University of Kentucky College of Medicine

2019 Neuroscience Clinical—Translational Research Symposium

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Platform Sessions

Neurotrauma

Understanding interleukin-1 receptor 1 localization after traumatic brain injury

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Student

Neurotrauma (TBI, spinal cord injury, etc.)

Neuroinflammation is increasingly regarded as a major pathology in various diseases affecting the central nervous system. Trauma to the central nervous system is one such area where neuroinflammation has become a major focus, and with over 10 million traumatic brain injuries (TBI) occurring worldwide, understanding the inflammatory reactions after these injuries may be crucial to the development of effective therapies. Following a TBI, it is known that there is a large increase in cytokine release, followed by persistent cytokine dysregulation. Interleukin-1 (IL-1) is a cytokine that is responsible for coordinating the immune response in the body but also affects a variety of cell types in the brain, ultimately altering CNS function, and the neuroimmune axis. IL-1 signals mainly through the IL-1 receptor 1 (IL-1R1). The localization of IL-1R1 in the CNS has been described in the healthy brain, but little is known about how an injury or disease effects localization of IL-1R1, and if there is a gain or loss of IL-1R1 expression on different cell types in the brain after injury. To answer these questions, we have employed a reporter transgenic mouse in which the IL-1R1 gene and protein are tagged to allow for the neuropathological assessment of IL-1R1 regional and cellular localization after a TBI. Using these approaches, we found robust expression of IL-1R1 on neurons, and blood vessels in the brain, with no apparent microglia expression of IL-1R1 at least at acute time points post-injury. These results suggest critical roles for IL-1/IL-1R1 in neuromodulation and the neuroimmune axis.

Examining CD44 as a Novel Marker for Reactive Astrogliosis Following TBI

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Student

Neurotrauma (TBI, spinal cord injury, etc.)

The current knowledge base regarding the pathobiological contribution of reactive astrogliosis in the brain following traumatic brain injury is poorly understood. To begin to understand astrocyte-specific response to TBI, we examined a time course spanning acute- subacute intervals following TBI; 1, 3, and 7 days post-surgery using 3-month-old C57BL6/J mice that received either sham or controlled cortical impact (CCI) contusion surgery. Two cohorts were generated; one for histological quantification and a second for gene expression profiling. Reactive astrogliosis was quantified using GFAP, S100beta, and Aqp4 as histological markers. Secondly, at the prescribed interval, ipsilateral neocortex and dorsal hippocampus was used for ACSA-2 magnetic bead astrocyte enrichment. Enriched astrocytes were subsequently processed for RNA isolation, cell enrichment validation, and finally gene expression. Astrocyte- specific response to TBI was profiled using a focused array of 46 genes. Our time course profiling revealed that the bulk of disparate responses occurred at the 3d interval, notably exacerbated expression of CD44, relative to sham. Gene profiling was examined using principle component analysis (PCA) and hierarchical clustering, revealing conserved groupings between each surgical cohort. As an ex-tension; to examine the molecular underpinnings of CD44+astrocytes, we used our novel astrocyte-specific conditional reporter strain (Ai9DAldh1l1CreERT2) which fluorescently labels all CNS astrocytes with tdTomato following tamoxifen administration. Using these reporter mice, 3 months old, we examined the 3d post-surgery interval (sham and TBI) via FACS of CD44+astrocytes (e.g. CD44+tdTomato+) versus CD44neg.tdTomato+astrocytes. Sorted cells were again processed for RNA isolation and analyzed on the same focused gene array as above. Our current data indicate that TBI-induced CD44+reactive astrocytes acquire a predominantly pro-inflammatory response (e.g. CCL2), with a concomitant decrease in genes associated with synaptic support (e.g. GPC6), compared to CD44neg.astrocytes. Collectively, our findings identify a novel subset of astrocytes that may play an integral role in the deleterious neurodegenerative sequelae following TBI, highlighting these cells as a potential therapeutic target.

Mitochondrial Uncoupling Prodrug Improves Mitochondrial Bioenergetics, Cognitive Outcome and Tissue Sparing after Traumatic Brain Injury

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Fellow

Neurotrauma (TBI, spinal cord injury, etc.)

Traumatic brain injury (TBI) results in cognitive impairment, which can be long-lasting after moderate to severe TBI. Currently, there are no FDA-approved therapeutics to treat the devastating consequences of TBI and improve recovery. This study utilizes a prodrug of 2,4-dinitrophenol (DNP), MP201, a mitochondrial uncoupler with extended elimination time, that was administered after TBI to target mitochondrial dysfunction, a hallmark of TBI. Using a model of cortical impact in male C57/BL6 mice, MP201 (80 mg/kg) was provided via oral gavage 2-hours post-injury and daily afterwards. At 25-hours post-injury, mice were euthanized and the acute rescue of mitochondrial bioenergetics was assessed demonstrating a significant improvement in both the ipsilateral cortex and ipsilateral hippocampus after treatment with MP201. Additionally, oxidative markers, 4-hydroxyneneal and protein carbonyls, were reduced compared to vehicle animals after MP201 administration. At 2-weeks post-injury, mice treated with MP201 post-injury (80mg/kg; q.d.) displayed significantly increased cortical sparing ($p = 0.0059$; 38% lesion spared) and improved cognitive outcome ($p = 0.0133$) compared to vehicle-treated mice. Additionally, vehicle-treated mice had significantly lower ($p = 0.0019$) CA3 neuron count compared to sham while MP201-treated mice were not significantly different from sham levels. These results suggest that acute mitochondrial dysfunction can be targeted to impart neuroprotection from reactive oxygen species, but chronic administration may have an added benefit in recovery. This study highlights the potential for safe, effective therapy by MP201 to alleviate negative outcomes of TBI.

Effects of repeated concussive brain injury on progression of tau hyper-phosphorylation

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Student

Neurotrauma (TBI, spinal cord injury, etc.)

Tau is a protein that stabilizes microtubules in neurons, astrocytes, and oligodendrocytes. In some neurological diseases, tau undergoes a greater degree of phosphorylation, known as hyper-phosphorylation, which may promote aggregation of insoluble tau into tangles resulting in the degeneration of neurons. Chronic traumatic encephalopathy (CTE), one of these diseases, is linked to repeated head injury. To better understand the effects of repeated mild traumatic brain injury (TBI) on the progression of tau pathology, a mouse model of closed head injury (CHI) was used to produce a concussive impact, similar to concussion observed in humans. Transgenic mice overexpressing human tau (rTg4510) and wild-type mice were given two CHIs at a 24h interval before euthanasia at 3d, 2wk, or 2mo. Using immunohistochemistry with a human tau specific antibody (HT-7), we confirmed the genotype of each animal. The pathology of phosphorylated tau was then examined using the antibody AT-8, which detects hyper-phosphorylation at Ser396/404, and caspase cleaved tau antibody, which detects truncated Asp 421. In rTg4510 mice, independent of injury, we observed the progression of phosphorylated tau deposition in an age-dependent manner. In the CA3 sub-region of the hippocampus, numbers of AT-8 positive cells significantly increased after CHI compared to sham injury in rTg4510 mice at 2mo post-injury. Numbers of caspase cleaved tau-positive cells in the dentate gyrus were significantly increased at 3d after CHI compared to sham. Further analysis needs to be done to determine if wild-type mice show a similar increase in tau cleavage after injury. Therefore, our model is a possible platform to examine pathology related to CTE and therapeutic targeting tau pathology for the goal of mitigating cognitive decline.

