan was an undergraduate researcher in the lab of Dr. Merritt Delano-Taylor where he studied the molecular underpinnings of midbrain dopaminergic neuron generation and began his career in developmental neurobiology.

Summary of work: Dan's research is focused on molecular and cellular mechanisms that underpin the development and wiring of neural circuits in the cerebral cortex and their dysregulation in neurodevelopmental disorders. In particular, his work aims to: 1) understand how the diverse cortical neuronal subtypes are generated from neural progenitor cells; 2) identify the cellular and molecular mechanisms essential to neocortical wiring and ultimately cortical function; and 3) define how disruption of these developmental mechanisms contributes to brain disorders such as autism spectrum disorder (ASD) and intellectual disability (ID) by leveraging mouse genetics, transcriptomics and functional genomics, circuit neurobiology, and molecular biology techniques. In line with these goals, he has shown that chromatin remodeler *Arid1a* is a multifaceted regulator of subplate neurons' transcriptome and their circuit wiring functions during cortical development, thereby mediating the formation of major neural circuits including the corpus callosum and thalamocortical connectivity.

Future aspirations: He is currently pursuing postdoctoral positions, and his career aspiration is to lead an academic laboratory exploring genetic mechanisms underlying the anatomical and circuit development of the cerebral cortex.

Founders Awards

(1:45-2:35 PM)

These awards are in honor of Montford F. Piercey and Duncan McCarthy for their contributions in organizing our chapter

Awardees are listed in alphabetical order

Sindhuja Koneru

Field Neurosciences Institute Laboratory for Restorative Neurology Program in Neuroscience
Central Michigan University
TREATMENTS USING GM1 GANGLIOSIDE AND CRISPR-CAS9- MEDIATED GENE EDITING REDUCE BEHAVIORAL AND NEUROPATHOLOGY DEFICITS IN MOUSE MODELS OF
HUNTINGTON'S DISEASE
Koner1s@cmich.edu

Bio: Sindhuja grew up in Guntur, India. She received her Master's of Science degree in Pharmacy from Manipal University in 2014. She did her internship at a clinical research organization, where she focused on the safety of new drug formulations in lung cancer clinical trials. She started her career at Novartis as a drug safety associate in phase IV and compassionate clinical trials on devastating diseases like Multiple Sclerosis and Glioblastoma. Sindhuja's growing interest in neurodegenerative diseases and their treatment methods led her to pursue a Ph.D. in Dr. Gary Dunbar's Neuroscience lab at Central Michigan University.

Summary of work: Sindhuja's research focused on delivery of the most promising novel drug GM1 from ovine-source in comparison to the more commonly used bovine-sourced GM1 to treat Huntington's disease (HD). She has shown the effects of these treatments on reducing behavioral and neurodegenerative processes, including inflammation, in the R6/2 mouse model of HD. In addition to her work with GM1 gangliosides, Sindhuja has been working on the use of CRISPR-Cas9 gene editing tool as a means of treating HD. The study results provide a proof-of-concept that the use of CRISPR-Cas 9 with carefully designed gRNAs can effectively reduce the neuropathology as well as reduction in behavioral deficits in the YAC 128 mouse model of HD.

Future aspirations: As she nears the completion of her PhD, she has accepted a position at Henry Ford Hospital in Detroit, Michigan as a Clinical Research Scientist for the Infectious Disease department. She will be tasked with running many clinical trials revolving around Monkeypox, COVID-19, Neurogenic bladder infections, HIV, *Clostridium difficile* and Bacteremia. Sindhuja's long term goal is to use the knowledge and experience she gains from preclinical- and clinical research studies to pursue a career in a pharmaceutical industry and contribute to provide adequate study designs in phases II/III to bring cost effective and most efficacious treatments for better health care.

Keynote Speaker

(3:15pm to 4:15pm)

Dr. Deborah A Shear

Director, Brain Trauma & Neuroprotection Branch
Center for Military Psychiatry & Neuroscience,
Walter Reed Army Institute of Research

"Gaps, Challenges, and Solutions to Diagnosing and Treating Traumatic Brain Injury"

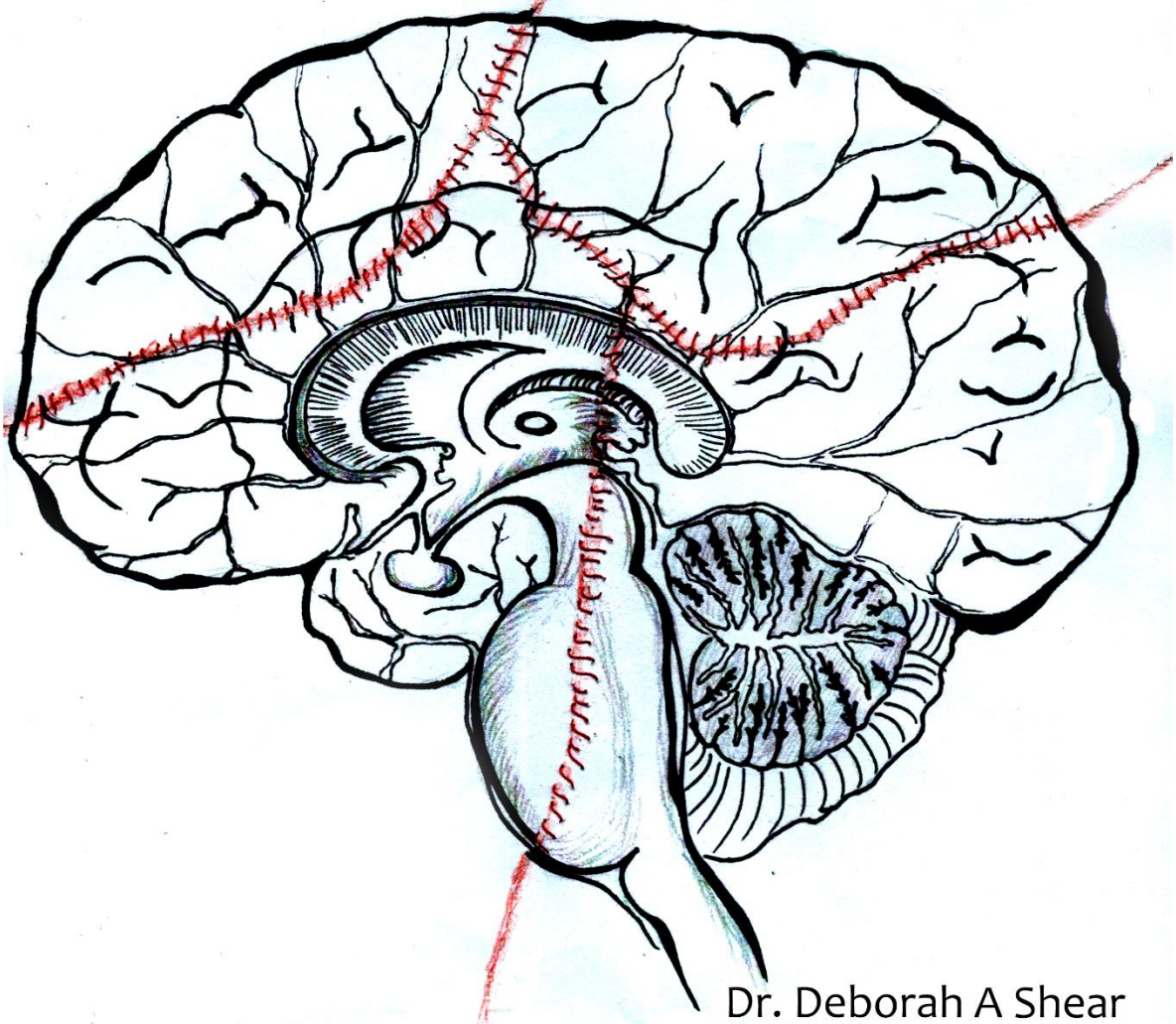
Dr. Deborah Shear earned her Ph.D. in experimental Psychology from Central Michigan University, Mt. Pleasant, MI while working as a Scientist and Lab Director at Field Neurosciences Institute (FNI), Ascension Health Medical System, Saginaw, MI. Her doctoral dissertation focused on the use of neural stem cell transplantation, biomedical engineering, and progesterone as therapeutic strategies for traumatic brain injury (TBI) and was conducted in collaboration with Emory University and Georgia Institute of Technology. Dr. Shear joined the WRAIR Brain Trauma Neuroprotection Branch as a GS Scientist in July of 2007. During the early years of her tenure, she served as Principle Investigator on Combat Casualty Care Research Program (CCCRP) Army core-funded projects focused on neural stem cell transplantation, neuroprotection, anti-seizure combination therapy studies, post-traumatic epilepsy (PTE) models, and selective brain cooling therapeutics for TBI.

In 2009, Dr. Shear was awarded the Applied Neurotrauma Research Award through the Psychological Health and Traumatic Brain Injury Research Program to address the problem of recognizing the markers and long-term effects of single and repeated concussion. Dr. Shear led her team investigated how cellular changes resulting from single and repeated concussion relate to clinically-relevant molecular (i.e. TBI biomarkers, bioenergetics, and metabolomics), neurobehavioral, and electrophysiological (EEG) outcomes. In support of these efforts, Dr. Shear's team developed the Walter Reed Army Institute of Research (WRAIR) Projectile Concussive Impact (PCI) model of concussion in rats. The PCI model generates a closed-head concussion that is comparable to injuries experienced by troops, allowing for the reproducible generation of closed-head mTBI across a wide spectrum of injury severities, and incorporating the use of a custom-designed helmet equipped with pressure sensor film. This contributed directly to a US patent: "Device and Method for Inducing Brain Injury in Animal Test Subjects" (US 8,973,565 B2); and a second patent subaward for the helmet sensor system.

In 2012, Dr. Shear was promoted to a GS15 Supervisory Biologist and in 2016 she was promoted to Director of the BTN Program. As Branch Director, Dr. Shear is responsible for leading and supervising a research team comprised of 10 Ph.D. scientists and 20 technical staff and provides critical research oversight and supervisory support essential to the planning, coordination and performance of the mission-approved and fully funded neuroscience research program to study the functional, cellular and molecular dynamics of central nervous system injury as it relates to TBI and the development novel neuroprotective and neurorestorative therapies for the soldier exposed to repeated concussive impact injury and penetrating (ballistic and non-ballistic) brain trauma.

Dr. Shear has received honorary awards from the Commanding General (MRMC), the WRAIR Commander, and the Department of Defense Army National Guard (Patriotic Employer Award). In addition, her research with the Operation Brain Trauma Therapy Consortium has been cited in the New England Journal of Medicine and by the FDA. She has published over 65 peer-reviewed manuscripts, book chapters, and has delivered numerous oral and poster presentation at national and international conferences. Dr. Shear also serves as the Capability Area Manager (CAM) for the Combat Casualty Care Research Program (CCCRP) TBI Portfolio, overseeing approximately $6.5M of Army core funds designated for TBI research at DoD intramural laboratories. During the past 3 years as Director, Dr. Shear has successfully pivoted her research program to align more directly with the changing requirements of CCCRP to include establishing partnerships focused on developing innovative point-of-injury therapeutics and establishing a large animal model of TBI/polytrauma in swine.

Gaps, Challenges, and Solutions to Diagnosing and Treating Traumatic Brain Injury



Dr. Deborah A Shear

Art by Delphine Rossignol

Posters

Odd #s are in Poster Session A and Even #s are in Poster Session B

#	Presenting author	Title	Institution	Theme
1	Durack, S	INVESTIGATING DORSAL HIPPOCAMPAL HISTONE DEACETYLASE ACTIVITY FOLLOWING FEAR CONDITIONING	Wayne State University	Cognition
2	Eichstaedt, JM	EVIDENCE OF INCREASED GLUTAMATE IN THE DORSAL ANTERIOR CINGULATE CORTEX DRIVEN BY INHIBITORY MOTOR CONTROL FUNCTION USING 1H fMRS	Wayne State University	Cognition
3	Fontana, B.D	THE DEVELOPMENT OF A MACHINE LEARNING MODEL TO ANALYZE DIFFERENCES IN INDIVIDUAL RESPONSE ON FEAR CONDITIONING RESPONSES IN ZEBRAFISH (DANIO RERIO)	Wayne State University	Cognition
4	Yahya, M	NOREPINEPHRINE DEPLETION LEADS TO A SEX-SPECIFIC IMPROVEMENT IN MEMORY CONSOLIDATION	Wayne State University	Cognition
5	Carpenter, C	THE IMPACT OF SLEEP-RELATED PROBLEMS ON ANXIETY SYMPTOMS IN ADOLESCENTS	Wayne State University	Development
6	Desai, S	GENETIC VARIATION IN ENDOCANNABINOID SIGNALING AND THREAT- AND REWARD-RELATED BRAIN FUNCTIONING DURING THE TRANSITION INTO ADOLESCENCE	Wayne State University	Development
7	Evanski, J	CANNABIDIOL (CBD): EFFECTS ON ANXIETY, SEIZURE FREQUENCY, AND ENDOCANNABINOID LEVELS IN PEDIATRIC EPILEPSY PATIENTS	Wayne State University	Development
8	Gowatch, LC	IMPACT OF THE PARENT-CHILD RELATIONSHIP ON FEAR EXTINCTION AND ANXIETY SYMPTOMS IN YOUTH	Wayne State University	Development
9	Lahr, J	IMPACT OF AN AUTISM-ASSOCIATED MUTATION IN GLUN2B ON THE CAMP RESPONSE ELEMENT-BINDING PROTEIN (CREB) SIGNALING PATHWAY	Central Michigan University	Development
10	Losiowski, J	EXTINCTION LEARNING AND OBSESSIVE-COMPULSIVE SYMPTOM SEVERITY IN YOUTH	Wayne State University	Development
11	Myers, AM	OPIOID ADMINISTRATION DURING PREGNANCY: EFFECTS OF MORPHINE COMPARED TO BUPRENORPHINE EXPOSURE ON OFFSPRING NEURODEVELOPMENTAL OUTCOMES IN A TRANSLATIONAL RODENT MODEL	Wayne State University	Development
12	Owens, Z	RACIAL DISCRIMINATION AND ASSOCIATIONS WITH ANXIETY SYMPTOMS IN DETROIT-AREA YOUTH	Wayne State University	Development
13	Paulisin, S	IS DIET ASSOCIATED WITH ANXIETY SYMPTOMS IN YOUTH?	Wayne State University	Development
14	Richardson, L	A TRANSLATIONAL RODENT MODEL OF OPIOID EXPOSURE DURING PREGNANCY: EFFECTS OF MORPHINE COMPARED TO BUPRENORPHINE ON MATERNAL BEHAVIOR, BRAIN, AND THE MICROBIOME	Wayne State University	Development
15	Zundel, C	LEAD EXPOSURE RISK AND FUTURE MENTAL HEALTH SYMPTOMS IN YOUTH: PRELIMINARY RESULTS FROM THE ABCD COHORT	Wayne State University	Development

16	Powell, K	SUBCHRONIC MITRAGYNINE CAUSES SEX-DEPENDENT CHANGES IN BRAIN CYTOKINE LEVELS IN RATS WITH MINIMAL COGNITIVE AND LOCOMOTOR EFFECTS	Western Michigan University	Integrative Physiology and Behavior
17	Haidar, S	BDNF-RELATED MECHANISMS OF NEUROPLASTICITY IN THE ROSTRAL VENTROLATERAL MEDULLA OF SEDENTARY VERSUS ACTIVE RATS	Wayne State University	Integrative Physiology and Behavior
18	Henry, M	CHEMOGENETIC INHIBITION OF OXYTOCIN RECEPTOR EXPRESSING NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS HAS SEX-SPECIFIC EFFECTS ON JUVENILE SOCIAL BEHAVIOR	Michigan State University	Integrative Physiology and Behavior
20	Hernandez, M	REGULATION OF TGF-β SIGNALING IN C. ELEGANS BY THE POST-TRANSLATIONAL MODIFICATION PROTEIN AMPYLATION	University of Michigan	Integrative Physiology and Behavior
21	Irshaid, A	ROLE OF MC3R IN ENERGY HOMEOSTASIS USING AN ADULT KNOCKOUT MOUSE MODEL	University of Michigan	Integrative Physiology and Behavior
22	Jiddou, H	LOW DOSE 1-(BENZOFURAN-5-YL)-N-METHYLPROPAN-2-AMINE (5-MAPB) DOES NOT ALTER ANXIETY-LIKE BEHAVIORS IN A RODENT ELEVATED PLUS MAZE	Western Michigan University	Integrative Physiology and Behavior
23	Johnson, C	LOCOMOTOR STIMULANT EFFECTS AND PERSISTENT SEROTONIN DEPLETION FOLLOWING BINGE-LIKE 1-(BENZOFURAN-5-YL)-N-METHYLPROPAN-2-AMINE (5-MAPB) TREATMENT IN SPRAGUE-DAWLEY RATS	Western Michigan University	Integrative Physiology and Behavior
24	Kowalski, C	THE EFFECTS OF PHYSICAL ACTIVITY ON PANAS-C SCORES IN YOUTH	Wayne State University	Integrative Physiology and Behavior
25	Lee, J	SEX DIFFERENCES IN LOCOMOTOR ACTIVITY AND BEHAVIORAL SENSITIZATION IN RATS ADMINISTERED FENTANYL	Wayne State University	Integrative Physiology and Behavior
26	LeVasseur, G	ESCALATING MORPHINE AND NALOXONE-PRECIPITATED WITHDRAWAL EFFECTS ON CONDITIONED FEAR LEARNING IN SPRAGUE DAWLEY RATS; A FEAR-POTENTIATED STARTLE (FPS) STUDY.	Wayne State University	Integrative Physiology and Behavior
27	Maharjan, S	DEVELOPMENTAL STIMULATION OF PYRAMIDAL NEURONS DURING A DEFINED TEMPORAL WINDOW ALTERS SINGLE UNIT ACTIVITY IN ADULT MICE	Central Michigan University	Integrative Physiology and Behavior
28	Murphy, A	ROLE OF OVARIAN HORMONES ON PROBDNF LEVELS IN THE ROSTRAL VENTROLATERAL MEDULLA: INFLUENCES RELATED TO NEURONAL PLASTICITY AND SYMPATHETIC CONTROL OF BLOOD PRESSURE	Wayne State University	Integrative Physiology and Behavior
29	Simpson, S	NOREPINEPHRINE REGULATION OF SPATIAL MEMORY USING BARNES MAZE IN MALE AND FEMALE RATS	Wayne State University	Integrative Physiology and Behavior
30	Steck, K	EVALUATION OF BENZOFURAN AND BENZOTHIOPHENE MOLECULES FOR MDMA-LIKE DISCRIMINATIVE STIMULUS EFFECTS IN SPRAGUE-DAWLEY RATS	Western Michigan University	Integrative Physiology and Behavior
31	Uprety, A	EFFECTS OF HYPEREXCITATION OF LAYER V NEOCORTICAL PYRAMIDAL NEURONS DURING EARLY POSTNATAL DEVELOPMENT ON ADULT BEHAVIOR	Central Michigan University	Integrative Physiology and Behavior

32	Davidson, C	EFFECTS OF A MIXTURE OF COCAINE AND FENTANYL ON LOCOMOTOR ACTIVITY ACROSS REPEATED EXPOSURES IN MALE WISTAR RATS	Wayne State University	Motivation and Emotion
33	Gheidi, A	DIAL PREFRONTAL CORTEX IS INVOLVED IN ANXIETY-LIKE BEHAVIOR IN RATS WITH A HISTORY OF BINGE COCAINE ADMINISTRATION AND DRUG-ABSTINENCE NEURONAL ENSEMBLE IN THE ME	Wayne State University	Motivation and Emotion
34	Maitland, A	EFFECT OF RATE OF INTRAVENOUS COCAINE INFUSION ON LOCOMOTOR SENSITIZATION IN FEMALE RATS USING DEEP ANIMAL TOOL KIT, A NEW AUTOMATED APPROACH TO BEHAVIORAL ANALYSIS	University of Michigan	Motivation and Emotion
35	Matsko, M	THE EFFECTS OF TREADMILL EXERCISE AND STRETCHING ON ANXIETY IN YOUTH	Wayne State University	Motivation and Emotion
36	Rogers, S	EFFECTS OF ACUTE EXERCISE VS. STRETCHING ON MOOD IN YOUTH	Wayne State University	Motivation and Emotion
37	Jennings, M	RIPPLE EFFECTS OF SCHOOL SHOOTINGS: IMPACT OF THE OXFORD SCHOOL SHOOTING ON ANXIETY SYMPTOMS, SCHOOL SAFETY, AND ATTITUDES TOWARDS GUNS IN A SAMPLE OF DETROIT ADOLESCENTS	Wayne State University	Motivation and Emotion
38	Harikumar Sheela, H	SYNAPTIC GLUTAMATE RECEPTOR SIGNALING ACUTELY REGULATES THE NEURONAL STRESS KINASE DLK	University of Michigan	Neural Excitability, Synapses, & Glia
39	Blanzy, A	SYMPATHETIC RESPONSE TO GLUTAMATE MICROINJECTIONS IN THE ROSTRAL VENTROLATERAL MEDULLA OF SEDENTARY AND ACTIVE FEMALE RATS	Wayne State University	Neural Excitability, Synapses, & Glia
40	Elvira, C	ANKYRIN-G IN DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY	University of Michigan	Neural Excitability, Synapses, & Glia
41	Kolanowski, M	EFFECTS OF MELATONIN ON STRIATAL DOPAMINE NEUROTRANSMISSION	Grand Valley State University	Neural Excitability, Synapses, & Glia
42	Tuppil, K	PREDICTED INCREASES IN BDNF IN THE RVLM OF HYPERTENSIVE VERSUS NORMOTENSIVE RATS	Wayne State University	Neural Excitability, Synapses, & Glia
43	Combs, B	MECHANISMS OF TAU-BASED REGULATION OF RETROGRADE FAST AXONAL TRANSPORT	Michigan State University	Neuro-degenerative Disorders & Injury
44	Gishto, R	QUANTIFICATION OF EXTRACELLULAR VESICLE MEMBRANE MARKERS AFTER PHOTOBIOMODULATION THERAPY AND TRAUMATIC BRAIN INJURY IN MICE	Wayne State University	Neuro-degenerative Disorders & Injury
45	Gregolynskyj, A	CHARACTERIZING AA-CRYSTALLIN'S ROLE IN THE REGULATION OF MÜLLER CELL TROPHIC SUPPORT AND THE RETINAL INFLAMMATORY RESPONSE	University of Michigan	Neuro-degenerative Disorders & Injury
46	Halfhide, C	COMBINING EMBRYONIC MESENCEPHALIC DOPAMINERGIC NEUROSPHERE TRANSPLANTATION AND ENCOURAGED MOVEMENT TO IMPROVE DOPAMINE RELEASE CONTROL AND RELATED RECOVERY FROM LIMB USE ASYMMETRY IN A UNILATERAL 6-OHDA LESIONED RAT MODEL OF PARKINSON'S DISEASE	Central Michigan University	Neuro-degenerative Disorders & Injury

