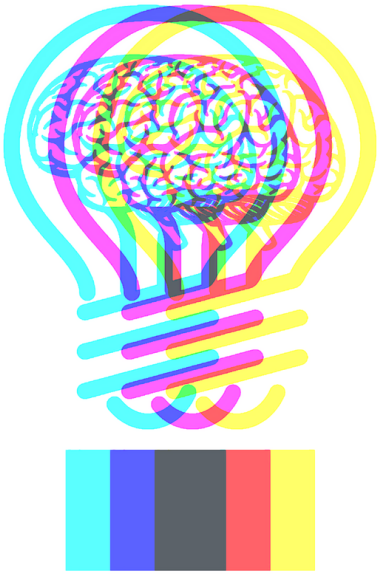
2022 NEUROSCIENCE CLINICAL-TRANSLATIONAL RESEARCH SYMPOSIUM



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Addiction

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Role of Neuregulin 3-ErbB4 Signaling in a Murine Model of Co-Morbid Nicotine Dependence and Schizophrenia

Student

Heritability of nicotine dependence is estimated at 40-75% and genome-wide association studies have identified a number of risk alleles. Our lab has recently shown that variations in the gene for Neuregulin 3 (NRG3), a member of the EGF superfamily and the cognate ligand for ErbB4 receptors are linked to smoking cessation outcomes and are of particular interest due to their therapeutic potential with downstream targets under development for several neuropsychiatric disorders. Interestingly, the Neuregulin signaling pathway contains some of the strongest genetic links contributing to schizophrenia risk. However, specific mechanisms underpinning NRG3's association with both increased nicotine withdrawal symptomology and increased schizophrenia risk are unknown.

We plan to utilize a novel genetic mouse model of both nicotine dependence and schizophrenia in a multidimensional approach to study how NRG3 mediates nicotine withdrawal-induced alterations on behavioral and molecular levels and how the alterations relate to schizophrenic-like dysfunction. To first begin these investigations, we examined the behavioral and molecular baseline effects of nicotine and aripiprazole, a long-acting atypical antipsychotic used to treat schizophrenia, in wildtype littermates. Animals treated chronically for nicotine and then undergoing spontaneous nicotine withdrawal were examined in the alternating Y-maze, social interaction test, and open field test for efficacy in reducing behavioral endophenotypes common to both nicotine withdrawal and schizophrenia in our model. Following behavioral testing, PFC tissues were analyzed to determine mRNA and protein expression of factors in the NRG3 signaling pathway. Preliminary results show differential changes in mRNA expression associated with glutamatergic and serotonergic transmission in the mPFC, OFC, and hippocampus. Ongoing experiments will extend these studies to include time- and site-specific genetic deletion of ErbB4 in the PFC and compare these results to those derived in wildtype littermates. Our findings will provide insight into the underlying mechanisms linking the NRG3 signaling pathway and nicotine dependence and may provide a novel link between nicotine use and its highly co-morbid psychiatric disorder, schizophrenia. Findings from these highly translational studies may lead to novel therapeutic development for both nicotine dependence and schizophrenia.

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Alpha2 Noradrenergic Receptor as a Target for Reversing Fentanyl-Induced Respiratory Depression

Student

Aim: Fentanyl is a high-potency synthetic opioid that causes profound motoric incapacitation and respiratory depression. In aerosolized form, high-potency synthetics have been identified by the U.S. Department of Homeland Security (DHS) to be potential chemical threats to civilians and military personnel. The most common antagonist used to reverse the effects of high-potency opioids is the mu opioid receptor (MOR) antagonist naloxone. However, recent reports suggest that the respiratory depressant effect of high-potency opioids are, at least in part, due to actions at non-MOR receptors, thus prompting the need to identify non-MOR adjunctive agents to combine with naloxone. The current study examined the potential role of the α2-noradrenergic receptor in decreasing fentanyl-induced locomotor incapacitation and respiratory depression.

Methods: Male and female Sprague-Dawley rats (n=27) were given saline or fentanyl (200 µg/kg; s.c.), followed 15 min later by a second injection of vehicle or one of the following: (1) naloxone (0.003-0.1 mg/kg; i.p.); (2) the α2-noradrenergic agonist clonidine (0.01-0.3 mg/kg; i.p.); or (3) the α2-noradrenergic antagonist yohimbine (0.3-10 mg/kg, i.p.). Rats were then immediately placed into a locomotor activity chamber for 15 min to measure distance travelled, followed by placement into a whole body plethysmography chamber for 30 min to measure minute ventilation. A within-subject dose response curve was generated for each drug administered after fentanyl, with the dose order counterbalanced across rats.

Results: As expected, naloxone reversed the locomotor and respiratory depressant effects of fentanyl in a dose-dependent manner. Clonidine produced a dose-dependent decrease in both locomotor activity and respiration when tested alone and it failed to reverse the locomotor and respiratory depressant effects of fentanyl. In contrast, while yohimbine did not alter fentanyl-induced motoric incapacitation, it increased minute ventilation when tested alone and also significantly decreased the respiratory depressant effect of fentanyl.

Conclusion: This study identifies the α2-noradrenergic receptor as a potential novel target for the development of an adjunctive rescue agent to be used as a counter measure for exposure to high-potency opioids.

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Impacts of chronic nicotine and withdrawal on contextual fear conditioning in male and female mice

While 90% of Americans will experience a traumatic event, only 8% will present with Post Traumatic Stress Disorder (PTSD) during their lifetime. Biological sex is a major risk factor for the development of PTSD, with women being twice as likely to suffer from PTSD than men. Additionally, there is a bidirectional relationship between smoking status and PTSD. People suffering from PTSD are twice as likely to smoke daily than healthy controls, and people who smoke daily are twice as likely to suffer from PTSD. The relationship between smoking and PTSD is sex specific. Nicotine dependence is positively correlated with PTSD symptoms in men, while no correlation was observed in women, suggesting a sex specific neurobiological response to trauma resulting in an increased risk of smoking. The hippocampus has a well characterized role in both memory and emotion making it a prime target for investigating the mechanist link between PTSD and nicotine dependence. Specifically, the dorsal hippocampus (DHIPP) is responsible for learning and contextual memory, while the ventral hippocampus (VHIPP) is integral in affective responding. To elucidate the hippocampus' regiospecific role in the link between smoking and PTSD, we chronically treated male and female 7 to 8-week-old mice with either saline or intermittent nicotine (18 mg/kg/day) via osmotic minipumps (Alzet 1002 model). On the twelfth day of treatment, the subjects were trained in a fear conditioning paradigm with two CS-US pairings (CS 89 dB tone and US 0.50 mA shock), followed by baseline test and minipump removal surgeries, then underwent 5 contextual fear extinction sessions. Following behavioral testing, the DHIPP and VHIPP were collected and assessed for transcriptomic and proteomic changes via qPCR and western blots. Preliminary results from these experiments suggest a baseline sex difference in contextual fear conditioning and extinction. Chronic nicotine did not impact the fear conditioning but had sex specific effects on contextual extinction likely related to differences in transcriptional control mechanisms. Further examination of the functional alterations underpinning treatment by sex effects in extinction behavior are ongoing.

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ErbB4 Transcription on PV Interneurons in the Ventral Hippocampus in Relation to Anxiety Effects of Nicotinic Withdrawal

Student

Around 80% of smokers relapse after an attempt to quit, leading to 480,000 deaths per year. Chronic smoking and early nicotine withdrawal can stimulate transcription of the NRG3 gene, and its correlated receptor ErbB4. These effects are thought to be driven by chronic nicotinic receptor desensitization and long-term alterations in synaptic plasticity within the brain. Of particular interest, ErbB4 is predominantly localized in parvalbumin positive interneurons of the ventral hippocampus, potentiating GABAergic tone in the CA1 and directly impacting anxiety-like endophenotypes of nicotine withdrawal. Our studies are examining the effects of cell-type specific knock-down of ErbB4 in the ventral hippocampus on (1) anxiety-like endophenotypes, (2) downstream signalling, and (3) potential interactions with neuroinflammatory responses. We will discuss these results and their potential for smoking cessation interventions in the US population.

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The Effects of Cocaine Withdrawal on Cognitive Flexibility and Claustrum Activity

Student

Cognitive flexibility is an organism's ability to adapt its behavior in response to changes in its environment. Withdrawal from prior cocaine use impairs cognitive flexibility, which can lead to relapse to drug use. 5-HT2A receptors have been shown to facilitate cognitive flexibility improvements, and cocaine may alter their activity via its effect on serotonin reuptake transporters. The underlying mechanisms by which cocaine withdrawal impacts cognitive flexibility are not well-understood, but we speculate that the claustrum, a subcortical brain region with an abundance of 5HT2ARs, may play a role in this phenomenon. To determine whether the excitability of neurons in the claustrum are responsible for cocaine-induced cognitive flexibility deficits, we tested the effects of cocaine withdrawal on cognitive flexibility, then examined how cocaine withdrawal has impacted the activity of claustral neurons. We also investigated the effects of ketanserin, a 5-HT2AR antagonist, on the claustrum activity of saline-exposed and cocaine-exposed groups. A cohort of 12 sprague-dawley rats (6 males, 6 females) was injected with an adeno-associated virus driving the expression of the GCaMP6f calcium sensor under the neuron-specific hSyn promoter. One group, consisting of 3 males and 3 females, received daily IP injections of cocaine (10 mg/kg) for 7 days, and the other group received daily IP injections of saline (0.9% NaCl, 1 mL/kg) for 7 days. We employed the strategy set-shifting task to test the cognitive flexibility of both groups after a 7-day withdrawal period, and within 24 hours of their testing, we used wide-field calcium imaging to record the activity of their claustrum in the absence and presence of ketanserin. We found no significant differences in cognitive flexibility between the cocaine-exposed and saline-exposed groups. The calcium imaging data analysis is still underway.

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A social peer renews cocaine seeking: potential role of oxytocin

Student

Aim: The purpose of this study was to determine if cocaine seeking is triggered by a cocaine-associated social peer using an ABA renewal paradigm and to examine a potential role for brain oxytocin (OT).

Methods: Male and female young adult Sprague-Dawley rats were trained in a dual-compartment operant conditioning chamber to self-administer cocaine using a 2-lever procedure with a same-sex/age peer (Context A). Following 21 days of self-administration, rats were randomly assigned to one of 2 groups: (1) ABA or (2) AAA. ABA rats underwent 10 extinction sessions in Context B (no peer) and then were tested for renewal of cocaine seeking in Context A (peer) or Context B (no peer). AAA rats were treated similarly, except extinction and renewal testing both occurred in Context A. Half of the ABA rats were then perfused 30 minutes after testing in Context A (n=8) and the other half were perfused 30 minutes after testing in Context B (n=8); AAA rats were all perfused 30 minutes after testing in Context A (n=6). Rats were perfused with 4% PFA and brains were removed for immunohistochemical analysis.

Results: Following acquisition of cocaine self-administration, both ABA and AAA rats decreased responding across extinction sessions, with ABA rats showing overall less responding than AAA rats collapsed across sessions. More importantly, ABA rats renewed cocaine seeking when reintroduced to Context A compared to Context B and compared to the AAA rats. Preliminary immunohistochemical analyses revealed that, while OT neurons in paraventricular nucleus of hypothalamus (PVN) were quiescent in AAA rats tested in Context A and ABA rats tested in Context B, ~12% of these OT neurons were co-labelled with c-Fos in ABA rats tested in Context A, indicating that ABA renewal of cocaine seeking was associated with increased activation of OT neurons.

Conclusion: Cocaine-associated social peers serve as powerful stimuli to trigger drug seeking, perhaps by activating OT neurons within the PVN.

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Claustrum serotonin and spike-timing plasticity associated with cognitive deficits after cocaine

Student

The claustrum (CLA), subcortical nucleus, is the most densely connected structure in the brain and has been understudied in neuroscience research. The CLA is implicated in attention salience and cognitive flexibility and has the highest density of the serotonin 2A receptor (5HT2AR) in brain. 5HT2ARs are the site of action for psychedelic hallucinogens and 5HT2AR agonists are gaining attention in clinical research with promising findings for treating psychiatric diseases such as substance use disorder. The mechanistic relationships between the CLA, 5HT2AR, and cognitive flexibility relevant to substance use remain largely uninterrogated. We propose that cocaine-induced cognitive flexibility deficits rely on the relationship between 5HT2ARs and glutamatergic synapses in the CLA with implications for long-term plasticity and the likelihood to relapse to cocaine use. We first found that cocaine extended access reduced performance on a set-shifting task. We then found that microinjections of the hallucinogenic 5HT2AR agonist, DOI, into the CLA also impaired set-shift performance, suggesting hindered cognitive flexibility. Next, we used whole cell recordings to observe effects of 5HT on CLA neurons that project to the anterior cingulate cortex (ACC). CLA-ACC neurons were recorded in the presence of 5HT and the 5HT2AR antagonist, ketanserin. 5HT caused a drastic inhibitory response. Significant decreases in sEPSC frequency and amplitude were observed in CLA-ACC neurons after application of 5HT. Blockade of the 5HT2AR with Ketanserin eliminated the synaptic effects of 5HT, indicating a regulatory role of the 5HT2AR in claustrocortical signaling. Next, we observed spike-timing dependent plasticity (STDP) in CLA-ACC neurons, revealing anti-hebbian long-term depression (LTD). DOI, reversed this LTD into long-term potentiation (LTP). These findings provide the first physiological evidence that the large population of CLA-ACC neurons are under inhibitory control from 5HT and the 5HT2ARs and suggest long-term synaptic plasticity which contribute to cognitive flexibility deficits following substance use.

Neurophysiology

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Examining the effect of iron (ferric) on proprioception and mitochondrial function using an invertebrate model

Student

Iron (Fe^{3+}) is an essential element for life in plants and animals and is found in soil, fresh waters and marine waters. The Fe^{3+} ion specifically is a vital prosthetic group and cofactor to mitochondrial electron transport complexes and numerous proteins involved in normal functioning. Despite its importance to life-sustaining processes, overexposure results in toxicity, and Fe^{3+} accumulation in the mammalian central nervous system is associated with various neurological disorders. Although current literature has investigated the long-term effects of Fe^{3+} overload, studies examining the acute effects are lacking. Using blue crab (*Callinectes sapidus*), the present study seeks to ascertain the effects of acute Fe^{3+} overload on proprioception within the Pd nerve as well as mitochondrial function. For proprioceptive studies, the effects of 10 and 20 mM ferric chloride (FeCl3) and ferric ammonium citrate ($(NH_4)_5[Fe(C_6H_4O_7)_2]$) solutions were investigated at 5 and 20 min exposure times. For mitochondrial studies, 20 mM ferric ammonium citrate was used. Exposure to 20 mM concentrations of ferric chloride and ferric ammonium citrate reduces excitability and function in proprioceptive neurons. Thus, Fe^{3+} likely blocks stretch-activated channels or voltage-gated Na^+ channels. The depressive effects of Fe^{3+} are partly reversible with acute exposure following saline washouts, indicating cells are not acutely damaged. Mitochondrial function remains present in nerve bundles after 10 min exposure to Fe^{3+} and suggests that Fe^{3+} does not rapidly permeate the cells. This study is relevant in demonstrating the dose-dependent effects of acute Fe^{3+} exposure on proprioception and provides a model to further investigate the mechanisms by which Fe^{3+} acts on the nervous system.