Stroke/Neurovascular

Circulating microRNAs as potential biomarkers for assessing vasospasm risk following aneurysmal subarachnoid hemorrhage

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Faculty

Disease biomarkers

Aneurysmal subarachnoid hemorrhage (aSAH) is a medically complex, life-threatening event caused by a ruptured intracranial aneurysm. Approximately one-third of aSAH patients develop cerebral vasospasm 4-10 days after aneurysm rupture, which results in extremely high morbidity and mortality. Currently, no validated biomarker is available in clinical practice to determine the risk for vasospasm in aSAH patients. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression post-transcriptionally. MiRNAs have been implicated in virtually all human diseases, including aSAH, and can be found in circulating biofluids including plasma, serum, and cerebrospinal fluid (CSF). MiRNAs are highly stable in these biofluids and are relatively easy to detect, both of which are ideal characteristics for biomarkers. We hypothesize that alteration of biofluid miRNAs may accurately reflect the pathophysiological events in aSAH patients who develop vasospasm. Therefore, CSF, plasma, and serum are rich sources for searching vasospasm biomarkers. The specific objective of our current study is to identify temporal changes of biofluid miRNAs in association with vasospasm risk following aSAH for rapid-assessment of vasospasm risk. We examined miRNA expression patterns in CSF and plasma specimens collected from patients with and without vasospasm at 3 days and 7 days after aSAH. Using a custom designed brain-injury related miRNA panel, we observed a striking differential expression pattern of these miRNAs in both CSF and plasma specimens collected from patients with and without vasospasm. Interestingly, the differential miRNA expression patterns were observed in samples obtained at an early time point (3 days after aSAH) prior to the onset of vasospasm (all occurred after day 5 in this study). Our study suggests that circulating miRNAs are excellent candidates as biomarkers in predicting vasospasm risk, and have a strong translational application for the diagnosis and treatment of vasospasm.

A Wearable Optical Sensor for Noninvasive and Simultaneous Measurements of Tissue Blood Flow and Oxygenation

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Student

Stroke/Neurovascular

Quantification of tissue blood flow and oxygenation provides vital information for diagnosis and therapeutic assessment of various diseases associated with tissue ischemia and hypoxia. We report an innovative, noninvasive, wearable, near-infrared diffuse speckle contrast flow-oximetry (DSCFO) technology for continuous monitoring of regional blood flow and oxygenation variations in relatively deep tissues (up to one centimeter). A wearable DSCFO probe, consisting of small laser diodes at two wavelengths (780 nm and 850 nm) as point sources and a tiny CMOS camera as a 2D detector array, was constructed and used to detect the reflectance spontaneous spatial fluctuations of laser speckles, resulting from the movement of red blood cells in deep tissues (i.e., tissue blood flow). Light intensity attenuations at the two wavelengths due to the absorptions of oxy-hemoglobin and deoxy-hemoglobin were detected for extracting tissue oxygenation information. The DSCFO system was tested and calibrated against established technologies in standard tissue-simulating phantoms and human forearm tissues with manipulated physiological changes. Consistent results were obtained between the concurrent measurements by different techniques. The wearable DSCFO technique has the potential to be used for continuous monitoring of tissue blood flow and oxygenation in conscious, freely moving subjects including animals and humans. We are currently testing this innovative DSCFO system for continuous cerebral monitoring in rodents and newborn infants. In the future, this DSCFO system will enable simultaneous measurement of cerebral blood flow, fluctuations in cerebral oxy- and deoxy-hemoglobin concentrations from newborn infants, which will provide prompt biomarkers for evaluating neuronal brain health and treatment effects. Our paper for this study is proceeding.

Protocol to Isolate Lymphocytes for Flow Cytometry from BACTRAC Samples

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Stroke/Neurovascular

Introduction: Since 2015, mechanical thrombectomy is the standard treatment for emergent large vessel occlusion stroke. Using standard techniques during mechanical thrombectomy, the Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol (clinicaltrials.gov NCT03153683) isolates distal blood within the artery immediately downstream from the clot, peripheral blood proximal to the clot, and the thrombus. BACTRAC is the first protocol utilizing the thrombectomy technique to collect local whole blood samples during brain infarction. We aimed to augment the current collection protocol to reproducibly obtain and study local leukocyte populations during human stroke.

Methods: We started with the established BACTRAC protocol previously published (PMID: 30064997). We modified the tissue collection protocol to isolate lymphocytes for flow cytometry and to bank the clot and plasma. We performed a flow cytometry panel that identifies CD3, 4, 8, 11b, 11c, 14, 19, 31, 45, 66b, 161, 183 markers to investigate populations of B-cells, T-cells, dendritic cells, NK cells, macrophages/monocytes, granulocytes, endothelial cells, and progenitors, respectively.

Results: The protocol was first established in healthy controls (n=9) by drawing venous blood and simulating a vial of distal blood and a vial of proximal blood. In healthy subjects, we were able to isolate on average 2x106 leukocytes/ml with a viability of 97.85%. Banked lymphocytes are also viable upon reconstitution and for flow cytometry. This protocol successfully isolates leukocytes in proximal and distal blood samples in subjects undergoing mechanical thrombectomy, with identification and analysis by flow cytometry currently ongoing.

Conclusions: This modification to the existing BACTRAC protocol provides the opportunity, for the first time, to study local leukocyte populations in the arteries undergoing ischemic stroke in the human condition. Efficient processing of these samples will provide insight into the neuroinflammatory microenvironment of the occlusion and accelerate translational stroke research to elucidate novel approaches for drug discovery and prognosis.

Can’t breathe, Can’t see, Can’t think: COPD Increases the Risk of Posterior Reversible Encephalopathy Syndrome

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Stroke/Neurovascular

Introduction: Several theories exist regarding the pathogenesis of posterior reversible encephalopathy syndrome (PRES). One theory suggests that PRES occurs when systemic blood pressure exceeds the upper limit of cerebral autoregulation. Endothelial dysfunction has been proposed as an alternative pathogenesis to account for PRES outside the setting of acute hypertension. This mechanism has been implicated in other conditions associated with PRES including autoimmune diseases, cytotoxic medications, sepsis, and eclampsia. The purpose of this study was to determine if COPD, a disease known to cause endothelial dysfunction, has a causative association with the development of PRES.

Methods: A single center retrospective, age-matched, case-control study was performed from January 2013 to June 2019 comparing patients discharged with a primary diagnosis of PRES to a control group of patients with acute ischemic stroke. Demographics, medical comorbidities, initial blood pressure, imaging findings, and clinical outcomes were compared between the two groups. For categorical variables, p-values were calculated using $\chi 2$ and Fisher’s exact tests. Continuous variables were reported using means and standard deviations, and p-values were calculated using two-sample t-tests. The effect of COPD and acute hypoxic respiratory failure on PRES status was investigated using multivariate logistic regression.

Results: A total of 94 PRES subjects and 109 control subjects were included for analysis. Mean age did not differ between the two groups; however, the PRES group was more likely to be female (78.7% vs. 49.5%, $p<0.001$). COPD was present in 26.6% (n=25) of cases and 11% (n= 12) of controls (odds ratio 4.12, p=0.003). Occurrence of hypertension did not differ significantly between the two groups (78.0% vs 86.2%). Among patients with PRES in the setting of COPD (n=25), 60% (n=16) did not meet criteria for hypertensive emergency. Controlling for hypertensive emergency status in a multivariate logistic regression analysis, patients with COPD were 3.21 times more likely to develop PRES (p= 0.004).

Conclusions: To our knowledge, very few reports of PRES in the setting of COPD have been described in the literature and no association of PRES and COPD has been defined to date. Our data support the role of COPD as a risk factor in the development of PRES.

Cognitive/Behavioral

Feasibility of dynamic sleep enhancement in a mouse model of Alzheimer's disease

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Student

Other neurodegenerative disorders

Alzheimer’s disease (AD) is a neural degenerative condition that is marked by memory impairment, cognitive deficits and changes in personality. Neurofibrillary tangles and amyloid plaque buildup in the brain are believed to play an integral role in AD pathology and are therefore active topics of research. However, disruptions or alterations in sleep commonly seen in this condition are less understood. There is growing evidence that disordered sleep is not merely a consequence of AD, but that it may also accelerate pathology. Therapeutic strategies for improving sleep quality may slow disease progression and are therefore desirable. To this end, we have developed a system to accomplish sleep enhancement in mice through dynamic control of ambient temperature (Ta), which influences sleep through thermoregulatory mechanisms. Building on previous experiments in which we established the feasibility of this approach in C57BL/6 and 5xFAD mice, we performed a pilot study on 3xTg-AD mice (n = 8, all female), in which Ta was gradually elevated to the thermoneutral zone during the light period alone for four weeks to promote sleep. The animals were instrumented for electroencephalogram (EEG) and electromyogram (EMG) recording in individual cages. In addition, motion signals from a noninvasive floor-mounted piezoelectric sensor (Signal Solutions, LLC) were monitored to better discriminate sleep and behavior. These experimental recordings have been completed and data analysis is ongoing. Future work will investigate the effects of sleep enhancement on molecular and cognitive disease markers in this and other experimental models of AD.