47	Ikefuama, E	NON-INVASIVE OPTOGENETIC STIMULATION IN A RAT MODEL OF SPINAL CORD INJURY	Central Michigan University	Neuro-degenerative Disorders & Injury
48	Kubik, M	ASSESSMENT OF THE NEUROPROTECTIVE POTENTIAL OF REPURPOSING TERAZOSIN IN RAT MODELS RECAPITULATING FEATURES OF PARKINSON'S DISEASE	Michigan State University	Neuro-degenerative Disorders & Injury
49	Nguyen, A	BENZENE EXPOSURE DURING PREGNANCY INCREASES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS SEVERITY IN OFFSPRING	Wayne State University	Neuro-degenerative Disorders & Injury
50	Poudel, A	MODULATION OF NOTCH, GSK-3B, AND BMP PATHWAYS TO CONVERT ASTROCYTES INTO NEUROBLASTS USING CRISPR/CAS9 GENE EDITING TOOL – AN IN VITRO STUDY	Central Michigan University	Neuro-degenerative Disorders & Injury
51	Rozofsky, J	POTENTIAL SEX DIFFERENCES IN MITRAL CELL DENDRITIC MORPHOLOGY FOLLOWING INJURY AND RECOVERY	Western Michigan University	Neuro-degenerative Disorders & Injury
52	Rukhsana, D	DECREASE IN PLASMA LEPTIN LEVELS ARE AGE AND SEX-RELATED IN THE 3XTG MOUSE MODEL OF ALZHEIMER DISEASE	Central Michigan University	Neuro-degenerative Disorders & Injury
53	Sluzala, Z	EVIDENCE OF REGULATION OF T148 PHOSPHORYLATION ON αA-CRYSTALLIN BY MTOR OR PI3K	University of Michigan	Neuro-degenerative Disorders & Injury
54	Srinageshwar, B	RECOVERY FROM MOTOR DEFICITS FOLLOWING DELIVERY OF TRANSCRIPTION FACTOR IN MCAO STROKE MODEL USING PAMAM DENDRIMERS	Central Michigan University	Neuro-degenerative Disorders & Injury
55	Bowers, Z	EFFECTS OF TART CHERRY EXTRACT AND OMEGA FATTY ACIDS IN THE 3XTG MOUSE MODEL OF ALZHEIMER'S DISEASE	Central Michigan University	Neuro-degenerative Disorders & Injury
56	Zahoor, I	DOCOSAHEXAENOIC ACID-DERIVED PRO-RESOLVING LIPID MEDIATOR MARESIN-1 AMELIORATES INFLAMMATION AND PREVENTS DISEASE PROGRESSION IN PRECLINICAL MODEL OF MULTIPLE SCLEROSIS	Henry Ford Health System	Neuro-degenerative Disorders & Injury
57	Ebendick-Corpus, B	THE EFFECTS OF IMMUNE MODULATION ON THE RECOVERY RATE OF ZEBRAFISH OLFACTORY GLOMERULI AFTER DEAFFERENTATION	Western Michigan University	Sensory Motor Systems
58	Lopez, A. J	MOTOR NEURON CELL BODY MORPHOLOGY WITH AGE, EXERCISE, AND SEX	Western Michigan University	Sensory Motor Systems
59	VanGyseghem, J	THE EFFECTS OF EXERCISE ON GDNF AND ESTROGEN CONCENTRATION IN MALE AND FEMALE RATS	Western Michigan University	Sensory Motor Systems
60	Woolman, B	SENSORIMOTOR ADAPTATION AND RETENTION IN MILD COGNITIVE IMPAIRMENT AND EARLY ALZHEIMER'S DISEASE	Michigan Technological University	Sensory Motor Systems
61	Gorantla, N	NOVEL LUCIFERASE - OPSIN COMBINATIONS FOR IMPROVED BIOLUMINESCENT OPTOGENETICS	Central Michigan University	Techniques
62	Matchynski, JI	QUANTIFICATION OF CORTICAL FOS NEUROACTIVITY AFTER CUED FEAR CONDITIONING USING PHOTOACOUSTIC IMAGING	VAMC, Wayne State University	Techniques

		IN VIVO WITH VALIDATION BY EX VIVO IMMUNOFLUORESCENCE		
63	McLean, L	CONTROLLING NEURON-MUSCLE COMMUNICATION WITH BIOLOGICAL LIGHT	Central Michigan University	Techniques
64	Patel, J	CELLULAR AND CIRCUIT EFFECTS OF CHEMOGENETIC NEURONAL STIMULATION	Central Michigan University	Techniques
65	Prakash, M	INTERLUMINESCENCE FOR SELECTIVE CONTROL OF SYNAPTICALLY CONNECTED PRE-AND POSTSYNAPTIC NEURONS	Central Michigan University	Techniques
66	Simkins, J	MOLECULAR EVOLUTION OF BL-OG COMPONENTS	Central Michigan University	Techniques
67	Slaviero, A	OPTIMIZING BIOLUMINESCENCE-INDUCED PHOTOACTIVATION OF TRANSCRIPTION	Central Michigan University	Techniques
68	Smith, J.E	DENDRIMER-DELIVERED NOCODAZOLE ATTENUATES GLIOBLASTOMA PROLIFERATION, MIGRATION, AND METABOLISM IN-VITRO	Central Michigan University	Techniques

Theme: Cognition

1

*Durack, S.[1], Zdun, J.[1], Glover, M.[1,2], Kallakuri, S.[2], & Perrine, S.[2]
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INVESTIGATING DORSAL HIPPOCAMPAL HISTONE DEACETYLASE ACTIVITY FOLLOWING FEAR CONDITIONING

The hippocampus is involved in memory formation and learning; therefore, it plays an important role in contextual fear conditioning. Histone deacetylases (HDACs) are enzymes involved in epigenetic regulation and have been shown to play an essential role in learning and memory. Therefore, class IIa HDAC5s within the dorsal hippocampus may play an important role in fear extinction. To investigate this, 12 rats were subjected to a contextual fear conditioning paradigm. Rats were split into three groups: no-shock control (n=3), extinction (n=5), and fear renewal (n=4). Context A was shown for habituation, conditioning, and fear renewal, and context B was shown for extinction. During habituation, rats were placed in fear conditioning chambers with no stimuli added. Extinction and fear renewal groups received 5 tone and shock combinations during acquisition while the control group only received 5 tones. During extinction and fear renewal, only a tone was played for all groups. Behavioral results show that rats exposed to the shock treatment displayed diminished fear behaviors 21 days after acquisition during extinction and renewal. Preliminary results from immunofluorescent-stained brain sections from the three groups show a qualitative difference in the amount of HDAC5 activated cells between the groups, with the fear renewal group having the highest amount of HDAC5 activity. This study provides a molecular and behavioral understanding of the involvement of the dorsal hippocampus in the regulation of fear memory extinction.

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EVIDENCE OF INCREASED GLUTAMATE IN THE DORSAL ANTERIOR CINGULATE CORTEX DRIVEN BY INHIBITORY MOTOR CONTROL FUNCTION USING 1H fMRS

Background: Inhibitory control operationalized as the ability to selectively withhold 'pre-potent' motor responses involves both general motor control and inhibition. Poor inhibitory control is implicated in many psychiatric conditions, including obsessive-compulsive disorder, substance use, and a poor ability to maintain weight loss. fMRI studies have demonstrated that the dorsal anterior cingulate cortex (dACC) is central to inhibitory control; however, the fMRI signal cannot illuminate neural mechanisms such as the interplay between glutamatergic and GABAergic neurons that may be important in inhibitory control. By comparison, proton functional magnetic resonance spectroscopy (^{1}H fMRS) can assess how task conditions effect shifts in the excitatory neurotransmission of glutamate and is therefore more proximate to fundamental neuronal processes. Here, in an initial attempt to develop experimental protocols for clinical application, we investigated whether fMRS can detect changes in dACC glutamate during inhibitory control,
Method: Single-voxel ^{1}H fMRS of the dACC (midline) at 3T was acquired in twenty healthy controls (mean age: 17.4±2.6 years; 8 females) using a visually guided inhibitory control task with distinct response modes related to "Non-Selective" (motor control) and "Selective" (motor control + inhibition) function. During the Non-Selective mode, participants tapped their right forefinger to flashing green or red probes (50/50% distribution, 100% responses). During the Selective mode, participants responded to green probes (80% responses) but inhibited responses to red probes (20% responses). Each response mode included a .1s probe stimulus duration and six 32s task epochs interspersed with 16s rest epochs. Additionally, the six epochs were subdivided into three epochs with periodically presented probes (Periodic) and three epochs with randomly presented probes (Random) to investigate effects on periodicity for both response modes. A task control-condition (visual fixation crosshair) was included as baseline. Nineteen consecutive ^{1}H MRS measurements were acquired during each mode (PRESS sequence, short echo time of 23ms, 6 averages per measurement). Glutamate was quantified using LCModel. Data was analyzed to assess changes in glutamate across task modes as well as interaction effects between response mode and periodicity (SAS GENMOD; SAS Institute).
Results: The task mode term failed to reach significance ($p=0.074$), but post hoc analysis showed increased dACC glutamate in both Non-Selective and Selective response modes (independent of periodicity) from the control-condition ($p=0.022$ and 0.042). The interaction term between response mode and periodicity failed to reach significance ($p=0.17$), but post hoc analysis for the Non-Selective mode showed an increase in dACC Glu during the random vs periodic ($p=0.020$).
Conclusion: Though preliminary, these results suggest that specially titrated inhibitory control task can be used in conjunction with ^{1}H fMRS to study the functional neurochemistry of the dACC. This framework can be extended to investigate potential dysfunctions in dACC glutamate modulation across a multiplicity of conditions characterized by poor inhibitory control.

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THE DEVELOPMENT OF A MACHINE LEARNING MODEL TO ANALYZE DIFFERENCES IN INDIVIDUAL RESPONSE ON FEAR CONDITIONING RESPONSES IN ZEBRAFISH (DANIO RERIO)

As observed across species, zebrafish (Danio rerio) exhibit individual differences in behavior which is an important evolutionary process that may affect individuals' fitness and survival in nature. Although it is known that strain and sex play a role in zebrafish individual differences in a novel environment and in response to stress, the study of both genetic background and sex was never studied for fear and memory in this species. However, one of the limitations of working with individual differences is that large sample sizes are necessary to have sufficient power to identify these differences. When working with fear-conditioning in zebrafish, a few behavioral parameters are often manually assessed such as freezing, erratic movement, burst swimming and backwards swimming. This raises the second limitation of working with individual differences in fear conditioning: The typical method of manually assessing the animals' behavior is subject to bias, can decrease data reproducibility, and is not feasible with large sample sizes. Here, we developed an approach to investigate individual differences in zebrafish responses to a fear conditioning task by automating the analysis of behavior using machine learning. Fish were recorded using an automated system (Zantiks AD), and tracked using DeepLabCut, a deep neural network to track 3 points on each animal (head, trunk, and tail). Using the frame posture information (x and y coordinates of the 3 points), we have labeled over fifty thousand frames containing the following behaviors: straight swimming, normal turn, freezing, erratic movement, burst swimming and backwards swimming. To allow us to analyze fish movement and train our model we created a sliding window of 45 frames (0.75s) containing 34 variables which included parameters such as absolute and net values of velocity, acceleration, fish turn angle, etc. We have used a Random Forest model to develop our machine learning model, and tested several parameters such as the number of variables randomly sampled (mtry) from 1 to 15 and different number of trees (ntree) 500, 1000 and 2000. We have found that a mtry of 12 and ntree of 1000 exhibit the lowest out of bag error (7.62%). Although both zebrafish normal swimming parameters (straight swimming and normal turn) showed the highest error values when predicting data (18% and 13%, respectively), these behaviors were mostly mistaken between each other. Thus, low error values were observed when looking at the abnormal fear-related behaviors exhibited by the animals such as freezing (9%), erratic movement (0.9%), burst swimming (1.9%) and backwards swimming (0.03%). Overall, the method developed here is a high throughput tool to assess fear conditioning responses in zebrafish and will facilitate the study of individual differences, decreasing human error and increasing data reproducibility.

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NOREPINEPHRINE DEPLETION LEADS TO A SEX-SPECIFIC IMPROVEMENT IN MEMORY CONSOLIDATION

Norepinephrine is largely synthesized and modulated by the locus coeruleus-norepinephrine (LC-NE) system; however, studies that examine this system's impact on the distinct phases of memory are scarce. This experiment selectively targeted the LC-NE system through the utilization of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4). DSP-4 works to rapidly and permanently deplete pre-synaptic norepinephrine (NE) stores within brain regions innervated by the LC-NE system. In this study, 16 male and 16 female Wistar rats were used, with half of each sex receiving an intraperitoneal injection of DSP-4 (50 mg/kg) or saline (control). Rats were kept in their home cages for 10 days following injections to allow for DSP-4 induced NE-depletion to occur. Following this period, rats were subjected to a 5-day Barnes maze assay. The Barnes maze apparatus is comprised of 20 uniformly spaced holes that line the perimeter of a circular table, with one of the holes leading to a hidden/goal zone. Rats were placed on the center of this maze and subjected to aversive auditory (80 dB white noise) and visual (bright light) stimuli that were terminated once they entered the hidden zone (for a total of two trials per day). Spatial learning was assessed using latency and distance traveled metrics. Following this 5-day Barnes maze paradigm, the rats remained in home cages for a two-day delay period. After this delay period, rats were subjected to a probe-trial (goal zone covered) allowing for assessment of memory consolidation. After probe-day, a two-day reversal learning assessment was conducted which utilized the same procedure as the original five-days; however, the open hole was shifted 90 degrees relative to its original location. After the first reversal was completed, a second was employed shifting the goal zone 90 degrees from the reversed location (180 degrees relative to its original position). In a previous study, we confirmed that NE was depleted following the last day of the 5-day Barnes maze training procedure. Overall, our behavioral results indicate that during the probe-day, male rats that received DSP-4 exhibited better memory consolidation in comparison to male control rats. Furthermore, during the reversal-learning period, male rats that received DSP-4 visited the location of the original open-hole more often than the male control group, suggesting that NE plays a significant role in regulating memory consolidation in male rats. Within the female cohort, no significant differences in memory consolidation or updating were observed. This indicates that the impact of NE depletion within the LC-NE system is sex-specific, but further investigations are needed to validate and extend these findings using multiple assessment paradigms.

Theme: Development

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THE IMPACT OF SLEEP-RELATED PROBLEMS ON ANXIETY SYMPTOMS IN ADOLESCENTS

Introduction: Recent studies indicate that sleep and anxiety symptoms share a close, bidirectional relationship. It's important that this relationship is further investigated given that anxiety disorders are the most common childhood mental disorders, affecting nearly one in three youth. Here, we assessed the relationship between sleep-related problems (SRPs; i.e., short sleep duration and poor sleep quality) and adolescent anxiety symptoms. We hypothesized that adolescents who experience more SRPs will report greater anxiety symptoms than those who report less SRPs.
Methods: This study included 23 adolescents (ages 10-16; M ± SD age = 12.87 ± 1.86; 56.5% male, 39.1% female, 4.3% genderfluid; 47.5% Black/Non-Hispanic, 47.5% White/Non-Hispanic, 4.3% White/Hispanic) from the Metro Detroit area. Data on SRPs were obtained from a self-report questionnaire, which inquired about participants' typical sleep patterns on school days and free days (e.g., weekends, holidays), as well as sleep quality and duration on the night prior to a study visit. Sleep duration was defined as the hours of sleep reported by the adolescent. Sleep quality was determined based on how well the participant reported sleeping the night prior on a Likert scale from 1 to 3 where 1 = bad and 3 = good. Participants' anxiety symptoms were assessed using the Screen for Child Anxiety Related Disorders, with higher scores indicating higher anxiety levels. Pearson bivariate correlations were used to evaluate the association between SRPs (i.e., sleep duration and sleep quality) and anxiety symptoms. Results were considered significant at a p-value of <0.05.
Results: A significant negative correlation between sleep duration on school days and anxiety symptoms ($r(19)=-0.483$, $p=0.036$) was observed; however, sleep duration on free days was not associated with anxiety symptoms ($r(21)=-0.096$, $p=0.677$). Average sleep duration the prior night was not significantly correlated with anxiety symptoms ($r(22)=-0.020$, $p=0.928$). There was also no significant correlation between sleep quality and anxiety symptoms ($r(22)=-0.291$, p=o.189).
Discussion: Here, we found that adolescents who sleep for shorter durations on school days report higher anxiety symptoms than adolescents who sleep for longer durations. Surprisingly, there was no significant association between sleep duration on free days, or the prior night's sleep, on overall anxiety symptoms. This could be because school days are more structured and mentally demanding than free days, and thus may require more rest. Further, our measure of anxiety symptoms captures longer-term mental health rather than state (i.e., in-the-moment) anxiety measures. Future research should examine this relationship with a larger sample size, and using daily sleep journals rather than retrospective reports.

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GENETIC VARIATION IN ENDOCANNABINOID SIGNALING AND THREAT- AND REWARD-RELATED BRAIN FUNCTIONING DURING THE TRANSITION INTO ADOLESCENCE

Introduction: The endocannabinoid (eCB) system plays a key role in modulating neural activity across the lifespan, and disruptions in eCB signaling are implicated in a variety of stress-related psychiatric disorders, including anxiety and depression. Neuroimaging studies in adults link a common genetic variant in fatty acid amide hydrolase (FAAH C385A) — the enzyme that regulates eCB signaling — to lower threat-related amygdala activity but higher reward-related ventral striatal reactivity. Further, FAAH genotype has been shown to modulate the association between amygdala reactivity and anxiety in adults. However, it is unclear whether similar patterns are observed earlier in development, particularly during preadolescence. Indeed, emerging cross-species data suggests that effects of FAAH genotype on anxiety and frontolimbic brain circuitry emerge during adolescence. Here, we leveraged neuroimaging data from the Adolescent Brain Cognitive Development (ABCD) study to test the impact of the FAAH C385A variant on threat- and reward-related neural activity in preadolescent children.
Methods: This study included longitudinal (Year [1-3]) neuroimaging, genetic, and behavioral data from 5,327 participants (46.7% female) from the ABCD study. We examined the effects of the FAAH C385A variant on anxiety and depressive symptoms using the Child Behavior Checklist (CBCL), on threat-related activity in the amygdala, reward-related activity in the nucleus accumbens (NAcc), and changes in brain-behavior associations.
Results: There were no main effects of FAAH genotype on threat-related amygdala reactivity, nor reward-related NAcc activity (p's > 0.3). However, FAAH genotype modulated the association between left amygdala reactivity to threat and anxiety symptoms ($F[7, 8,223] = 47.44$, $p < 0.001$; $t = X$, $p < 0.05$). In particular, there was a positive association between amygdala reactivity to threat and anxiety symptoms in youth with the A-allele during Year 1 only. In addition, There was a significant FAAH x time interaction for anxiety, which was associated with an increase in anxiety symptoms in A-alleles (but not CC genotypes) from Year 2 to Year 3 only ($p < 0.05$).
Conclusions: Our findings add to emerging evidence that the effects of the eCB system on the brain vary across development. In particular, main effects of the FAAH C385A variant on threat- and reward-related neural activity may emerge during adolescence or adulthood. However, the FAAH genotype modulates brain-behavior associations during preadolescence. Future studies, including longitudinal studies in the ABCD study, should explore the role of eCB signaling in modulating adolescent neurodevelopment and risk of stress-related disorders.