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THE EFFECT OF POSTSYNAPTIC RECEPTOR DESENSITIZATION DURING REPETITIVE SYNAPTIC ACTIVATION

Student

The process of synaptic transmission is generally described as synaptic vesicles fusing to the presynaptic membrane during evoked stimulation. This is due to an action potential opening voltage gated Ca2+ channels. Transmitters bind to postsynaptic receptors to mediate the postsynaptic response. Postsynaptic receptors will initially open and then desensitize with glutamate still bound. As glutamate is released from the receptors and cleared from the synaptic cleft, the receptor changes conformation but remains closed until glutamate again binds to it. The notion of the desensitization is to allow time for the transmitter to be cleared from the synapse to avoid re-binding as the initial glutamate is released from the receptor and cleared away. The potential degree of postsynaptic desensitization during short term facilitation (STF) remains elusive to determine. We are using the neuromuscular junctions (NMJs) of crayfish and *Drosophila* to address question by altering the degree of desensitization with background glutamate (increase desensitization) and Con-A (decrease desensitization) while examining the effect of STF. The larval *Drosophila* preparation shows a greater STF with 0.1 mM than for 1 mM Ca2+ and are dampened by application of glutamate (0.05 mM). However, the crayfish NMJ of opener muscle shows a greater STF with higher Ca^{2+} from 5 to 20 mM. The amplitude of the EJPs are dampened by application of glutamate (0.05 mM). The shape in the decay of the last EJP in a train is used as an index of desensitization. We will compare the treatments by looking at the average amplitude and average time to decay of the EJP.

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The effect of Doxapram, a K2p channel blocker, on proprioceptive neurons: Invertebrate model

Student

The resting membrane potential enables neurons to rapidly initiate and conduct electrical signals. K2p channels are key in maintaining this membrane potential and electrical excitability. They allow K+ ions to leak through, directing the membrane potential toward the K+ equilibrium potential. Researchers are working to describe the physiology and pharmacology of K2p channels; there is still much to learn. Doxapram is utilized clinically as a respiratory stimulant. This compound is a known blocker for a subset of K2p channels that are pH sensitive, including TASK1 and TASK3 channels. Using blue crabs as a model, we assess the effects of 0.1 and 5 mM preparations of Doxapram on the neuronal activity within a proprioceptive sensory organ. Results indicate that 0.1 mM Doxapram enhances excitation while the higher concentration 5 mM may over-excite the neurons and promote a sustained absolute refractory period until the compound is removed. The effect of 5 mM Doxapram mimics the effect of 40 mM K+ exposure. These findings are notable as they demonstrate Doxapram has acute effects on types of neurons other than those targeted to increase respiratory drive in mammals. This project was an integral part of a neurophysiology Course-based Undergraduate Research Experience (CURE).

Novel Methodology

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Single cell transcriptome analysis of zebrafish periocular mesenchyme during anterior segment development

Staff

Periocular mesenchyme (POM) is a subgroup of neural crest cells, important for forming the anterior segment (AS) of the eye. Despite their importance for eye development, our understanding of developmental mechanisms governing this cell group is limited. The purpose of this study is to characterize, at the single cell level, the transcriptomic regulation of POM cells as they form the zebrafish AS. We employed scRNA analysis over the course of zebrafish AS development. Larval eyes of transgenic zebrafish Tg(*Foxc1b:GFP*) and Tg(*Lmx1b*:GFP) were collected every 24 hours between 48hpf and 144hpf. GFP+ cells were isolated via FACS cell sorting and processed with the 10x genomics chromium single cell transcriptome kit. The resulting Illumina sequencing single cell transcriptomes were processed with the Cell Ranger pipeline and analysis was done with Monocle3. We collected over 31,000 GFP+ cells, from two biological replicate at each time point. Clustering analyses showed the cells were organized in re-occurring clusters, representing specific AS structures, including the cornea and the iridocorneal angle, as well as the retina. Additional trajectory analyses enabled us to follow the development of individual cell groups throughout development. We identified several genes, previously not associated with the AS, with specific AS expression patterns and importance for eye development, as proven by genetic knockout. These genes include *hgd*, *si:ch211-251b21.1*, *slc22a7a* and *nusap1*. Our results provide the first single cell transcriptome atlas of zebrafish AS development. This data reveals not only previously unknown genetic markers, but also gives the first insight into potential genetic interactions necessary for AS development. Future investigation into the function of novel AS regulators could ultimately provide new screening options for clinicians working with families suffering from congenital AS disorders, as well as potential avenues for therapy or prevention strategies.

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Reverse Engineering of MultiBrain® Technology for Embedding and Slicing Multiple Brain Samples for Histological Analysis

Staff

Immunohistochemistry (IHC) is a form of histology that uses antibodies to detect specific antigens within tissues and cells. Tissue must first be sectioned with a microtome, typically one sample at a time, in order to perform IHC and other staining procedures on it. The company NeuroScience Associates developed and patented the MultiBrain® Technology with which they can embed up to 40 mouse brain hemispheres in a gelatin matrix, allowing them to section them all simultaneously and significantly speed up the process of histological analysis. Multiple labs have published procedures that attempt to recreate this process. The purpose of this study is to reverse engineer the MultiBrain® technology and create a similar working procedure to use in future studies. Beginning with previously published procedures, many trials were conducted to adjust and improve processes to embed multiple mouse brain hemispheres in a gelatin matrix, slice a created matrix on a microtome, and mount sections on slides for analysis. Through these trials, a working procedure was created and is outlined in this paper for the creation and sectioning of a gelatin matrix for embedding tissue, specifically mouse brain tissue. This procedure is a usable and viable method to increase the speed of slicing, staining, and analyzing tissue for histological analysis.

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Improved detection of microglia immunohistochemical stains by an optimized method of antigen retrieval on free-floating PFA-fixed mouse brain tissue

Student

Microglia are the brain's tissue-resident macrophage, and immunohistochemical staining of microglia is essential to understanding the spatial patterning of microglia morphological changes in response to injury or disease. Tissue fixation is necessary for the post-mortem preservation and stabilization of the brain for neuropathological evaluation. Formaldehyde preservatives, such as formalin or paraformaldehyde (PFA), cross-link proteins to prevent their degradation but also mask antigens. Fortunately, this process can be reversed through heat-induced antigen retrieval. While antigen retrieval is commonly used on formalin-fixed, paraffin-embedded (FFPE) tissue, it is less commonly used for tissue fixed in 4% PFA for only a short time. In the process of developing a new multiplex staining method, we used a heat-induced antigen retrieval with a new mouse decloaking buffer on 4%PFA fixed free-floating mouse brain sections. We found that heat-induced antigen retrieval profoundly increased the microglia immunohistochemical staining. Since antigen retrieval is not typically used for PFA-fixed tissue, our goal was to optimize the antigen retrieval process and determine which antibodies would benefit from the treatment. To determine optimal conditions, free-floating sections of PFA-fixed mouse brain tissue were heated in a heat block at various temperatures and times. Trials were run at 60, 65, 70, 75, and 80 degrees Celsius to determine an optimal temperature. At temperatures higher than 80 C the tissue wrinkled significantly. At temperatures higher than 90 C, the tissue started to dissociate completely and was unusable. At 75 C, trials were run for 10, 15, 20, 25, and 30 minutes to determine optimal time. After determining preferable treatment conditions, trials were run on common antibodies: IBA1, GFAP, and CD45. The results of this study show that optimizing time and temperature are important to increase the immunohistochemical staining while maintaining the integrity of the tissue so it can be easily mounted on microscope slides.

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In viligo imaging: a new frontier in two-photon neuronal calcium imaging

Student

Over the past 30 years, the calcium (Ca^{2+}) hypothesis of brain aging has suggested that neuronal Ca^{2+} dysregulation is a key biomarker of aging. Indeed, age-dependent Ca^{2+}-mediated changes in intrinsic excitability, synaptic plasticity, and environmental mapping activity have helped identify some of the mechanisms engaged in memory and cognitive decline. However, most of this work has been done at the single-cell level in slice preparations. While more recent work from our lab has highlighted age-related calcium-centric changes in the cortex of the anesthetized animal, novel techniques are needed to investigate potential neuronal Ca^{2+} changes in the awake animal. Here, we used *in viligo* two-photon (2P) imaging techniques in ambulating mice, paired with a genetically encoded, fluorescent Ca^{2+} nanosensor (GCaMP8f) to characterize neuronal Ca^{2+} networks in the primary somatosensory cortex (S1), an area involved in proprioception and tactile discrimination. A Morse continuous wavelet transform (CWT) analysis was developed (MATLAB) to extract network communication variables (overall activity, connectivity, synchronicity & connection length) and graph correlogram data, in order to detect both aging- and surface-related changes in the network performance across aging. In young and aged mice, and compared to smooth surfaces, a surface that provided more points of contact on the paw pad yielded greater network activity. Importantly, we have successfully adopted a complex, yet valid, routine for obtaining neuronal calcium network dynamics in the young (4 months) and aged (24 months) mouse, providing a new frontier for brain calcium-centric research. The work here highlights central differences in neuronal Ca^{2+} network encoding with age in S1 *in vigilo* and may reflect on the reduced locomotor stability seen in aging. Future mechanistic work investigating gait characteristics in these same animals will reveal the impact of altered network communication as well as potential therapeutic targets to offset the increase falls with aging.

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Optimizing RT-QuIC assay for detection of aggregation-prone alpha-synuclein in Parkinson's disease

Staff

Background:

Nearly a million of people in the USA are affected with Parkinson's Disease (PD), a neurodegenerative movement disorder involving pathological aggregation of alpha-synuclein (α-syn). Recently, the development of the real-time quaking induced conversion assay (RT-QuIC) has allowed the detection of α of α-synuclein seeding activity from clinical available biospecimens of PD patients. In this assay, proteopathic α-syn aggregates present in patient samples induce aggregation of recombinant α-syn monomer under cycles of shaking and rest. The presence of Thioflavin T in the reaction mixture binds to the β-sheets of α-syn in these aggregates producing a measurable fluorescence.

Objective:

Using the RT-QuIC assay, we aimed to determine whether there are kinetic differences in the pathological aggregation of α-syn in PD and a different synucleinopathy, multiple system atrophy (MSA), derived from participant brain tissue. We then tested whether it is possible to detect aggregation prone α-syn in liquid biopsies from PD and control VA participants. The end goal is optimiztion of this assay for early diagnosis of PD and differentiation from MSA cases.

Methods:

Soluble and insoluble fractions were obtained by serial homogenization of brain tissues obtained from PD, MSA and non-synucleinopathy control patients. Blood from VA patient volunteers was collected and processed for plasma, erythrocyte and platelet isolation. Fractions were tested for α-syn aggregation activity by RT-QuIC assay using a BMG CLARIOstar plate reader and represented as the time required to reach fluorescence intensity threshold values obtained using the MARS software. Molecular methods such as Western and dot blot were utilized to measure a-syn levels in biofluids and tissues.

Results:

We observed an increased α-synuclein activity in the PD cases in both soluble and insoluble fractions compared to MSA in brain tissue and suggest an underlying structural or conformational difference in α-syn between these synucleinopathies. We have observed differences in detection of a-syn from human samples by varying buffers, detergents, and types of beads utilized in the assay. Optimization of the RT-QuIC assay for detection of α-syn aggregation activity from platelets, erythrocytes and plasma from PD cases is presented.

Conclusions:

We have successfully optimized the RT-QuIC assay in our lab using brain tissue from PD and MSA cases and observed differences in the aggregation activity in presence of different α-syn mutations. We further demonstrated the use of peripheral tissues and make progress toward the use of liquid biopsies as a potential tool to aid in the diagnosis of synucleinopathies.

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Development of a Wearable Fluorescence Imaging Device for Intraoperative Identification of Brain Tumors

Student

Introduction: The most common type of primary malignant brain tumor is malignant glioma (MG), accounting for ~40% of all intracranial tumors. Surgical resection remains the cornerstone of therapy and the extent of resection correlates with patient survival. A limiting factor for resection, however, is the surgeon's ability to differentiate the tumor from normal tissue. Fluorescence imaging is an emerging technique for real-time intraoperative visualization of brain tumors and their boundaries. 5-aminolevulinic acid (5-ALA) is one of the clinical fluorescent contrast agents for generating visible wavelength and tumor-specific fluorescence. The goal of this study is to develop and validate a low-cost, eye-loupe based, wearable fluorescence imaging device for detecting 5-ALA contrasts in MGs to guide brain tumor removal.

Materials and Methods: The wearable eye loupe fluorescence device includes a battery-drive headlight with a narrow-band, high-intensity, blue LED (405 nm) for 5-ALA excitation and a small CMOS video camera with a customized, flippable, long-pass filter (> 550 nm) for fluorescence imaging and recording. One pair of long-pass filters (> 550 nm) sitting in a custom-designed flippable frame is attached to the eye loupes for easily changing from fluorescence to color vision and vice versa. Two male patients (30 and 66 years old) with MG received 5-ALA at a dose of 20 mg/kg body weight three hours before induction of anesthesia. Patients were imaged by our customized wearable fluorescence eye loupe device and compared to an expensive and large florescence operative microscope (BLUE 400™ module + ZEISS KINEVO® 900) for intraoperative identification of MG margins.

Results: Two florescence imaging modalities generated consistent results in the visualization of MGs. The fluorescent tumorous tissues appeared more vivid with our wearable eye loupe device as compared to the KINEVO® 900 with BLUE 400™ module.