Association of multiple proteinopathies, cognitive decline, and dementia in a community-based autopsy cohort

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Epidemiology

Background: Abnormal accumulation of amyloidβ (Aβ) plaques and neurofibrillary tangles in the brain are the hallmark of Alzheimer's disease (AD). However, it is important to recognize that AD pathology is frequently accompanied by additional neurodegenerative pathologies, specifically TDP-43 and α-synuclein. We hypothesized that the co-occurrence of four misfolded proteins is common but under recognized in old age. This study aimed to determine the frequency of multiple proteinopathy among cases with at least one misfolded protein; evaluate demographic, neuropsychological, neuropathological characteristics; as well as evaluate cognitive trajectories over time.

Methods: Data were analyzed from elderly, longitudinally evaluated participants in a community-based cohort study of aging and dementia who had undergone autopsy and satisfied criteria of having at least a misfolded tau. Cases definitions were based on the presence of the misfolded proteins: tau alone n=14, tau+TDP43, n=19, tau+Aβ, n=138, tau+Aβ+α-synuclein, n=59, tau+Aβ+TDP43, n=68, tau+Aβ+tdp43+α-synuclein, n=45. We examined the association between case groups and neuropsychological test scores, sex, age, years of education, clinical diagnosis and APOEε4. Data analyses included descriptive statistics, multinomial logistic regression, and group-based multi-trajectory models.

Results: A total of 343 autopsied participants were included. All 4 misfolded proteins were detected in 13.11%, 3 misfolded proteins were detected in 37% and 2 misfolded proteins in 46%. The participants with more proteinopathies had the lowest neuropsychological scores at death. The lowest MMSE scores, consistent with severe dementia, were observed in cases with all 4 proteins. Presence of 3 or 4 misfolded proteins was associated with most severe cognitive decline in the 10 years prior to death.

Conclusion: Multiple proteinopathy is common in aged brains. It appears that overlapping presence of multiple proteins plays a major role in cognitive decline in these subjects. This has significant implications for public health, since strategies to prevent/cure AD may be complicated by the unrecognized presence of additional neurodegenerative pathologies.

The Effects of βeta-hydroxybutyrate (BHB) on Cognitive Performance in Healthy Populations

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Student

Other

βeta-hydroxybutyrate (BHB) is a ketone body produced by the liver in a process known as ketogensis. During periods of fasting or carbohydrate caloric restriction, ketones are used as the main fuel source throughout the body, including in the brain. Although glucose is the brain's principal energy source, when limited, ketones derived from fats become the major energy source. Exogenous BHB is safe to administer orally, and enhances energy and physical performance. While growing evidence from basic science indicates significant cognitive improvement in animal models following ketone elevation, and in clinical human samples such as in Alzheimer's disease, episodes of epilepsy, and severe traumatic brain injury, there is limited literature demonstrating beneficial neurocognitive effects of exogenous administration of ketones in healthy populations. As a proof of concept pilot, we present twelve healthy participants who underwent a single exogenous administration of 11.7g of BHB. After ingestion, healthy participants performed significantly better in attentional accuracy compared to baseline scores ($p < 0.05$; $d = 0.65$). The results of this pilot study suggest exogenous administration of BHB may have positive effects on attention in healthy participants. Currently, we are expanding on the pilot data by running a randomized control study and subject recruitment is underway.

Improving use of MoCA in assessing cognitive impairments in older adults with low formal education

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Cognitive/Behavioral disorders

BACKGROUND: Lower education is more common among rural older adults. The Montreal Cognitive Assessment (MoCA) is a widely used screening tool for cognitive impairment. It is unclear if the 1-point adjustment for lower education (i.e., ≤12y) is accurate when using the recommended cutoff of <26.
METHODS: This study examined the association of formal education and MoCA scores using a large national sample of unimpaired older adults with age ≥60 and non-elevated brain amyloid. Education was split into lower (≤12y) and higher (>12y). MoCA scores were adjusted by +1 point for ≤12y education. Logistic regression was used to examine classification based on the cutoff score of <26 for impairment and to identify MoCA items sensitive to low education.
RESULTS: Of 323 participants included, 270 had higher and 53 had lower education. Participants with lower education were more likely to score below the MoCA cut-off of <26 for cognitive impairment (adjusted OR=3.31, p<0.001). Backwards selection identified four MoCA items significantly associated with lower education: cube copy, sentence repetition, abstraction, and delayed recall. Comparing MoCA scores excluding cube copy, sentence repetition, abstraction eliminated the difference in adjusted-MoCA means, t(45.33)=1.45, p=0.34, between low (21.98) and high (22.39) education groups.
CONCLUSIONS: These findings suggest that the +1 point adjustment for lower education may be inadequate if using the MoCA to classify cognitive impairment using the < 26 cutoff. Computing adjusted MoCA scores while excluding three of four sub-items that demonstrate educational bias neutralized this effect in the sample. Practitioners serving older adults with lower education may need to use other means of diagnosis other than cognitive tests.

Epilepsy/Brain Metabolism

VAL-1221: Testing a Novel Pompe Disease Therapy in Lafora disease Mouse Models

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Epilepsy/Brain metabolism

Pompe disease is caused by deficiency of α-glucosidase (GAA) and characterized by cytosolic and lysosomal glycogen accumulations in muscle and other tissues. Depending on the severity, the disease can lead to death in infancy or childhood. Recombinant human GAA (rhGAA, Alglucosidase alf) is an FDA approved drug for Pompe disease, but this enzyme only targets lysosomal glycogen, and cytosolic glycogen also contributes to disease progression. Valerion Therapeutics has developed an enzyme delivery platform that uses an anti-DNA antibody Fab fragment fused to the enzyme for cytoplasmic delivery through the ENT2 nucleotide salvage pathway. For Pompe disease, Valerion has fused rhGAA to the Fab fragment to build a therapy that penetrates cells both to the cytoplasm and lysosomes to degrade abberant glycogen deposits. Fab-GAA (VAL-1221) Is currently in a phase I clinical trial for Pompe disease and results are promising (NCT02898753).
Lafora disease (LD) is the most common form of progressive myoclonus epilepsy. LD manifests in adolescence with rapidly progressing epilepsy, neurodegeneration and dementia, leading to a vegetative state. Death ensues ten years after onset. Insoluble carbohydrate deposits known as Lafora bodies (LBs) are present in all tissues of LD patients and contain an aberrant form of glycogen. LD is caused by mutations in EPM2A and EPM2B, genes that respectively encode laforin, the glycogen phosphatase, and malin, an E3 ubiquitin ligase. Epm2a- and Epm2b-deficient mice have LBs, neurodegeneration, and seizures, and multiple groups have shown that the reduction or ablation of LB formation and rescues LD in mice, demonstrating that LBs drive disease progression.
VAL-0417 is an antibody-enzyme fusion (AEF) therapy developed by Valerion Therapeutics specifically for LD, VAL-1221 is an AEF already showing success in clinical trials to treat Pompe Disease. Therefore, if VAL-1221 is able to degrade LBs, then this promising Pompe disease therapeutic can be reformulated and repurposed for treatment of LD patients and we may have a therapy for LD on a shorter timeframe. We show herein that VAL-1221 is capable of degrading LBs in vitro, penetrating neurological cells in vitro and in vivo, and reducing carbohydrate load in the brains of LD mouse models. These promising preclinical results provide evidence supporting repurposing the Pompe disease therapy, VAL-1221, for LD patients.

Membrane palmitoylated proteins regulate synapse density and function in C. elegans.