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CANNABIDIOL (CBD): EFFECTS ON ANXIETY, SEIZURE FREQUENCY, AND ENDOCANNABINOID LEVELS IN PEDIATRIC EPILEPSY PATIENTS

Epilepsy is the most frequent chronic neurologic condition in childhood. Cannabidiol (CBD, brand name Epidiolex) is one of the current Food and Drug Administration (FDA)-approved treatments for three severe forms of epilepsy for children ages 2+. However, the mechanism of CBD in the treatment of epilepsy is not clear. Interestingly, although preclinical data suggest that CBD affects the endocannabinoid (eCB) system and impacts mental health (i.e., reduces anxiety), few studies have examined the impact of CBD on peripheral circulating eCB concentrations or anxiety symptoms. Here, we present preliminary data from our ongoing 4-6 week observational study of epilepsy patients on clinically-indicated CBD treatment. We examined weekly change in seizure frequency, anxiety symptoms, and eCB concentrations in plasma, from baseline to the end of the study. To date, nine participants (ages 3-20 years; M ± SD age = 13 ± 5.2) have completed the study at the Children's Hospital of Michigan. The final sample size included 9 participants (N=1 removed for incomplete data). Paired-samples t-tests were used to compare outcomes between visits. Exploratory mediation analyses tested whether changes in anxiety mediated changes in seizure frequency or vice versa. Preliminary two-sample t-tests were used to compare eCBs at baseline between youth with epilepsy vs. healthy controls from another ongoing study (n = 3/group). Seven out of nine participants showed a reduction in seizure frequency and anxiety symptoms from baseline to the end of study. Across the sample, no significant difference was observed in seizure frequency ($p = 0.062$) nor anxiety ($p = 0.098$) between baseline and end visit. There was, however, a significant decrease in seizure frequency from baseline to Week 1 ($p = 0.016$). Change in anxiety scores did not mediate the change in seizure frequency over time, or vice versa ($p > 0.05$). Compared with age-matched healthy controls, youth with epilepsy had higher baseline eCB levels which reduced over time after starting treatment. However, these effects did not reach statistical significance. These preliminary data suggest that CBD is effective for reducing seizure frequency and anxiety symptoms in youth, with most youth showing reductions from baseline to the end of the study. Future analyses with this study cohort or a larger randomized controlled trial may help to understand the effects of CBD on anxiety, seizure frequency, and the eCB system. Although preliminary, these results are consistent with prior studies showing that CBD is beneficial for reducing seizures in youth with epilepsy. Further, our data suggest that CBD may also have effects on mental health, particularly anxiety.

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IMPACT OF THE PARENT-CHILD RELATIONSHIP ON FEAR EXTINCTION AND ANXIETY SYMPTOMS IN YOUTH

Introduction: Deficits in extinction learning and its recall have been implicated in the etiology of childhood anxiety disorders. Prior research has shown that anxious youth display higher conditioned fear responses, e.g., skin conductance responses (SCRs), than their non-anxious counterparts during extinction learning, indicating a hindered ability to extinguish fear. Importantly, recent publications suggest that parents can impact youth's risk of anxiety and fear learning. Indeed, studies have shown that youth can acquire fear vicariously by observing their parents undergoing fear conditioning, suggesting a transmission of fear/anxiety disorders from parent to child. Despite the salient role of parents in youth's fear regulation and anxiety, few studies have examined whether key aspects of the parent-child relationship, such as conflict and closeness, moderate the association between fear extinction and anxiety among youth. Here, we tested the hypothesis that close/conflicted parent-child relationships are related to better/worse extinction learning and recall and lower/higher anxiety in youth, respectively. We further predicted that both parent-child conflict and closeness moderate the link between extinction and anxiety.
Methods: This study included 25 10–16-year-old Metro Detroit youth (M ± SD age = 13.16 ± 1.95, 52% male, 52% White non-Hispanic). Youth completed a well-validated two-day fear extinction paradigm that involved fear conditioning, extinction, and recall phases. Conditioned fear was measured using SCRs and unconditioned stimulus (US) expectancy ratings. Extinction learning and retention indices were calculated, such that higher values indicate better extinction learning and recall, respectively. Youth completed self-reported measures of anxiety and conflict/closeness in parent-child relationships: Screen for Child Anxiety Related Disorder (SCARED) and Child-Parent Relationship Scale Short Form (CPRS-SF), respectively. First, Pearson correlation was used to test for associations among conflict, closeness, extinction learning, extinction recall, and anxiety. Then, the moderating effects of conflict and closeness on the association between extinction and anxiety was tested using linear regression analysis. Age was included as a covariate in all analyses.
Results: Overall, there were no significant associations between conflict/closeness and extinction learning, extinction recall, or anxiety (p's > 0.05). A regression analysis did not find a significant moderating effect of parent-child closeness or conflict on the relationship between extinction learning or recall and anxiety (p's > 0.05).
Conclusion: The present study did not find any significant correlations between parent-child conflict/closeness and extinction learning, extinction recall, or anxiety, nor any moderating effect of conflict or closeness on the association between extinction and anxiety. These results are inconsistent with the existing literature, which has established links between parents, anxiety, and extinction learning. Limitations of this study include a small sample size and a relatively wide participant age range. Future studies should improve on these shortcomings to further illuminate the role of parent-child relationships in fear extinction and anxiety in youth.

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IMPACT OF AN AUTISM-ASSOCIATED MUTATION IN GLUN2B ON THE CAMP RESPONSE ELEMENT-BINDING PROTEIN (CREB) SIGNALING PATHWAY

De novo mutations within the gene that encodes the N-methyl-D-aspartate receptor (NMDAR) subunit, GluN2B, have been associated with the emergence of autism spectrum disorder (ASD). The first mutation identified in ASD patients was a truncation that removes the cytoplasmic tail, fourth transmembrane domain, and part of the extracellular loop of GluN2B. Previous research in our lab has found that this mutation alters NMDAR function and dendrite development in cortical neurons. While we know that this mutation disrupts neuronal development, the exact mechanisms that link GluN2B truncation to abnormal dendrite development remain unknown. This project aimed to determine if mutant GluN2B alters the cAMP response element-binding protein (CREB) signaling pathway by using quantitative immunofluorescence imaging to test whether the ASD-associated GluN2B mutation reduces CREB activation.

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EXTINCTION LEARNING AND OBSESSIVE-COMPULSIVE SYMPTOM SEVERITY IN YOUTH

Obsessive-compulsive disorder (OCD) is a psychiatric disorder characterized by unwanted, obsessive thoughts or urges, typically followed by compulsive acts, rituals, or behaviors to combat the fear/anxiety derived from the obsessions. The fear/anxiety strongly tied to the obsession(s) is suggestive of a deficit in extinguishing learned fear. In addition, fear extinction is a core component of exposure response prevention, which is a form of cognitive behavioral therapy used to treat OCD. OCD affects 1-2% of children, and 80% of OCD cases begin in childhood, which is a sensitive developmental period when fear extinction mechanisms mature. Few studies, however, have examined fear extinction in youth with OCD diagnoses. These studies have yielded inconsistent results. Even fewer studies have examined the impact of obsessive-compulsive (OC) symptoms on fear extinction in an at-risk community sample of youth. The present study tested the hypothesis that youth with higher OC symptoms will show poorer fear extinction than youth with low OC symptoms. Forty-three children participated in this study (M+SD age=8.88+1.43 years, 51% female). The parent-reported Child Behavior Checklist was used to measure OC symptom severity and children were split into low OC (t-score=50) and high OC (t-score>50) groups. Participants completed a validated fear conditioning and extinction paradigm. During conditioning, participants are shown repeated presentations of conditioned stimuli (CS), either paired with an unconditioned stimulus (US) or not. The CS paired with the US was considered the fear cue (CS+) and the CS not paired with the US was considered the safety cue (CS-). During extinction, both cues were shown but neither paired with the US. Skin conductance responses (SCRs) and US expectancy ratings were measured during the experiment as physiological and subjective measures of conditioned fear, respectively. Independent samples t-tests were used to compare low vs. high OC groups in conditioned fear. The significance level was set at p=0.05. The high OC group showed significantly higher SCRs to the CS- during the second half of extinction as compared to the low OC group (p=0.027). There were no group differences to the CS+ during extinction or to either cue during fear conditioning. There were no group differences in US expectancy ratings during either phase. Our results show that children with higher OC symptoms exhibit altered extinction learning, such that an elevated fear response was still present for the safety (but not fear) cue at the end of the extinction session. These results suggest that youth at heightened risk of OCD exhibit uncertainty that a safety cue is indeed safe. Future research should explore whether altered fear responding to safety cues is relevant for identifying youth who are at risk, or can be used to improve treatments for OCD.

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OPIOID ADMINISTRATION DURING PREGNANCY: EFFECTS OF MORPHINE COMPARED TO BUPRENORPHINE EXPOSURE ON OFFSPRING NEURODEVELOPMENTAL OUTCOMES IN A TRANSLATIONAL RODENT MODEL

Opioid use during pregnancy has increased drastically within the last several years. To avoid harmful opioid-induced effects on the fetus, pregnant women who use opioids are often prescribed Medications for Opioid Use Disorder (MOUDs), including buprenorphine (BUP) or methadone. However, exposure to synthetic opioids during pregnancy is known to negatively impact offspring neurodevelopment. Importantly, it is not yet understood how gestational BUP exposure may affect fetal brain development. In the current study, we used a translational rodent model to investigate offspring neurodevelopmental outcomes following discontinued or continued administration of morphine (MS; to mimic opioid use disorder) or BUP (to mimic MOUD treatment). Seven days prior to pregnancy, female rats were administered either MS (3-6.0mg/kg, b.i.d., s.c.) or BUP (1.0mg/kg, q.d., s.c.) until gestational day (GD) 19 ('discontinued', mimicking withdrawal before parturition) or until postnatal day 2 (PN2, 'continued' through parturition). Pups were sacrificed on PN2, and brains and trunk blood were collected for subsequent analysis. Both continued and discontinued BUP exposure resulted in higher pup mortality, reduced body weight, and increased neonatal withdrawal symptoms compared to MS-exposed pups and controls. On PN2, discontinued BUP exposure resulted in fewer visible milk bands in pups compared to other groups, while discontinuation of both opioids resulted in an increase in righting latency compared to the continued groups. Preliminary results indicate that BUP levels were very low in pups on PN2, especially in the discontinued group; while pups exposed to continued MS seemed to accumulate drug in their system (i.e., elevated serum levels compared to dams'). Offspring brains are currently being analyzed for neurotransmitter levels (dopamine, serotonin, norepinephrine, and common metabolites DOPAC, 5-HIAA, HVA & 3-MT) in the prefrontal cortex, hypothalamus, and hippocampus. This will allow us to measure the impact that gestational exposure to these opioids has on neurotransmitters during early fetal development. Taken together, our results suggest that BUP use during pregnancy negatively influences pup survival despite very low levels of BUP in the pups' system after birth. More research is crucial to investigate the underlying mechanisms associated with offspring outcomes following prenatal BUP exposure with the goal of improving clinical outcomes in pregnant women undergoing treatment for opioid use disorder.

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RACIAL DISCRIMINATION AND ASSOCIATIONS WITH ANXIETY SYMPTOMS IN DETROIT-AREA YOUTH

Abstract:
Experiences of racial discrimination have been associated with disparities in physical and mental health. Indeed, research on Black adult women suggests that women who experience more racial discrimination are at increased risk of physical and mental disorders, including depression and anxiety. Importantly, recent research shows that these effects may also extend to youth, for example, elevated depression, anxiety, and low self-worth. This is important because mental disorders, including anxiety disorders, typically begin during adolescence. Environmental exposures, such as racial discrimination, may increase the risk of mental health issues in youth. The present study tests the hypotheses that 1) adolescents who experience more racial discrimination will report higher anxiety symptoms compared to adolescents who experience less discrimination and 2) this association will be stronger among Black, as compared to White youth. Twenty-three Detroit-area 10-16 year-olds completed this study (mean age= 12.87 + 1.87 years), and the sample was 47.8% Black and 52.2% White. Racial discrimination was assessed using the 10-item Perceptions of Racism in Children and Youth (PRaCY) questionnaire. PRaCY scores range from 0-10 where 0 = no racial discrimination, 10 = highest racial discrimination. Adolescents also self-reported their anxiety symptoms using the 41-item Screen for Childhood Anxiety-Related Disorders (SCARED). SCARED scores range from 0 to 82, where 0 = no anxiety symptoms, 82 = highest possible anxiety. Pearson Bivariate correlations were used to assess the relationship between racial discrimination and anxiety symptoms in youth across the entire sample, and among Black youth separately. Additionally, a two-sample t-test was used to compare discrimination between Black and White youth. Overall, 69.6% of youth reported experiencing racial discrimination, with the average PRaCY score of 2.52 (SD = 2.78). Youth who experienced more racial discrimination reported higher anxiety scores, $r(23) = 0.51$, $p = 0.012$. Within Black youth, 90.9% reported experiencing racial discrimination, with the average PRaCY score of 3.73 (SD = 3.03). Black youth reported experiencing more racial discrimination than White youth, $t(21) = 2.15$, $p = 0.043$. Black youth who experienced more racial discrimination reported higher anxiety scores, $r(11) = 0.64$, $p = 0.033$. Overall, Black youth experienced more racial discrimination as compared to White youth, and this experience was positively related to anxiety symptoms. Our results are consistent with data from previous cross-sectional and prospective studies in adults and suggest that exposure to racial discrimination also impacts youth and may increase the risk for developing subsequent mental disorders, like anxiety. Together, these findings provide additional evidence on the harmful effects of racial discrimination on mental health in youth. Future studies on coping strategies specific to racism-related stress and anxiety are needed to inform approaches to intervention.

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IS DIET ASSOCIATED WITH ANXIETY SYMPTOMS IN YOUTH?

Introduction: Dietary patterns can lead to metabolic pathologies (e.g. inflammation) within the gut microbiome. Further, changes in the gut microbiota have been shown to affect the brain through the gut-brain axis, which may, in turn, increase risk of mental disorders. However, studies examining the link between diet and mental health have focused primarily on adult populations. This is a critical gap given that mental disorders often begin during childhood and adolescence, which coincides with rapid changes in brain development. While few studies have begun to examine the link between diet and mental health in youth, these studies have focused primarily on symptoms of externalizing disorders, such as ADHD. Less is known about how diet relates to symptoms of internalizing disorders, such as anxiety disorders, which are the most common childhood mental disorders. The objective of the present study is to examine the impact of diet on anxiety symptoms in youth.
Methods: Participants included 11,876 children (45.1% female, 25.9% non-white race, mean ± SD age 10.92 ± 0.64 years) from the Adolescent Brain Cognitive Development (ABCD) study, which is a large study of child development across 21 sites in the US. Diet was measured using the parent-reported Child Nutrition Assessment, which inquires about what children eat in a typical week. We focused on questions assessing the consumption of (1) leafy green vegetables (7 or more times/week), (2) other vegetables (1 or more times/day), and (3) fast food (less than 1 time per week). Anxiety symptoms were measured using the parent-reported Child Behavior Checklist (CBCL). Independent samples t-tests were used to test for significant ($p < 0.05$) differences in anxiety symptoms between youth who did vs. did not regularly eat vegetables or abstain from eating fast food.
Results: Overall, 44.5% of youth eat green leafy vegetables six or more times per week, 82.8% eat other vegetables one or more times per day, and 65.4% eat fast food less than once per week. Youth who regularly eat green leafy vegetables and other vegetables have significantly lower anxiety scores as compared to youth who do not regularly eat vegetables, p's < 0.001. In addition, youth who do not regularly eat fast food had significantly lower anxiety scores than youth who do ($p < 0.001$).
Conclusion: Our results show that youth who eat a healthy diet consisting of green leafy vegetables, other vegetables, and less fast food, have significantly lower anxiety symptoms than youth who consume a more unhealthy diet. These findings fit with prior studies showing that diet can impact mental health outcomes in both adults and youth. Diet and nutrition modifications should be evaluated as an intervention to reduce risk of mental disorders.

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A TRANSLATIONAL RODENT MODEL OF OPIOID EXPOSURE DURING PREGNANCY: EFFECTS OF MORPHINE COMPARED TO BUPRENORPHINE ON MATERNAL BEHAVIOR, BRAIN, AND THE MICROBIOME

The recent opioid epidemic has resulted in many opioid-dependent pregnant women receiving opioid maintenance therapies. Buprenorphine (BUP) or methadone are pharmacological treatments used to reduce negative effects of misused opioids on the mother and developing fetus. Clinically, BUP produces preferable outcomes for exposed infants as compared to methadone or opioid misuse. However, a dearth of knowledge remains for BUP's effects on neural networks that are critical during the transition to motherhood and for maternal caregiving behaviors. BUP's mechanism of action (partial mu-agonist/kappa antagonist) varies significantly from morphine's (MS; full mu-agonist), which may result in a different impact on the maternal brain during a critical neuroplasticity period. In the current study, we used a translational rodent model to mimic chronic opioid (mis)use (MS exposure, 3-6mg/kg/day, b.i.d.) or opioid maintenance drug (BUP exposure, 1mg/kg/day) to investigate the behavioral and neurochemical consequences of gestational opioid exposure on dams and their offspring. Opioid or saline administration to female rats (N=50) via subcutaneous injections began 7 days prior to mating and continued daily throughout pregnancy until postpartum day 2 (PD2) or was discontinued on gestational day 19 to allow for drug clearance before parturition. Dams' maternal behaviors were monitored through detailed observations of pup-directed and non-pup directed behaviors. Dams were also evaluated with several behavioral tests, including a pup retrieval test, a hunting task, and two-chamber pup-odor preference test. Our preliminary findings indicate that continued and discontinued BUP exposure resulted in more maternal care deficits, increased postpartum pup mortality, and maternal deficiencies in the pup retrieval and pup-odor preference test, but not in the hunting task, as compared to our control group. Conversely, MS may have resulted in fewer pregnancies (despite obvious sperm in vaginal lavage samples), but care behavior and survival rates of the MS groups varied little from controls. Interestingly, each opioid also resulted in a unique change in the microbiome profile of the gut. Using high performance liquid chromatography, we will further analyze neurotransmitter levels and their metabolites in the maternal brain (collected on PD2) to investigate potential neurological effects of these opioids on the maternal brain network. More research is critical to elucidate how BUP mechanistically interacts with the neural network during the transition to motherhood to help alleviate possible negative consequences.

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LEAD EXPOSURE RISK AND FUTURE MENTAL HEALTH SYMPTOMS IN YOUTH: PRELIMINARY RESULTS FROM THE ABCD COHORT

Introduction: The neurobehavioral consequences of childhood lead exposure, such as decreased IQ, poor academic achievement, and hyperactivity, are well documented. However, less is known about the impacts on mental health. Lead exposure in adulthood, even at levels below standard guidelines, has been associated with increased prevalence of both depression and anxiety disorders in adults. Recent research has shown that childhood lead exposure is also associated with future mental health issues into adulthood. However, what remains unknown, is 1) whether childhood lead exposure is associated with mental health issues in childhood and adolescence and 2) when this impact on mental health first occurs. This longitudinal study will characterize lead exposure risk on the emergence of mental health symptoms during the transition from childhood to adolescence.
Methods: The study sample is comprised of 5,553 youth from the NIH's Adolescent Brain and Cognitive Development (ABCD) longitudinal nationwide study, starting at 9-10 years old, with follow-ups every year. The sample was predominately male (53.1%) and White (59.0%). The mean age at baseline and year 3 follow up was 9.98 and 12.90 years, respectively. The lead risk scores were estimated from each participant's census tract. The Washington State Department of Health derived scores using a weighted sum of the ages of homes and poverty rates. The lead risk scores range from 1=low risk to 10=highest risk. Mental health symptoms were measures at baseline and each follow-up using the parent-reported Child Behavioral Checklist (CBCL). A causal Directed Acyclic Graph was used to identify the following potential confounders to include in analyses: air pollution, neighborhood safety, trauma exposure, family income, green space, and baseline age. Linear mixed effects models were used to examine the association between lead risk and mental health symptoms.
Results: No significant associations between lead risk scores and changes in mental health symptoms over time were observed ($p > 0.05$). CBCL withdrawn/depressed T score significantly decreased over time, however this association no longer reached significance following correction for multiple comparisons.
Discussion: Our results suggest that increased lead risk is not associated with the development of mental health symptoms during childhood and early adolescence (9-13 years). While prior studies have found significant associations between childhood exposure and mental health in adulthood, our null findings in early-adolescence suggest that these symptoms may develop in mid- to late- adolescence. As the ABCD study continues yearly follow-ups, we will extend these investigations to timepoints in mid to late adolescence (15-17 years), to identify when the impact on mental health first occurs. Additionally, future studies should investigate the neurobehavioral mechanisms underlying this association, so that strategic interventions may be devised to counteract the negative effects of lead exposure on adolescent health outcomes.

Theme: Integrative Physiology and Behavior

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SUBCHRONIC MITRAGYNINE CAUSES SEX-DEPENDENT CHANGES IN BRAIN CYTOKINE LEVELS IN RATS WITH MINIMAL COGNITIVE AND LOCOMOTOR EFFECTS

Mitragyna speciosa (Kratom) is a medicinal plant indigenous to Southeast Asia and Africa with a long history of use for a variety of ailments, including diarrhea, pain relief, cough suppression, and amelioration of opiate withdrawal symptoms. Despite its status as an herbal remedy, Kratom is banned in some countries due to a myriad of negative health risks. Preclinical psychopharmacology studies of mitragynine (MG), the main alkaloid of Kratom, indicate it has opioid-like effects, with lower potency than morphine. Lower doses reportedly produce psychostimulant-like effects. Few studies have evaluated the neurobehavioral effects of repeated MG exposure. The current study utilized a repeated measures design to evaluate the effects of repeated MG (0, 1, 10 mg/kg) exposure on locomotor activity and on the acquisition of a spatial memory task in Wistar-Han rats (24 M, 24 F, N=8 per group). For the locomotor activity assay, rats were injected every other day for five days and activity was recorded in an open field for 1 h immediately before and 1 h immediately after injections. Activity measures assessed included total distance, stereotypy, and time in center. The same rats were assessed one week later for spatial memory acquisition in an eight-arm radial maze while injections continued daily after each learning trial for eight days. Maze acquisition was assessed by latency to complete each trial, total arm entries, and repeat arm entries. Twenty-four hours after the last injection, rats were euthanized and brains were harvested and stored at -80 º C for tissue analysis. The amygdala, hippocampus, periaqueductal gray (PAG), and prefrontal cortex (PFC) were dissected and assessed by Luminex (IL-1β, IL-6, IL-10, IL-17A, CCL2/MCP-1, CCL5/RANTES) or ELISA (CXCL12/SDF-1α) to determine tissue cytokine/chemokine levels. Both MG doses produced stronger psychomotor stimulant effects in females compared to males, though neither dose produced locomotor sensitization in either sex. RAM acquisition was unaffected by mitragynine treatment in either sex. In male brains, most cytokines were significantly reduced in the amygdala, hippocampus, and PAG after repeated exposure to 10 mg/kg MG, and 1 mg/kg produced significant reductions in all cytokine levels in the amygdala. In female brains, similar results were obtained in the amygdala. However, hippocampal cytokine levels were elevated by 1 mg/kg MG in females. Interestingly, cytokine levels in the PFC were not impacted in male or female rats. In summary, despite nonsignificant effects in behavioral assessments of activity and spatial memory, brief intermittent MG exposure caused region- and sex-dependent changes in brain cytokine levels.