Discussion and Conclusions: This pilot case study demonstrates the feasibility of an innovative low-cost, wearable, fluorescence imaging device that can be attached to the standard surgical eye loupes for helping neurosurgeons to easily identify brain tumors for safe and maximal resection. With no interference of operation, this wearable ergonomic device allows the neurosurgeon to view the tumor with more flexibility of angles and distances compared to the stationary microscope, thus providing a novel way to image fluorescing brain tumors without a large expensive operative microscope. More patients are being studied and will be reported during the meeting.

Disclosure and Acknowledgements: In collaboration with Bioptics Technology LLC, this work is supported by the National Institutes of Health Small Business Technology Transfer Phase-I Grant (R41CA243600).

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Long-read sequencing and complete telomere to telomere human reference genome provide deeper insight into human brain transcriptomics

Student

Background: RNA-seq experiments have traditionally been done with short-read sequencing technologies that, by nature, collapse all RNA isoforms for a given gene into a single expression measurement—a major oversimplification of the underlying biology. Collapsing all RNA isoforms for a single gene severely limits our ability to characterize all RNA isoforms and determine their individual downstream functions. While computational approaches for assembling short reads into full transcripts exist, these methods are inherently structurally inaccurate, especially when compared to full-length sequencing possible with long-reads. Long-read sequencing technologies can sequence entire RNA molecules, allowing researchers to accurately quantify expression for the complete set of RNA isoform species, including *de novo* RNA isoforms. Long-read sequencing is especially well suited for discovering novel isoforms and genes in the recently released telomere-to-telomere (T2T) human reference genome (CHM13). The new CHM13 genome assembly resolved highly homologous regions that are challenging to study with short-reads. Here we sequenced post-mortem human brain tissue with long-reads and aligned them to CHM13 to explore novel gene bodies and transcript isoforms.

Methods: We sequenced frontal cortex tissue - Broadman area 9/46 - from four postmortem human brain samples using Oxford Nanopore Technologies long-read sequencing for PCR amplified cDNA. Each sample was sequenced with two PromethION flow cells. Data were basecalled using Guppy 6.0.7, reads were aligned to the CHM13 human reference genome using minimap2, and transcripts were assembled and quantified with the Bambu package in R.

Results: Among other findings, we discovered 81 new high-confidence gene bodies expressed with an average Counts Per Million (CPM) > 1. We also found 456 new high-confidence RNA isoforms in previously annotated gene bodies. Of these 456 novel isoforms, 49 aligned to medically relevant genes such as *MAOB*, *BCAN*, *ABO*, *HLA-DQA1*, and *WNT3*. Lastly, our samples had 853 genes containing 2 or more highly expressed transcript isoforms with CPM > 5, including *MAPT*(3), *CLU*(4) *APP*(4), *SNCA*(6), and *PARK7*(2).

Conclusions: Our results suggest long-reads aligned to the CHM13 reference genome have the potential to reveal novel gene bodies and transcript isoforms that were missed in previous studies. More accurate RNA quantification paired with more sensitive and precise transcript/gene discovery could lead to findings that help us better understand the mechanisms driving neurodegenerative diseases. Our goal moving forward is to use these methods in a case-control study of postmortem Alzheimer's brains to determine if differential transcript isoform expression is associated with the disease.

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Surgical Outcome of Long-segment Spinal Fusion in Osteoporotic Patients Undergoing Treatment with Teriparatide

Student

Spinal fusions have higher rates of failure (vertebral fracture, pseudoarthrosis, and reoperation) in osteoporotic patients, especially in those undergoing long-segment fusion. Teriparatide, a parathyroid hormone analog, has been successfully used to treat osteoporosis; its efficiency in long-segment spinal fusions has not been well studied. The objective of the study was to determine whether the use of teriparatide injections before and after long-segment (4 levels and more) posterior spinal fusion decreases vertebral fracture, pseudarthrosis, and reoperation rates. Adult osteoporotic patients undergoing long segment posterior spinal fusion within the 2016-2019 time period (case group) were compared with a group of patients without osteoporosis undergoing the same surgery (control group). Only patients with more than a year of post-op follow-up were included. Cases were treated with teriparatide for at least 6 weeks prior to surgery and continued post-op. In total, 15 cases and 41 controls met the inclusion and exclusion criteria. At baseline, age, number of fused levels, and rate of extension to the pelvis were different in cases vs. controls (71.3 vs. 65.4, 6.6 vs. 5.3, 53.3% vs. 17.1%; p values = 0.03, 0.01, 0.01, respectively), while gender, race, and number of interbody devices were statistically similar. There were no vertebral fractures in either group. Moreover, no statistically significant differences were found between the 2 groups for pseudoarthrosis and reoperation rates.

In conclusion, our study showed no significant differences in pseudoarthrosis, vertebral fracture, and reoperation rate between the osteoporotic group receiving teriparatide and the patient population that was not diagnosed with osteoporosis, despite more unfavorable baseline in our case groups. This suggests patients with osteoporosis needing long-segment posterior spinal fusion can get comparable outcomes after proper treatment with teriparatide.

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Exploring the Virtual Reality as a Neurorehabilitation Aid: A Systematic Review

Faculty

Background: Over the past couple of decades there has been increasing interest in using virtual reality (VR) technologies as rehabilitation aids. VR has the potential to positively impact rehabilitation outcomes for upper and lower extremity deficits and gait and balance dysfunction for a broad variety of patient populations. With this interest comes the recognition of the challenges associated with access and reimbursement for clinical utilization.

Purpose: We investigated the recently published literature (within the past five years) using VR as a rehabilitation aid for three categories of conditions: upper and lower extremity deficits, and gait and balance dysfunction. Furthermore, we examined potential solutions associated with cost reimbursement to address identified limitations connected to the regular clinical use of the technology.

Method: Three undergraduate researchers conducted independent literature reviews within four databases: PubMed, MedLine, Academic Search Complete, and PsyInfo. Search terms included: (virtual reality) AND (upper extremity OR lower extremity OR gait OR balance) AND (rehabilitation). Regular check-in meetings helped establish interrater reliability with regard to articles appropriate for inclusion in this review. Clinical trials, original research articles, and case studies were included. Researchers compiled the study population (age range, reason for rehab), intervention details (VR platform used, intervention duration and frequency), outcome measures, and adherence to the intervention.

Results: One hundred and twenty-four articles were included in the rehabilitation review and fifteen articles were included to examine potential solutions using telehealth as a model. Analyses are still ongoing to further comment on the potential utility of VR as a rehabilitation aid and to develop recommendations for improving the likelihood of reimbursement.

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Bioprocessing and analysis of human peripheral nerve cells for in vitro modeling and regenerative medicine

Other

We herein describe an optimized and scalable workflow to prepare and analyze cultures of human donor-derived Schwann cells (hSCs) from mature and developing nerves. We suggest implementing the following steps to achieve optimal recovery, growth and purity of the isolated primary hSCs: (1) dissecting perineurium-free nerve fascicles and pre-culturing them for at least 10 days to facilitate the enzymatic release of highly viable, *in vitro*-activated hSCs; (2) plating the initial cell suspensions as individual droplets on a laminin-coated substrate to expedite and increase the recovery of adherent cells; and (3) using mitogen- and serum-supplemented medium for the culturing of tissues and cells to accelerate the dedifferentiation and mitogenesis of the hSCs both before and after tissue dissociation. The hSCs obtained in this manner are suitable for a variety of downstream applications in basic and translational research. The cultured hSCs are proliferative, metabolically active and capable to degrading myelin debris even after undergoing 3 to 5 successive rounds of expansion. With the appropriate adaptations, patient-relevant hSC cultures can be prepared from fresh and postmortem biospecimens of a wide range of types and sizes, including fascicles from long spinal nerves, nerve roots and ganglia.

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Innovative, pulsed, coaxial laser speckle contrast imaging (C-LSCI) of cerebral blood flow in mice

Student

Introduction: Laser speckle contrast imaging (LSCI) is a powerful tool for 2D mapping of cerebral blood flow (CBF) in small animals [1]. Conventional LSCI illuminates wide-field coherent light onto tissue surface from the side of CMOS/CCD camera at an angle (i.e., side-illumination). The camera captures the temporal/spatial speckle pattern fluctuations on tissue surface, resulting from motions of red blood cells (i.e., blood flow). In this project, we reported a novel coaxial LSCI system that shares the illumination and detection paths (i.e., share-path) via utilizing a pellicle beam splitter. This new LSCI setup obviated the need to align the light source and camera to the same field-of-view (FOV). We also replaced the continuous-wave (CW) laser with a picosecond-pulsed laser, which has the potential to be integrated with other imaging techniques using a pulsed illumination.

Materials and Methods: We customized a coaxial LSCI system that combined source and imaging paths into a pellicle beam splitter. A CW laser at 785 nm and a picosecond pulsed laser at 775 nm were used as sources for comparisons. A CMOS camera (1440 × 1080 pixels) was used to collect an intensity image, which was converted to a spatial speckle contrast (K_s) image. K_s is defined as the ratio of intensity standard deviation (σ) to its mean (μ) in a 5 × 5 pixel window. A blood flow index is approximated as the inverse square of K_s [2]. CBF index map was then obtained by shifting the pixel windows over the selected FOV. The new system was tested in a mouse model of bilateral occlusion of common carotid artery (CCA).

Results and Discussion: We compared baseline CBF maps using 785 nm CW laser and 775 nm picosecond pulsed laser (20 MHz) with a camera exposure time of 5 ms. CBF variations were observed before, during, and after a bilateral CCA ligation using the pulsed laser and side-illumination. Utilizing the pulsed laser and share-path illumination also confirmed the CBF variations before, during, and after bilateral CCA ligation. The bilateral ligations resulted in substantial variations in CBF, which agreed with clinical expectations.

Conclusions: We have developed an innovative coaxial LSCI that provides more uniform illumination and easier operation compared to the conventional LSCI . We have also compared the results obtained by CW laser and pulsed laser as the LSCI source. Preliminary results in mice demonstrate consistence using both CW and pulsed lasers in the side-illumination and share-path LSCIs. This new LSCI system has the potential to be integrated with other imaging techniques using pulsed illumination such as photoacoustic imaging [3].

References: [1] Boas and Dunn et al. JBO. 2010;15(1):011109. [2] Patel et al, Translational vision science & technology. 2021;10(9):19. [3] Zhao, Y., et al., J Biomed Opt., 2020. 25(5): p. 056005.

Other Neurologic Conditions

Geetanjali Gera, PhD [1] • Fay Horak, PhD [2]
Physical Therapy University of Kentucky [1] • Neurology Oregon Health & Science University [2]

Balance Training in individuals with Cerebellar versus Sensorimotor Multiple Sclerosis: Case Reports

Faculty

Objective: Why is postural perturbation training quickly successful for some versus slow/poor for others with multiple sclerosis (MS)? One possibility is that many (but not all) people with multiple sclerosis have involvement of the cerebellum. The cerebellum is critical in trial-and-error based motor learning, a commonly-used approach in physical rehabilitation. The purpose of this case report is to illustrate the importance of determining cerebellar deficits when deciding on a balance rehabilitation plan for individuals with MS, even when patients show similar overall disease severity and overall balance impairments.

Methods (case description): Case I (SensorimotorMS): Clinical scores: ICARS: 9/100 (little or no cerebellar ataxia); EDSS MS disease severity: 4/10, (no deficits in the cerebellar system); MiniBESTest balance: 17/28. Unlike Case I, Case II (CerebellarMS) ICARS score: 43/100 (significant cerebellar ataxia); similar to Case I, Case II EDSS: 4/10 (but with more deficits in both the cerebellar and sensory systems) and similar overall MiniBESTest score: 16/28.The patients were exposed to 25 exposures of trial and error-based surface perturbation training with objective outcomes reflecting anticipatory control of postural responses.

Results: Case I with SensorimotorMS made improvements in postural control after the trial-and-error-based perturbation training and quckly learned to anticipate the surface perturbations. In contrast, Case II with CerebellarMS did not make improvements in postural control with perturbation training, they did not learn to anticipate the surface perturbations.

Conclusion: Patients with SensorimotorMS may show improvements in balance from trial-and-error-based surface perturbation training. In contrast, patients with CerebellarMS may benefit from other rehabilitation approaches, such as reinforcement training, since the cerebellar involvement may impair the ability to improve reactive balance control with trial and error.

Impact Statement: Clinicians are encouraged to incorporate assessment of cerebellar systems using tests like the ICARS to determine the presence of cerebellar deficits and develop targeted rehabilitation.

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Impact of Risk Factors on Retinopathy of Prematurity in Preterm Infants

Student

Introduction: Retinopathy of prematurity (ROP) is an eye disease that causes abnormal blood vessels to grow in the retina in preterm babies. Despite advancements in early detection and treatment, it remains a leading cause of visual impairment worldwide. Gestational age (GA), birth weight (BW), and small for gestational age (%SGA) are common risk factors for ROP [1]. Intermittent hypoxia (IH), defined as episodic drops in blood oxygen saturation (SpO_2), often occurs in preterm infants and may impact the development, progression, and severity of ROP. This study investigated the effects of these risk factors on ROP development.

Materials and Methods: Data were collected from 210 preterm infants (23 0/7 to 30 6/7 weeks gestation) at the Kentucky Children's Hospital. Cases were defined as Type 1 ROP, the stage beyond which ROP treatment is initiated. The cohort included 30 with Type 1 ROP and 180 without. Both males (108) and females (102) are included. SpO_2 was continuously recorded using high-resolution pulse oximeters in this study. IH primary outcome measures were percent time in hypoxemia ($SpO_2 < 80\%$) and average duration of IH events. IH events were quantified daily for each infant and reported cumulatively over weeks. The logarithmic transformation was applied to all data. Statistical analysis was performed using SPSS program. Univariate comparisons of risk factors between the groups without and with ROP were evaluated using Student's t-test for normally distributed data (BW and average duration of IH events) and the Mann-Whitney U test for non-normally distributed data (GA, %SGA, and percent time in hypoxemia). Results were considered statistically significant with $p < 0.05$ at a 95% confidence level.

Results and Discussion: The group differences in mean values of GA, BW, and %SGA indicate that both GA and BW significantly impact the ROP. The group differences in mean values of percent time and average duration of IH events show the average duration and percent time of IH events are higher in the ROP group compared to the non-ROP group which indicates the impact of IH events on the ROP.

Conclusions: Both GA and BW are significantly associated with the ROP. The percent time and average duration of IH events over the first 8 weeks of life are also significantly associated with the ROP. Unlike GA and BW, IH is a modifiable risk factor that when addressed can reduce the occurrence rate of Type 1 ROP.