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Student

Epilepsy/Brain metabolism

Neural circuit function requires precise synaptic connectivity and robust synaptic signaling. Disruption of circuit function is associated with multiple neurological disorders. To better understand how neural circuits are regulated at the molecular level, we are using the motor circuit of Caenorhabditis elegans (C. elegans). C. elegans locomotion is modulated by a precise balance between cholinergic excitation and GABAergic inhibition. By assessing locomotion behavior, we identified new proteins that regulate excitation-inhibition balance that is disrupted by an epilepsy-associated mutation in an acetylcholine receptor. A subset of these proteins regulates intercellular signaling between neurons and non-neuronal cells, which function similar to mammalian glia. Here, we focused on how a family of membrane-associated guanylate kinases, the membrane palmitoylated proteins (MPPs), regulates synaptic function. In mammals there are seven members of the MPP family; C. elegans contains only three: MAGU-1, MAGU-2, and MAGU-3. Using the C. elegans motor circuit, we first investigated synaptic function of the cholinergic neuromuscular junctions using aldicarb, an inhibitor of acetylcholinesterase. We found that magu-3 mutants were hypersensitive to aldicarb, but magu-1 mutants were comparable to wild type. Interestingly, the magu-3 hypersensitivity was suppressed by a mutation in zig-10, a cell adhesion molecule that regulates interactions between neurons and non-neuronal cells.. When challenged with aldicarb, magu-2 mutants exhibited a relatively extreme resistance, indicative of a reduction in cholinergic activity. Furthermore, the reduction of cholinergic activity in magu-2 mutants prevented magu-3 mutant-associate hypersensitivity in magu-2; magu-3 double mutants. To further understand the changes in synaptic function, we examined the cholinergic synapses in magu-2 mutant animals. Surprisingly, we observed that magu-2 mutant animals contained more cholinergic neuromuscular junctions. In mammals, MPPs function in cell polarity pathways in neurons and epithelial cells. Therefore, we investigated whether two homologs of Crumbs, a cell polarity protein that binds to mammalian MPPs, also modulated motor circuit function. We found that the Crumbs homologs, CRB-1 and EAT-20 displayed an increase in cholinergic synapses, but did not alter cholinergic activity¬. These results suggest that MPPs regulate neural circuit function through two distinct mechanisms: a cell polarity-dependent pathway that regulates synapse number and a cell polarity-independent pathway that regulates synaptic activity. Understanding of how MPPs regulate motor circuit function will illuminate new mechanisms that regulate neural circuit function and may provide additional insights into how non-neuronal cells regulate circuits during health and disease.

Toward high-throughput, non-invasive seizure detection using piezoelectric motion sensors

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Student

Epilepsy/Brain metabolism

Outcomes in preclinical models of epilepsy–i.e., the likelihood and timing of epileptogenesis, seizure yield and frequency—can be quite variable. It is often desirable for experimental studies to commence only after spontaneous seizures have been verified and their frequency has stabilized. This determination is complicated by the diversity of seizure-related behaviors and the unpredictability of seizure recurrence. While invasive electroencephalography (EEG) is the standard for accurate seizure detection, it is resource-intensive and impractical in large cohorts. Visual observation or video analysis, while non-invasive, are labor-intensive and tedious. Thus, convenient non-invasive automated methods for seizure screening in animal models are desirable. Here, we investigate the utility of a piezoelectric (“piezo”) motion sensor for noninvasive seizure screening in two rodent epilepsy models: mice and rats. Mice and rats of both sexes were treated with pilocarpine i.p. to induce acute status epilepticus (SE). In this model, seizures typically subside within 1-2 hours post-injection and spontaneously recurring seizures—the hallmark of chronic epilepsy—may emerge after a latent period of several weeks. Animals that survived SE (12 mice; 7 rats) were monitored continuously for 12 weeks in individual cages, where signals from the piezo sensors and continuous video were collected. Piezo signals were screened weekly for seizures using an algorithm that responded to significant deviations in piezo signal features from a moving baseline. Video review of the detections enabled timely identification of animals that developed chronic epilepsy. This demonstrates the feasibility of noninvasive epilepsy screening without exhaustive review of video. Animals with good seizure yield were then instrumented with EEG headmounts and, after recovery from surgery, monitored for four more weeks with simultaneous EEG, piezo, and video recording. Seizures detected from the EEG were used as the truth set to validate the performance of the piezo detection algorithm. In mice, the piezo detector gave a maximum sensitivity of 80% to EEG-verified seizures but at the cost of low precision (11%). Nevertheless, this greatly reduced the amount of data to be reviewed to about 20 minutes per 24-hour period. Using a supervised logistic classifier for the rats, sensitivity and precision reached peaks of 68 and 62% respectively. Efforts to improve these preliminary results, such as feature selection and algorithm development, are ongoing.

Discrimination of High Frequency Oscillations from Sharp Transients in the Electrocorticogram of Patients with Refractory Epilepsy

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Student

Epilepsy/Brain metabolism

High frequency oscillations (HFOs) are potentially useful biomarkers of epileptogenic brain tissue. In diagnostic use for surgical treatment, true HFOs first need to be identified and their region of activity demarcated to predict the location and extent of cortex to be resected for a patient to become seizure-free. HFOs co-occurring with spikes are potentially stronger markers of epileptogenic cortex than HFOs alone. But HFOs masked by spikes may be rejected as artifacts (false negatives). The purpose of this study is to develop an algorithm that retains such events while rejecting genuine artifacts based on rhythmicity criteria. With IRB approval, eight epilepsy patients admitted for invasive presurgical evaluation were monitored using intracranial EEG (iEEG) with 1000 Hz sampling rate. HFO candidates were first identified using a slightly modified version of a well-known algorithm (Staba et al., 2002), which is highly sensitive to HFOs but admits spikes and other artifacts which, when filtered, look like genuine HFOs. This deficiency is addressed here by modeling the transient baseline (see figure) of the iEEG around a detection. When a spike fitted thus is subtracted from the signal the residual does not produce a false ripple when sent through a highpass filter. An HFO riding on the spike remains in the residual after the spike is eliminated. If the baseline does not contain a spike, it is unaffected. This approach decouples HFOs from spikes and other artifacts in the iEEG. A superset of 2520 detections made by the standard algorithm were selected at random from 1-3 channels in each recording and further screened using our two-step algorithm. True HFOs were distinguished from spikes with a sensitivity of 80.5%, specificity of 81%, positive prediction value of 92.5%, and Cohen's kappa of 51%. This procedure mostly rejected spikes while retaining highly rhythmic HFOs and HFOs co-occurring with spikes whose site of origin is strongly correlated with the seizure onset zone (SOZ) demarcated by the physician. Acknowledgements: Scholarship support from University of Babylon in Iraq to AA and National Science Foundation Grant No. 1539068.

Poster Session Abstracts

Neurotrauma

Longitudinal analysis of gut microbiome composition after experimental cervical spinal cord injury

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Student

Neurotrauma (TBI, spinal cord injury, etc.)

Following an experimental C2 spinal cord (SC) hemisection in rats, there is a gradual spontaneous recovery process that can take place over time. Additionally, interventions at later time points are more effective chronically after injury. What is not known is the mechanism mediating this observation. To begin to answer this question, we investigated the role of the gut microbiome after injury. Recent studies have emerged suggesting that the gut microbiome has critical implications on the proper functioning of the central nervous system (CNS). Indeed, gut dysbiosis, or a microbiome imbalance, can occur which can negatively impact the CNS. Neurotrauma, including spinal cord injury (SCI), can lead to acute gut dysbiosis leading to impaired recovery. It is our hypothesis that the composition of the gut microbiome is dynamic after injury with dysbiosis improving over time from an acute post-SCI state. In this study, we build upon these initial studies and investigate the impact of cervical SCI on the gut microbiome over time and up to chronic timepoints, as well as its impact on respiratory motor function and plasticity. In order to test our hypothesis, we performed a C2 hemisection on adult female rats and collected fecal samples from these animals, as well as from non-injured animals in order to assess microbiome composition at various timepoints post injury. Preliminary results suggest that following cervical SCI (up to 12 weeks post injury), robust differences in gut microbiome are apparent compared to non-injured animals. Future studies will classify bacterial identities and assess the impact of the post-injury gut microbiome on respiratory motor function and plasticity. As a majority of the SCI population are at the cervical level and at post-acute stages, this study is both important and translationally relevant.

Investigating the Link Between Cervical Spinal Cord Injury and Acute Lung Injury

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Student

Neurotrauma (TBI, spinal cord injury, etc.)