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BDNF-RELATED MECHANISMS OF NEUROPLASTICITY IN THE ROSTRAL VENTROLATERAL MEDULLA OF SEDENTARY VERSUS ACTIVE RATS

Overactivity of the sympathetic nervous system has been implicated in cardiovascular disease (CVD), the leading cause of death globally. Sympathetic nerve activity is regulated by a region in the brainstem known as the rostral ventrolateral medulla (RVLM). The RVLM contains sympathoexcitatory neurons, which when overactive, likely contribute to hypertension, a leading risk factor for CVD. RVLM neurons undergo structural and functional neuroplasticity. For example, our laboratory has reported that RVLM neurons of sedentary animals have increased dendritic branching, suggesting a mechanism toward greater sympathoexcitation observed following sedentary conditions. Since regulation of dendritic branching occurs through a balance between pro- and mature forms of brain-derived neurotrophic factor (BDNF), it's not surprising that sedentary conditions result in higher mBDNF/proBDNF expression in subregions of the RVLM associated with increased dendritic branching. Conversion of proBDNF to mBDNF is facilitated by tissue plasminogen activator (tPA), suggesting that differences in the expression of tPA could contribute to physical (in)activity-induced neuroplasticity. The purpose of this study was to determine whether tPA expression in the RVLM is regulated differently under sedentary versus active conditions. We hypothesized that tPA levels are higher in more rostral regions of the RVLM of sedentary versus active animals. Four-week-old male Sprague-Dawley rats were divided into two groups (n=4 each), Active (in-cage running wheels) or Sedentary (no running wheel) for 12 weeks. Rats were sacrificed for fresh tissue removal under deep anesthesia, brainstems were cryosectioned serial sections (80 µm) were collected. Bilateral tissue punches of RVLM were retrieved. Post-punched brain sections were stained with cresyl violet to determine the rostrocaudal boundaries of the RVLM. Punches were pooled for gel electrophoresis and expression levels of tPA were detected using validated antibodies. Immunoblots were captured using a Typhoon. Bands representing tPA and GAPDH were analyzed using ImageQuant software. Our results demonstrate that tPA/GAPDH increased in a caudal to rostral fashion only in sedentary animals, with the most rostral region having significantly higher tPA/GAPDH than two caudal regions ($p=0.0006$, for both). tPA/GAPDH remained constant in the RVLM of active animals ($p>0.05$, between all levels) and levels in the most rostral region appeared to be lower compared to sedentary rats ($p=0.07$). Interestingly, tPA/GAPDH was significantly higher in the most caudal region of the RVLM in sedentary versus active rats (1.70± 0.22 versus 1.03± 0.60, respectively; $p=0.017$). Our data suggests that increased tPA in sedentary individuals may drive a greater conversion of pro-BDNF to mBDNF. However, higher tPA expression in more caudal portions of the RVLM suggest additional factors influence dendritic branching in sedentary rats. Thus, additional studies are necessary to clarify the role of tPA and other factors involved in (in)activity-induced neuroplasticity of RVLM neurons involved in blood pressure regulation. (1R01-HL161233)

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CHEMOGENETIC INHIBITION OF OXYTOCIN RECEPTOR EXPRESSING NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS HAS SEX-SPECIFIC EFFECTS ON JUVENILE SOCIAL BEHAVIOR

Social animals have innate behaviors that promote peer-to-peer familiarity. Two such behaviors are juvenile social investigation, an individual's tendency to approach and explore their peers, and social play, a behavior seen in juveniles involving two individuals reciprocally engaging each other physically with no harmful intent. These behaviors are also displayed by human children but differ between neurotypical children and children with social deficit disorders, such as autism. Exploring the underlying causes of these differences gives us the opportunity to improve social experiences for individuals with social deficits disorders. These disorders are often correlated with differences in the activity of oxytocin (OXT), a neuropeptide involved in many social behaviors. The Bed Nucleus of the Stria Terminalis (BNST) is a brain area involved in similar social behaviors, including social investigation. Research in rats showed that the BNST contains a high density of OXT receptors (OXTR) with males having a higher density than females. In contrast to adults, the role of OXTR in the BNST in juvenile social behavior remains largely understudied. We aim to understand the role of OXTR-expressing neurons in the BNST on social behavior in juvenile rats. We utilized juvenile male and female OXTR-iCre rats to determine the effects of chemogenetic inhibition of OXTR-expressing neurons in the BNST on social investigation and social play behavior. We infused a Cre-dependent inhibitory DREADD into the BNST of OXTR-iCre and Wild-type rats at postnatal day 23. This was followed by intraperitoneal infusion of the DREADD ligand Clozapine-N-Oxide (CNO) or saline 30-minutes prior to testing for social investigation and social play behavior on postnatal days 33 and 34. Rats received CNO and saline in a counter-balanced way across the two days of testing. A sex-matched conspecific was placed into the homecage of the test animal for 4-minutes. Test sessions were video recorded for behavioral analysis. We observed a sex difference in the effect of chemogenetic stimulation of OXTR-expressing neurons in the anterior BNST: Juvenile female OXTR-iCre rats showed an increase in the duration of social play, while social investigation and overall social behavior (social play + social investigation) levels remained unchanged. In contrast, juvenile male OXTR-iCre rats showed a decrease in overall social behavior. Chemogenetic stimulation of the posterior BNST had no effect on social behavior in either male or female juvenile OXTR-iCre rats. From these findings, we can conclude that inhibition of OXTR-expressing neurons in the anterior BNST of female juvenile rats increases play behavior and decreases overall social behavior in male juvenile rats. This study provides a better understanding of this sex difference in the role of OXT and the BNST in social behavior. These findings could help us understand why social deficit disorders like autism are more prevalent in male versus female children.

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REGULATION OF TGF-β SIGNALING IN C. ELEGANS BY THE POST-TRANSLATIONAL MODIFICATION PROTEIN AMPYLATION

Post-translational protein modifications (PTMs) are fundamental components of the proteome that enhance functional diversity and play critical roles in the regulation of several biological processes. Our work suggests that the emerging post-translational modification, AMPylation, plays a role in the regulation of TGF-β signaling in C. elegans. In C. elegans there are five TGF- β -related ligands (dbl-1, daf-7, unc-129, tig-2, and tig-3) in which only two TGF-β ligands (DAF-7 and DBL-1) have been well characterized. DAF-7 ligand-dependent signaling regulates metabolic changes associated with the diapause stage, and DBL-1 ligand-dependent signaling regulates body morphology through the activity of the sma (Small) genes, as well innate immunity, and reproduction aging. Here, we show that over-expression (OE) of the constitutive AMPylase FIC-1(E274G) contributes to the regulation of adult body growth, and larval entry into dauer stage; all processes controlled by TGF-β signaling. Our data also shows FIC-1(E274G) OE inhibits pathogen avoidance behavior by selectively suppressing the production of DAF-7 and DBL-1 ligands in ASI sensory neurons. These results suggest a new avenue in which TGF-β signaling can be post-translationally regulated.

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ROLE OF MC3R IN ENERGY HOMEOSTASIS USING AN ADULT KNOCKOUT MOUSE MODEL

The central melanocortin system is essential for the control of energy homeostasis. The germline MC3R knockout mice exhibit a mild increase in adipose mass and loss of lean mass under normal growth and dietary conditions. Further, using the germline MC3RKO, the melanocortin-3 receptor has been shown to be involved in the control of both the upper and lower boundary conditions of energy homeostasis; these mice lose more weight compared with WT mice when exposed to behavioral or pharmacologic anorexic challenges, yet gain more weight when provided high fat diet. To circumvent possible development effects that may be present in the germline deletion of the Mc3r gene, we used mice carrying a Cre-recombinase-Estrogen-Receptor-T2 (Cre-ERT2) allele targeted to the ubiquitously expressed ROSA26 locus and enabled temporal control of Mc3r expression by tamoxifen induction in vivo. We injected male and female mice with either 100mg/kg tamoxifen (KO; N=40) or vehicle (100µl, corn oil, N=40). Body composition was measured weekly using a Bruker minispec and all food and body weights were measured daily. We also studied anorexic behavioral stressors, including social isolation anorexia and novelty suppressed feeding. We found that social isolation had no effect on food intake in male mice. In contrast, female mice showed significant reduction in food intake after 6 hours of being singly housed when Mc3r was deleted in the adult mouse. Additionally, we observed that adult MC3RKO female mice significantly took longer time to consume food in a novelty suppressed feeding paradigm compared to control females. We did not observe any differences in fat mass between the oil and tamoxifen treated animals, but we did detect a trend in lower lean mass for the treated mice. Overall, we have found that the adult MC3RKO recapitulates the behavioral phenotypes identified in the germline MC3RKO. We are currently collecting additional data on feeding and body composition and conducting additional experiments to study other phenotypes to rigorously compare the many reported phenotypes in the germline MC3RKO with hormonally-induced adult MC3R knockout mice.

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LOW DOSE 1-(BENZOFURAN-5-YL)-N-METHYLPROPAN-2-AMINE (5-MAPB) DOES NOT ALTER ANXIETY-LIKE BEHAVIORS IN A RODENT ELEVATED PLUS MAZE

3, 4-methylenedioxymethamphetamine (MDMA) is an amphetamine derivative with demonstrated clinical efficacy for the treatment of post-traumatic stress disorder (PTSD). As a popular recreational drug, MDMA also poses a public health risk, particularly when misused at high doses in uncontrolled settings. Preclinical studies are essential to the development of safer alternative therapeutic agents. Benzofurans comprise a group of synthetic phenethylamines with similar pharmacological actions to MDMA. Several recent studies have evaluated the neurochemical and behavioral effects of selected benzofuran molecules, though no published studies have investigated these drugs in animal models of anxiety. Contrary to clinical reports, preclinical screening for anxiety in the mouse elevated plus maze (EPM) indicated MDMA exerts anxiogenic effects at low doses, but may have anxiolytic effects at higher doses. The current study employed the EPM to assess behavioral effects of acute low dose treatment with 1-(Benzofuran-5-yl)-N-methylpropan-2-amine (5-MAPB) in rats. Twenty-four experimentally naïve adult male Sprague-Dawley rats were randomly assigned to three treatment groups (0, 0.31 mg/kg, 1.24 mg/kg 5-MAPB, N=8). Following a 15 min acclimation to the test environment, rats received an intraperitoneal injection of saline or 5-MAPB 30 min prior to placement in the EPM for 5 min. Each rat was individually placed in the EPM, while an overhead camera recorded maze activity. Three independent observers blind to treatment assignments scored video recordings for number of entries, explorations, and time spent in open and closed arms. A two-way repeated measures ANOVA (arm type, dose) indicated a statistically significant difference between open and closed arms for all dependent variables, though the treatment effect was not statistically significant. A one-way ANOVA was also conducted on difference scores calculated between closed and open arms for each dependent variable. This analysis yielded a significant treatment effect on closed-open arm time, but not on closed-open arm entries or explorations. Tukey multiple comparisons revealed statistical significance only between the 0.31 mg/kg and 1.24 mg/kg treatment groups and neither of these groups differed significantly from saline controls. These results indicate that at low doses, 5-MAPB does not alter anxiety-like behaviors in the rat EPM. Additional investigations with a wider dose range and other behavioral assays are required to fully characterize the behavioral effects of 5-MAPB and related benzofurans. Such investigations will serve to inform further development of these molecules for therapeutic potential.

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LOCOMOTOR STIMULANT EFFECTS AND PERSISTENT SEROTONIN DEPLETION FOLLOWING BINGE-LIKE 1-(BENZOFURAN-5-YL)-N-METHYLPROPAN-2-AMINE (5-MAPB) TREATMENT IN SPRAGUE-DAWLEY RATS

The emergence of novel psychoactive substances (NPS) on the illicit drug market is cause for public health concern. Benzofuran molecules with structural similarities to the entactogen, 3,4-methylenedioxymethamphetamine (MDMA), have been examined in recent preclinical studies of NPS for both abuse liability as well as medication development. Microdialysis studies in mice indicate 1-(Benzofuran-5-yl)-N-methylpropan-2-amine (5-MAPB) may pose a higher risk for acute toxicity compared to equimolar amounts of MDMA. 5-MAPB has been marketed as a less neurotoxic analogue of MDMA, but no studies have addressed whether 5-MAPB can cause the long lasting serotonergic changes seen with high or repeated MDMA dosing. The current study employed locomotor activity screening followed by neurochemical analyses to evaluate the effects of repeated 5-MAPB dosing in rats. Forty-eight male Sprague Dawley rats were randomly assigned to one of three treatment conditions (0, 1.2, or 6 mg/kg 5-MAPB, n=16). Intraperitoneal injections were administered every two hours for three injections over the course of a single day to mimic binge-like use. Locomotor activity was monitored in an open field for one hour prior to injections and for two hours after each successive injection. Rats were euthanized 24 hours or two weeks after the last injection (n=8 per group), and brain tissues were rapidly dissected and immediately frozen for later analysis. Prefrontal cortex, striatum, and hippocampus were analyzed for monoamine content via High-Performance Liquid Chromatography (HPLC) with electrochemical detection. Acute injections of 6 mg/kg 5-MAPB produced a statistically significant increase in distance traveled, time in center, and stereotypy counts following the first injection, though these effects were reduced after subsequent injections. Increases in stereotypy counts, time in center, but not distance traveled were significantly higher following 1.2 mg/kg 5-MAPB compared to saline-treated rats, and time in center showed a cumulative increase with repeated injections of this dose. Neurochemical analyses indicated a statistically significant reduction in 5 HT and 5-HIAA in all brain regions assessed 24 hours and two weeks after 6 mg/kg 5 MAPB, with no statistically significant differences in monoamine levels between 1.2 mg/kg and saline-treated rats. There were also non-significant trends for reductions in striatal dopamine at both time intervals after 6 mg/kg 5-MAPB. These results show that 5-MAPB can dose-dependently produce persistent changes in 5-HT and 5-HIAA that appear analogous to those produced by MDMA.

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THE EFFECTS OF PHYSICAL ACTIVITY ON PANAS-C SCORES IN YOUTH

Purpose: Positive and Negative Affect Schedule for Children (PANAS-C) is a scale used to measure changes in youths' emotions after engagement in common daily activities. Exercise is an activity known to positively influence factors of emotion yet there is limited research in youth that uses the PANAS-C to investigate associations between exercise and changes in emotion. Therefore, the current study aims to identify the effects of a 30-minute acute exercise session (treadmill walking/running or stretching) on PANAS-C scores. An exploratory aim for this study is to investigate the relationship between perceptions of physical activity (PA) engagement and PANAS-C scores. We hypothesized that the completion of a 30-minute exercise session will 1) increase positive affect schedule scores and 2) reduce negative affect schedule scores in youth across exercise modalities. It is also hypothesized that positive perceptions of PA engagement will be significantly related to positive affect schedule scores in youth. Methods: The study sample included 14 participants residing in an urban setting, aged 9-17 years old. Participants were randomly assigned one of two conditions, treadmill exercise (N=9) or stretching (N=5). PANAS-C and perceptions of PA were measured before and after the 30-minute exercise session. Bivariate Correlations were used to explore associations between PA and PANAS-C scores among participants. A repeated-measures ANOVA was used to determine the effect of time (pre and post for positive and negative affect) and condition (treadmill or stretching) on PANAS-C scores in youth. All analyses considered p-value, $p<0.05$ significant. Results: Significant associations between PANAS-C positive affect scores and participants finding PA enjoyable ($p=0.679$), pleasurable ($p=0.691$), or exciting ($p=0.735$) were identified at the 0.05 level. A significant correlation was observed between pre and post positive affect scores ($p=0.854$), however, no relationship was observed in pre and post negative affect scores($p=0.474$). No significant relationship between time or exercise condition for PANAS-C scores was detected, however, there was a significant increase in positive affect scores and a significant decrease in negative affect scores from pre- to post- intervention Conclusions: Findings from this preliminary and ongoing study suggest that acute exercise increases positive affect and reduces negative affect in youth regardless of the condition (i.e., treadmill, stretching). Results also suggest that positive perceptions of PA are significantly related to positive affect schedule scores. These results may be useful for future intervention development aiming to improve the mood of youth populations. Future research should examine the longitudinal impact of PA on PANAS-C scores in youth. Future studies should also examine exercise intensity and duration to further identify how PANAS-C scores may be affected.

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SEX DIFFERENCES IN LOCOMOTOR ACTIVITY AND BEHAVIORAL SENSITIZATION IN RATS ADMINISTERED FENTANYL

Opioid overdoses have continued to increase, and women have experienced a greater rate of increase than men. Preclinical studies demonstrate marked sex differences in addiction-related behaviors, with females being more vulnerable due to a potential role of estradiol. We sought to understand how the estrous cycle, as a proxy measure of estradiol, influences sex differences in the sensitizing effects of fentanyl. In this ongoing study, we used male and female rats to investigate potential sex effects of fentanyl (20μg/kg subcutaneously) administration for 14 days and a forced abstinence period of 13 days on behavioral sensitization via locomotor activity (LMA) following a fentanyl challenge on day 28. Vaginal lavage samples were collected on days 1, 14, and 28, and cytological characterization was used to determine the estrous stage for estrus versus non-estrus comparisons. Consistent with previous research, fentanyl induced significantly higher levels of LMA compared to the control groups in both sexes. Further, female subjects exposed to fentanyl experienced significantly greater LMA on day 14 (last day of repeated administration) and day 28 (challenge after abstinence) compared to fentanyl exposed males. The day 1 estrus group exposed to fentanyl demonstrated a significant increase in LMA from day 1 to 14 and from day 1 to 28. The day 1 non-estrus group exposed to fentanyl showed a significantly greater LMA from day 1 to day 14 but not from day 1 to day 28. There was no significant difference in LMA from day 1 to 14 or from day 1 to 28 between estrus and non-estrus groups. Our data validate previous investigations showing a greater impact of fentanyl on LMA and behavioral sensitization in females; however, our current data does not support the estrous stage impacting the change in LMA acutely or following an abstinence period. More research into the effect of the estrus cycle and corresponding estradiol fluctuations on fentanyl sensitization is needed.

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ESCALATING MORPHINE AND NALOXONE-PRECIPITATED WITHDRAWAL EFFECTS ON CONDITIONED FEAR LEARNING IN SPRAGUE DAWLEY RATS; A FEAR-POTENTIATED STARTLE (FPS) STUDY.

Opioid use disorder (OUD) is highly comorbid with stress. National surveys have shown that up to 33% of patients with OUD met the criteria for posttraumatic stress disorder (PTSD) diagnosis vs. only 7% of the population. Great effort has been made to investigate the effects of extreme stress on the risk of opioid use. Fewer investigations have explored the opposite: how opioid use affects threat perception and reactions to extreme stress. Opioids can produce profound changes in associative learning mechanisms, leading to dependence and altered fear learning following stress exposure. Fear conditioning and its sequelae are laboratory models of the associative learning processes that appear to underlie PTSD development and symptomatology. In this study, we used 47 male Sprague Dawley rats to: 1) develop a replicable FPS paradigm that allowed for the study of fear learning outcomes (n=32) and 2) determine the effect of morphine withdrawal on FPS learning outcomes, including fear expression, extinction learning, extinction recall, and subsequent reinstatement (morphine: n=7, saline: n=8). Escalating doses of morphine were administered twice daily for 10 days (5 mg/kg + 5 mg/kg/day) to a final morphine dose of 50 mg/kg. Morphine injections were vehicle controlled by a saline group. All animals received a single naloxone injection (1.5 mg/kg) after the last dose of morphine and prior to fear conditioning.
RESULTS: (all F tests listed are 2way ANOVA)
1) For development of FPS parameters, we found a main effect of fear conditioning trial type ($F(4, 116) = 83.3$, $P<0.0001$). Tukey's multiple comparisons showed significant increase in startle in the presence of the conditioned stimulus (CS) as compared to noise alone ($p<0.0001$), a significant within-session decrease in FPS during extinction ($P<0.0001$), and no significant difference in FPS between extinction end vs. extinction retention test. 2) Morphine withdrawal experiments showed robust fear expression in both morphine and saline groups ($F(2, 14) = 34.7$; $p<0.0001$). The extinction retention test revealed both morphine and saline groups reliably extinguished response to the CS, showing no main effect of trial type ($F(2, 14) = 2.29$, $p=0.14$) and no main effect of drug ($F(1, 7) = 4.16$, $p=0.08$). Interestingly, a test of fear reinstatement revealed a robust return of fear in the saline group that was blocked in the morphine group ($F(2,12) = 14.7$, $p=0.0006$).
These findings and future investigations may be helpful for identifying neurobiological changes relevant to both chronic opioid administration and later exposure to extreme stress, and may ultimately clinically inform our understanding of OUD and traumatic stress comorbidity.