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Suppression of microglial p38α MAPK signaling in APPswe/PSENdE9 mice impairs spatial performance but does not alter the pro-inflammatory profile

Fellow

While amyloidβ and tau accumulation are the primary biomarkers associated with Alzheimer's disease (AD), other pathological processes, such as neuroinflammation, are also involved. The mitogen activated protein kinase p38α (p38α) is expressed in multiple cell-types throughout the brain. Accumulation of amyloid is associated with elevated p38α signaling, which leads to increased tau phosphorylation, excitotoxicity, and altered synaptic plasticity. In microglial cells, amyloid-mediated p38α activity induces pro-inflammatory cytokine release which further elevates p38α signaling in other cell-types. Thus, targeting this pathway may be a relevant therapeutic approach to ameliorate AD-associated neuroinflammation. Here, we investigated the impact of p38α suppression on cognitive function, microglial morphology, and pro-inflammatory cytokine expression in WT (C57BL/6J) and amyloidogenic AD model (APPswe/PSENdE9) mice. Knock out of p38α in microglial cells was induced using a Cre-Lox system. Briefly, WT and APPswe/PSENdE9 control ($p38^{+/+}$) and floxed (p38 KO) mice were fed a tamoxifen diet for 28 days beginning around 3 months of age, followed by 3 months of standard chow to allow for replenishment of p38α-expressing peripheral cells and progression of amyloid pathology. Animals then underwent behavioral assessment using the open field (OF), Y-maze novel spatial recognition (NSR), and radial arm water maze (RAWM) tests. Amyloid burden, pro-inflammatory cytokine levels, and the microglial transcriptome in hippocampus and cortex were also assessed. Analysis of OF results showed that APPswe/PSENdE9 mice had increased locomotion compared to WT, though no impact of p38α KO was detected. However, p38α suppression did significantly alter the transcriptional profile in microglia while also increasing the frequency of larger plaques in the cortex of p38 KO mice, suggesting a potential correlation between microglial morphology and amyloid deposition. Neither genotype nor abrogation of p38α signaling impacted RAWM or NSR performance. While there was a significant genotype effect on some of the pro-inflammatory cytokines, this was unchanged by loss of microglial p38α. Although somewhat surprising, it could be that suppression of p38α in microglia only is insufficient to modify plaque burden or neuroinflammatory processes at this early point in the pathological progression (*i.e.* prior to the onset of memory impairments). Future work will further characterize plaque-associated microglial morphology as well as investigate the impact of p38α signaling in other cell-types across age and sex, as well as different stages of disease.

Meet Patel [1] • Jakub Famulski, PhD [1]
Biology University of Kentucky [1]

Examining the absence of CDHR1 – a Photoreceptor specific cadherin in larval zebrafish

Student

Inherited retinal blindness affects millions of people worldwide. Cone rod dystrophy is a type of retinal disorder caused by the degeneration of photoreceptors (PRCs) in the retina. Over 30 genes have been associated with cone-rod dystrophy. Loss of function in CDHR1, a photoreceptor-specific cadherin has been found to be associated with the incidence of cone-rod dystrophy. Functionally, CDHR1 has been shown to localize at the junction of the Inner segment and the Outer segment (OS) of Photoreceptors (PRCs). Recent work in our lab using zebrafish embryos has suggested that Cdhr1a (a homolog of CDHR1 in zebrafish) may play a role in the development of PRCs. However, the functional role of CDHR1 during the development of photoreceptors and/or retinal progenitor cells remains unknown. As such my primary goal intends to examine the functional role of Cdhr1a during early photoreceptor development in larval zebrafish. Using Whole Mount In Situ Hybridization (WISH), wildtype spatial-temporal patterning of Cdhr1a was analyzed. Temporally, Cdhr1a expression initiates around 62hpf (hours post fertilization), which is preceded by the emergence of the first few nascent outer segment discs at around 60hpf. The absence of Cdhr1a could potentially affect OS assembly and thereby dismantle the phototransduction process of the OS discs thus inducing apoptosis in PRCs. To test this hypothesis, we have created an Alt R CRISPR-induced Cdhr1a^{D173} mutant zebrafish line harboring a 173bp deletion in the coding sequence. WISH analysis of the mutant line shows little to no Cdhr1a mRNA as well as no genetic compensation from its ortholog Cdhr1b. Further analysis via WISH for PRC progenitor markers such as Crx and Nr2e3 do not show any significant change in expression. Future experiments will be focusing on examining rod and cone phenotypes and their ability to maintain homeostasis. Inferences from these experiments will be vital in understanding how CDHR1 leads to blinding disorders such as cone-rod dystrophy at both developmental and adult stages.

Neurological Deficits and Metabolic Impairment in CLN3 Disease
Faculty

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CLN3 disease (*aka.*, juvenile Batten disease or juvenile neuronal ceroid lipofuscinosis) is a devastating pediatric neurodegenerative disease. Children with CLN3 disease appear normal until reaching 4-5 years old when they quickly develop progressive blindness, seizures, sleep abnormalities, and cognitive and motor failures. They later become wheelchair-bound and need feeding tubes prior to premature death. Despite the devastation of this disease for both affected children and their families, a cure is not available. CLN3 disease is caused by mutations in the *CLN3* gene; however, how *CLN3* mutations result in disease phenotypes is yet to be fully understood.

Our published work [1, 2] showed that *Cln3*-deficient (*Cln3KO*) mice had late vision impairment (as detected by electroretinography) with retinal pathologies in both neurons and retinal pigment epithelial (RPE) cells, suggesting that both may contribute to CLN3 disease-associated vision loss. Also, knocking-down *CLN3* in human RPE-1 cell line led to cell death, and signaling cascades and transcription alterations that enhanced the autophagy-lysosomal system – a system critical for cellular metabolism. Our Stable Isotope Resolved Metabolomics (SIRM) study on these cells further revealed that knocking-down *CLN3* led to extensive metabolic impairment including compromised glycolysis, tricarboxylic acid (TCA) cycle, oxidative phosphorylation (OXPHOS), pentose phosphate pathway, and *de novo* nucleotide synthesis.

In our recent unpublished work (see another abstract submitted to this symposium by Iradukunda *et al*), we characterized sleep and epileptic activity in *Cln3KO* mice. Here, we further expanded our work to determine behavioral abnormalities, including those related to vision, anxiety, and learning and memory, in *Cln3KO* mice. To understand the molecular mechanism underlying these phenotypes, we also determined metabolic impairment and its spatial distributions in the *Cln3KO* mouse brain and retina, using imaging mass spectrometry and histology. Our work will facilitate both the understanding of disease etiology and the development of therapeutics that rescue CLN3 disease-associated neurological deficits by targeting cell metabolism.

GYeon Oh, PhD [1] • Daniela Moga, MD, PhD [2] • David Fardo, PhD [3] • Jordan Harp, PhD [4] • Erin Abner, PhD [1]
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The association of gabapentin initiation and neurocognitive changes in older adults with cognitive impairment

Fellow

Background

Although gabapentin has been increasingly prescribed to older adults, the relationship between gabapentin and neurocognitive change is not well studied. Therefore, we aimed to examine the association of gabapentin use and neurocognitive change (e.g., cognitive decline, functional status decline, and motor function change) in older adults with cognitive impairment.

Methods

A retrospective cohort study was conducted using National Alzheimer's Coordinating Center Uniform Data Set (2005-March 2021). Gabapentin new users with with cognitive impairment at the visit of gabapentin initiation (i.e., index visit) were included. Non-users were randomly selected using the incidence density sampling method. The cognitive status decline was measured by any increase of Clinical Dementia Rating global score (CDRGLOB) and 1-point increase of Clinical Dementia Rating sum of boxes (CDRSUM). The functional status decline was measured by 3-point increase of sum of Functional Activities Questionnaire (FAQ) and 0.3-point increase of mean of FAQ. The change in motor behavior was defined as new clinician reports of gait disorder, falls, and slowness. To mitigate confounding and selection bias, stabilized inverse probability of treatment weights and stabilized inverse probability of censoring weights were used. All analyses were conducted comparing index to index+1 and index+2 visits.

Results

We included 472 new-users (mean age [SD]: 78.8 [7.5]; male 45.3%) and 4,248 nonusers (79.2 [7.6]; 49.2%) with cognitive impairment at index. Gabapentin initiation was significantly associated with improving CDRGLOB (odds ratio [95% confidence interval]: 0.71 [0.51, 0.99]) at index+1 visit. The results from other outcomes were not significant; however, they were in the same direction. At index+1 visit, gabapentin initiation was associated with improving CDRSUM (0.82 [0.63, 1.06]), sum of FAQ (0.94 [0.65, 1.37]), and mean of FAQ (0.90 [0.68, 1.19]). Also, at index+2 visit, gabapentin initiation was associated with improving CDRSUM (0.79 [0.57, 1.09]), sum of FAQ (0.88 [0.55, 1.41]), and mean of FAQ (0.83 [0.59, 1.16]). After excluding new-users with motor dysfunction (n=146) at index, we identified 326 new-users (78.3 [7.3]; 43.3%) and 2,934 nonusers (78.6 [7.3]; 47.4%); in this sample, gabapentin initiation was associated with increased falls at the index+2 visit (2.37 [1.28, 4.38]).

Conclusions

Gabapentin use was associated with improving cognitive and functional status and motor function decline among older adults with cognitive impairment. Further studies are needed to examine the risk and benefit of prescribing gabapentin in older adults.

Geetanjali Gera, PhD [1] • Fay Horak, PhD [2]
Physical Therapy University of Kentucky [1] • Neurology Oregon Health & Science University [2]

Balance Training in individuals with Cerebellar versus Sensorimotor Multiple Sclerosis: Case Reports

Faculty

Objective: Why is postural perturbation training quickly successful for some versus slow/poor for others with multiple sclerosis (MS)? One possibility is that many (but not all) people with multiple sclerosis have involvement of the cerebellum. The cerebellum is critical in trial-and-error based motor learning, a commonly-used approach in physical rehabilitation. The purpose of this case report is to illustrate the importance of determining cerebellar deficits when deciding on a balance rehabilitation plan for individuals with MS, even when patients show similar overall disease severity and overall balance impairments.

Methods (case description): Case I (SensorimotorMS): Clinical scores: ICARS: 9/100 (little or no cerebellar ataxia); EDSS MS disease severity: 4/10, (no deficits in the cerebellar system); MiniBESTest balance: 17/28. Unlike Case I, Case II (CerebellarMS) ICARS score: 43/100 (significant cerebellar ataxia); similar to Case I, Case II EDSS: 4/10 (but with more deficits in both the cerebellar and sensory systems) and similar overall MiniBESTest score: 16/28.The patients were exposed to 25 exposures of trial and error-based surface perturbation training with objective outcomes reflecting anticipatory control of postural responses.

Results: Case I with SensorimotorMS made improvements in postural control after the trial-and-error-based perturbation training and quckly learned to anticipate the surface perturbations. In contrast, Case II with CerebellarMS did not make improvements in postural control with perturbation training, they did not learn to anticipate the surface perturbations.

Conclusion: Patients with SensorimotorMS may show improvements in balance from trial-and-error-based surface perturbation training. In contrast, patients with CerebellarMS may benefit from other rehabilitation approaches, such as reinforcement training, since the cerebellar involvement may impair the ability to improve reactive balance control with trial and error.

Impact Statement: Clinicians are encouraged to incorporate assessment of cerebellar systems using tests like the ICARS to determine the presence of cerebellar deficits and develop targeted rehabilitation.

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Selective Glucocorticoid Receptor (GR) Antagonist, PT150 as a Potential Therapeutic Candidate for Depression in Long Haul COVID-19 Patients

Faculty

The novel Coronavirus disease 2019 (COVID-19) outbreak is a major health threat caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 has caused a wide range of disease severity among the patients. COVID-19 affects approximately 45 million Americans, out of that 10-30% are estimated to be “long haulers.” A growing body of clinical and preclinical evidence implicates hypothalamic-pituitary-adrenal (HPA) axis dysfunction as the neurologic and psychological aspects of long COVID-19. In support of Palisades Therapeutics and a multiple collaborative research team, a multicenter, randomized, double-blind, paroxetine-controlled, flexible dose study with PT150 in patients with major depressive disorder was performed. PT150 is an oral dose clinical stage glucocorticoid receptor (GR) antagonist with antiviral activity against COVID-19 with an active IND. We found that, PT150 treatment for 4-weeks equally reduced the symptoms of depression (measured by HDRS score), compared to the selective serotonin reuptake inhibitor (SSRI), paroxetine. The HDRS (Hamilton Rating Scale for Depression) is the most widely used clinician-administered depression assessment scale of 21 items. However, PT150 showed significantly increased efficacy in subsets of depressed patients displaying HPA-axis dysfunction (as measured by high levels of serum cortisol), compared to the paroxetine. Similarly, in another clinical trial, we also observed that the treatment of PT-150 is particularly effective in depressed patients with HPA-axis dysregulation, compared to the antidepressant, clomipramine. Importantly, our collaborators have demonstrated for the first time a significant inhibitory antiviral activity in *in vitro* COVID-19 model using human bronchial epithelial lining cells (Theise et al., 2020), and in vivo COVID-19 infection model using Syrian hamster (Rocha et al., 2022). Moreover, the reported beneficial effects of SSRI’s for long COVID-19 neuropsychiatric conditions, further support the potential candidature of PT150.

Spinal Cord Injury

Felicia Michael, PhD [1] • Sajeev Kaur, PhD [1] • Samir Patel, PhD [1] • Hope Vaught, Other [1] • Job Tharappel, PhD [1] • Alexander Rabchevsky, PhD [1]
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Reversible DREAAD-mediated silencing of ascending propriospinal neurons mitigates autonomic dysreflexia

Fellow

Complete high thoracic spinal cord injury often leads to autonomic dysreflexia (AD), a condition that manifests as acute, episodic hypertension with concurrent bradycardia. It is characterized by massive discharge of sympathetic preganglionic neurons (SPN) in the intermediolateral cell column (IML) that are reflexively activated by noxious stimuli below the injury level. In addition to loss of supraspinal modulation, the neural circuitry involved in AD includes nociceptive primary afferent C-fibers, the SPN that trigger the sympathetic response, and ascending propriospinal neurons (APN) that relay the information from the afferent terminals to the SPN.