The global incidence rate of traumatic spinal cord injury (SCI) is an estimated 768,473 persons per year, the majority of which occur at the cervical level. These high-cervical SCIs disrupt respiratory pathways and disable autonomous breathing, which introduce morbidity and mortality to SCI individuals. It has been shown that SCI individuals are at risk for developing acute lung injury (ALI). ALI is a life-threatening disease of the lung. It is primarily characterized by respiratory failure, widespread lung inflammation, intra-alveolar edema, and alveolar-capillary membrane damage. In SCI individuals, the presence of a lung injury predicts substantial mortality rates. While the correlative relationship between ALI and SCI has been explored in retrospective clinical studies, the causal relationship is not well understood. Elucidating the relationship between SCI and ALI may enable new ALI treatment via techniques previously reserved for high-cervical SCI individuals. The present study attempts to explore the potentially causal relationship between SCI and ALI in the rat model. We performed a C2 hemisection injury to the rat model and assessed ALI at various timepoints. Our preliminary studies found heightened levels of interstitial edema and an increased bacterial presence in the lung microbiome of SCI rats. This has been observed in human ALI patients and is suggestive of an increased alveolar membrane permeability that is susceptible to bacterial translocation. Changes to the lung microbiome and alveolar-capillary membrane after SCI are being investigated in continuing studies. Additionally, we will verify the presence of ALI by characterizing lung inflammation. Collectively, this study and future directions will verify the mechanistic link between ALI and SCI. This will be used to identify potential therapies to ameliorate respiratory distress and lung injury after SCI.

Photo- and Phono- sensitivity Following mTBI is Associated with Emotional Distress Identified in the Beck Youth Inventory- 2nd Edition for Children

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Staff

Neuropsychology

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Background/ Objectives
Millions of concussions (mild traumatic brain injuries or mTBI) occur each year in the United States. About 70% of concussions treated in the emergency departments are pediatric patients, and they often report experiencing physical and affective post- concussion symptoms longer than adults. Identification of the relationship between emotional distress and other physical symptoms, such as photo- and phono- sensitivity could help clinicians diagnose and treat patients for mTBI symptoms. Prior pilot findings revealed potential associations between photo- and phono-sensitivity and mood after mTBI. The purpose of this analysis was to examine the association between clinical measures of affective and mood disruption following mTBI and the reporting of photo- and phono- sensitivity in a more robust pediatric sample.

Methods
A retrospective chart review of pediatric (N=160, 6-17 years of age, mean age 13.99±2.72 years, 65.3% male) patients was conducted from individuals who sought neurodiagnostic testing services through the Kentucky Neuroscience Institute (KNI) at the University of Kentucky, between 1 July 2010 and 31 August 2018, following mTBI. Data was de-identified, and affective assessments from the Beck Youth Inventory- 2nd Edition (BYI-2) were included as dependent measures. Specifically, t-scores of the Beck Depression Inventory for Youth (BDI-Y); Beck Anxiety Inventory for Youth (BAI-Y); Beck Anger Inventory for Youth (BANI-Y); Beck Disruptive Inventory for Youth (BDBI-Y); and Beck Self-Concept Inventory for Youth (BSCI-Y) from patients' first visits. Additionally, patients were further divided into who clinically report of photo- and phono-sensitivity symptoms. Group differences between BYI scores and photo- and phono-sensitivity were determined by one-way ANOVAs.

Results
Results demonstrated that patients with photo-sensitivity had significantly higher BYI scores in anxiety ($F(1,159) = 10.16$, $p=0.002$), depression ($F(1,159) = 13.56$, $p<0.001$), and anger ($F(1,158) = 12.49$, $p=0.001$) domains compared to patients without photo-sensitivity. Additionally, patients with photo-sensitivity had significantly lower self-concept scores ($F(1,159) = 8.24$, $p = 0.005$) compared to patients without photo-sensitivity. Similarly, patients with phono-sensitivity had significantly higher BYI scores in anxiety ($F(1,159) = 8.04$, $p=0.005$), depression ($F(1,159) = 10.77$, $p=0.001$), and anger ($F(1,158) = 5.60$, $p=0.02$) domains compared to patients without phono-sensitivity. Patients with phono-sensitivity had significantly lower self-concept scores ($F(1,159) = 4.21$, $p = 0.04$) compared to patients without phono-sensitivity.

Conclusion
Our findings suggest that pediatric patients with mTBI who report photo- or phono- sensitivity are more likely to have lower self- concept, higher anxiety, depression and anger post- injury. Given the prevalence and duration of such symptoms posing potentials for disruption in activities of daily living (e.g., schoolwork, social and psychological health), current findings help in providing early treatment decisions for different aspects of neurotrauma symptoms.

Stroke/Neurovascular

Prediabetic Hypersecretion of Amylin Alters Oxygen Sensing and Accelerates Aging

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Staff

Stroke/Neurovascular

Capillary function and oxygen-carrying capacity of red blood cells (RBCs) decline in type-2 diabetes exacerbating the risk of hypoxia and organ malfunction. Amylin is a β-cell hormone that forms pancreatic amyloid in patients with type-2 diabetes and its blood level is elevated in prediabetes. Given the amyloidogenicity of human amylin, we hypothesized that hyperamylinemia increases the risk of hypoxia by provoking microcirculatory disturbances. Using rats with pancreatic overexpression of human amylin (HIP rats) and transfusion with RBCs from diabetic HIP rats into normal rats, we show that the transition from prediabetes to diabetes is associated with amylin deposition in capillaries and RBCs, which increases RBC to endothelial cell adherence, decreases RBC hemoglobin and activates hypoxia-inducible factors in endothelial cells leading to arginase-nitric oxide dysregulation. Prediabetes-induced amylin dyshomeostasis accelerates aging in HIP rats with multi-organ impairments and increased mortality. Upregulation of epoxyeicosatrienoic acids, which are lipid mediators formed by endothelial cells, mitigates amylin deposition in capillaries and hypoxia. In humans, amylin deposition in RBCs increases with aging in association with type-2 diabetes, heart failure, cancer and stroke. Thus, prediabetes-induced amylin dyshomeostasis impairs capillary function and oxygen-carrying capacity of RBCs; amylin-loaded RBCs can initiate pathological processes that are involved in pathological aging.

Diabetes-Related Amylin Dyshomeostasis Impairs Amyloid-β Clearance by miRNA-Mediated LRP1 Downregulation in brain capillaries

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Fellow

Stroke/Neurovascular

The brain blood vessels of patients with type-2 diabetes and Alzheimer's disease (AD) have deposition of amylin, an amyloidogenic hormone co-secreted with insulin. Here, we tested the hypothesis that vascular amylin deposition contributes to dementia by impairing the clearance of amyloid-β (Aβ) from the brain. We found that the incubation of primary rat brain microvascular endothelial cells (RBMVECs) with aggregated human amylin downregulated the expression of low density lipoprotein receptor-related protein 1 (LRP1), a key mediator of the transport of Aβ across the blood-brain barrier (BBB). The interaction of aggregated amylin with RBMVECs upregulated microRNA (miR)-103 and miR-107 expression, which were shown to bind at 3´ UTR region of LRP1 downregulating the protein expression post-translationally. Indeed, we found that the transfection of miR-103 and miR-107 in RBMVECs downregulated LRP1 expression. In functional in-vitro BBB model studies, we found that aggregated amylin reduced the trans-endothelial electrical resistance and decreased Aβ clearance from the brain side to blood side. Consistently, in the brain capillaries of rats that overexpress human amylin in the pancreas, the miR-103 and miR-107 expression was upregulated, whereas the LRP1 expression was downregulated. The human amylin-expressing rats accumulated more Aβ in the brain compared to wild type rats that express non-amyloidogenic rat amylin. In conclusion, amylin dyshomeostasis exacerbates Aβ accumulation in the brain through impairing the LRP1 expression. The results could help to identify novel therapeutic targets to reduce the BBB impairment in AD.