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DEVELOPMENTAL STIMULATION OF PYRAMIDAL NEURONS DURING A DEFINED TEMPORAL WINDOW ALTERS SINGLE UNIT ACTIVITY IN ADULT MICE

The malformation of neuronal circuitry in the developing neocortex leads to a variety of psychiatric disorders later in the organism's life. By investigating alterations in circuitry though hyperexcitation in the developing brain, adult behavior can be assessed for phenotypic examples of psychiatric disorders. To test the impact of hyperexcitation of neuronal circuitry during early postnatal development, we took advantage of Bioluminescent Optogenetics (BL-OG), where light stimulation of an optogenetic element is achieved either through bioluminescence emitted from a tethered luciferase upon application of a chemical substrate or through physical light via fiber optics. Mice conditionally expressing LMO3, a fusion of sbGluc luciferase and the blue light sensing opsin VChR1, were crossed with Emx1-Cre transgenic mice, thus limiting expression of LMO3 to cortical pyramidal neurons. Overexcitation of developing pyramidal neurons was achieved chemogenetically by administering the luciferase substrate coelenterazine intraperitoneally during postnatal days 4-14. During adulthood, in vivo extracellular recordings of neocortical circuits revealed functional deficits in excitability, both in spontaneous firing rates and in response to temporally precise LED stimulation. Additionally, alterations in excitatory and inhibitory balance were seen. Here we analyzed the spike dynamics of neurons using single-unit activity with high temporal resolution. Using high temporal resolutions allow us to dive deeper into our understanding of these neurons and this understanding will help us to find strong correlation and/or causation factors.
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ROLE OF OVARIAN HORMONES ON PROBDNF LEVELS IN THE ROSTRAL VENTROLATERAL MEDULLA: INFLUENCES RELATED TO NEURONAL PLASTICITY AND SYMPATHETIC CONTROL OF BLOOD PRESSURE

Introduction: Cardiovascular disease (CVD) is the leading cause of death worldwide. CVD is lower in females of reproductive age compared to age-matched males, but the onset of menopause marks the beginning of an exponential rise in CVD in females. Post-menopausal increases in CVD indicate that ovarian hormones such as estrogen may have cardioprotective effects, possibly due to ovarian hormone receptors in the brain. One brain region that plays a key role in maintaining the cardiovascular system via its impact on sympathetic nerve activity (SNA) is the rostral ventrolateral medulla (RVLM). Like other brain regions, the RVLM contains receptors for ovarian hormones but also brain-derived neurotrophic factor (BDNF), a neurotrophin that influences neural growth and branching. In its mature form, mBDNF promotes dendritic branching and may enhance the RVLM's excitability, increasing SNA. In contrast, mBDNF's precursor, proBDNF, can oppose dendritic outgrowth. A high proBDNF:mBDNF ratio in the RVLM could therefore be "cardioprotective." Purpose and Hypothesis: Given what is known about the roles of ovarian hormones and the RVLM in cardiovascular health, the purpose of this study is to examine ovarian hormones in relation to proBDNF levels in the RVLM. We hypothesized that ovarian hormones increase proBDNF levels in the RVLM, minimizing dendritic branching and imparting cardioprotective effects. Methods: Female Sprague-Dawley rats received an ovariectomy (OVX) or sham surgery at 4 weeks of age (n=5 and n=4, respectively). Rats were sacrificed at 16 weeks old and the hindbrain was cut into 80µm sections from which tissue punches of the RVLM were isolated. The sections containing the rostrocaudal boundaries of the RVLM were identified and punches were pooled into 4 different subregions. Western blotting was performed to determine proBDNF levels in the RVLM relative to GAPDH (loading control). Predicted Results: Since our studies are ongoing, we predict lower proBDNF levels in the RVLM of ovariectomized rats compared to sham controls. Anticipated Conclusions: If proBDNF is lower in OVX rats, it will be consistent with our hypothesis that the elimination of the estrous cycle relates to decreased proBDNF in the RVLM; however, we will interpret these results cautiously. In addition to studying proBDNF, examining the influence of ovarian hormones on mBDNF levels in the RVLM could address potential differences in the conversion of proBDNF to mBDNF, which is essentially the central question of how ovarian hormones affect BDNF in the RVLM. We could also examine proteins involved in the conversion of proBDNF to mBDNF in the presence/absence of ovarian hormones. Future studies, together with our study, will add to current knowledge about the cardioprotective targets of ovarian hormones via mechanisms within the brain and provide insight into the interrelationship between ovarian hormones and neural control of the cardiovascular system via the RVLM. (R01-HL161233)

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NOREPINEPHRINE REGULATION OF SPATIAL MEMORY USING BARNES MAZE IN MALE AND FEMALE RATS

The role of norepinephrine (NE) in learning and memory has been extensively studied, yet its contribution remains to be clarified. This study aimed to investigate the role of NE on spatial learning and memory in female and male rats using a Barnes maze assay. We used N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), a specific noradrenergic neurotoxin that can cross the blood brain barrier, to deplete NE stores. We hypothesized that brain NE ablation would attenuate spatial learning and memory in rats. Loss of NE by DSP-4 was determined by measuring NE (and dopamine and serotonin) levels in several brain regions using HPLC. For the Barnes maze learning, 32 male (n=16) and female (n=16) Sprague-Dawley rats were trained to reach a hidden goal box using aversive visual and auditory cues with 3 trials per day for 5 days. Rats were administered 50 mg/kg/i.p of DSP-4 or saline 10 days prior to Barnes maze training. Results indicate learning via a reduced latency to reach the goal box with progressive training in both sexes over 5 days. There were no significant differences in latency to the goal box between saline and DSP-4 cohorts. Interestingly, levels of NE were significantly lower in the dorsal hippocampus, cingulate cortex, and the striatum indicating DSP-4 depleted NE levels. These data suggest that norepinephrine's role in spatial memory may be limited in simple tasks and non-stressed conditions. We are currently exploring whether increasing the task's behavioral demand via reversal learning will result in memory impairment in DSP-4 cohorts that have suppressed NE.

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EVALUATION OF BENZOFURAN AND BENZOTHIOPHENE MOLECULES FOR MDMA-LIKE DISCRIMINATIVE STIMULUS EFFECTS IN SPRAGUE-DAWLEY RATS

Illicit novel psychoactive substances (NPS) present a global health concern. Benzofurans, the third most prominent group of NPS on the market, are structurally and pharmacologically similar to 3, 4-Methylenedioxymethamphetamine (MDMA). The cardiovascular and neurobehavioral risks of benzofurans may also be similar to those of MDMA, particularly when misused at higher doses. Sulfur-containing analogues of benzofurans, benzothiophenes, are less studied but have recently been proposed as potential therapeutic agents. Characterizing the neurobehavioral effects of NPS is crucial to understanding their abuse liability and/or potential medicinal uses. Drug discrimination is a widely accepted preclinical behavioral assay utilized to discern the in vivo pharmacological activities of psychoactive substances. Previous studies utilizing this assay demonstrated full substitution and higher potency of select benzofurans compared to MDMA. The present study evaluated several novel benzothiophene [5-EAPBT, 5-MAPBT, 6-MAPBT] and benzofuran molecules, [(R)-BK-5-MAPB, (S)-BK-5-MAPB, (R)-BK-6-MBPB, (S)-BK-6-MBPB] in rodent drug discrimination studies. Sixteen male Sprague-Dawley rats were trained to discriminate MDMA from saline in a standard food-reinforced operant drug discrimination procedure and the aforementioned compounds were assessed for substitution at a range of doses. With the exception of (R)-BK-5-MAPB, and (R)-BK-6-MBPB, the novel molecules produced dose-dependent increases in MDMA lever responses and fully substituted at the highest dose assessed. These findings support previous reports that benzofurans share similar psychoactive effects to MDMA and they extend these findings to benzothiophenes. Further assessment of these compounds for abuse liability is warranted.

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EFFECTS OF HYPEREXCITATION OF LAYER V NEOCORTICAL PYRAMIDAL NEURONS DURING EARLY POSTNATAL DEVELOPMENT ON ADULT BEHAVIOR

Patterns of neuronal activity during early development are believed to guide the assembly of neural circuits. Thus, alterations of neuronal activity in this time window may cause structural and functional changes that persist into adulthood. Specific alterations may lead to unique patterns of aberrant circuit formation, a mechanism which has been implicated in neurodevelopmental disorders such as autism. Findings from human and animal studies suggest that multiple etiologically distinct forms of autism alter the physiology of specific deep-layer (layer V) prefrontal cortical neurons that project to subcortical targets. These neural populations play an important role in both normal and abnormal social behavior. To probe a potential functional link between development of cortical circuitry and adult behavior, we hyperexcited layer V cortical neurons during early postnatal development and assessed the effects on adult behavior. Mice conditionally expressing the excitatory luciferase-opsin fusion LMO3 (sbGluc fused to VChR1) were crossed with Rbp4-Cre mice, enabling LMO3 expression in layer V pyramidal neurons in offspring carrying both alleles. The luciferin coelenterazine (CTZ) was delivered to all pups in the litter intraperitoneally once per day during post-natal days 4-14. In presence of CTZ, light emission from sbGluc drives activation of VChR1 to depolarize the cell and evoke action potentials in Rbp4-LMO3 pups. Starting at postnatal day 60, we examined the behavior of all mice across several testing paradigms, including open field, water T maze, sociability, novel object, and rotarod. Where applicable and to standardize our quantification of behavioral data we implemented DeepLabCut, an open-source machine learning tool that leverages recent advances in computer vision to allow accurate, markerless tracking of animals across behavior testing videos.
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Theme: Motivation and Emotion

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Effects of a mixture of cocaine and fentanyl on locomotor activity across repeated exposures in male Wistar rats

There has recently been a rise in mortality from opioid overdose deaths disproportionally resulting from drug supplies being contaminated with fentanyl, such as fentanyl-laced cocaine. Since 2010 the mixture has been reported to have doubled the rate of increase in deaths compared to cocaine alone (3x combination vs 1.5x alone) and the rate of non-prescribed fentanyl in cocaine positive urinalysis between 2013 (0.9%) and 2018 (17.6%) increased 1850%. These findings demonstrate that the rate of mixed use has and is increasing with roughly 1 in 6 cocaine users knowingly or unknowingly consuming the combination of cocaine and fentanyl. The lack of fundamental understanding of this combinations' impact to behavioral and neurochemical functioning along with increases in the proportion of people exposed necessitates investigation. Here, we investigated the locomotor activity (LMA) caused by cocaine or cocaine and fentanyl mixture. Male Wistar rats were grouped into control (saline; n = 6), cocaine (20 mg/kg; n = 6), or cocaine and fentanyl mixture (20 mg/kg coc + 5mcg/kg; n = 6) and were handled and exposed to the LMA chamber prior any drug exposure (baseline D0). Exposures occurred once per day, 5 days per week for two weeks with 2 days of homecage abstinence between the exposures (5 on, 2 off, 5 on; 10 total exposure days). LMA was collected immediately following injections on the first, third, and fifth day of exposure each week (7 total exposures; 1 baseline, 6 drug). Rats underwent transcardial perfusion 90m following the last injection and brains were collected. Exposure to cocaine and the mixture of cocaine and fentanyl increased LMA compared to control (saline) but the drug exposure groups did not significantly differ. Brain tissues from these investigations are currently being sectioned for Fos analysis via immunofluorescence microscopy. Future directions related to explorations of the cocaine and fentanyl mixture and neuronal ensembles will be discussed.

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DIAL PREFRONTAL CORTEX IS INVOLVED IN ANXIETY-LIKE BEHAVIOR IN RATS WITH A HISTORY OF BINGE COCAINE ADMINISTRATION AND DRUG-ABSTINENCE NEURONAL ENSEMBLE IN THE ME

Cocaine abuse remains a public health problem with no FDA-approved medications to treat cocaine use disorder. Previous results show that rats given chronic binge-pattern cocaine administration display a disinhibited hyper-exploratory phenotype in novel environments, indicative of anxiety-like behavior. A growing body of evidence also suggests that targeted neuronal ensemble ablation in the medial prefrontal cortex (mPFC) can affect cocaine seeking and taking behaviors. Therefore, we assessed the contribution of mPFC neuronal ensembles in the disinhibited hyper-exploratory phenotype observed in a rat model of chronic binge cocaine administration. Female and male heterozygote Fos-LacZ Wistar rats were administered either cocaine (15 mg/kg/injection) or saline (1 ml/kg/injection) for 14 days using a binge-pattern administration paradigm (3 times daily at 1-hour intervals) followed by 14 days of forced abstinence (in the home cage). Injections took place in locomotor activity chambers (LMA) on days 0, 1 and 14 and their respective home cages on other days. Rats were then assessed for novelty induced exploration in a black plexiglass open field box. After 90 minutes, the time point of maximum Fos expression and hence □-galactosidase expression in Fos-LacZ rats, Daun02 or vehicle was intracranially-injected bilaterally into the mPFC. After 3 days of rest in the home cage, all rats were tested for cocaine-induced hyper-exploratory behavior on an elevated plus maze consisting of closed/open arms. Our results show that a sexually dimorphic LMA response to cocaine occurred over the 14 days of cocaine administration, with females showing greater behavioral sensitization. Additionally, male, but not female, rats treated with cocaine displayed higher anxiety-like behavior by spending less time in the center of open field following 14 days of cocaine withdrawal. In the elevated plus maze, cocaine-treated male rats who received vehicle injections continued to show anxiety-like behavior, but Daun02 chemoablation of mPFC neuronal ensemble attenuated cocaine-induced anxiety-like behavior by increasing time/entries in open arms. The finding that ensemble ablation of one context carries over to a new context is an interesting finding and suggests a shared neuronal ensemble of the mPFC to anxiety provoking stimuli regardless of the specific context. Furthermore, neuronal ensembles that underlie anxiety-like behavior during cocaine withdrawal can contribute to dysfunction of the mPFC, which is known to play a role in drug-relapse.

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EFFECT OF RATE OF INTRAVENOUS COCAINE INFUSION ON LOCOMOTOR SENSITIZATION IN FEMALE RATS USING DEEPANIMAL TOOLKIT, A NEW AUTOMATED APPROACH TO BEHAVIORAL ANALYSIS

The speed at which addictive drugs reach the brain influences the magnitude of their neurobehavioral effects and increases abuse liability. For example, in rats fast rates of i.v. cocaine infusion (5 s) induce greater cocaine self-administration, and enhance psychomotor sensitization compared to the same dose of drug delivered slowly (90 s). In addition, rapid cocaine infusion produces stronger c-fos activation within nucleus accumbens (NAc). However, most rate of infusion (ROI) behavioral studies used only males and examined effects of just one or two drug infusions. Furthermore, ROI-dependent c-fos induction has not been examined in females. Therefore, we examined locomotor sensitization and c-fos expression in female Long-Evans rats given either fast (5 s) or slow (90 s) cocaine (50 µl, 2.0 mg/kg i.v.) or saline infusions (1 mL/kg). Rats were given one infusion per session, three sessions per week, for 8 total infusions. Videos were recorded throughout each session. To examine long-lasting effects, some rats were then re-exposed to cocaine (fast or slow) after 14 days of withdrawal, and cocaine-induced c-fos expression in the nucleus accumbens and dorsolateral striatum (DLS) were examined. We applied a novel approach to measure locomotion using DeepAnimalToolkit (DpA),a recently developed computer vision toolbox to track animals in low lighting conditions (Kaul and Eban-Rothschild, in preparation). This was directly compared to traditional hand scoring measures. DpA measures of locomotor activity strongly correlated with hand-scored locomotor measures, providing an unbiased and reliable method to quantify this behavior. Initial results show that fast repeated cocaine infusions induced locomotor sensitization, whereas slow repeated infusions did not. To our knowledge, this is the first demonstration that repeated slow infusions of cocaine do not produce sensitization in either sex, and suggests that although total drug exposure is identical, the speed of drug delivery strongly influences cocaine-induced behavioral plasticity in females. Ongoing studies are examining cocaine-induced c-fos expression. Based on previous studies in males, we expect more robust induction in the 5 s group, but that this effect may be more pronounced in DLS than NAc of females. Together these data reveal how varying pharmacokinetics influence cocaine-induced neurobehavioral plasticity in females, and validate a new method for automated quantification of locomotor sensitization behavior.

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THE EFFECTS OF TREADMILL EXERCISE AND STRETCHING ON ANXIETY IN YOUTH

Introduction:
Exercise is an intervention that plays a critical role in leading and maintaining a healthy lifestyle. Recent studies show beneficial effects of exercise on mental health in the adult population, specifically the improvement of symptoms of anxiety, depression, ADHD, and other disorders. However, there are limited data on the effects of exercise, specifically different modality types (e.g., stretching, walking or running), on mental health in youth. The current study used a randomized controlled trial with treadmill exercise and stretching interventions to compare state anxiety symptoms before and after the physical activity in youth. We hypothesize that 1) acute exercise will reduce anxiety symptoms in youth across modalities; 2) there will be no significant difference in anxiolytic effects between modalities.
Methods:
The study sample included 14 participants from our ongoing randomized controlled trial of acute exercise. The sample was 64.3% male and 35.7% female, ages 9-17 years old (M=12.93±1.98), and was racially and ethnically diverse (28.6% White, 28.6% Black, 21.4% Multi-racial, 14.3% Hispanic or Latinx, and 7.1% Other). Participants were randomly assigned to a one 30-minute session of either treadmill exercise (N=9) or stretching (N=5). State anxiety was measured before and after the 30-minute session using the self-reported State-Trait Anxiety Inventory (STAI). A repeated measure ANOVA was used to assess the impact of both time (pre and post) and condition (treadmill vs. stretching) on anxiety in youth. All analyses were considered significant at a p value of < 0.05.
Results:
Prior to the exercise intervention, the mean anxiety score in our sample was 29.667±0.953; post intervention—the mean anxiety score was 26.300±1.107. Overall, there was a significant decrease in anxiety scores from pre- to post- intervention, regardless of condition type ($p=0.015$). There was no significant main effect of the condition, nor significant time by intervention interaction in anxiety scores ($p=0.978$).
Discussion:
Our results demonstrate that acute exercise reduces state anxiety in youth, and these effects are independent of condition (i.e., treadmill, stretching). Compared to psychotherapy or pharmacotherapy, exercise may be a relatively low-cost, low-risk behavioral intervention to combat the risk of mental disorders in youth. This is a preliminary analysis of an ongoing study. Future analyses will incorporate an additional meditation group and examine whether main effects of condition or time by condition interactions emerge. Future research should examine the longitudinal impact of exercise on anxiety symptoms.

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Effects of Acute Exercise vs. Stretching on Mood in Youth

Physical activity (PA) is essential for growth and development and plays a critical role in mental health. However, a reduced focus on school PA programs has contributed to an overall decrease in youth PA and health outcomes. Additionally, mental health is becoming a more prominent concern as rates of mental disorders (e.g., anxiety, depression) have increased in youth following the onset of the COVID-19 pandemic. Previous studies have assessed correlations between PA and mental health, such as mood state. However, most research has been conducted on adults or focused on the effects of chronic exercise programs (e.g., 10 weeks). Here, we report preliminary data from an ongoing randomized controlled trial of acute, moderate-intensity (treadmill walking/running) vs. light-intensity (stretching) exercise on mood in youth. We hypothesized that 1) both acute forms of exercise (e.g., walking, stretching) would improve mood states in youth and 2) that the moderate-intensity condition (i.e., treadmill) would lead to greater improvements in mood compared to the light-intensity condition (i.e., stretching). Participants between the ages of 9 and 17 were recruited from the Metro Detroit area. The sample reported here (N =14: 31% female, mean age =12.93, SD + =1.98) is racially and ethnically diverse. Participants were randomly assigned to a single 30-minute treadmill walking/running or stretching session. Mood state was measured before and after exercise conditions using the self-reported Mood and Feelings Questionnaire. A repeated-measures ANOVA with time (pre, post) and condition (treadmill, stretching) tested for differences in mood state over time and by condition. A Pearson bivariate correlation tested for correlations between pre-mood state and change in mood state over time. A two-sided p value of <0.05 was considered significant. No significant interaction between time and condition was observed for mood, meaning there was no difference in change of mood between the conditions ($p = 0.291$). There was also no significant main effect of time nor main effect of condition (p's > 0.05). However, poorer baseline mood was significantly associated with greater improvements in mood over time ($p < 0.001$). This study demonstrates that youth with poorer mood initially may benefit more from the antidepressant effects of exercise than those with better baseline mood. These data are part of an ongoing study; thus, the sample size is relatively limited. Future research should examine how different durations (e.g., 15 vs. 30 vs. 45 mins) of acute exercise affect mood and whether baseline characteristics (e.g., baseline mood, physical fitness) predict response to exercise.

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RIPPLE EFFECTS OF SCHOOL SHOOTINGS: IMPACT OF THE OXFORD SCHOOL SHOOTING ON ANXIETY SYMPTOMS, SCHOOL SAFETY, AND ATTITUDES TOWARDS GUNS IN A SAMPLE OF DETROIT ADOLESCENTS

Introduction:
School shootings have a profound impact on students and the community that is directly related to the incident. What is less documented is the ripple effect that the event has on communities and students who are not directly related to such incidents. In November of 2021, a Metro Detroit suburb, Oxford, Michigan, experienced a school shooting that devastated the community. The present study uses a unique longitudinal design to test for changes in attitudes about guns, anxiety symptoms, and perceptions of school safety before vs. after the Oxford school shooting, in a sample of adolescents living in the city of Detroit.

Methods:
Eleven participants had complete survey data before (T1) and after (T2) the Oxford school shooting (ages 14-18 years, M + SD = 15.6 + 1.3, 81.8% Black, 9.1% Hispanic, 9.1% South Asian, 63.6% male, 36.4% female). Participants completed self-reported questionnaires about attitudes towards guns, anxiety symptoms, and school safety at T1 and T2. Attitudes about guns were measured using 13 Likert-style questions (1=agree, 2=unsure, 3 = disagree), such as “I wish everyone would get rid of all their guns”. Perceived school safety was measured using one question from the Things I Have Seen and Heard questionnaire with the following Likert question (1-4, 1 = never, 4 = four or more times): “Do you feel safe at school?”. Anxiety symptoms were measured using the Screen for Child Anxiety-Related Emotional Disorders (0=not true, 2=very true, e.g., “When I feel frightened, it is hard to breathe”).