Maladaptive plasticity of nociceptive afferent C-fibers is associated with AD development, but they do not directly excite SPN, instead interneurons aid in modulating the SPN. Using chemogenetic tools, we aimed to correlate the contribution of APN sprouting with hemodynamic responses during AD. Putative APN reside in the lumbosacral cord with terminals projecting rostrally to thoracic SPN, thus contributing to AD. Accordingly, we selectively and reversibly silenced APN with inhibitory designer receptors exclusively activated by designer drugs (DREADD) at their terminals in the IML, and the severity of colorectal distension (CRD)-induced hypertension was evaluated before, during and after silencing APN in rats with complete T4 spinal transection. Because of the temporal development of AD, we further compared responses of APN transfected before and after the onset of injury-induced sprouting (immediate vs delayed 2 weeks). mCherry+ APN in injured spinal cords were denser compared to naïve, but no significant difference between immediate and delayed APN labelling were noted. A significant increase in APN labeling was observed when axonal terminals at both T7 and T13 were targeted, indicating the presence of more lumbosacral APN axonal terminals between those spinal levels. Increase in mean arterial pressure (MAP) following CRD was suppressed in animals which had a greater number of labeled neurons. This physiological effect persisted for up to 4 hours in most animals, and reverted back to pre-CNO levels only 24-hours later. These hemodynamic changes in response to CRD appeared correlated with the number of mCherry+ neurons that were silenced in response to CNO. We also monitored the number of spontaneous AD (sAD) events weekly for 24hrs to show that when animals received CNO towards the end of the experiment, no sAD events were detected with inhibitory DREADD activation, irrespective of the injection group (i.e. immediate or delayed). Taken together these results show that APN are key in relaying the afferent input from the lumbosacral afferent fibers to the SPN, and that modulating their activity may help to prevent/mitigate the incidence and severity of AD, although not entirely.

Supported Craig H. Neilsen Postdoctoral Fellowship #651019 (FMM), SCoBIRC Chair Endowment (AGR).

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Mitochondrial transplantation to the injured spinal cord via engineered erodible hydrogels

Faculty

Traumatic spinal cord injury (SCI) triggers a cascade of secondary complications that propagate pathophysiology thwarting regeneration and functional recovery. Mitochondrial dysfunction is a primary contributing factor that exacerbates this response by leading to energy failure and cell death. Mitochondrial loss during the acute phase of SCI is accompanied by inflammatory and immunomodulatory responses. Employing our novel approach to transplant healthy isolated mitochondria, we found that acute intraspinal nano-injections of mitochondria isolated from rat soleus muscle significantly preserved bioenergetics of injured tissues 48hr after contusion SCI. However, focal accumulation of mitochondria at the injection sites resulted in compromised tissue integrity, limited mitochondrial spread and reduced viability of transplanted mitochondria. We developed the overall hypothesis that, compared to naked mitochondria, enclosing exogenous mitochondria within a thermal gelling erodible hydrogel matrix will prolong their bioenergetic integrity at 37°C, and thus aid in localized delivery around the lesion epicenter of the injured spinal cord for extended periods. The current study employed human SH-SY5Y cells (neuroblastoma cell line) and rat PC-12 (pheochromocytoma cell line) to optimize an ideal hydrogel composition to preserve the integrity and bioenergetics of exogenously applied mitochondria. The erodible hydrogel was composed of 1% methylcellulose (MC) and 1% hyaluronic acid (HA) that facilitate subdural mitochondrial delivery and release for uptake into recipient cells. Results showed that ~70% of mitochondria are released from the hydrogel within 60 minutes at 37°C, and that the hydrogel maintained higher mitochondrial oxygen consumption rates (OCR) compared to those without gel. We also documented that mitochondria that are genetically tagged with red fluorescence protein (RFP) is a reliable marker for real-time spatiotemporal tracking of exogenous mitochondria. Additionally, xenogeneic mitochondrial DNA serves as an unequivocal surrogate marker to verify mitochondrial uptake after transplantation into host cells, by qPCR. Ongoing and future experiments are assessing the dose- and time-dependent effects of intraspinal vs subdural mitochondrial delivery on cellular bioenergetics and cell-specific internalization.

Support: SCoBIRC Chair Endowments (AGR & PGS); DoD W81XWH2010347 (AGR); NIH-R01 NS119337 (AGR & SPP)

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Astrocytes promote acute survival of CNS macrophages and motor recovery after SCI

Fellow

Spinal cord injury (SCI) results in permanent disability and affects more than 250,000 individuals in the US. Astrocytes, together with microglia and monocyte-derived macrophages (referred to as CNS macrophages), coordinate wound healing after SCI. Much of how these cells orchestrate wound compaction remains to be understood. We found that astrocytes produce colony stimulating factor 1 (CSF1), which is vital for microglia and macrophages' proliferation, survival, and phagocytic activity. In this work, we aim to examine the effects of astrocyte-derived CSF1 on the cellular dynamics of astrocytes, microglia, and macrophages at the lesion site in acute and chronic stages of SCI, modeled by complete crush at thoracic level T8. At 7 days after SCI, mice with CSF1 deletion in adult astrocytes showed a trend towards reduction in microglia, macrophage, astrocyte and lymphocyte cell numbers. At the chronic time point of 60 days after SCI however, astrocytic CSF1 knockout mice trended towards higher cell numbers of all three cell types, suggesting a mechanism that compensated for their early loss. Basso Mouse Scale and regular horizontal ladder testing over 8 weeks showed worsened hindlimb motor recovery in astrocytic CSF1-knockout mice. These results indicate a positive role of astrocyte-derived CSF1 in the acute survival of CNS macrophages and functional recovery following SCI.

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Leucine Zipper-bearing Kinase (LZK) regulates astrocyte cell migration

Staff

After Spinal cord injury, reactive astrocytes migrate toward the injury site to form the astrocytic scar. Our lab discovered leucine zipper-bearing kinase (LZK) as a major positive regulator of astrocyte reactivity to injury. This study examines the role of LZK in the regulation f astrocyte cell migration. Astrocytes were isolated from tamoxifen-inducible, astrocyte-specific LZK knockout (KO) mice and *4-Hydroxytamoxifen was applied to induced gene deletion in vitro*. We assessed cell migration by scratch assay, lamellipodia characterization, microtubule acetylation, and filamentous to globular actin ration. Astrocytes lacking LZK showed decreased cell migration, reduced length of lamellipodia, and lower levels of polymerized actin and acetylated tubulin. These results suggest that LZK promotes astrocyte migration by regulating tubulin and actin dynamics in cytoskeleton rearrangement. Pathways through which LZK causes these cytoskeletal changes are under investigation.

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Role of reactive astrocytes in axon sprouting and motor recovery after SCI

Student

Injured axons in the adult mammalian central nervous system (CNS) lack the ability to regenerate, resulting in often permanent paralysis following CNS injury including spinal cord injury (SCI). However, a form of axon growth called axon sprouting, defined as injury-induced growth of spared axons, can occur in undamaged tissue distal to the injury site and facilitate functional recovery. In this study, we seek to determine the role of reactive astrocytes in axon sprouting and associated functional recovery after SCI. Our lab has previously identified leucine zipper-bearing kinase (LZK) as a positive regulator of astrocyte reactivity in the injured spinal cord. By deleting or overexpressing LZK in adult astrocytes, we modulate the level of astrocyte reactivity to examine its effect on corticospinal tract (CST) axon sprouting at the lumbar enlargement and hindlimb motor recovery after lateral hemisection of the spinal cord at thoracic level T8. By Basso Mouse Scale and regular horizontal ladder, we found that LZK overexpression in astrocytes resulted in an improvement of motor recovery after SCI, supporting a positive role of astrocyte reactivity in motor recovery. Axon sprouting analyses are currently underway.

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Neuronal-specific deletion of PTEN using retrograde AAVs induces axon regeneration and restores motor functions in both acute and chronic SCI

Faculty

Spinal cord injury (SCI) is hallmarked by damage to long spinal tracts carrying motor and sensory information to and from the brain. Long-distance spinal tracts originate throughout the neuroaxis with several spinal-projecting nuclei located in vital brainstem regions. There is a need to advance regenerative strategies capable of regrowing damaged axons in a manner specific and expansive enough to affect only and all axons involved in the SCI pathology. To date deletion of the phosphatase and tensin homologue protein (PTEN) from neurons in the motor cortex has demonstrated a limited ability to induce axon regeneration after SCI from this singular spinal tract. Our project has utilized PTEN knockout (KO) as an actionable target to stimulate regeneration and has utilized spinal injections of adeno-associated viruses capable of retrograde transport (AAVrg) to target a breadth of neurons throughout the neuroaxis using a single spinal injection. We demonstrate that a single spinal injection of AAVrg can target neurons throughout the motor cortex, red nucleus, various brainstem motor neuron pools, as well as neurons throughout the spinal cord, effectively conferring a more expansive gene-therapy effect compared to previous methods targeting a single spinal tract. When AAVrg was used to knockout PTEN in mice with acute severe SCI, we observed a robust improvement in hind-limb function with several mice regaining weight supporting stepping. When AAVrg was used to knockout PTEN in mice with chronic severe SCI, mice with lower functional abilities at the time of treatment improved hind-limb function while mice with weight supporting abilities at the time of treatment lost the ability to weight support. PTEN-KO using AAVrg resulted in a 2-fold increase in axon densities within the lesion confirming the regenerative effects. Analysis of regenerating serotonergic (5-HT) axons and corticospinal tract axons labeled with neural tracers is currently underway in both acute and chronic conditions. We observed that using the neuronal specific promoter, hSyn1, to drive reporter genes in affected neurons interacted with the PTEN-KO treatment. Specifically, we found that the red fluorescent protein reporter gene was down regulated in mice receiving PTEN-KO. To validate that PTEN-KO was indeed suppressing the hSyn1 promotor rather than inducing neuronal toxicity we performed retrograde tracing using Fluorogold and identified the perseverance of neurons throughout the motor cortex. To better understand the relationship between PTEN and synapsin expression, we derived adult neural stem cells which were differentiated into neurons in vitro and treated the cultures with a PTEN inhibitor. The effects of PTEN inhibition on the gene expression of mature synaptic markers is currently underway. Collectively our work has identified that using AAVrg can affect neurons throughout the neuroaxis, enhance axon regeneration, and restore functions in both acute and chronic severe SCI.

The intraspinal adaptive immune response varies between males and females after spinal cord injury across multiple species

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Sex differences in pathophysiology and recovery outcomes have been observed post spinal cord injury. However, sex is understudied in SCI pathophysiology. We examined intraspinal inflammatory responses between male and female pigs after T10 contusion injury. The pig SCI model has important similarities to humans in anatomic and physiologic characteristics including inflammation. Briefly, adult (gonad-intact) male and female Yucatan miniature swine were subjected to either a SCI or laminectomy only control. Females were injured on proestrus, estrus and diestrus stages to consider the effects of estrous cycle. Animals were sacrificed at 48hr or 6 weeks after SCI and spinal cord sections were analyzed for astrocytes, microglia, macrophages, B-lymphocytes, d T-lymphocytes (T cells) by immunohistochemistry. Neutrophils were counted based on H&E morphology. No histopathological abnormalities were identified in control cases. All data were analyzed on Halo v2.2.1870. No differences were seen for astrocytes, microglia, macrophages, B cells, and neutrophil infiltration between males and females. Individual intraspinal T cell counts and T cell clusters were significantly higher in females (independent of estrus stage) and in females injured at proestrus (in case of T cell counts only) compared to males by Welch's t test. Interestingly, we observed a similar increase in intraspinal t-cell accumulation in female vs. male mice at 6 weeks post SCI. Our observation indicates that sex is a potential biological variable for T cell infiltration and may contribute to sex-based differences in SCI pathophysiology and recovery outcomes. Our data implicates sex as a potential factor for immune-focused SCI therapies.

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University of Kentucky- SCoBIRC Endowed Chair #5

Sleep

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Characterizing sleep and epileptic activity in a mouse model of juvenile Batten disease

Student

CLN3 disease, also known as juvenile Batten disease, is a rare pediatric inherited neurodegenerative disease caused by mutations in the *CLN3* gene. Children with CLN3 disease develop progressive blindness, cognitive and motor deficits, speech difficulties, sleep disturbances, and seizures that worsen over time after 4 to 6 years of seemingly normal development. Seizures are known to be correlated with poor sleep in many epilepsies and other neurodegenerative conditions. In animal studies using an electroencephalogram (EEG), increased spiking activity has been correlated with reduced delta power; this suggests a possible interaction with NREM sleep, which is characterized by slow delta oscillations. However, no study of the mechanism by which poor sleep may correlate with – and perhaps promote – epileptic activity in CLN3 disease has been conducted. Here, we employ a mouse model to investigate sleep and epileptic aspects of CLN3 disease, which are highly disruptive to patients' everyday life.

All animal procedures were performed with IACUC approval at the University of Kentucky. A non-invasive piezoelectric motion sensor was utilized to generate a "PiezoSleep" signal to distinguish between sleep and wakefulness for *Cln3* knockout (*Cln3KO*; Jax#029471; n=6F, 2M) and WT (C57BL/6J, Jax#000664; n=7F, 5M) mice (4-7 months). A subset of these mice, including nine adult female mice (*Cln3KO*, n=4; WT, n=5; 5–16 months) were instrumented for EEG and monitored for an average of 7 days each on a 12h:12h light/dark cycle. Epileptiform spikes were detected from the recorded EEG signals as voltage deflections greater than 5 standard deviations above the mean of the signal and full width at half maximum amplitude of 5-200 ms.

Our PiezoSleep data show a difference in both sleep percentage and sleep bout length between *Cln3KO* and WT mice. Our EEG data further demonstrate that spikes are more prevalent in *Cln3KO* mice compared to WT mice ($p<0.05$) regardless of age, confirming that *Cln3KO* mice exhibit epileptiform activity. In further analysis of the collected EEG data (ongoing), we expect to determine whether spiking activity is modulated by changes in sleep composition and architecture in *Cln3KO* mice.

Characterization of sleep and epileptic activity in the animal model of CLN3 disease will lead to a better understanding of the correlation between sleep disturbances and seizures in CLN3 disease, with possible implications for disease management strategies.