ACTIVATION OF JAK2/STAT1 PATHWAY BY IFN GAMMA INDUCES CXCR3 SIGNALING AND INFLAMMATORY RESPONSE BY MOUSE CEREBRAL ENDOTHELIAL CELLS AND ASTROCYTES

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Student

Stroke/Neurovascular

Emergent large vessel occlusion (ELVO) is the deadliest form of stroke and is caused by a blockage within a major a cerebral artery, usually the middle cerebral artery. This condition triggers edema, which occurs due to the movement of water into the brain. Cerebral edema can produce massive brain damage which can result in disability and death. Leukemia inhibitory factor (LIF), a neuroprotective and anti-inflammatory cytokine, decreases neurodegeneration and increases survival after intraluminal middle cerebral artery occlusion (MCAO) model, a rat model of ELVO. Previously we have reported that IFNγ is the primary inflammatory mediator in the MCAO model. Treatment with LIF decreases its expression and prevents the upregulation of CXCL10, an IFNγ-inducible chemokine. This study examined the presence of IFNγ on mouse endothelial cells and astrocytes by measuring the expression of CXCL9, another IFNγ-inducible chemokine, and determined the ability of LIF to block IFNγ signaling. Exposing cerebral microvascular endothelial cells to 1 ng/ml IFNγ significantly induced expression of CXCL9 under both normoxic conditions and oxygen-glucose deprivation (OGD). Cells exposed to normoxic conditions were plated and allowed to mature for 7 days, then stimulated with 1 ng/ml IFNγ. At 6, 24, 48 and 72 hours after stimulation, supernatants were collected and ELISA was used to measure the amount of CXCL9 released. Cells subjected to OGD were allowed to mature for 7 days. On day 7, glucose-free DMEM was added to cells and they were placed in an oxygen-free chamber for 6 h. After 6 h, the glucose-free DMEM was replaced with glucose-containing DMEM containing 1 ng/ml IFNγ. Supernatants were collected at the previously mentioned time points d, and the concentration of CXCL9 was determined via ELISA.

To determine whether IFNγ upregulates CXCL9 via the JAK2/STAT1 pathway, cells were treated with 1 ng/ml IFNγ, 50uM fludarabine (STAT1i) and 100uM AG490 (JAK2i). Treatment with JAK2 and STAT1 inhibitors significantly blocked the induction of CXCL9. Also, these cells were treated with LIF (200 ng/ml) and IFNγ under normoxic conditions and OGD. Surprisingly, LIF + IFNγ significantly increased the release of CXCL9 by endothelial cells exposed to OGD. One potential explanation for these results is that LIF induces CXCL9 expression to open the BBB. LIF is known to promote an anti-inflammatory phenotype in CD4+ T cells and macrophages. Thus, the opening of the BBB could be to allow anti-inflammatory leukocytes to enter the ischemic brain and promote repair after stroke.

Impact of Substance Use Disorder on Stroke Outcomes

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Student

Stroke/Neurovascular

Background:
Substance abuse is a major health crisis in the US, with an estimated 20 million people suffering from substance use disorders (SUD). In addition to rising rates of SUD, Kentucky is located in the northern region of the stroke belt and has one of the highest rates of stroke hospitalizations in the US. Substance use may cause stroke by various mechanisms, including vasoconstriction, endothelial dysfunction, drug-induced vasculopathies, advanced rates of atherosclerosis, and infective endocarditis. We sought to examine the relationship between SUD and stroke outcomes.

Methods:
This is a single center, retrospective chart review of adults age >18 years with a diagnosis of ischemic or hemorrhagic stroke, and SUD based on either urine drug testing or medical record history, admitted between 12/6/2015 and 5/10/2019. We collected length of stay (LOS), admission/discharge NIHSS, discharge modified Rankin Scores, ICH scores, and discharge status and compared them to controls of ischemic stroke without SUD.

Results:
A total of 197 cases were identified [M=147 (74.6%)]. The most common illicit substances identified by testing were stimulants (42.6%, n=84), opioids (32.5%, n=64), and benzodiazepines (28.4%, n=56). Nearly all subjects had multiple substances present on screening. 13.8% (n=27) and 5.6% (n=11) received thrombolysis with either IV alteplase or mechanical thrombectomy, respectively. Compared to a control group of 176 ischemic stroke patients that did not test positive for illicit substances, cases (n= 139, ischemic stroke + SUD) were younger (mean=54.94+/-12.01 vs 66.15 +/- 14.38 yrs , p=0.0137), had a longer LOS (n=139, mean=8.44+/-10.84 vs 5.06 +/- 5.74, p=0.0006), higher admission NIHSS (mean=9.87+/-9.08, p=0.00012), and higher discharge NIHSS (mean=6.51+/-7.13 vs 4.19 +/- 5.73 , p=0.000512).

Conclusion:
Patients with SUD and stroke had longer LOS and worse discharge NIHSS compared to ischemic stroke patients without SUD. This could be due to the different mechanisms that cause strokes in substance users or could be a reflection of the effects of specific substances present at the time of admission. Future directions will include evaluating a hemorrhagic control population and examining a subpopulation of infective endocarditis.

Noninvasive Noncontact Optical Imaging of Cerebral Blood Flow in Rats

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Faculty

Disease biomarkers

Introduction: Continuous and longitudinal monitoring of cerebral blood flow (CBF) variations provides crucial information for investigating the pathophysiology of various cerebrovascular diseases. A novel speckle contrast diffuse correlation tomography (scDCT) system has been recently developed in our laboratory for 3D imaging of blood flow distributions in relatively large and deep tissues. The goal of this study was to adapt and optimize this noninvasive scDCT system for transcranial imaging of CBF distributions in rats through intact skull and scalp. The continuous imaging capability of our system was demonstrated by imaging global CBF increases during CO2 inhalations and regional CBF decreases across two hemispheres during sequential ipsilateral and bilateral common carotid artery (CCA) ligations. Ultimately, we expect to provide a noninvasive noncontact cerebral imager for basic neuroscience researches in small animal models and clinical applications in human neonates.
Materials and Methods: The scDCT system uses a galvo mirror to remotely deliver focused point near-infrared light to source positions and CCD/sCMOS cameras to detect spatial diffuse speckle fluctuations resulting from the moment of red blood cells (i.e., blood flow). Thousands of pixels provided by the camera significantly improve the temporal/spatial resolutions. Our scDCT technique extracts boundary blood flow indices from the spatial diffuse speckles detected by the camera. In this study, an adjustable iris diaphragm was integrated into the source pathway to optimize the light spot size. This modification allowed us to have enough number of source positions distributed over a small region of interest on the small rat head. The normalized boundary blood flow data were then input into a modified NIRFAST program, developed previously for expedient tomographic reconstructions of blood flow distributions. Furthermore, we have modified the exposure time and effective source-detector separations based on phantom tests. The modified scDCT was then used for continuous imaging of relative changes in CBF (rCBF) distributions during 10% CO2 inhalation and CCA ligations in 9 rats. One additional rat was imaged by scDCT for longitudinal monitoring of CBF over 14 days after unilateral stroke induced by a transient middle cerebral artery occlusion (MCAO) in left hemisphere.
Results: As a vascular dilator, CO2 induced significant global increases in rCBF from the baseline of 100%: +18% (118 ± 8%) in scalps/skulls and +19% (119 ± 8%) in brains (n = 9). Significant differences in rCBF were observed at all stages of ligations in both hemispheres (n = 9). The unilateral left-CCA ligation created significant decreases in ipsilateral rCBF: -15% (85 ± 3%) in scalps/skulls and -65% (35 ± 9%) in brains (n = 9). The sequential bilateral CCA ligation induced greater decreases in rCBF in left and right hemispheres, respectively: -39% (61 ± 7%) and -35% (65 ± 10%) in scalps/skulls (n = 9); -68% (32 ± 11%) and -66% (34 ± 10%) in brains (n = 9). In the rat for longitudinal monitoring, a substantial decrease in rCBF (-70%) was observed in the brain layer of ipsilateral hemisphere during a 60-minute left-MCAO (n = 1). From 9 to 14 days after the stroke, rCBF values in different layers of two hemispheres tended to be similar, although they were still lower than the pre-stroke values.
Conclusions: We have downscaled, optimized, and tested an innovative scDCT system for noninvasive, continuous, and longitudinal imaging of CBF distributions in rat brains through intact scalps and skulls. The continuous dynamic imaging capability of the system was proven by imaging global CBF increases during CO2 inhalations. The regional imaging capability was demonstrated by imaging CBF distributions across two hemispheres of the brain during sequential unilateral and bilateral CCA ligations. The longitudinal imaging capability was shown

by imaging CBF variations over a long recovery period of 14 days after MCAO-induced stroke. All results meet physiological expectations and agree with those measured previously with other technologies and similar experimental protocols. Ultimately with more future investigations, we expect to provide a unique, noninvasive, noncontact cerebral imager for basic neuroscience research in small animal models and translation studies in human neonates.