Results:
Overall, there was no difference in attitudes about guns, anxiety, or school safety ratings between T1 and T2 (p’s > 0.05).
Discussion:
We did not find evidence of significant changes in anxiety, school safety, nor attitudes about guns following the Oxford school shooting in a sample of Detroit adolescents. One possible reason for these findings is that Detroit youth may have more exposure to violence in the community and school at baseline as compared to Oxford youth. Future studies should include larger sample sizes and a more diverse sample of youth. More research is needed to understand the ripple effects of school shootings on mental health in youth.

Theme: Neural Excitability, Synapses, & Glia

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SYNAPTIC GLUTAMATE RECEPTOR SIGNALING ACUTELY REGULATES THE NEURONAL STRESS KINASE DLK

Dual Leucine Zipper kinase (DLK/MAP3K12) is a MAPKKK (mitogen-activated protein kinase kinase kinase) that is stimulated by a range of neuronal stresses including axonal injury, excitotoxicity, trophic factor withdrawal, and cytoskeletal disruption. Conversely, DLK inhibition rescues synapse loss and learning defects in mouse models of Alzheimer's disease, prevents neuronal death following excitotoxicity, and mitigates chemotherapy-induced neuropathy. Recent unpublished work from the Collins lab suggests that DLK signaling is tuned to synaptic connectivity and triggers the loss of synapse from axotomized neurons. As a neuronal stress response pathway that becomes activated in many scenarios, the cellular pathways which regulate and that are governed by DLK are important to understand. However, while there are many studies that document important functions for DLK, there is only limited understanding of the cellular mechanisms which regulate or are triggered by DLK signaling. To address this gap, I have established a cellular model to study DLK signaling activation in mouse primary embryonic cortical neuron cultures. Induction of synaptic activity in these cells using bicuculline or glutamate receptor agonists (glutamate, NMDA or DHPG) leads to a rapid increase (within 10 minutes of treatment) of DLK protein levels, as well as downstream signaling effectors. This in vitro model provides an ideal platform for interrogating the molecular mechanisms that regulate DLK signaling and its function at synapses. This understanding of both the upstream and the downstream of DLK signaling will give us a holistic picture of how this kinase affects the cellular functions and can ultimately be instrumental in developing strategies to pharmacologically manipulate DLK functions for the alleviation of multiple disease conditions such as neuropathy and dementia

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SYMPATHETIC RESPONSE TO GLUTAMATE MICROINJECTIONS IN THE ROSTRAL VENTROLATERAL MEDULLA OF SEDENTARY AND ACTIVE FEMALE RATS

Introduction: Cardiovascular disease (CVD) is the leading cause of mortality worldwide with hypertension and physical inactivity as leading risk factors for CVD. Males have higher blood pressure than reproductive-age females. However, the incidence of hypertension in females drastically increases after the onset of menopause, suggesting female reproductive hormones have cardioprotective effects. It is unknown whether a sedentary lifestyle offsets the anti-hypertension effects of reproductive hormones. In the brain, control of blood pressure is regulated by the Rostral Ventrolateral Medulla (RVLM). Previously, we reported that female rats have a lower resting mean arterial pressure (MAP) than male rats but had similar increases in blood pressure in response to activation of the RVLM. Our study was not powered to account for stages of the reproductive cycle as a primary outcome or to examine interactions between sedentary conditions and the estrous cycles (analogous to the human menstrual cycle). Purpose and Hypothesis: The purpose of this study is to determine if active versus sedentary conditions impact blood pressure regulation by the RVLM during the high hormone stage of the estrus cycle (proestrus/estrus). We hypothesized that female sedentary rats would have higher sympathetic nerve activity and blood pressure responses to the activation of the RVLM compared to physically active females. Methods: Ten female Sprague-Dawley rats (16 weeks old were housed either in sedentary conditions (no running wheel) or physically active conditions (running wheels) (n=5 ea.). Under Inactin anesthesia, vaginal lavages were also obtained to validate high hormone status (proestrus/estrus). Sympathetic nerve activity (SNA) was recorded via electrodes implanted on the splanchnic nerve. Mean arterial pressure (MAP) was recorded through a femoral arterial catheter. Rats then received glutamate microinjections (1, 10, & 100mM) into the RVLM. Results: Vaginal lavages confirmed all animals were in high hormone stages. Microinjections of glutamate into the RVLM produced dose-dependent increases in MAP and SNA in both groups ($p<0.001$, for both; main effect of dose by 2-way mixed ANOVA). The dose-dependent increases in MAP were not different between groups ($p=0.229$, main effect of group). In contrast, increases in SNA (% change) appeared to be higher in sedentary rats, with somewhat low sample size limiting differences from becoming significant ($p=0.051$, main effect of group). Conclusions: Our results suggest that physical activity may enhance sympathoexcitatory responses to activation of the RVLM. We interpret these findings cautiously due to a somewhat small sample size. Nonetheless, our study provides insight into future studies to determine whether sedentary versus active conditions impact blood pressure regulation in females during different stages of the estrous cycle. (R01-HL161233)

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ANKYRIN-G IN DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

Developmental and epileptic encephalopathies (DEEs) are a group of devastating disorders affecting tens of thousands of infants and children. These patients experience seizures and developmental impairments, and are at an increased risk of Sudden Unexpected Death in Epilepsy (SUDEP). To date, there is no known cure for DEE and seizures are often highly resistant to current therapeutics, with approximately 90% of DEE patients remaining untreated. Thus, there is an urgent need to better understand the etiology of the disease. The majority of DEEs are linked to de novo variants in genes encoding several ion channels, including voltage-gated sodium (NaV) and potassium (KV) channels, and GABAA receptors. These mutations in ion channel genes contribute to imbalances in excitatory and inhibitory (E/I) electrical brain activity characteristic of DEEs. Interestingly, many DEE-associated ion channels are binding partners of ankyrin-G, a scaffolding protein encoded by the ANK3 gene, critical for the proper localization of these ion channels to neuronal excitatory domains, such as the axon initial segment (AIS) and nodes of Ranvier (noR). Recently, a novel role for ankyrin-G has been identified in the development of inhibitory domains, including GABAergic synapses. However, it remains unknown if ANK3 dysfunction contributes to DEE etiology. Our collaborators have identified three DEE patients with compound heterozygous ANK3 variants of unknown significance (VUS). Understanding the impact of these VUS on ankyrin-G-dependent ion channel localization and GABAergic signaling could provide a mechanistic link between ANK3 dysfunction and abnormal neuronal activity seen in DEE. To determine how these ANK3 VUS may impact ion channel localization and GABAergic synapse formation, I will knock out ankyrin-G in cultured mouse cortical neurons and rescue expression with a wild-type or variant form of ankyrin-G. Immunocytochemistry will be used to probe for ankyrin-G-dependent ion channels and key inhibitory synaptic components. Additionally, to determine the impact of ankyrin-G loss-of-function on seizure susceptibility, I will induce febrile seizures in a mouse model lacking ankyrin-G function at GABAergic synapses (Ank3 p.W1989R) and in a mouse model lacking ankyrin-G function at GABAergic synapses, AIS, and noR (Ank3 exon37 flox/flox; □-actin-Cre). It is expected that in cultured mouse neurons, ANK3 VUS will result in deficits in DEE-associated ion channel localization and impaired GABAergic synapse development, and mutant mouse models with ankyrin-G loss-of-function at inhibitory domains will result in the increased seizure susceptibility observed in DEE. These studies may reveal a novel pathway underlying DEE etiology, paving the way for the development of effective DEE treatments.

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EFFECTS OF MELATONIN ON STRIATAL DOPAMINE NEUROTRANSMISSION

Melatonin, a serotonin-derived neurohormone, is a regulator of sleep and immune function, as well as a modulator of other neurotransmitter systems such as glutamate, acetylcholine, and GABA. Melatonin's effects on the sleep-wake cycle have made it an effective addition to therapeutic regimens for treating the sleep-disorders associated with neurodegenerative diseases such as Parkinson's Disease, Alzheimer's Disease, and Huntington's Disease. Studies of these therapeutic regimens suggest that melatonin can modulate the neurotransmission of dopamine. Here, we used various methods of fast-scan cyclic voltammetry and amperometry, both in vivo and ex vivo, to record and characterize the effects of melatonin on dopamine neurotransmission in the striatum in real time. In both the in vivo and ex vivo models, our data suggest that melatonin suppresses the release of dopamine. Our work provides evidence that, in addition to regulating sleep and immune function, melatonin is also responsible for the modulation of dopamine release in the striatum.

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PREDICTED INCREASES IN BDNF IN THE RVLM OF HYPERTENSIVE VERSUS NORMOTENSIVE RATS

Heart disease is the leading cause of death in adults in America. Thus, it is important to investigate the pathophysiology of hypertension (HTN), a prominent risk factor for heart disease. HTN affects over 47% of adults in America. Dysregulation of brain regions such as the rostral ventrolateral medulla (RVLM), which controls sympathoexcitation of the cardiovascular system, has been implicated in the pathophysiology of HTN. In many animal models of HTN, there is increased glutamatergic activation of the RVLM, a form of neuroplasticity. However, it is unclear what drives the observed neuroplasticity in the RVLM of hypertensive animals. One candidate is brain derived neurotrophic factor (BDNF), a growth factor that is expressed in the RVLM. BDNF is a prime growth mediator of neuroplasticity in other brain regions such as the hippocampus and of acute cardiovascular responses originating from the paraventricular nucleus of the hypothalamus. Whether BDNF mediates neuroplasticity in the RVLM associated with HTN is unknown. In this prospective study, we hypothesize that BDNF levels in the RVLM will be higher in hypertensive conditions. To test this hypothesis, we will study the two- kidney one clip (2K1C) renovascular model of HTN in Sprague-Dawley rats and compare BDNF levels in the RVLM of 2K1C to sham-operated, normotensive rats. We will quantify BDNF levels using western blotting and antibodies against the mature form of BDNF. We predict that BDNF will be expressed in the RVLM and that BDNF levels will be elevated in hypertensive animals. If our hypothesis is confirmed, the results would suggest that BDNF mediates and/or modulates the increases in sympathoexcitation of the RVLM in hypertensive rats. In the case that BDNF is a key neurotrophic growth factor in the RVLM of hypertensive animals, it could become a prime target for novel pharmaceutical therapies to combat the high prevalence of HTN. Future studies could also investigate whether variables such as sex and activity levels affect BDNF expression in the RVLM.

Theme: Neurodegenerative Disorders and Injury

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MECHANISMS OF TAU-BASED REGULATION OF RETROGRADE FAST AXONAL TRANSPORT

Alzheimer's disease and related dementias are characterized by neuronal and axonal degeneration that may be caused by disruption of microtubule-based axonal transport. Pathological forms of tau, including tau aggregates as well as some phosphorylated and/or mutant monomeric forms of the protein, are linked to these axonal transport defects. For example, several pathological tau modifications disrupt bidirectional fast axonal transport in mammalian primary neuron models. The anterograde effect occurs through activation of a protein phosphatase 1 (PP1)-mediated signaling pathway that alters kinesin behavior. However, the mechanisms mediating the effects on retrograde transport, involving the cytoplasmic dynein motor complex, are not yet known. We sought to characterize tau's potential regulatory role in bidirectional fast axonal transport and identify mechanisms by which pathological tau forms alter retrograde axonal transport. Using lentiviral-mediated protein expression in tau-knockout (TKO) mouse primary hippocampal neurons we determined how wild-type or FTDP-17 mutant tau altered the activation of kinases implicated in regulating transport via dynein. Pathological tau expression resulted in activation of the p38 kinase but not others. We also found that the presence of tau altered relative amounts of specific cargo proteins associated with the dynein complexes by comparing human tau knock-in (hTau KI) mice to TKO mice. Together this implicates tau in modulating specific aspects of axonal transport regulation and provides further avenues of study into how these changes result in disruptions to this critical neuronal process in disease.

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QUANTIFICATION OF EXTRACELLULAR VESICLE MEMBRANE MARKERS AFTER PHOTOBIOMODULATION THERAPY AND TRAUMATIC BRAIN INJURY IN MICE

Traumatic brain injury (TBI) is a prevalent diagnosis among both civilians and service personnel, with around 1.5 million cases every year in the US. Pain symptoms are common after TBI and can significantly impair life quality. Even though opioid-based therapies are contraindicated due to risk of opioid misuse or further complications, they are currently the most commonly used treatment for post-injury pain management. While the mechanism behind chronic pain in TBI is still unclear, we do know that it involves an increase in oxidative stress which promotes neuroinflammation. Photobiomodulation (PBM) light therapy, which interacts with oxidative and inflammatory mediators, is a potential non-opioid based treatment that has shown some promise in reducing pain sensitivity in TBI patients and in our preclinical studies. However, the mechanism by which systemic (whole body) exposure to PBM affects neural mechanisms related to TBI and pain symptoms is unclear. In the present study, we hypothesize that extracellular vesicles (EVs), cell secreted lipid-bound particles containing content varying from nucleic acids, proteins and lipids, as a form of cell-to-cell communication, may be the mechanism by which systemic PBM imparts its central antioxidant/anti-inflammatory effects. To test this hypothesis, C57Bl/6 mice were anesthetized and subjected to a single controlled impact (5 mm rounded tip; 2 mm impact, 5 m/s velocity) or sham (control) surgery. One day after TBI, a subset of sham and TBI mice received PBM therapy through the use of a handheld LED panel (670 nm. 50 mW/cm2) light source for 90 sec/day over 7 consecutive days inside an individual chamber. Brain sections along with serum were collected following decapitation on day 7 or day 30 after injury/surgery. The levels of two EV biomarkers, CD63 and CD81, were measured using enzyme-linked immunosorbent assays (ELISA). In serum, CD63 levels were significantly lower 7 days after the injury, regardless of PBM status, when compared to controls, with no effect of TBI or PBM at 30 days post-injury. At the 7-day timepoint, no significant differences in the concentration of CD81 following injury or PBM therapy were observed in either the prefrontal cortex or the dorsal hippocampus. Thus far, the results do not support the hypothesis that PBM mediates pain sensitivity through EV-based mechanisms, although there were limitations to our study such as small sample sizes and technical issues with quantifying CD63 in brain tissue and CD81 in serum. The current study does indicate that TBI significantly reduces CD63 levels in serum in the subchronic post-injury period, which will be further explored in future studies to expand our understanding of EV action after TBI.

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Characterizing αA-Crystallin's Role in the Regulation of Müller Cell Trophic Support and the Retinal Inflammatory Response

During diabetes, the small heat shock protein αA-crystallin (cryAA) is highly upregulated in the retina. Despite this upregulation, cryAA appears to lose its chaperone function due to prolonged stress, evidenced by its diminished ability to sequester the pro-apoptotic protein Bax. This loss of function is correlated with a decrease in phosphorylation of cryAA's threonine 148 (T148) residue. Our lab has shown that T148 phosphorylation controls cryAA's protective function by using phosphomimetic (T148D) and non-phosphorylatable (T148A) cryAA mutants. Indeed, overexpressing WT cryAA or the phosphomimetic T148D mutant in retinal neurons reduces metabolic stress-related cell death, while overexpressing the non-phosphorylatable T148A mutant has no impact. We recently reported that Müller glial cells (MGCs) express high levels of cryAA, especially in response to stress. The objective of the present study is to fully characterize the regulatory role of cryAA in MGCs. Our recent work demonstrates that overexpressing WT or T148D cryAA in MGCs significantly reduces pro-inflammatory cytokine induction, suggesting cryAA modulates MGC activity. Along with their involvement in the inflammatory response, MGCs are vital for providing trophic support to the retina. We have shown that retinal neuron survival increases when treated with conditioned media from glial cells transfected with WT or T148D cryAA. In addition to being protective on its own, cryAA may increase neuroprotection by enhancing MGC function. As chaperone proteins have previously been shown to regulate trophic factors, we hypothesize that cryAA promotes neurosurvival by modulating MGC-mediated trophic support.

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COMBINING EMBRYONIC MESENCEPHALIC DOPAMINERGIC NEUROSPHERE TRANSPLANTATION AND ENCOURAGED MOVEMENT TO IMPROVE DOPAMINE RELEASE CONTROL AND RELATED RECOVERY FROM LIMB USE ASYMMETRY IN A UNILATERAL 6-OHDA LESIONED RAT MODEL OF PARKINSON'S DISEASE

Our laboratory has been interested in ways to encourage and document more efficient incorporation of dopamine (DA) producing transplantable cells fostering behavioral restoration in our preclinical Parkinson's Disease (PD) research. Major unilateral nigrostriatal DA depletion (> 90%) using the neurotoxin 6-OHDA results in very apparent limb use deficits that are expressed during different forms of locomotion. It is our goal to document the establishment of both behavioral recovery from these limb-use deficits and related DA release control measured using in vivo microdialysis from freely moving animals in a swim apparatus. It is our belief that early animal activation, following transplantation, encourages the transplant to integrate into the host tissue. This poster will display our current findings of the relationship between DA producing embryonic (D14) mesencephalic neurosphere transplants and limb-use symmetry recovery that depends on the incorporation of such transplants as our primary behavior focus is encouraged swimming. Our current results display microdialysis data indicating DA level changes associated with the swimming experience of from PD model rats. Our goal is to explore and demonstrate evidence indicating that transplantations support behavioral recovery better, as well as establishing release control conducive to such recovery, in response to behavioral challenges offered while transplants incorporate into the host tissue.

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

The ability to manipulate specific neuronal populations of the spinal cord following spinal cord injury (SCI) could potentially prove highly beneficial for rehabilitation in patients through maintaining and strengthening still existing neuronal connections and/or facilitating the formation of new connections. A non-invasive and highly specific approach to neuronal stimulation is bioluminescent-optogenetics, where genetically expressed light emitting luciferases are tethered to light sensitive channelrhodopsins (luminopsins, LMO); neurons are activated by the addition of the luciferase substrate coelenterazine (CTZ). This approach takes advantage of utilizing ion channels for current conduction while activating the channels through application of a small chemical compound, thus allowing non-invasive stimulation and recruitment of all targeted neurons. We previously showed the efficacy of this approach in improving locomotor recovery following severe spinal cord contusion injury in rats expressing the excitatory LMO3 under control of a pan-neuronal and motor neuron specific promoter; CTZ was applied through a lateral ventricle cannula (Petersen et al., Front Neurol 2022). Here we transduced spinal cord neurons with a synapsin promoter-driven LMO that contains an opsin with higher light sensitivity. In this construct, the Gaussia luciferase variant sbGLuc is fused to CheRiff, a Channelrhodopsin from Scherffelia dubia. Taking advantage of the high light sensitivity of CheRiff, we stimulated transduced lumbar neurons after thoracic SCI by intraperitoneal application of CTZ, allowing for a less invasive treatment. The efficacy of this non-invasive bioluminescent optogenetic approach was confirmed by improved open-field locomotion (BBB) scores, BBB subscores, and catwalk subscores. This study demonstrates that peripheral, intraperitoneal injection of the luciferin CTZ can be used to activate LMOs expressed in spinal cord neurons that employ an opsin with increased light sensitivity, CheRiff. Activation of sbGLuc-CheRiff after a severe thoracic level contusion injury resulted in ameliorated motor deficits.
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Assessment of the neuroprotective potential of repurposing terazosin in rat models recapitulating features of Parkinson's disease

Parkinson's Disease (PD) is the second most common neurodegenerative disorder, predicted to affect ~1% of the U.S. population over 65 years of age. Pathological hallmarks of PD include the accumulation of Lewy bodies (LBs) or intracellular inclusions containing the protein alpha-synuclein (a-syn), and the progressive degeneration of the nigrostriatal dopamine neurons. Susceptibility of the nigrostriatal dopamine neurons in PD in part has been suggested to be influenced by their high energy requirement, due to the rhythmic firing pattern, and long, highly branched, unmyelinated axons of these neurons. Other contributing factors to energy burden in PD include dysregulation of glucose metabolism/glycolysis and mitochondrial function in the brain, which can result from advanced age, and mitochondrial deficits induced by the "pathological form" of a-syn, which is phosphorylated at serine 129 (pSyn) and is the main form of a-syn in Lewy bodies. Many of the genes associated with familial PD (PINK1, PRKN, DJ-1, LRRK2, and SNCA) also have functions related to the mitochondria. Recently, repurposing of terazosin, an FDA approved drug for benign prostatic hyperplasia, has been shown to enhance glycolysis and increase ATP in humans and rodents. Terazosin has also been reported to show motor restorative and neuroprotective benefits in neurotoxicant models of PD, and reduce a-syn protein. However, terazosin has yet to be examined in the context of a synucleinopathy.

In studies to replicate the reported neuroprotective benefits of terazosin in a neurotoxicant model, three-month-old male Fischer 344 rats received unilateral intrastriatal injections of 6-OHDA or vehicle. Two weeks post-surgery, rats received daily injections of terazosin (70 µg/kg; i.p.) or equal volume of vehicle and were assessed at 4 weeks post-surgery. Terazosin was sufficient to halt soma degeneration in the substantia nigra pars compacta (SNpc) and restore motor function based on the cylinder test. However, we did not observe recovery in the axon terminals.

Currently, we are examining the effects of terazosin in the rat a-syn preformed fibril (PFF) synucleinopathy model. In the rat PFF model, intrastriatal injections of PFFs result in the formation of pSyn-containing inclusions in the SNpc, followed by loss of dopaminergic phenotype and ultimately neurodegeneration at 5-6 months post-injection. In this ongoing study, three-month-old male Fischer 344 rats received unilateral intrastriatal injections of PFFs or vehicle, followed by 2 or 6-months of daily injections of terazosin (70 µg/kg; i.p.) or equal volume of vehicle. Tissue processing for the 2-month timepoint is in progress to determine if terazosin can reduce total a-syn protein, and the number of pSyn-containing inclusions in the SNpc.