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Sleep fragmentation leads to a sex-specific increase in sleep debt in the chimeric humanized APPxPS1 knock-in mouse model of Alzheimer's disease

Student

Considerable evidence supports that disruption of sleep and circadian rhythms in patients with Alzheimer's disease (AD) exacerbates neuropathology, which further impairs sleep quality. Altered sleep behavior is a leading cause of institutionalization for AD patients. The increased incidence of AD among women necessitates investigating sex differences in sleep disruption and its effects on the progression of AD pathology. Thus, we examined an APPxPS1 knock-in (KI) mouse model that better mimics AD without using transgene over expression. To assess the impact of sleep disruption on AD, we chose to focus on sleep fragmentation (SF) rather than sleep restriction, similar to the transient arousals seen in AD patients. Therefore, WT and KI female and male mice (avge 9.16 ± 1.38 mo) were either allowed to sleep undisturbed (US) or were subjected to SF consisting of sleep deprivation for 4 intervals (1 h each) evenly distributed during the light phase of the daily light-dark cycle. The US and SF treatments were conducted from Monday to Friday for 3 weeks. Sleep-wake patterns were recorded with a non-invasive PiezoSleep system, detecting vibrations that are categorized as sleep or wake at a 2sec resolution. Then, the cortex and hippocampus were dissected and Aβ40 and 42 levels were quantified in both aqueous soluble and non-soluble fractions. All SF mice had 20% less sleep compared to the US during the light phase. In the dark phase, KI females had significant rebound sleep, a 70% increase compared to its control US group ($p<0.001$). Interestingly, sleep percentage differed little across the weeks. Sleep bouts were unaffected by SF, genotype, or sex in the light phase but varied greatly in the dark phase, with the sleep bouts of the KI females exposed to SF increasing by 49.7% compared to their US group ($p < 0.001$). SF did not affect Aβ levels in this mouse line. Aβ pathology in KI mice typically begins in the cortex, and we were able to detect this in in some fractions. A modest and marginally significant increase in Aβ was observed in females versus males in some assays. However, no other comparisons showed significant differences, perhaps due to young age of the mice. We are currently investigating Aβ in older mice to confirm this trend. Disruption of sleep during the normal rest phase led to a significant increase in sleep during the active phase, with KI females exhibiting the most robust sleep rebound. This suggests that females incur increased sleep debt during sleep disruption, which may be relevant to the increased risk and severity of AD in women.

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Thermoneutral temperature exposure increases slow-wave sleep in the 3xTg-AD mouse model of Alzheimer's Disease.

Student

Introduction: There is growing evidence that disordered sleep, which is known to be associated with Alzheimer's disease (AD), may accelerate neuropathology, thus promoting a vicious cycle. Strategies for improving sleep quality may slow disease progression. Here we investigate the feasibility of sleep enhancement through ambient temperature regulation and examine the effect on amyloid pathology.

Method: Female 3xTg-AD mice (~12 m.o.) were instrumented for EEG/EMG monitoring. After a week-long baseline, one half of the mice (n=8, EXPT) were exposed to stepwise diurnal increases in ambient temperature (Ta) to reach 30^{o}C (thermoneutral for mice) during the light phase while the rest (n=8, CTRL) remained at room temperature (22^{o}C). Vigilance state – i.e., Wake, REM, NREM, and slow wave sleep (SWS) within NREM – was scored in 4-second epochs and sleep metrics were computed.

Results: SWS percentage became significantly greater ($p<0.05$) in the light phase for EXPT mice over the course of treatment. These effects suggest better sleep consolidation and greater sleep depth with thermoneutral warming. After four weeks of treatment, the animals were euthanized, and the brains removed to assay amyloid pathology by ELISA. We found that thermoneutral warming caused a significant reduction in both Aβ40 and Aβ42 in the hippocampus, but not in the cortex.

Conclusion: These data imply that thermoneutral warming might have some regional specificity in its effects. The effects appear to be specific to some brain areas more than others, with implications for the cognitive and neuropathologic changes found in AD. Furthermore, since SWS and REM support memory, future studies should investigate the effects of thermoneutral enhancement of SWS and REM on cognition.

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Stroke/Neurovascular

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Amylin, a diabetes-associated amyloid-forming peptide, accumulates in thrombi and on red blood cells – a new biomarker for stroke?

Student

Emergent large vessel occlusions result in severe ischemic stroke without appropriate treatment with thrombolysis and/or mechanical thrombectomy. Type-2 diabetes mellitus (T2DM) is a major risk factor in stroke, with 25% of ischemic attacks occurring in individuals with T2DM, and T2DM diagnosis is associated with poorer functional outcomes and increased risk of recurrent stroke. Amylin, a peptide co-secreted with insulin from pancreatic β-cells, is hypersecreted in T2DM and readily forms neurotoxic oligomers which deposit in brain parenchyma.

Due to amylin's role in T2DM and T2DM's relationship to stroke, we anticipated an increased level of amylin would be deposited on red blood cells (RBCs) of stroke patients when compared to non-stroke patients. Additionally, we anticipated an increased level of amylin immunoreactivity (AIR) in clot lysates when compared to RBC lysates and plasma.

Blood samples and thrombi (n=47) were collected from patients undergoing mechanical thrombectomies for stroke while blood samples (n=21) were collected from patients with non-stroke neurological conditions. Samples were lysed and assayed for total protein concentration and intensity of AIR. Amylin uptake coefficients (AUCs) demonstrating the proportionality of amylin deposited on RBCs compared to total circulating amylin were calculated.

After normalizing to total protein concentration, analysis revealed a significantly increased level of AIR in stroke clots when compared to stroke and non-stroke plasma and RBC lysates ($p<0.001$ for each). Additionally, a significant increase ($p<0.0073$) in AUC was found in stroke versus non-stroke.

In summary, amylin accumulates in thrombi and deposits on RBCs of stroke patients. Further research into amylin's potential role in thrombus formation is justified. Future studies are also needed to determine if stroke severity is associated with amylin level in thrombi and if T2DM exacerbates amylin-stroke pathology.

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Medicine University of kentucky [1] • Center for Advanced Translational Stroke Science University of Kentucky [2] • Neurology Beth Israel Deaconess Medical Center [3] • Biostatistics University of Kentucky [4] • Radiology University of Kentucky [5] • Neurosurgery University of Kentucky [6]

Investigating Ischemic Stroke Biomarkers Utilizing a Novel Cerebrovascular Disease Control Group

Student

Every year approximately 795,000 people have a stroke in the United States. Intraluminal retrieval of a thrombus by mechanical thrombectomy and a thrombolytic agent, tissue plasminogen activator are the only treatments for ischemic stroke. Though these interventions have improved clinical outcomes, stroke remains a leading cause of death and a major cause of disability, demonstrating a need for predictive biomarkers for functional and cognitive outcomes. These biomarkers are also potential therapeutic targets for treatments. The BACTRAC Tissue Bank at the University of Kentucky collects blood distal and proximal to a thrombus from ischemic stroke patients during the mechanical thrombectomy procedure. For control comparisons, arterial blood samples from cerebrovascular disease (CVD) patients undergoing a diagnostic angiogram are collected and banked. The clinical data retrieved includes demographics and comorbidities for each patient. This study analyzed differences in the proteomic expression of proximal blood of stroke patients compared to CVD control patients. Stroke and CVD control patients were matched for age, sex, BMI and other comorbidities. Proteomic analyses of 184 proteins from proximal stroke and control plasma samples were performed by Olink Proteomics. Proteomic differences were analyzed using unpaired T-tests. We also investigated correlations between proteomic changes with stroke outcome metrics. Our results indicate proteins associated with inflammation increased during stroke, while proteins related to growth and survival decreased during stroke. IL6, a major inflammatory cytokine, showed a significant increase in stroke patients and was positively correlated to National Institute of Health Stroke Scale (NIHSS) scores at patient discharge. Hepatocyte growth factor (HGF), which has angiogenic and anti-inflammatory properties, decreased in stroke and was negatively correlated to Montreal Cognitive Assessment (MoCA) scores at 90 days. Human patient data is notoriously variable, thus matching stroke patients with a demographic and diseased matched control may offer an improved approach to identify predictive biomarkers and therapeutic targets.

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Novel data showing Age-Associated B cells in aged mice cortex, hippocampus, and cerebellum in post-stroke and healthy mice brains

Student

Age-Associated B cells (ABCs) were first named in 2011 by Hao and colleagues in terms of autoimmune disease. These cells express the transcription factor T-Bet, which normally aids T cell differentiation. ABCs can be anergic or act as APCs, secrete antibodies and cytokines, and contribute to immunosenescence. However, the role that ABCs play in the aging brain and during post stroke recovery remains unexplored.

Methods: Histology is being completed via immunohistochemistry. Male (M, n=21) and female (F, n=20) mice aged 19-28 mos were sacrificed according per animal protocol at 3 wks & 6 wks post-30-minute-tMCAo. Out of 41 mice, 30 received 30 minute-tMCAo surgery with n=22 (53.7%) surviving. Spleens were removed and processed for flow cytometry. An extracellular panel with antibodies for CD45, CXCR5, CD23, TCRβ, CD27, CD11b, CXCR4 (CD184), IgG2a/c, IL-21R, CD11c, CD80 (B7-1), CCR7, Mouse IgD, IgM, MHC II, CD138, and CD21/25 was used to identify ABCs. Brains were perfused first with phosphate buffered saline and heparin followed by fixation (4% paraformaldehyde). Brains were removed from the skull, then cryopreserved in 15% and 30% sucrose. Brains were cryosectioned (40 μm width) and serially collected into 6-well plates to give cortical and hippocampal regions. Free floating sections were stained with B220 (CD45) to identify B cells, T-Bet for ABCs, and DAPI for nuclei. Imaging will occur on a Nikon Ti2 epifluorescent microscope and Zeiss Axioscan Z.1, with confirmation of ABC presence via z-stack on a Nikon confocal microscope. HALO software will be used for image analysis. These mice also underwent Rotarod, open field, and Catwalk tests to examine functional recovery post-stroke. Rotarod required 2 weeks of training prior to tMCAo.

Results: Aged male and female mice demonstrate the presence of T-Bet+ ABCs in cortical, hippocampal, and cerebellar regions post-stroke but also in healthy brains, with no discernable pattern for diapedesis yet identified. UMAP flow cytometric analysis will be used to identify ABC numbers in the spleen and brain using FlowJo software. Initial rotarod analysis showed no significant difference between baseline training and 1, 2, or 3-weeks post-stroke. Catwalk and open field are still being analyzed. We currently have a second cohort of n=18 post-stroke mice underway that will be sacrificed after 6 weeks. But our data are the first to confirm this B cell subset in the rodent brain post-stroke by immunohistochemistry, in the cortex, hippocampus, and cerebellum.

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Extracellular vesicle-associated proBDNF is increased during emergent large vessel stroke.

Faculty

Background: Mechanical thrombectomy (MT) and intravenous tissue plasminogen activator are standard of care ischemic stroke treatments that assist in restoring blood flow to the brain, but do not guarantee good outcomes. Emerging as plasma biomarkers for disease prognostication and targeted therapeutics, extracellular vesicles (EVs) are nanoparticles released from all cells that readily cross the blood brain barrier. EV-associated cargo, (e.g. lipids, proteins, and nucleic acids) is stimuli-specific and a direct response of the cellular micro environment.

Aim: We hypothesize that an EV protein of interest, pro brain derived neurotrophic factor (proBDNF) can be clinically relevant and used to predict stroke outcomes/progression.

Method: Human plasma (8F/ 5M) collected during MT in “Blood And Clot Thrombectomy Registry And Collaboration” (BACTRAC; NCT03153683) and control plasma (4F/ 7M) were unbanked. EVs were quantified with Zetaview-NTA analysis, following isolation with Exoquick, before proBDNF protein was quantified via ELISA.

Results/Conclusions: There was no significant difference in the size (~107 nm) or concentration (10^{13} particles /mL) of EVs between controls (average age 34.5 years) and ischemic stroke subjects (average age 73.7 years). EV proBDNF expression was higher in ischemic stroke subjects compared to healthy controls (p=.0021). During MT, female subjects with higher EV proBDNF expression correlated to lower time to recanalization (i.e. infarct time / last known normal; R2=0.657, p=0.0147). In males, higher EV proBDNF expression correlated with higher Modified Rankin Scores (mRS; i.e. more disability) at discharge (R2=0.812, p=0.0988). No significant sex differences were observed in proBDNF EV expression, recanalization time, or mRS. These data suggest that EV proBDNF expression is differentially regulated with sex in response to stroke and should be explored further for translational applications.

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Differences in proteomic response in Appalachian stroke patients undergoing thrombectomy

Faculty

The Appalachian region of North America is a subpopulation of the country that has become an area of great interest regarding access to care, health disparities, and health outcomes. Appalachia has a high proportion of rural, Caucasian, socioeconomically and medically underprivileged individuals. As reported by the Appalachian Regional Commission in 2017, individuals from Appalachia are more likely to have stroke-related comorbidities including diabetes, obesity, and increased tobacco usage. Patients in rural areas are less likely to receive thrombectomy treatment and clinical outcomes are poorer among rural patients when compared to their urban counterparts. Our institution is optimally positioned for research in this field as it provides ischemic stroke interventions including mechanical thrombectomy (MT) to both Appalachian (63%) and non-Appalachian (37%) counties. Our institution has developed a human stroke biospecimen tissue bank and registry, the Blood And Clot Thrombectomy Registry And Collaboration (BACTRAC). For each thrombectomy subject, the BACTRAC registry obtains an intracranial (distal to thrombus) arterial blood sample, a systemic (carotid) arterial blood sample, and the thrombus for research purposes. The objective of this study is to utilize BACTRAC proteomic data to identify Appalachia-specific predictors of neurologic function, cognitive function, and radiographic outcome after MT. Interestingly, no differences in demographics or co-morbidities arose between Appalachia and non-Appalachia patients. As expected, time to treatment was significantly longer for Appalachian patients. Our analysis of 184 cardiometabolic and inflammatory proteins revealed expression values of 22 proteins were significantly different between Appalachian and non-Appalachian groups. The functions of these proteins are linked to signaling to extracellular stimuli and to responses to chemicals and organic substances. A few of these proteins were associated with time to treatment, functional and cognitive recovery, and radiographic outcomes. This study is suggestive that environmental factors are involved in differences in the proteomic response to stroke in patients from Appalachia compared to non-Appalachia.