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Inhibition of BMP-Smad1 Signaling and Neural Stem Cell Transplantation for Cerebral Ischemia-reperfusion Injury of Mice

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Faculty

Stroke/Neurovascular

Stroke is a leading cause of disability and mortality in adult population worldwide. Smad1, the downstream of BMP signaling, has been implicated in the stroke pathology and neural stem cells' (NSCs) activation, proliferation and differentiation. Therefore we investigate whether a strategy combining Smad1 inhibition with pharmaceutical approach and NSCs transplantation through carotid artery can attenuate stroke-induced brain injury and promote neurologic recovery. C57B6 mice at the age of 10-12weeks received 1h middle cerebral occlusion-induced ischemia using a silicone rubber coated filament nylon suture, and then were randomly assigned into 4 groups: 1. i.p. injection of vehicle solution for 10days (control group); 2. Injection of DMH1 (Smad1 inhibitor, 5mg/kg); 3. Injection of vehicle solution + intra-arterial NSCs delivery (at 3days post-stroke); and 4. Injection of DMH1 solution + NSCs delivery. NSCs were isolated from embryonic cortex of E18 eGFP (+) mice and expanded until delivery. At day3 post-stroke, 106 eGFP(+) NSCs in single cell suspension was injected through ipsilateral carotid artery to mice in group 3 and 4. The stroke volume, motor function, survival of NSCs were examined at 10days and 30days post-stroke. Our results showed that inhibition of Smad1 signaling with DMH1 or transplant of NSCs mitigate brain injury; while in the group4, DMH1 treatment also prompted NSCs' survival and neuronal differentiation in the stroke area, although we did not observe synergic protective effects. Taken together, our results suggest the translational potential of a regimen for stroke including both Smad1 inhibition and NSCs therapy.

Combination of DMH1 treatment and intra-arterial NSCs delivery improved ischemic stroke outcome

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Faculty

Other

Stroke is a leading cause of disability and mortality in adult population worldwide. Smad1, the downstream of BMP signaling, has been implicated in the stroke pathology and neural stem cells' (NSCs) activation, proliferation and differentiation. Therefore we investigate whether a strategy combining Smad1 inhibition with pharmaceutical approach and NSCs transplantation through carotid artery can attenuate stroke-induced brain injury and promote neurologic recovery. C57B6 mice at the age of 10-12weeks received 1h middle cerebral occlusion-induced ischemia using a silicone rubber coated filament nylon suture, and then were randomly assigned into 4 groups: 1. i.p. injection of vehicle solution for 10days (control group); 2. Injection of DMH1 (Smad1 inhibitor, 5mg/kg); 3. Injection of vehicle solution + intra-arterial NSCs delivery (at 3days post-stroke); and 4. Injection of DMH1 solution + NSCs delivery. NSCs were isolated from embryonic cortex of E18 eGFP (+) mice and expanded until delivery. At day3 post-stroke, 106 eGFP(+) NSCs in single cell suspension was injected through ipsilateral carotid artery to mice in group 3 and 4. The stroke volume, motor function, survival of NSCs were examined at 10days, 30days and 3months post-stroke. Our results showed that inhibition of Smad1 signaling with DMH1 or transplant of NSCs mitigate brain injury; while in the group4, DMH1 treatment also prompted NSCs' survival and neuronal differentiation in the stroke area, although we did not observe synergic protective effects. Taken together, our results suggest the translational potential of a regimen for stroke including both Smad1 inhibition and NSCs therapy.

Cognitive/Behavioral

Stress Effects on Simple Learning and Memory: A Rodent Model

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Student

Neuropsychology

Inability to handle stress is closely associated with impaired physiological functions and maladaptive behaviors. Recent studies indicate that males and females handle stress differently, leading to different behavioral deficits. Using a rodent model, this study compared stress-induced behavior of male and female rats in simple learning. Initially, Wistar rats were trained on a fixed ratio 5 (FR5) schedule, which required five lever-presses for a food pellet (45 mg) until they reached a behavioral criterion. On the day of testing, rats were either placed in a restraint for 30 minutes or kept in their home cages prior to testing on FR5. The first response latency (time to make the first lever-press) and runtime (time to complete 5 lever-presses) were measured. Overall, stressed rats showed markedly slower responses in pressing the lever and took longer to complete the response requirement. However, males and females showed different patterns of behavior. Females and males took longer to make the first lever-press, but made a significantly slower response than males across 4 days, reflecting no adaptation to stress in initiating first response. Runtime was not affected in stressed males, whereas stressed females took longer to complete five lever-presses compared to controls. Such impairment in females decreased substantially across four days, reflecting adaptation. Our data suggest that appetitive behaviors are differentially affected by stress, and that susceptibility to stress differs between sexes. Given that stress-induced changes are likely mediated via the limbic system, such as the amygdala, and subsequent activation of the hypothalamus-pituitary-adrenal (HPA) axis, examining sex differences in pharmacologically-induced stress would provide further information.

The role of eIF5A in TDP-43 pathology in FTD; the tip of the iceberg?

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Fellow

Other neurodegenerative disorders

TAR DNA-binding protein 43 (TDP-43) is a nuclear RNA/DNA binding protein that associates with Frontotemporal disorders. The clinical manifestations include motor neuron degeneration (ALS) and cognitive decline (FTD-TDP-43). FTD remains the second most common form of early-onset dementia after Alzheimer Disease. The hallmark of TDP-43 proteinopathy is nuclear loss-of-function and accumulation of nuclear and cytoplasmic TDP-43 inclusions, which acquire toxic gain-of-function. The unique post-translational modification of eIF5A; hypusination (eIF5AhypK50), within the hypusination loop denotes its activation and cytoplasmic localization where it further interacts with specific RNA binding proteins. eIF5A is implicated in translational elongation and translation silencing of certain mRNA in stress granules (SG). Together with our findings we posit that active eIF5A is position as a stress-response protein. Our data shows aberrant increase in enzymes responsible for hypusination in brain tissue from AD patient, TDP-43 animal models and arsenite-induced stress cellular models, suggesting that aberrant hypusination underlies the progression of disease. Further, we show that arsenite-induced stress induces interactions between eIF5AhypK50 and cytoplasmic TDP-43. We also find that eIF5AhypK50 binds TDP-43 and stress granule protein TIA-1 during pathology and arsenite-induced stress. Importantly, we find that pharmacological inhibitor of hypusination and sited-directed mutagenesis induces acetylation of eIF5A at lysine 47 (eIF5AacK47) resulting in significant reduction of phosphorylated and total TDP-43 in the cytoplasm and SG. We further confirm that potentiation of spermidine/spermine N1-acetyltransferase 1 (SSAT1) acetylates eIF5A and reduces the TDP-43 phenotype in cellular models. Hence, we argue that post-translational modifications specifically, hypusination vs. acetylation increases or subverts TDP-43 pathology, respectively. We predict that eIF5AhypK50 regulates TDP-43 fate via several potential mechanisms, including protein-protein binding properties, increasing TDP-43 cytoplasmic retention or perturbing the nucleocytoplasmic shuttling of TDP-43 via affecting the nuclear transport machinery. Here, we will discuses the strategies and approaches that we have employed to dissect the mechanism of action through which eIF5A affects TDP-43 pathology in FTD disorders and related dementia.

CSF Amylin – Effect Modifier of the Aβ-AD Relationship

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Fellow

Disease biomarkers

Background: Accumulating evidence from several laboratories indicates that patients with Alzheimer's disease (AD) have cerebral mixed deposits formed by β amyloid (Aβ) and amylin, a pancreatic hormone that crosses the blood-brain barrier and has amyloidogenic properties. In contrast to amylin from humans, rodents have non-amyloidgenic amylin, which does not accumulate in the brain or other tissues. Here, we speculated the difference in amyloidogenicity between human and rodent amylin species to test the hypothesis that chronically elevated levels of amylin in cerebrospinal fluid (CSF) promote mixed amylin-Aβ pathology worsening the behavior changes in a rat model of mixed amylin-Aβ pathology. Methods: For studying amylin-Aβ pathology, we crossed rats expressing human amylin in the pancreas (HIP) rats with AD rats to generate ADHIP rats. Littermate AD and wild-type (WT) rats expressing the non-amyloidogenic rat amylin served as negative controls for brain amylin deposition. Behavior was tested in ADHIP, AD and WT littermate rats at 8 months of age (when all rats have normal behavior), at 12 months of age (when HIP and ADHIP rats develop amylin pathology) and at 16 months of age, by the Novel Object Recognition (NOR) and Morris water maze (MWM) tasks. Amylin-Aβ interaction in CSF was assessed by immunoprecipitation of amylin (1 ml CSF/rat; n=4 rats/group) followed by ELISA for amylin. The formation of mixed amylin-Aβ pathology in the brain was tested by immunohistochemistry. Results: In ADHIP rats, behavior changes have developed at ~12 months of age, which was four months earlier than in AD littermate rats. Brain dysfunction in ADHIP rats correlated with elevated blood levels of aggregated amylin. The lower performance in ADHIP rats compared with age-matched rats in the other groups correlated with the development of mixed Aβ-amylin oligomers in CSF and mixed Aβ-amylin plaques in the brains of ADHIP rats. Conclusions: Finding mixed amylin-Aβ oligomers in ADHIP rats indicates that CSF amylin level is effect modifier of the Aβ-AD relationship. The formation of "mixed" amylin-Aβ oligomers in vivo is consistent with in vitro and in silico studies showing that amylin-Aβ interaction can promote robust growth of mixed amylin-Aβ amyloids.