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BENZENE EXPOSURE DURING PREGNANCY INCREASES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS SEVERITY IN OFFSPRING

Although Multiple Sclerosis (MS) is one of the most common autoimmune demyelinating diseases, its exact etiology is still unknown. Research in the field of Developmental Origins of Health and Disease has shown environmental toxin exposure during pregnancy leads to maternal inflammation, which can adversely impact fetal neurodevelopment and cause long-term effects on offspring health. Benzene in particular, a growing concern in urban areas, has been associated with developmental delays, psychiatric disorders, and hyperinflammation in the brain. Microglia, the primary immune mediators of the central nervous system, have been heavily implicated in pathogenesis of these conditions. We hypothesize benzene-induced in utero inflammation may predispose offspring to greater disease severity in the murine experimental autoimmune encephalomyelitis (EAE) model of MS. In this study, pregnant C57BL/6J mice were exposed to benzene (50 ppm) in full-body inhalation chambers for 5 hours/day from embryonic day 0.5-17.5. To induce EAE, female and male offspring were challenged at 6-8 weeks of age with a single subcutaneous emulsion (200ug/200ul) of myelin oligodendrocyte peptide (MOG35-55) and Complete Freund's Adjuvant (CFA). Control animals were given CFA only. All animals were assessed daily using an established clinical score for disease symptomology. On post-immunization day 16 (PID 16), all animals were sacrificed when one group required euthanasia due to moribund paralysis. Brain samples were collected for flow cytometry and qRT-PCR. We found that benzene-exposed male offspring developed disease at an earlier rate and at a higher severity, but there were no differences in benzene versus control females. At baseline, benzene exposure during pregnancy led to reduction of M2 microglia and increased cortical mRNA expression of chemokine CXCL17 in male offspring. After EAE induction, benzene-exposed males showed significantly decreased IL-17B gene expression and significantly increased TGFβ and Aif1 in cortical tissue compared to controls. There were also no differences in inflammatory gene expression between control and benzene females. Our results suggest that inflammatory in utero environments may induce dysregulation in the developing neuroimmune system in an exposure- and sex-specific manner.

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MODULATION OF NOTCH, GSK-3B, AND BMP PATHWAYS TO CONVERT ASTROCYTES INTO NEUROBLASTS USING CRISPR/CAS9 GENE EDITING TOOL – AN IN VITRO STUDY

In stroke, disruption of blood flow in the circle of Willis deprives brain tissues of oxygen eventually killing the neurons and causing neuroinflammation. The neuroinflammation activates astrocytes into reactive astrocytes for defensive functions. Previous studies have shown that inhibition of Notch, GSK-3 β, and BMP pathways can convert astrocytes into functional neurons. In this study, genes that regulate the above-mentioned pathways (such as Hes5, NFkB1, and Blc2 for Notch; NLRP3 inflammasome for GSK-3β and Smad1,5, and 8/9 for BMP) were knocked out using the CRISPR/Cas9 gene-editing tool. The goal of the study was to knock out the above-mentioned genes using the CRISPR/Cas9 gene-editing technology in cultured astrocytes to confirm the gene knockout and reduction in their protein expression as a proof of principle for treatment in an animal model of stroke. Lipofectamine CRISPRMAX Cas9 reagent was used to transfect with sgRNA that were designed specifically to target genes in the pathways. Astrocytes were extracted from adult rat brains and HEK T293 cells were thawed from stock, both were maintained in culture using appropriate media. These cells underwent 3- and 6- days of forward and reverse transfection. Then the DNA and proteins were extracted. Sanger sequencing on transfected HEK T293 showed 100% transfection efficiency. Sanger sequencing on DNA extracted from transfected astrocytes showed the knockout of genes. Western blot was performed to analyze protein expression and results showed a significant reduction in the expression of Hes5, Nfkb1, Bcl2, Smad1, and Smad5 protein, suggesting the knockout of Hes5, Nfkb1, Bcl2 genes Notch pathways, and Smad1 and Smad5 genes in BMP pathways. MTT proliferation assay showed reduced proliferation of transfected astrocytes after transfection with all combined 7sgRNA. Here, in this study, we confirmed the knockout of six genes in three pathways, and the knockout was confirmed by reduction in protein regulated by the aforementioned genes in Notch and BMP pathways. Although no reduction in Nlrp3 protein expression was observed, Sanger sequencing indicatedNlrp3 knockout. This study represents the first step to confirm the feasibility of the reprogramming of astrocytes into neuroblasts.

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POTENTIAL SEX DIFFERENCES IN MITRAL CELL DENDRITIC MORPHOLOGY FOLLOWING INJURY AND RECOVERY

Recovery after neuronal injury remains an enigmatic dilemma to the scientific community. The zebrafish (ZF) olfactory system provides an excellent model to address this issue due to its inherent plasticity. Mitral cells (MC) of the olfactory bulb (OB) serve as the primary relay neurons for transmitting odorant information from the olfactory epithelium to output targets. Our lab has developed a novel methodology to quantify the extent of injury and recovery of MC dendritic arborization as a result of chronic deafferentation, which was achieved through the repeated application of the detergent Triton-X 100 to the right olfactory epithelium. The left side remained untreated to serve as an internal control. ZF were then allowed to recover for 3 or 8 weeks, and morphological measures were quantified based on number of tips, total length of dendritic branches, size of dendritic field, and distribution of fine processes.

Consideration of sex as a biological variable has become increasingly important in scientific research. Previous work has shown that female animals of some species have a propensity to recover more quickly than males following neuronal injury. We hypothesize that sex differences may extend to ZF, which could lead to further understanding of the differences between male and female neuronal recovery. Current work aims to decipher potential differences that may exist within OB structures during growth as well as following injury and recovery between males and females.

Control measurements of MC dendritic arbor features show potential differences in male and female ZF with males possessing fewer number of tips and a decreased optical density at the 8-week timepoint. However, this significant difference appears to attenuate at 16 weeks within control animals. Combined data of males and females shows that following 8 weeks of repeated damage, MC dendritic morphology within the deafferented OB significantly decreased in number of tips, total length of dendritic branches, size of dendritic field, and distribution of fine processes. When ZF are allowed to recover for 3 or 8 weeks these significant differences are alleviated as shown by a return of the analyzed morphological structures to near internal control levels. Interestingly, preliminary results appear to show quicker recovery of branch length in males while the number of tips appears to recover more quickly in females at the 8-week recovery time point.

Although skepticism is warranted due to small sample sizes, this research furthers our understanding of the neurogenic processes in the ZF olfactory system, as well potential neurogenic differences between males and females. Understanding the morphological changes that take place within neuronal structures following injury and recovery is essential to demystifying the processes underlying neural regeneration and may lead to potential avenues for therapeutics in human populations.

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DECREASE IN PLASMA LEPTIN LEVELS ARE AGE AND SEX-RELATED IN THE 3XTG MOUSE MODEL OF ALZHEIMER DISEASE

Alzheimer disease (AD) is the most common cause of dementia. The characteristic neuropathological symptoms of AD include loss of neurons, especially in the hippocampus of the brain, as well as chronic inflammation and the accumulation of amyloid beta (Aβ) and hyper-phosphorylated tau. Leptin is a hormone and adipokine that regulates satiety and immune functions in the brain. Evidence suggests that lower circulating leptin or leptin-signaling defects may play a role in AD pathophysiology. However, there are no reports that correlate plasma leptin levels with age, sex, or fasting conditions in either AD patients or in mouse models of AD. This study used enzyme-linked immunosorbent assay (ELISA) to measure the plasma leptin in the fasting and non-fasting conditions in triple transgenic (3Tg) mouse model of AD. Plasma samples were drawn from the blood of groups of 13- or 18-month old male and female 3Tg or wild-type (WT) mice in either a fasting (for 16 hours) or non-fasting conditions. A total of 64 samples of plasma were used for both age groups and the leptin levels were measured using ELISA, with each measurement repeated. Fasting blood was taken between 0830 and 1100 hours after an overnight fast (16 hours). In the fasting condition, the leptin concentration in both age groups revealed no significant differences between male and female 3Tg [13-month: Male (M) = 2.71± 0.06 pg/ml, Female (F) = 2.97 ± 0.11 pg/ml; 18-month: M = 5.51± 0.17 pg/ml, F = 5.11 ± 0.10 pg/ml] or WT (13-month: M = 3.08 ± 0.29 pg/ml, F = 3.42 ± 0.44 pg/ml; 18-month: M = 5.19 ± 0.08 pg/ml, F = 5.25 ± 0.19 pg/ml) mice. Leptin concentrations were significantly lower in fasting and non-fasting-13-month (Non-fasting: M = 2.12 ± 0.23 pg/ml, F = 2.68 ± 0.30 pg/ml) and 18-month (Non-fasting: M= 5.45 ± 0.37 pg/ml, F = 5.89 ± 0.57 pg/ml) old-3Tg mice than in WT [(13-month-non-fasting:M = 3.92 ± 0.07 pg/ml, F = 4.25 ± 0.09 pg/ml; 18-month-non-fasting: M = 8.62 ± 0.14 pg/ml , F = 9.18 ± 0.24 pg/ml) counterparts. However, leptin concentrations of 18-month-old-fasting and non-fasting-3Tg mice were markedly higher than the 13-month-old-fasting and non-fasting 3Tg mice, although they were considerably lower than their WT counterparts. These results indicate that leptin levels were reduced as early as 13 months of age in the 3Tg mice, suggesting that they are more susceptible to subsequent leptin-signaling defects or leptin resistance. These results indicate that leptin levels may be reduced during AD progression and, therefore, may be a viable target for AD therapies.

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EVIDENCE OF REGULATION OF T148 PHOSPHORYLATION ON αA-CRYSTALLIN BY MTOR OR PI3K

α-crystallin proteins have recently gained increased interest due to their demonstrated roles in neurodegenerative disorders, including diabetic retinopathy. Our lab has shown that one member of the α-crystallin family, αA-crystallin, is upregulated in the retina of diabetic donors. This upregulation is accompanied with a substantial decrease in T148 phosphorylation, and impairment of αA-crystallin's neuroprotective abilities. While the molecular mechanisms underlying this T148 phosphorylation-dependent protection are getting clearer, the mechanisms of its regulation remain unknown. In the present study, we aimed to identify the kinase(s) responsible for T148 phosphorylation. R28 retinal neurons and MIO-M1 Müller glial cells were transfected with plasmids encoding either 3x-FLAG-tagged wild-type (WT) or 3x-FLAG-tagged T148C αA-crystallin and exposed to "diabetes-like" stress (2h incubation with 100ng/ml TNFα and 25mM glucose). These cell lines were chosen in part to elucidate differences in neuronal and glial mechanisms of cell protection. Kinase identification was performed using the chemoproteomic PhAXA assay which requires a chemical crosslinker to stabilize interactions formed between the newly introduced cysteine residue of the substrate and phosphorylating kinases. Kinase/substrate complexes were then immunoprecipitated and analyzed via western blot (WB) and liquid chromatography with tandem mass-spectrometry (LC-MS/MS). Specific complexes were identified by WB in the T148C+crosslinker condition, and several candidate kinases were identified in R28 retinal neurons by LC-MS/MS including mTOR, PI3K, WNK1, CK1δ, Map4k4, Mapk3, and STK4. Of these, mTOR and PI3K demonstrated the greatest fold-change increase in protein abundance and peptide-spectrum matches between the WT+crosslinker and T148C+crosslinker conditions. Our lab has also previously shown decreased mTOR gene expression in human retina samples from patients with diabetic retinopathy in comparison to non-diabetic patient samples, and both mTOR and PI3K have been shown to be involved in diabetes and cell survival pathways, making these two candidate kinases particularly promising. These data demonstrate, for the first time, evidence of specific kinases regulating T148 phosphorylation on αA-crystallin.

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RECOVERY FROM MOTOR DEFICITS FOLLOWING DELIVERY OF TRANSCRIPTION FACTOR IN MCAO STROKE MODEL USING PAMAM DENDRIMERS

Stroke is one of the leading causes of death in the United States, with ischemic stroke accounting for about 80% of the cases. Current treatment strategies involve the use of tissue plasminogen activator (tPA). However, a major drawback of using tPA is that the treatment is time sensitivity and must be administered within 3 h of the patient's first symptoms to achieve maximum therapeutic efficacy. Alternative treatment plans include finding a strategy that is less time-sensitive. It is well known that the brain becomes highly inflamed with reactive astrocytes around the area of the infarct. Previous studies have shown that these reactive astrocytes can be re-programmed into neuroblasts to combat the cell death in the stroke brain. The present study utilizes in vivo reprogramming of resident reactive astrocytes around the infarct to replace the lost neurons in sufficient numbers by delivery a transcription factor, hSOX2, using nanomolecules. PAMAM dendrimers are 3-dimensional nanomolecules which can deliver a large cargo of drugs and biomolecules to the cells in vitro and in vivo. In the past, we used the generation-4 surface-modified PAMAM dendrimers with mixed-surface having 90% hydroxyl groups and 10% amine groups (G4-90/10), which can deliver the cargo into the cells in vitro and in vivo. Unlike some of the viral vectors, these dendrimers can carry plasmids that are more than 10 kb in size. In the current study, we utilized G4-90/10 PAMAM dendrimers to deliver hSOX2 (under GFAP promoter to target astrocytes) to the infarct region in a 90-min MCAo stroked rat brain. Our initial behavioral analysis indicated that, following PAMAM dendrimer-based delivery of hSOX2, there was recovery of motor functioning in the stroked rats compared to the vehicle-treated rats. In addition, we performed a parallel study to optimize the in vivo reprogramming, in which we analyzed astrogliosis in the stroked brain at three different time-points following 90-min MCAo. Our results showed that the reactive astrocytes peaked at day 4 following ischemic stroke compared to other time points such as day 1 and day 7. These results indicate that 4 days post-MCAo should be an optimal time to target the hSOX2 transformation process.

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EFFECTS OF TART CHERRY EXTRACT AND OMEGA FATTY ACIDS IN THE 3XTG MOUSE MODEL OF ALZHEIMER'S DISEASE

Nutraceuticals are treatments of dietary supplements that have positive outcomes in human disease. Both antioxidants and omega fatty acids provide therapeutic benefits for normal aging and neurodegenerative disorders, such as Alzheimer's disease (AD), which is the most common neurodegenerative disorder today and is the leading cause of dementia in the elderly. The major symptoms of AD neuropathology included reduction in brain size, chronic inflammation associated with gliosis, as well as the accumulation of amyloid beta (Aβ) and hyper-phosphorylated tau. The latter of these symptoms, Aβ and hyper-phosphorylated tau have been the primary targets of past research attempting to treat AD.

In a previous study from our laboratory, we have demonstrated that a proprietary combination therapy of tart cherry extract and omega fatty acids in the nutraceutical, Total Body Rhythm (TBR), significantly reduced memory and neuropathological deficits in the chemically induced 192 IgG-saporin mouse model of AD. In addition, we recently have shown that TBR can reduce cognitive deficits, spare neurons, reduce gliosis, and inhibit binding of Aβ in the5xFAD mouse model of AD.

The goal of the present project was to test whether the effects of TBR would generalize to the 3xTg mouse model of AD, which contains both Aβ plaques and tangles of hyper-phosphorylated tau. Specifically, we assessed the ability of TBR to reduce cognitive deficits, lower brain inflammation, and protect against both Aβ and tau accumulations in the 3xTg mouse model. This project consists of three studies to assess the effects of TBR on behavioral and neuropathological deficits in different age and genotypes of 3xTg mice, including 12-month-old heterozygous mice (study 1), 12-month-old homozygous mice (study 2) and 20-month-old heterozygous mice (study 3). Both 3xTg and wild-type control mice were given oral administration of TBR or the equivalent amount of vehicle (0.5% methylcellulose) every other day for two months.

Following treatment, the mice were tested on the open-field test (OF) as a measure of spontaneous motor activity and the novel-object-recognition test (NOR) for recognition memory as well as the Morris water maze (MWM) task for spatial memory. Twenty-four hours after behavioral testing the mice were euthanized, and the brains were prepared for histology or western blot (WB). Western blot was used to quantify changes in Aβ, hyper-phosphorylated tau, immunological activity. The results of these studies revealed that TBR reduced levels of Aβ and phosphorylated tau in 12-month-old heterozygous and homozygous 3xTg mice, and reduced deficits in the MWM reversal trials in 20-month-old 3xTg mice, suggesting that TBR has significant potential for reducing some of the hallmark symptoms of AD.

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DOCOSAHEXAENOIC ACID-DERIVED PRO-RESOLVING LIPID MEDIATOR MARESIN-1 AMELIORATES INFLAMMATION AND PREVENTS DISEASE PROGRESSION IN PRECLINICAL MODEL OF MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is one of the most common inflammatory and neurodegenerative diseases in young adults leading to a build-up of neurological defects with an irreversible disability. Unresolved inflammation represents its pathological hallmark; however current therapeutic options fail to adequately suppress the ongoing inflammation, resulting in chronic inflammation. Studies suggest that the endogenous mechanisms to resolve inflammation are intact but become defective in patients due to deficiency of downstream resolution metabolites, thus resulting in disease progression and continued neuronal damage.

Docosahexaenoic acid (DHA) metabolism being defective in MS, we hypothesize that supplementation of downstream metabolite of DHA, maresin 1 (MaR1) will resolve inflammation and demyelination in its preclinical animal model, experimental allergic encephalomyelitis (EAE). We performed a comparative metabolite profiling using targeted metabolipidomics in serum samples from 29 relapsing-remitting (RRMS) patients and 29 age and gender-matched healthy controls (HC). For therapeutic effect of MaR1, we induced EAE in SJL mice, followed by intraperitoneal treatment with 300ng of MaR1 from day1 post-disease induction. We evaluated the effect on disease severity and inflammation by monitoring disease course of EAE, recall response by ELISA, cytokine expression analysis by qPCR and western blotting, and immune profiling by flow cytometry. Also, the neuroprotective effect of MaR1 through myelination was assessed by single molecule array (SIMOA) assay and histopathology. Statistical analysis was done using Graph-Pad Prism. Metabolite profiling revealed significant imbalance ($p<0.05$) between inflammatory response and resolution process in MS, confirming the metabolic dysfunction of lipid mediators including MaR1. Restoration of MaR1 prevented disease progression and reduced disease severity in EAE by inhibiting the infiltration of immune cells (CD4+IL17+ and CD4+FNγ+) in CNS as shown by intracellular staining ($P<0.001$). Recall response showed that MaR1 significantly inhibited pro-inflammatory cytokine IL17 ($P<0.01$) and promoted IL10 and IL4 production ($P<0.001$). Also, it exerted neuroprotective effects as we found lower levels of NFL ($P<0.01$) in the plasma of treated mice compared to control which was further confirmed by higher expression of MBP in brain from MaR1 treated group. Overall, our targeted metabolipidomics in MS patients identified MaR1 deficiency, whose supplementation exerts anti-inflammatory and neuroprotective effects in preclinical animal model, suggesting MaR1 could be a new therapeutic molecule in MS.

Theme: Sensory Motor Systems

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THE EFFECTS OF IMMUNE MODULATION ON THE RECOVERY RATE OF ZEBRAFISH OLFACTORY GLOMERULI AFTER DEAFFERENTATION

Full recovery after neuronal damage is elusive for many organisms due, in part, to limited neurogenesis in adulthood. Zebrafish, however, are renowned for their persistent neurogenesis and regenerative ability. Although the brain's primary defense cells, microglia, play a vital role in both pro- and anti-inflammatory stages of recovery, the role of the immune system in Zebrafish injury response is not known. In under a week, Zebrafish are able to recover completely from olfactory epithelium chemical lesions. Since recovery depends on immune cell activity, we explored how modulating the microglial population would affect recovery rate of neuronal structures in the olfactory bulb.

We hypothesized that reducing the immune cell population prior to damage will slow the process of regeneration. Our lab previously established a baseline recovery rate following chemical lesioning via detergent application to the right olfactory epithelium, with the left side acting as an internal control. This baseline characterization allows for time-matched comparisons with fish lesioned after exposure to the apoptotic drug L-clodronate which specifically targets and reduces the population of phagocytic cells. Locally injecting clodronate greatly diminishes the microglial population prior to lesioning. Using confocal microscopy to identify three glomerular structures labeled with anti-KLH after 4hr, 12hr, 24hr, 4 days, and 7 days post-lesioning, we compared the clodronate-treated recovery rate to baseline recovery rate based on changes in glomerular morphology.

Previous work demonstrated that most fish fully recover in 7 days, with partial recovery evident at 4 days. We expected a delayed recovery after reducing microglial populations; however, clodronate-treated fish appeared to recover glomerular structure at the same rate or faster than baseline. Glomeruli were fully recovered in 7 days and many recovered by 4 days, several days sooner than untreated fish. While it is unclear if drug mechanism or pre-stimulating the immune system affected the rate, future projects will address this with three additional treatment groups: saline pretreatment, zymosan pretreatment, and clodronate treatment concurrent with damage.

In addition to structural analysis, behavioral experiments will address functional recovery. This work-in-progress will use perception of an odorant that maps to one of the assessed glomerular regions, as a marker for recovery. Detergent damages sensory neurons, thereby reducing sensitivity to some odorants, and recovered behavioral responses indicate restoration of neuronal function. Using both morphological and functional approaches in Zebrafish can better inform us of how conserved immune system features promote complete recovery in the nervous system of adult mammals.