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Prediction and Confirmation of Effort Associated with Graded Finger Extension in Individuals with Hemiparetic Stroke and Healthy Controls

Student

Strokes are a leading cause of lifelong disability (e.g., impaired hand function). Therefore, stroke survivors must undergo extensive therapies to attempt to regain motor control. Currently, technologies are being developed to help improve these rehabilitation strategies. For example, brain-computer interfaces (BCIs) offer disabled individuals the means to interact with external devices by decoding electroencephalogram (EEG) signals. In the case of hand rehabilitation, integrating a BCI system with a sensor glove can provide vital information on fine motor control in individuals with large cortical lesions. Here we investigate the feasibility of predicting motor effort from the EEG associated with graded finger extension as measured by a motion capture glove in stroke patients with left-hand paresis and healthy controls of similar age. Following an IRB-approved protocol, participants extended fingers of one hand in response to visual cues to one of four levels: low, medium, high, or “no-go” (i.e., none). Hand, extensor muscle, and brain activity were monitored using motion capture, electromyography (EMG), and EEG (32 channels), respectively. Event-related desynchronization (ERD) of the sensorimotor EEG was measured as the 8-30 Hz signal power at each scalp location relative to a pre-trial reference period. A quadratic classifier was trained on this ERD feature vector to predict the level of finger extension and its accuracy assessed using k-fold cross-validation in each participant. In both stroke and control groups, the ERD classifier gave 46-67% accuracy per target on either hand—much greater than chance (25%)—despite severe spasticity in the stroke-impaired hand. In contrast, an EMG power classifier gave only 34-44% accuracy per target on the stroke-impaired hand but 44-97% in the unimpaired hand and in controls. Hence, the EEG captures the effort or intent associated with finger extension regardless of success or failure in the task. The motion capture glove provided confirmation of attempted or accomplished movement. Work is ongoing to extend this protocol to include hand contraction and grip force using a custom-designed sensor glove. This system could be useful in rehabilitative BCI protocols that emphasize fine control.

Disclaimer: This work was performed while Lumy Sawaki was employed at University of Kentucky. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

Title: Using UMAP to identify unique populations in SAH CSF patient samples

Thomas Ujas
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I would like to attend both Thursday and Friday

Poster abstract submission:

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Stroke/Neurovascular

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Title:
Using UMAP to identify unique populations in SAH CSF patient samples

Background:
Prior clinical research used flow cytometry to identify an active presence of innate and adaptive immune cells in cerebrospinal fluid (CSF) of patients with aneurysmal subarachnoid hemorrhage (aSAH). The purpose of this study was to expand on this work to identify novel leukocyte using an un-biased algorithm that self-identifies unique populations through clustering in high-dimensional space.
Methods:
CSF samples (n=23) were collected from an extra ventricular drain (EVD) placed in aSAH patients (n=6). Samples were obtained at days 3, 5, 7, and 10 post-aSAH. All samples were processed and stained using a general immunophenotyping panel containing a viability stain (Ghost Dye 780), plus antibodies targeting CD45, CD3, CD4, CD8, CD11b, CD11c, CD14, CD19, CD66b, CD138, CD161, and CXCR3. Gating was performed in FlowJov10 and samples run through FlowAI to clean data of deviations

during acquisition. All CD45+ live cells were downsampled and concatenated into a single file and analyzed using uniform manifold approximation projection (UMAP). Six samples below threshold for concatenation were excluded from UMAP and manually gated. After UMAP performed a non-linear dimensionality reduction, FlowSOM was used to create self-organizing maps (SOM) of the data. FlowSOM runs an unsupervised clustering algorithm that also serves as a visualization aid to gain insight on subpopulations. Cell populations were identified using phenotypic characteristics based on the dimensionality reduction plot generated by UMAP in conjunction with the unique clusters identified by FlowSOM. The data was further analyzed for statistical significance using Graphpad PRISM.

Results:

Unsupervised gating in addition to the dimensionality reduction yielded 12 distinct CD45+ populations, including three distinct neutrophil clusters with one expressing CXCR3. Neutrophil populations significantly increased through 10 (all $p<0.05$ vs. day 3). Three CD19+ B cell clusters where identified, with two clusters expressing CD138. All B cell populations decreased from day 3 to day 10. Finally, two CD4 T cell populations were identified and all CD4 T cells significantly decreased from day 3 to day 5 ($p<0.05$). These data suggest that UMAP analysis of longitudinal CSF sampling can identify unique subpopulations of both innate and adaptive immune cells that may be lost using traditional gating analyses.

TBI

Sex Differences in Adult Hippocampal Neurogenesis

Ashley Glover, Hannah Williams, Kathryn Saatman

Abstract

Following a traumatic brain injury (TBI), adult hippocampal neurogenesis is stimulated. Hippocampal neurogenesis is linked to memory and learning performance. Traditionally TBI studies have almost exclusively focused on male mice. Sex differences exist in the regulation of neurogenesis and need to be examined as a physiological variable in posttraumatic hippocampal neurogenesis. We used transgenic reporter mice to genetically label neural progenitor cells to examine sex differences in the regulation of adult neurogenesis and in neuron morphology. Naïve mice received tamoxifen injections at 8 weeks of age. After waiting 6 weeks to allow neurons to mature, mice were euthanized. Here we investigate the number of mature neurons in the dentate gyrus of male and female mice. There is no significant difference in the overall numbers of mature neurons between male (n=6) and female (n=10) mice. To assess dendritic complexity we will conduct Scholl analysis to examine dendrite length and branching complexity. Conclusions for this study indicate thus far that there is no sex difference in the number of mature granule cell neurons. This study is an important baseline for future studies examining sex differences with neurogenesis post TBI.

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Effect of 17β-Estradiol on Mitochondrial Dysfunction in a Mouse Model of Traumatic Brain Injury.

Student

Traumatic brain injury (TBI) is caused by a blow, jolt, or penetrating injury to the head resulting in abnormal brain function. A large percentage of clinical and pre-clinical studies that included both males and females have found females experience better outcomes compared to male counterparts following severe TBI. Our research seeks to identify the underlying mechanism behind this apparent resilience. One strong possibility is the difference in sex-related hormones. One major female sex hormone is 17β-estradiol (E2) and published data identifies mitochondria as a target of E2 action. As mitochondrial dysfunction is a hallmark of TBI, we look to examine the role of E2 on mitochondrial homeostasis following TBI. These studies explore the hypothesis that E2 is protective for mitochondrial outcomes after the severe controlled cortical impact (CCI) model of TBI.

First, we sought to understand how E2 interacts with isolated cortical mitochondria from naïve male and female mice. Total mitochondria were treated *ex vivo* with doses of E2 that were shown to reduce mitochondrial peroxide production *in vitro* (0.2, 20, or 2000nM E2) to determine whether acute (30m) incubation improves bioenergetics, Ca^{2+} buffering, or reactive oxygen species (ROS) production compared to vehicle. The results showed no significant differences in mitochondrial bioenergetics between sexes nor E2 dose, though mitochondria from female mice had non-significant increases compared to males. Mitochondrial ROS production was observed under two conditions: high mitochondrial membrane potential ($\Delta\psi_m$) which typically produces more ROS, and low $\Delta\psi_m$ which typically produces less ROS. 2000nM E2 increased ROS production in both sexes at high $\Delta\psi_m$ compared to vehicle. Interestingly, at low $\Delta\psi_m$, mitochondria from female mice had significantly lower ROS production compared to males in a low dose-dependent manner. Ca^{2+} buffering was not significantly different between sex nor E2 dose.

We next utilized ovariectomized (OVX) mice implanted with a physiological dose of E2 (180µg/mL) 3d prior to CCI to determine whether prophylactic E2 administration would protect cortical synaptic and nonsynaptic mitochondria from CCI-related bioenergetic impairment. The results showed that nonsynaptic mitochondria from E2-implanted CCI mice had significant bioenergetic impairment 24h post-CCI compared to E2 sham mice, while the blank-implanted CCI group was not significantly different from blank sham. Alternatively, synaptic mitochondria from E2 CCI mice did not show bioenergetic impairment compared to E2 sham, while blank CCI mice did show significant bioenergetic dysfunction compared to blank sham. These results indicate E2 protects synaptic mitochondria, but not nonsynaptic mitochondria, from bioenergetic impairment. Ongoing studies will examine the effect of *ex vivo* E2 on injured cortical mitochondria from male, OVX female, and intact female mice to determine whether E2 provides therapeutic benefits after CCI.

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LRP1 knockout cells are more resilient to oxidative stress induced mitochondrial dysfunction

Staff

Millions of individuals suffer from from cognitive deficits after traumatic brain injury (TBI). However, to date, there is no approved treatment for TBI. Compelling experimental data demonstrate that strategies restoring mitochondrial bioenergetics and mitochondrial-derived reactive oxygen species (ROS) homeostasis following TBI are neuroprotective. Low-density lipoprotein receptor-related protein 1 (LRP1), a large endocytic molecule, significantly increased during ischemic brain injury and TBI. However, how LRP1 regulates mitochondrial dysfunction and ROS, two hallmarks of TBI pathophysiology, following brain injury is unknown. We mimicked the oxidative TBI environment *in vitro* by treating the mouse embryonic fibroblast (MEF) cells with the extensively-used free radical generator, 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH). Under normal conditions, LRP1 knockout (LKO) itself does not alter mitochondrial function in MEF cells as compared to WT cells. However, AAPH treatment significantly decreased mitochondrial bioenergetics, measured as oxygen consumption rate using the Seahorse XFe96 analyzer, in WT cells, though AAPH did not affect mitochondrial function in LKO cells. Oxidative stress-mediated mitochondrial fragmentation, measured by Mitochondrial network analysis (MiNA) by MitoTracker Green, was lesser in LKO cells compared to WT cells. In addition, LKO cells were found to have higher ROS buffering capacity compared to WT cells when treated with AAPH. Hydroxyl radicals are known to cause lipid peroxidation and the production of 4-Hydroxynonenal (HNE). AAPH treatment significantly increases HNE conjugates in WT cells, though does not increase HNE in LKO cells. Overall, cells deficient in LRP1 are more resilient to oxidative stress and ROS-induced mitochondrial dysfunction. To further explore the function of LRP1 in TBI, future studies will include neuron-specific conditional LRP1 knockout mouse model as well as LRP1 inhibitors to explore LRP1 mechanism following TBI.

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MicroRNA-223 regulated inflammatory pathways exhibit sexually dimorphic characteristics in bone marrow-derived macrophages

Student

Traumatic brain injury (TBI) is often followed by secondary injury cascades that can be acute or chronic. Neuroinflammation is a key player in secondary response following TBI and involves both resident microglia and recruited macrophages. While acute inflammation is essential for cellular repair, chronic dysregulation leads to excessive release of pro-inflammatory cytokines by resident and recruited myeloid cells. MicroRNAs (miRNAs) are small non-coding RNAs and are important regulators of post transcriptional gene expression. MiRNAs serve as key regulators in many secondary injury events. Our previous studies showed that miRNAs are dysregulated in injured brain and may regulate the neuroinflammatory response. However, the underlying mechanisms remain elusive. We recently reported that miR-223-3p exhibits a sex-specific differential neuroinflammatory response in mouse brain CD11b+ cells and has a general favorable effect in females following TBI. While miR-223-3p plays a key role in limiting myeloid cell pro-inflammatory signaling, its role in neuroinflammation is not known. Here we demonstrate that deficiency of miR-223-3p in bone marrow-derived macrophages (BMDMs) results in elevated inflammation with a higher inflammatory state in females versus males. Specifically, pro-inflammatory genes such as TNFα and CCL17 were generally up-regulated and anti-inflammatory genes such as ARG1 were down-regulated in female miR-223-3p knockout (KO) verse male KO BMDMs. At the protein level, the miR-223-3p target, NLRP3, a key molecule regulator of inflammasome pathway, was increased in KO BMDMs. Finally, immunofluorescent cytochemistry studies showed that major histocompatibility complex II (MHCII), an inflammatory protein marker, was significantly elevated in BMDMs of both female and male KO BMDMs, with the elevation being significantly higher in females relative to males. These results strongly suggest the existence of previously undescribed role for miR-223-3p in the sexual dimorphic inflammatory response of BMDMs, which may contribute to sexual differential neuroinflammation following TBI.

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Insights into necessary function of IL-1 in regulating neuroinflammatory gene expression following a closed-head traumatic brain injury in mice.

Student

Neuroinflammation is a major mechanism contributing to secondary injury cascades following a traumatic brain injury (TBI). Interleukin-1 (IL-1), often called a master cytokine, is elevated in the brain after a TBI, and likely drives a significant amount of the inflammatory response to the TBI. However, it is often assumed that significant redundancies in the cytokine communication network can overcome the loss of a single cytokine. In contrast, we hypothesize that specificity in the neuroinflammatory responses depends on IL-1 signaling. Using a diffuse mouse model of mild traumatic brain injury (mTBI) caused by a closed-head injury (CHI), we tested our hypothesis using a global genetic knockout of the receptor for IL-1 (IL-1R1). The neuroinflammatory response to a TBI is not stable over time but occurs in activation waves and then resolution. Therefore, we also evaluated IL-1R1s role in regulating the pattern of the inflammatory response. The study used wild-type (WT) male mice (n=56) and IL-1 Receptor 1 (IL-1R1) global knock-out (gKO) male mice (n=51) subjected to either a midline CHI (WT n=29, gKO n=29) or sham procedure (WT n=27, gKO n=22). Mice were sacrificed at 3 hrs, 9 hrs, 24 hrs, and 72 hrs post-injury. RNA was extracted from the neocortex, most proximal to the area of injury. Gene expression changes were measured using the Nanostring neuroinflammatory panel. Loss of IL-1R1 signaling blunted the inflammatory response to injury at the early (3 hrs, 9hrs) post-injury time point compared to the WT mice. However, the temporal pattern of gene expression changes was also altered in the IL-1R1 gKO mice with some genes showing a delayed upregulation compared to the WT mice. Out of 757 genes analyzed, 56 WT and 20 gKO genes were found significant at the 3 hr time-point, 41 WT and 0 gKO genes were found significant at the 9 hr time-point, 4 WT and 8 gKO genes were found significant at the 24 hr time-point, and 1 WT and 0 gKO gene was found significant at the 72 hr time-point. IL-1R1 was found to have specificity in regulating aspects of the neuroinflammatory cascade, including chemokine upregulation, while having limited effects on cytokines, such as TNFalpha. This study provides further insight into the necessary function of IL-1R1 in amplifying the neuroinflammatory cascade.

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QUANTIFYING ASTROCYTES AND PERICYTES WITHIN THE CORTEX AND HIPPOCAMPUS AFTER MILD TBI

Student

Individuals in the military can suffer from a traumatic brain injury (TBI), which can cause several physical and mental symptoms. About 80% of TBI within the population is mild and many; mild TBI injuries are not reported. Mild TBI is of concern because damage to the blood-brain barrier (BBB) and fluid-filled spaces of the brain can be the cause of long-term effects or severe symptoms. The BBB is made of both astrocytes, which express the glial fibrillary acidic protein (GFAP), and pericytes, which express platelet-derived growth factor receptor alpha (PDGFR alpha). Our hypothesis is that, levels of GFAP and PDGFR alpha decrease within the hippocampus and the prefrontal cortex region of the brain after mild TBI.A single blast wave of 11 psi peak overpressure was administered to Sprague Dawley rats and at 24 hours post-injury, GFAP, and PDGFR alpha were measured in the hippocampus and prefrontal cortex using the Western Blot technique. At 24 hours post-injury, there were no significant changes in GFAP and PDGFR alpha levels for the hippocampus or prefrontal cortex as compared to sham groups. A single mild TBI may not alter these regions acutely, therefore it may be necessary to quantify GFAP and PDGFR alpha at 7 days after a single mild TBI.