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MOTOR NEURON CELL BODY MORPHOLOGY WITH AGE, EXERCISE, AND SEX

The number of individuals of old age has continued to increase within the last few years, accompanied by an increase in senescence - an increased risk for diseases and ailments, one of the most common being sarcopenia, or loss of muscle mass. In aged rat models, the neuromuscular junction shows morphological changes such as decreased end plate areas, pointing to possible changes in the muscle's associated motor neuron and proposes an interest in the motor neuron cell bodies in the ventral horn of the spinal cord. Exercise has been shown to exhibit neuroprotective effects on motor systems with age. Prior studies in our laboratory have shown a 13% decrease in the number of motor neurons in old male rats compared to younger male rats (Cintron-Colon, 2022). Males only make up 50% of the population and have distinctive features from females regarding certain health risks and effects of aging. The focus of this study is to examine the motor neuron cell bodies in sedentary vs exercised aged male and female rats, including the number, area, and area distribution of cell bodies. Time points of 8, 12, 52, and 78 weeks were analyzed to correlate to major developmental markers of the Sprague-Dawley rat model. The lumbar region (L4-5) of the spinal cord was cryoprotected and sectioned at 20 µm, followed by immunohistochemical staining with anti-choline acetyltransferase, a neuronal marker, and DAPI. Sections were viewed under a confocal microscope to identify, count, and measure motor neurons in lamina 9 of the ventral horn. A comparison of average cell body sizes showed no significant ($p<0.05$) difference between 8-week male vs female (667.61 ± 0.08 µm2 and 463.068 ± 0.11 µm2, respectively), between 52-week vs 78-week females (423.26 ± 0.12 µm2 and 326.76 ± 0.09 µm2, respectively). However, there was a significant difference between 12-week (531.91 ± 0.08 µm2) vs 52-week females, and between 12-week vs 78-week females. Exercised females showed a significantly larger average cell body size compared to their sedentary counterpart. Following histogram analysis, 12-week females showed a higher frequency of cell bodies >1000 µm2in size, while 52-week and 78-week females showed a higher frequency of cell bodies between 100 and 500 µm2in size. The average number of cell bodies showed a decreasing trend with age, and there was no significant difference in the average number of cell bodies per section between 52-week (19.56 ± 0.19) vs 78-week (17 ± 0.17) females. The decrease in frequency distribution of cell bodies >1000 µm2 in size may be attributed to an overall decrease in size of all cell bodies with age or may suggest a loss of fast-type motor neurons with age since larger cell body sizes are observed in lower numbers in aged rats.

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THE EFFECTS OF EXERCISE ON GDNF AND ESTROGEN CONCENTRATION IN MALE AND FEMALE RATS

Males and females differ hormonally and neurologically as they age yet are given the same treatment for many neurological diseases. As aging occurs there is a loss of motor neurons, which could be explained by the reduced neurotrophic factor concentration. A possible way to maintain neuroprotection would be the production and release of a target-derived neurotrophic factor, such as glial cell line-derived neurotrophic factor (GDNF). Previous studies have shown that GDNF concentration has increased in skeletal muscle after exercise. However, this study has only been done in male rats. While exercise has been shown to mitigate the rate of development of degenerative loss of skeletal muscle mass, quality and strength called sarcopenia it has additionally been shown to increase estrogen receptors on skeletal muscle and increase levels of GDNF in skeletal muscle. Furthermore, it has been shown that GDNF and estrogen signal through similar intracellular pathway, and through such pathways, estrogen can enhance intracellular GDNF signaling. Our hypothesis is that GDNF concentration will be lower in sedentary rats when compared to exercised. In order to gain a more complete picture of what is happening neurologically as we age, I will be looking at female rats and comparing them to male rats of the same age. Hindlimb skeletal muscle was taken from Sprague-Dawley rats, from both sedentary and voluntarily exercised males and females. Their ages ranged from 4 weeks to 18 months. Sedentary muscle was taken at 4 weeks, 6 weeks, 8 weeks, 12 weeks, 12 months and 18 months. Exercised muscle was harvested from 4 week old rats that had access to running wheels for 2 weeks, from, 8 week old rats exercised for 4 weeks, and from 12 month old rats who voluntarily exercised for 6 months. ELISA was used to measure GDNF concentration, while immunohistochemistry was used to visualize the motor neuron, end plates, and GDNF. There were differences in GDNF concentration between male and females as they aged and if they were sedentary vs. exercised. Motor end plates showed more complexity and larger surface area from skeletal muscle in exercised rats as compared to sedentary. Future studies will investigate if estrogen concentration changes in the same trend in these groups as GDNF concentrations. Understanding the role that neurotrophic factors play in neuroprotection as we age and exercise between different sexes, may help develop novel pharmacological treatments and could impact our healthcare system, as we can think about it in a more nuanced way.

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SENSORIMOTOR ADAPTATION AND RETENTION IN MILD COGNITIVE IMPAIRMENT AND EARLY ALZHEIMER'S DISEASE

Learning new motor skills relies on declarative memory processes for initial performance improvements, and this likely allows us to retain the motor skill over time. Amnestic mild cognitive impairment (MCI) and Alzheimer's disease (AD) lead to impairments in declarative memory resources for learning and retention of motor skills. We investigated whether the early stages of motor learning are affected by MCI and early AD, and whether those patients exhibit additional impairments in short-term (i.e., within session) and long-term (after a 24-hour delay) retention of a newly acquired motor skill. Participants included patients diagnosed with MCI and early stages of AD, and control groups of cognitively healthy older and younger adults who all performed a force-field adaption task using a specialized robotic device (KINARM, B-kin Technologies). Participants were instructed to reach for visual targets, and while their arms moved, the robot would apply a velocity-dependent force perpendicular to the direction of the target. The mechanical load hinders smooth movements toward the target, but over time participants adapt by applying forces to counter the load. Short-term retention of force-field adaption was assessed in a final block of error-clamp trials on Day 1. Participants returned a day later to perform the same motor task to assess long-term skill retention over a 24-hour delay. Older participants were also tested with a standard neuropsychological assessment of cognitive function to quantify their current level of cognitive functioning. We compared the speed of skill acquisition, short-term retention, and long-term retention between all four groups. This work aims to determine whether acquisition, short- and long-term retention measures in a motor task can identify differences between MCI, AD and healthy aging individuals. Highlighting these differences could supplement as a measure to diagnose Alzheimer's Disease.

Theme: Techniques

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NOVEL LUCIFERASE - OPSIN COMBINATIONS FOR IMPROVED BIOLUMINESCENT OPTOGENETICS

BioLuminescent-OptoGenetics (BL-OG) is a bimodal approach for controlling the activity of optogenetic elements. Opsins can be activated by light from a physical source or by applying a small chemical, a luciferin, that is oxidized by a luciferase enzyme thereby emitting bioluminescent light. Various luciferases have been tethered to opsins in blue light utilizing luminopsins (LMOs), and these have been applied for excitation and inhibition of targeted neuronal populations in vivo. To expand the utility of BL-OG we generated a series of combinations of light emitters and light sensors and tested their efficacy for modifying neural activity in multi electrode arrays (MEAs). Light emitters were variants of Gaussia, Renilla, and Oplophorus luciferases, either alone or in combination with fluorescent proteins to leverage Förster resonance energy transfer (FRET) for bright light emitters. Light sensors resulting in neural excitation or inhibition were native or molecularly evolved channelrhodopsins or pumps, recently described super-sensitive channelrhodopsins, blue- and red-light sensors, and light-sensing G-protein coupled receptors. We also tested different configurations of LMOs, with the luciferase tethered to the opsin via the N-terminus, C-terminus, or both. LMOs were respectively analyzed to gauge effects of bioluminescent response (luciferin application) and optogenetic response (LED Stimulation). This set of novel LMOs expands the toolbox for controlling neural activities in the brain.

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QUANTIFICATION OF CORTICAL FOS NEUROACTIVITY AFTER CUED FEAR CONDITIONING USING PHOTOACOUSTIC IMAGING IN VIVO WITH VALIDATION BY EX VIVO IMMUNOFLUORESCENCE

Current functional neuroimaging techniques, such as functional magnetic resonance imaging, rely on indirect consequences (the hemodynamic response) to quantify neural activity. With the development of a photoacoustic (PA) imaging system and use of transgenic rodent technology, we propose a more direct method to map and quantify activated neurons using a Fos-LacZ transgene reporter system in rats. Fusion of Fos with the lacZ gene gives active (Fos+) cells the ability to cleave pro-chromogenic substrates, such as X-gal, into PA-detectable products. In this study, we quantified the neuroactivity-based PA signal in Fos-LacZ transgenic rats following cued fear conditioning or chemical and behavior control conditions following intrathecal administration of X-gal. Immunohistochemistry for Fos, NeuN, and beta-galactosidase was quantified for validation.

Homozygous Fos-LacZ Wistar female and male rats (N = 80 total) underwent acquisition of cued fear conditioning and intrathecal X-gal administration 60 minutes after the conclusion of fear conditioning. Twenty-four hours later, rats were anesthetized and a cranial window was created in the skull. PA imaging of Fos-dependent neuroactivity in the medial prefrontal cortex (mPFC) in vivo was immediately conducted and the signal strength was quantified. All PA imaging used an 18.5 MHz, 128 element L22-14v linear array ultrasound transducer to record PA signal produced by pulsed laser illumination directed with a custom optical fiber bundle. A second group of animals, identically prepared (N = 48), was perfused 90 minutes after fear conditioning or control conditions. Brain tissues were triple labeled with antibodies against NeuN, Fos, and beta-galactosidase for ex vivo validation using cell count analysis and colocalization. Images were quantified using Matlab and the FIJI version of ImageJ.

We report and compare fear response and Fos-expression from quantified in vivo PA images of the mPFC after X-gal administration and quantified cell count from triple-labeled confirmatory ex vivo immunofluorescence images acquired. Our data demonstrate significant differences in PA signal strength, and cell counts between both fear and sham conditioned animals when compared to vehicle or behaviorally naive controls in both the ex vivo immunofluorescence and in vivo PA groups.

Using this novel functional molecular photoacoustic approach, we demonstrate an ex vivo validated method of monitoring activated (Fos+) neurons, i.e. neuronal ensembles, in vivo with high spatial resolution and specificity.

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CONTROLLING NEURON-MUSCLE COMMUNICATION WITH BIOLOGICAL LIGHT

Interluminescence is a method for selective modulation of synaptic communication between genetically defined partners. Bioluminescent light from a pre-synaptic released luciferase activates a post-synaptic localized optogenetic channel, thereby allowing synapse-specific modulation of selected partners under experimenter-controlled introduction of a luciferin. Here we applied the Interluminescence strategy to control neuron-muscle communication at the neuro-muscular junction (NMJ-Int) via this 'optical synapse'.

The NMJ is a specialized peripheral synapse that translates the action potential of the presynaptic motor neuron to the contraction of the postsynaptic muscle fiber through the release of the neurotransmitter acetylcholine (ACh) and is thus essential for our physical mobility and daily life. Deficits in NMJ formation and maintenance cause several NMJ disorders (NMJDs), including Lambert–Eaton Myasthenic Syndrome (LEMS), Congenital Myasthenic Syndrome (CMS), Duchenne muscular dystrophy (DMD) and Myasthenia Gravis (MG). In a translational context, the NMJ-Int application carries the potential to rescue trans-synaptic neuro-muscular events when the traditional NMJ synaptic milieu is compromised by therapeutically correcting pathologically diminished or overactive muscle responses to neural input.

To test bioluminescence control of neuron-muscle communication we set up an in vitro system by co-culturing embryonic spinal cord explants transduced with viral vectors expressing the luciferase and cells from an immortalized mouse myoblast line (C2C12) engineered to stably express the excitatory step-function opsin ChR2(C128S). We then tested the impact of Interluminescence on communication between spinal cord motor neurons and C2C12 skeletal muscle cells by recording the muscular contractions of opsin expressing myofibers before and after application of the luciferin coelenterazine (CTZ).

Images were acquired using an inverted microscope (Zeiss Axio Observer). The contractions of myotubes were recorded using a high-speed camera at 20X magnification under phase-contrast microscopy (Hamamatsu Orca-Flash4.0 V2 sCMOS camera). Myotube contractions were analyzed before and after LED stimulation or CTZ stimulation; vehicle addition was used as our control. The image analysis tool MUSCLEMOTION was used to analyze recordings in the open-source software Fiji. Using an automated open-source software tool increases the efficiency of image analysis compared to manual human analysis and enables quantitative analysis of spontaneous and stimulated myotube contractions.

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CELLULAR AND CIRCUIT EFFECTS OF CHEMOGENETIC NEURONAL STIMULATION

Chemogenetic modulation of neuronal populations allows for precise control over cells engineered to express an exogenous receptor that responds exclusively to specific molecules. Chemogenetic approaches have steadily gained use for behavioral studies with experimental animals requiring circuit-wide manipulation over longer periods of times. There are several chemogenetic platforms for manipulating neuronal activity that are built from either ligand-gated ion channels (i.e., PSAMs, pharmacologically selective actuator modules) or G-protein coupled receptors (i.e., DREADDs, designer receptors exclusively activated by designer drugs). An orthogonal chemogenetic approach is BL-OG (BioLuminescent-OptoGenetics), where an opsin is activated by bioluminescent light emitted from a tethered luciferase (luminopsin, LMO); light emission occurs only in the presence of a luciferin oxidized by the enzyme luciferase. While the effects of chemogenetic activation of neurons on animal behavior in both acute and long-term treatments is well documented, information is lacking on the cellular and functional effects of chemogenetic stimulation technologies on the neurons expressing the actuators. We initiated in vitro studies designed to gain insight into how chemogenetic stimulation affects neurons by characterizing their electrophysiological attributes and their morphological features. Primary rat cortical neurons were cultured on multi electrode arrays (MEAs) and on coverslips in 24 well plates. These neurons were transduced with viral vectors each containing an excitatory chemogenetic actuator (hM3Dq, PSAM4-5HT3, and LMO7) or a control plasmid (EYFP). Cultures of mature neurons were stimulated once daily over 5 days with their respective effectors (CNO, PSEM, CTZ, or vehicle). Control cultures either expressed the actuator and were exposed to 5 days of vehicle treatment, or neurons did not express the actuator and were exposed to 5 days of effector treatment. MEA recordings before and after acute electrical stimulation revealed that chemogenetically stimulated neurons have decreased excitability compared to vehicle treated cultures. The extent of arborization and dendritic branching was assessed by Sholl analysis to determine if chronic chemogenetic stimulation altered neuronal morphology. The data showed differences between chemogenetic platforms in the degree of their dendritic arborization. This information on how chemogenetic stimulation affects cells is important because the causality between neuronal activity and behavior remains obscured without knowledge about the effects of stimulation modalities on inherent neuron properties. Identification of key parameters significantly altered through chemogenetic stimulation will guide interpretation of past and future chemogenetic experiments and will be instructive for users and tool builders of chemogenetic platforms.
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INTERLUMINESCENCE FOR SELECTIVE CONTROL OF SYNAPTICALLY CONNECTED PRE-AND POSTSYNAPTIC NEURONS

In BioLuminescent OptoGenetics, a genetically encoded light source, a luciferase, activates a light-sensing optogenetic element, a channelrhodopsin or a pump. Here, we leveraged this coupling strategy and developed a tool platform, Interluminescence, for experimental control of synaptic transmission between genetically defined neuronal partners by creating an optical synapse. 'Interluminescence' means 'bioluminescent light in between', here between a sender cell and a receiver cell. When the sender is a presynaptic neuron expressing luciferase and the receiver is its postsynaptic partner expressing opsin, we essentially create an optical synapse. Upon administration of the luciferase substrate, luciferin, bioluminescence emitted from a presynaptic neuron activates light-sensing opsins in a postsynaptic neuron. We previously demonstrated the effects of Interluminescence electrophysiologically at postsynaptic population level both in vitro and in vivo. Here we tested Interluminescence by patch clamp recordings from individual postsynaptic neurons.
We tested two different scenarios of optical synapse. First, when the presynaptic neuron expresses luciferase targeted to synaptic vesicles and second, when luciferase is tethered to the presynaptic membrane. The availability of luciferase in the former is dependent on presynaptic activity while the latter has persistent presence of luciferase in the cleft regardless of presynaptic activity. To test these conditions, rat cortex and striatum neurons were nucleofected with a luciferase-dTomato construct and an excitatory opsin-EYFP construct respectively, and were plated on glass coverslips as mixed culture. Synaptic pairs were located by visualizing the reporter expression for the luciferase (dTomato) on presynaptic cortical neurons and for the opsin (EYFP) on postsynaptic striatal neurons at the time of patch. Whole cell patch configuration was achieved on the opsin-expressing postsynaptic neurons and activity was recorded in continuous current clamp mode. Depolarization in postsynaptic neurons was robustly elicited with bioluminescence from presynaptic partners in the presence of synaptic blockers, and was likely due to trans-synaptic communication independent of traditional neurotransmission. We observed significant differences between traditional neurotransmission-induced versus Interluminescence-induced postsynaptic action potentials. The information gained about optical synapses at single neuron level with different luciferase and opsin combinations will be insightful in planning circuit-specific in vivo experiments.
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MOLECULAR EVOLUTION OF BL-OG COMPONENTS

Bioluminescent optogenetics (BL-OG) uses light emitted from a luciferase in the presence of its luciferin to activate light sensing molecules, including channelrhodopsins and light-sensitive transcription factors. Modifications to all components utilized in this approach, luciferases, luciferins, and light sensors, can aid in further research by improving, refining, and expanding the optogenetic toolbox. While luciferins are being modified chemically, light emitters and sensors can be improved by molecular evolution. Depending on available information about the protein structure, an initial step is to introduce mutations in a directed fashion. Alternatively, molecular evolution can proceed immediately or following rational mutagenesis, through random mutagenesis. We have developed a workflow for molecular evolution in mammalian cells, as the BL-OG approach is designed to be applied in mammalian brains. We tested the initial steps of molecular evolution using the fluorescent protein GFP.
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OPTIMIZING BIOLUMINESCENCE-INDUCED PHOTOACTIVATION OF TRANSCRIPTION

To understand how groups of neurons participate in neural circuits underlying behavior, technologies are needed for observing and controlling the ensembles employed during behavior across the brain. We are developing a platform technology for optical genetic activation using bioluminescence. In this system, light emitted from a luciferase in the presence of its substrate, luciferin, acts as the light source to drive light-sensing transcription factors. By using a Ca2+ dependent split luciferase we can take advantage of the unique possibility to make light emission itself dependent on neuronal activity. This technology allows acute capture of activated cells during a behavior across layers and brain regions and manipulation of these same cells experimentally by activity-driven transcription of actuator molecules. The precise temporal window of optical genetic activation is experimenter-controlled through the application of luciferin.

For this platform to work optimally it is critical to use photosensing transcription factors with high light sensitivity and robust signal-to-noise ratios. We initially explored bioluminescent activation in four distinct systems: FKF1/GI, EL222, GAVPO, and CRY2/CIB. Using NanoLuc luciferase (NLuc) for blue light emission, we found all four to be equally efficient in bioluminescence-induced transcription of the reporter protein Firefly Luciferase (FLuc) in HEK293 cells.

EL222 was chosen for further optimization because it is a single component factor and is also a relatively small molecule. To improve its sensitivity to light and decrease its basal activity levels, we are molecularly evolving wildtype EL222. We first used rational design, employing a multiple sequence alignment created via the software MUSCLE, to facilitate the identification of regions of functional significance across the protein using the program ConSurf. This design produced 97 EL222 variants that each contain a single amino acid change. All variants will be cloned using site-directed mutagenesis. The mutants will be screened by LED stimulation of transiently transfected HEK293 cells harboring the EL222 specific promoter 5xC120 driving the FLuc reporter gene. The best candidates identified in this screen will undergo repeated cycles of random mutagenesis and screening to further improve the transcription factor.

Transcription factor-based systems are advantageous because unlike chemical inducers of gene expression, transcription factors do not diffuse freely away from target regions. In addition to location specificity, transcription factors allow precise switching of genes from off to on in a timeframe that cannot realistically be achieved by chemical induction. The incorporation of transcription factors controlled by light not only increases the spatiotemporal resolution of gene activation but also applies an inducer (i.e. blue light) that is nontoxic, easy to acquire, and extremely tunable. Further improvement of EL222 will better these desirable characteristics of light controlled gene expression and make this transcription factor optimally inducible by bioluminescence.

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DENDRIMER-DELIVERED NOCODAZOLE ATTENUATES GLIOBLASTOMA PROLIFERATION, MIGRATION, AND METABOLISM IN-VITRO

Glioblastoma (GB) is the deadliest known central nervous system tumor. Using the current standard of treatment, the overall median survival for GB patients is only 12-14 months subsequent diagnosis, and this has remained virtually unchanged since the emergence of the Stupp Protocol in 2005. Thus, a new approach to GB treatment is needed. Nocodazole is a promising antineoplastic treatment, as it reversibly inhibits microtubule polymerization and mitosis by binding to beta-tubulin. This induces apoptosis, thereby inhibiting tumor progression. In this study, we encapsulated nocodazole in the novel G4 70/30-cystamine Poly(amidoamine) dendrimer, which increases the solubility and bioavailability of the drug. We then investigated the impact of this treatment on U87 human GB cell metabolism, migration and proliferation in-vitro. We quantified the metabolic impact of dendrimer encapsulated nocodazole (D-Noco) using an MTT assay, where U87 cells were treated with different concentrations of D-Noco for 72 hours. We treated cells with nocodazole alone as a treatment control, and used HEK293 cells as control cells to determine the impact of D-Noco on a non-cancerous cell line. Following D-Noco treatment, U87 cells were less viable than HEK293 cells at an optimal D-Noco concentration of 200 mM. D-Noco also reduced U87 cell metabolism to a greater degree than nocodazole alone did, demonstrating that the encapsulation of nocodazole increases the efficacy of the treatment. Additionally, we used a scratch assay to quantify the migration and proliferation of U87 cells subsequent treatment with 200 mM of D-Noco for 72 hours. HEK293 cells were used as a non-cancerous cell line control. The scratch assay results indicate that D-Noco significantly inhibits U87 cell growth ($p<0.001$). Additionally, no significant discrepancy was found between untreated HEK293 cell growth and the growth of HEK293 cells treated with D-Noco, suggesting D-Noco may confer a U87-specific treatment effect. Confirmation in vivo on animal models is ongoing.

See you all next year!!

MISFN Annual meeting 2023 will be hosted by Michigan State University