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Establishing the pharmacokinetics of ketone esters as a novel therapeutic to elevate beta-hydroxybutyrate for use following traumatic brain injury

Student

Traumatic Brain Injury (TBI) is a leading cause of neurological injury resulting from falls, vehicle collisions, war-sustained injuries, sports accidents, and intimate partner violence. Among the pathophysiological changes caused by TBI are hyperexcitability, excitotoxicity, and inflammation of the brain. As well as causing long-term neurological impairment, TBI increases the risk of neurodegenerative diseases like Alzheimer's disease (AD). Preexisting proteinopathies, such as amyloid beta (Aβ), exacerbate the effects of a TBI by causing deficits in energy metabolism and increased neuroinflammation. Beta-hydroxybutyrate (βHB; 3-hydroxybutyric acid) has been identified as a therapeutic agent which can reduce neuroinflammation and mitochondrial dysfunction. The HCA2 receptor is activated once levels of βHB produced in the liver reach levels above 0.5 mM – 1.0mM. The use of exogenous βHB as a neuroprotective agent following a TBI has never been investigated. In this study, various doses of ketone esters (1.5, 3, and 6 mg/kg) were administered via oral gavage every 15 minutes for one hour. The blood pharmacokinetics of βHB and glucose after KE ingestion were established to obtain a kinetic profile. βHB levels were evaluated by using a β-ketone plus glucose monitoring system. The optimal dose of KE needed following a TBI to act as a neuroprotective agent needs to be determined by further studies.

QUANTIFYING ASTROCYTES AND PERICYTES WITHIN THE CORTEX AND HIPPOCAMPUS AFTER MILD TBI

Abstract

Jaida Garrett, Gopal V. Velmurugan, Emily P. Brown, & W. Brad Hubbard

Individuals in the military can suffer from a traumatic brain injury (TBI), which can cause several physical and mental symptoms. About 80% of TBI within the population is mild and many; mild TBI injuries are not reported. Mild TBI is of concern because damage to the blood-brain barrier (BBB) and fluid-filled spaces of the brain can be the cause of long-term effects or severe symptoms. The BBB is made of both astrocytes, which express the glial fibrillary acidic protein (GFAP), and pericytes, which express platelet-derived growth factor receptor alpha (PDGFR alpha). Our hypothesis is that, levels of GFAP and PDGFR alpha decrease within the hippocampus and the prefrontal cortex region of the brain after mild TBI. A single blast wave of 11 psi peak overpressure was administered to Sprague Dawley rats and at 24 hours post-injury, GFAP, and PDGFR alpha were measured in the hippocampus and prefrontal cortex using the Western Blot technique. At 24 hours post-injury, there were no significant changes in GFAP and PDGFR alpha levels for the hippocampus or prefrontal cortex as compared to sham groups. A single mild TBI may not alter these regions acutely, therefore it may be necessary to quantify GFAP and PDGFR alpha at 7 days after a single mild TBI.

Vascular Cognitive Impairment and Dementia

Oxidative stress associated cerebrovascular pathology in Alzheimer's disease

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Oxidative stress is a key mechanism in pathogenesis and pathophysiology of neuronal disorders such as Alzheimer's disease (AD) and also plays roles in vascular injury. Cerebrovascular lesions highly comorbid with AD pathology and may worsen disease progression and reduce treatment efficacy. Oxidative stress markers including nitration of macromolecules are increased in AD. Here, we aim to investigate nitration status of fibronectin, a multi-function extra cellular matrix protein that present in the bloodstream and brain parenchyma where it maintains vascular and perivascular integrity. Immunolabeling was performed to investigate levels of fibronectin and nitrotyrosine in postmortem AD brain specimens confirmed with vascular pathology obtained from the UK –ADC brain bank. We found several lesions that link to different stages of vascular and brain pathology. Immunoreactivity of fibronectin and nitrotyrosine surround multiple arterioles and venules indicates a cute vascular leakage. Levels of fibronectin and nitrotyrosine are increased in reactive astrocyte surrounding the vessels suggesting that oxidative stress is involved in astrocyte activation. Our results provide evidence that oxidative damage of fibronectin could be used as a new biomarker and strengthen association of oxidative stress and vascular complication in AD which could benefit future studies of combine pathology

In vivo downregulation of pancreatic amylin improves brain function and reduces brain β-amyloid pathology in APP/PS1 mice

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Abstract Text:

Background:

Amylin is a systemic hormone that is co-secreted with insulin from pancreatic β-cells. Amylin co-aggregates with brain parenchymal and vascular β-amyloid in persons with Alzheimer's dementia. Cross-sectional data show higher CSF and blood amylin levels are associated with increased frequency of cognitive impairment. The present study sought to determine how *in vivo* downregulation of amylin influences brain function during the development of Aβ pathology.

Method:

Because mouse amylin is nonamyloidogenic, we developed an APP/PS1 mouse model in which the mouse amylin gene is replaced by the human amylin gene and is conditionally regulated by tamoxifen (TMX) injection, intraperitoneally. At 3 months of age, human amylin-expressing male mice were randomly assigned to either amylin downregulation group (maintained amylin expression) (n=10/group). Two months later, we assessed brain function with the novel object recognition test and performed comparative immunochemical Aβ analyses of hippocampal tissue by using MSD ELISA and immunohistochemistry (IHC).

Result:

Mice with downregulated human amylin show enhanced recognition memory index ($p < 0.001$) and lower plasma amylin levels ($p < 0.001$) compared to those that continued to express human amylin. This was associated with decreased hippocampal levels of $A\beta_{42}$ ($p < 0.05$) measured by MSD ELISA and decreased number of plaques ($p < 0.01$) measured by IHC.

Conclusion:

Amylin downregulation in APP/PS1 mice improves memory. Molecular processes associated with improved memory involved decreased hippocampal Aβ pathology. Further studies are needed to understand how altered secretion of pancreatic amylin may influence the balance between brain Aβ accumulation and Aβ elimination from the brain.

Evaluation of pathologies associated with hyperhomocysteinemia in human autopsy brain tissue

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Background: Vascular contributions to cognitive impairment and dementia (VCID) are one of the leading causes of dementia; VCID affects roughly 10-40% of all dementia patients. A major, yet underrecognized, modifiable risk factor for VCID is hyperhomocysteinemia (HHcy). Defined as elevated levels of plasma homocysteine (a non-protein-forming amino acid), most late-life HHcy is caused by impaired B vitamin absorption. Although HHcy has been recognized as a risk factor for VCID, studies aimed at identifying pathologies associated with HHcy have been lacking.

Methods: To determine pathologies associated with HHcy, we identified 31 autopsied research volunteers with antemortem homocysteine levels; 13 cases had normal plasma homocysteine levels (>14μmol/L) and 18 had high plasma homocysteine levels (<14μmol/L). We then measured levels of homocysteine and related metabolites in both plasma samples taken closest to autopsy and frontal cortex. Next, we determined whether the level of plasma homocysteine was associated with several markers identified via immunohistochemistry, including Aβ, PHF-1, IBA-1 and GFAP. Plasma and brain protein markers for inflammation and angiogenesis were also measured to determine associations with plasma homocysteine. Finally, we used the Human Neuroinflammation NanoString panel to determine gene expression changes of inflammatory markers associated with high homocysteine levels in the frontal cortex and occipital lobe.

Results: Plasma metabolite analysis showed patients who had elevated levels of homocysteine also had increased levels of several homocysteine cycle metabolites such as cysteine, S-adenosyl-homocysteine, cystathionine and choline. Flt1, an angiogenic marker, and IL5, an inflammatory marker, had a positive correlation with increased plasma homocysteine. No correlation between IBA-1 or GFAP immunohistochemistry with plasma homocysteine was found. Gene expression showed that most genes were downregulated in the presence of high plasma homocysteine, including many significant genes involved in apoptosis, growth factor and cytokine signaling, and the innate and adaptive immune response.

Conclusions: These preliminary data show that increased plasma homocysteine correlates with protein inflammatory and angiogenic markers and with a significant downregulation of inflammation-related gene expression markers in the brain. Overall, this could reflect impaired normal immune function, providing possible mechanisms by which hyperhomocysteinemia induces cognitive deficits and cerebrovascular damage.

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Enlarged perivascular space counts are negatively associated with Uniform Data Set Version 3 executive function composite scores in older adults

Student

Cerebral small vessel disease (cSVD) is an important risk factor leading to the development of vascular contributions to cognitive impairment and dementia (VCID). cSVD is characterized by several in-vivo neuroimaging biomarkers including enlarged perivascular spaces (ePVS). PVS are fluid-filled spaces believed to play a role in the glymphatic system's removal of waste from the brain. Reduced clearance may cause backup and enlargement of the PVS and subsequent accumulation of toxic solutes characteristic of neurodegeneration, including Aβ. Evidence supports the role of ePVS in aging and dementia, but the relationship between ePVS and cognitive function remains unclear**.** We hypothesized that quantitative, baseline ePVS counts in older adults would be associated with baseline executive function, a multifaceted cognitive domain critical to daily life that is impacted by cSVD and VCID. We explored the relationship between ePVS and the Uniform Data Set (v3.0) executive function composite scores (UDS3-EF) in 73 older adults (36F) ranging in age from 56-86. The UDS3-EF is a previously validated composite measure of seven tests which includes category fluency, letter fluency, digit span backwards, and trail making tasks. Participants were scanned on a 3T Siemens Prisma scanner with a 64-channel head coil. All ePVS counts were performed on T1 MPRAGE and T2 FLAIR images by an experienced rater blinded to participant demographics and under the direction of a trained neuroradiologist. In line with previously established guidelines, ePVS were defined as regions of hypointensity less than 3mm in diameter on T1 imaging and were distinguished from lacunar infarcts by the absence of T2 FLAIR hyperintensity. ePVS were individually and manually counted in a single, representative slice in the axial plane of the white matter centrum semiovale, basal ganglia, hippocampus, and midbrain. Regression analyses controlling for age, gender, estimated total intracranial volume, and years of education demonstrated a significant, negative relationship between UDS3-EF scores and ePVS counts in the basal ganglia ($\beta = -0.277$, $P = 0.011$) and in the midbrain ($\beta = -0.216$, $P = 0.042$). These cross-sectional findings suggest that ePVS burden is associated with executive dysfunction. Ultimately, our results support the continued investigation of ePVS as a potential early neuroimaging biomarker of cSVD-related cognitive dysfunction. Future work is required to determine if ePVS burden predicts later UDS3-EF scores.

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White matter hyperintensity (WMH) longitudinal volume changes (WMH growth/regression) within-subjects: implications for clinical trials in VCID

Faculty

Background: One of the major risk factors for late-life cognitive impairment and dementia (VCID) is small vessel ischemic disease (SVID). White matter hyperintensities (WMH) are a common SVID biomarker that are frequently seen as a bright white signal on the MRI FLAIR imaging sequence. Previous studies showed WMH volume dynamic changes: increase (growth) and decrease (regression) within subjects over time. We recently reported a method to quantify discrete WMH volumes (growth and regression) within individuals over 12 months with instrumental validation performed within the MarkVCID consortium. The present study provides preliminary evidence for the clinical relevance of this methodology.

Method: 3D MRI T1-weighted (MPRAGE) and FLAIR images at baseline and 12-month follow-up visits for sixty-one participants (mean age 74 ± 5.9 years, 30 men, University of Kentucky) were analyzed. The Stroop test was administered using the CANS-MCI computerized battery. WMH volumes growth and regression were quantified for each participant. A linear regression model was used to assess the association of dynamic WMH changes with Stroop performance.

Results: WMH growth, but not WMH regression or total WMH volume changes, adjusted for age and sex, was associated with Stroop performance at the 12-month ($\beta = -0.24$, $P = 0.02$).

Conclusions: Our results show that WMH growth was associated with a decline in performance on the Stroop test over 12 months, but we could not see that with WMH regression or total WMH volume change. These findings highlight the importance of studying dynamic (within-subject) WMH growth and regression rather than solely focusing on the longitudinal changes in the total WMH volume measurements. Therefore, WMH growth measurement may help reduce sample sizes, facilitate clinical trial conduct, and optimize the potential to identify interventions intended to mitigate SVID contributions to VCID.

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Cerebrovascular Accumulation of Amyloid-Forming Amylin Secreted from The Pancreas Induces Brain Hypoxia

Faculty

Background:

Islet amyloid polypeptide (amylin is a β-cell hormone co-secreted with insulin. Histological analysis of human brains identifies amylin deposits co-localized with parenchymal and vascular β-amyloid in humans with vascular cognitive impairment and Alzheimer's' dementia. Because circulating amyloid-foaming amylin triggers systemic hypoxia signaling, we sought to determine the relationship between circulating amylin levels and brain hypoxia markers in a transgenic rat model of amylin-mediated neurological deficits.

Method:

To determine amylin-related brain hypoxia signaling, we conducted a 16-month longitudinal study in which rats that express amyloid-forming human amylin in pancreatic β-cells (HIP rats) were compared to wild type (WT) littermates that express non-amyloidogenic amylin (n=10 rats/group) in pancreas.

Results:

Blood amylin levels measured by ELISA were ~2 fold higher in 16-months old HIP rats compared to WT littermates ($P<0.01$). The Thioflavin T (Th T) fluorescence signal intensity in blood lysates from HIP rats increased compared to that In WT littermates indicating the presence of amyloid-forming in the circulation in HIP rats ($P<0.01$). This was associated with higher amylin concentration in brain micro vessels lysates ($P<0.01$) and amylin immunoreactivity signal intensity in brain sections. Plasma erythropoietin, a marker of systemic hypoxia was higher in HIP rats than WT littermates ($P<0.05$), which correlated with brain accumulation of HIF-2α ($p<0.05$) and HIF-1α($P=0.07$), and with altered mitochondrial DNA brain tissues ($P<0.05$).

Conclusions

Increased circulating levels of amyloid-forming amylin promoted cerebrovascular amylin deposition leading to brain hypoxia-ischemic injury. Future studies need to address functional effects of amylin-induced brain hypoxia signaling in the brain.