Amylin dyshomeostasis disrupts white matter structural integrity and modulates amyloid composition and pathology distribution

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Student

Other neurodegenerative disorders

Background: Recent data shows that the brains of individuals with Alzheimer's disease (AD) contain deposits of amylin, an amyloidogenic peptide that makes up the pancreatic amyloid in type-2 diabetes. In the animal model of amylin dyshomeostasis (rats overexpressing human amylin in the pancreas - HIP rats), amylin accumulation in the brain white matter is associated with white matter injury. We hypothesize that amylin dyshomeostasis injures the brain white matter and modulates the amyloid composition and distribution within the grey matter in AD.
Method: Temporal lobe samples from non-diabetic familial Alzheimer's disease (fAD) patients with presenilin (n=20) or amyloid precursor protein mutations (n=7) and healthy individuals (n=12) were tested for brain amylin accumulation. Amylin-Aβ interaction and the anatomical distribution of the pathology were assessed by immunohistochemistry. To further test the hypothesis that amylin dyshomeostasis modulates AD pathology, we crossed TgF344-19 AD rats (which overexpress human APPSwe/PS1dE9 from a PrP promoter) with HIP rats. The newly generated ADHIP rats were compared with AD, HIP and wild-type littermates (males; n=10/group). Amylin-Aβ pathology in the brain was assessed with immunohistochemistry.
Result: In the brain of fAD patients, amylin has higher tendency to accumulate in the white matter compared to Aβ, suggesting that amylin preponderantly induces pathology in the brain white matter in fAD. In the brain grey matter, both independent amylin deposits and mixed amylin-Aβ plaques are found in fAD individuals. Similar to the pathology distribution observed in the brains of fAD patients, ADHIP and HIP rats (overexpressing human amylin in the pancreas) show a higher tendency of amylin accumulation in the brain white matter when compared to Aβ. Amylin deposits and amylin-Aβ mixed plaques are found in the grey matter of ADHIP brains.
Conclusion: Amylin dyshomeostasis induces white matter pathology and modulates the amyloid composition in familial AD.

Bisphenol A: A Trigger of Neurovascular Dysfunction and Cognitive Decline in Alzheimer's disease?

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Student

Cognitive/Behavioral disorders

Bisphenol A (BPA) is an endocrine disruptor found in a variety of plastics including food packaging, beverage containers, medical devices and other consumer products. Human exposure to BPA is inevitable and biomonitoring studies showed that 93% of the urine samples from more than 2,500 Americans six years and older have detectable BPA levels. Recent studies indicate that BPA can impact neuronal development and impair learning and memory in rodents and non-human primates as well as in children and adults. One factor that contributes to neurodegeneration and cognitive impairment is neurovascular dysfunction, a condition recognized as both a cause and consequence of the pathological cascade that leads to cognitive decline in Alzheimer's disease (AD). In this regard, we have first evidence that BPA accelerates neurovascular dysfunction in a mouse AD model.
We exposed 8-week old wild type (WT) and 5xFAD mice to BPA through their diet (0.1 mg BPA per 1 kg diet) for 1 and 6 months. We observed significantly reduced P-glycoprotein transport activity levels in brain capillaries isolated from BPA-fed mice compared to control mice after 1 month. In addition, we found that BPA-fed mice displayed neurovascular leakage compared to controls. Furthermore, cognitive performance tests (Y-maze and Morris water maze) revealed that BPA intake for 6 months worsened cognitive impairment in 5xFAD mice compared to WT control mice and BPA-fed WT mice.
Together, our data show that BPA triggers neurovascular dysfunction, a factor that increases the progression of cognitive decline in Alzheimer's disease.

Epilepsy/Brain Metabolism

Laforin nanobodies for the unnderstanding and treatment of lafora disease

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Student

Neurotherapeutics

Nanobodies are a type of Camelid antibody and more valuable that canonical antibodies for a variety of reasons. Nanobodies are easy to grow and modify by genetic fusion. Chemical or enzymatic conjugation makes them versatile tools for perturbation studies, microscopy, flow cytometry, mass cytometry, mass spectrometry, or non-invasive imaging methods such as immuno-PET. They have been shown to pass the blood-brain barrier. Nanobodies also facilitate protein crystallization and are widely used in structural biology. I have developed several nanobodies to a glucan phosphatase called laforin (involved in a fatal pediatric epilepsy called lafora disease) and am producing a comprehensive analysis of their utilities.

Movement/Neurodegenerative

Neurotransmitter levels in PFAS-treated Northern Leopard Frogs

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Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting over 10 million people. Environmental factors have been repeatedly linked to PD. Importantly, ~90% of cases are sporadic. PFAS (perfluorinated alkyl substances) are chemicals used in water repellents, carpets, food packaging, lubricants, surfactants, and fire extinguishers, and have been found in most humans' blood. Previously, we reported that PFAS selectively decrease dopamine and subsequent loss of nigrostriatal dopamine neurons, which is a key characteristic of PD. Perfluorooctane sulfonic acid (PFOS), one of the most common PFAS, accumulates in the brain. We hypothesized that exposures to PFOS or PFAS mixture would decrease dopamine levels in the brain. Northern Leopard frogs were exposed to control, PFOS (10ppb), or PFAS mixture (4ppb PFOS, 3ppb PFHxS, 1.25ppb PFOA, 1.25ppb PFHxA, 0.5PPB PFPeA). 20 frogs per treatment were sacrificed at 30 days after exposure. Snout-vent length, mass, and Gosner stage were measured and the brain was removed to measure neurotransmitters. HPLC was used to quantify dopamine and its metabolites DOPAC and HVA, serotonin and 5-HIAA, and norepinephrine. Acetylcholine was measured using an Invitrogen Amplex Red Acetylcholine/Acetylcholinesterase kit. The remaining 20 frogs under each treatment were sacrificed at metamorphosis and the brains were removed. None of neurotransmitters nor their metabolites showed significant changes in frogs after 30 days of exposure to PFOS or PFAS mixture. Currently, frogs that have completed metamorphosis are being examined for long term neurotoxicity. [Cali Clark is an undergraduate participant in the Summer Research Opportunities Program (SROP) at Purdue University]

A Novel Treatment for Olivopontocerebellar Atrophy

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Student

Movement disorders

Case presentation:

72 year-old male presented to the clinic with significant truncal and appendicular ataxia. Gait ataxia was noted, with dysphagia and vocal cord ataxia. The patient had been previously diagnosed with Parkinson's Disease. Neurologic examination revealed appendicular ataxia and tremor with normal strength and deep tendon reflexes. All sensation was intact. Finger-to-nose testing demonstrated significant ataxia and past-pointing. Severe truncal ataxia was noted, making it difficult for the patient to sit upright in a chair. On gait testing, significant ataxia and difficulty with balance were present. Cranial nerve exam revealed no deficits.

The patient had continuous tremors in his head and neck. Ataxic and scanning speech were elicited on exam. The patient possessed higher cortical function and recent/ remote memory appeared normal. The patient was begun on 4-aminopyridine, a potassium channel blocker commonly used in the treatment of lambert-eaton syndrome and multiple sclerosis. Upon follow up, he reported significant improvement in symptoms, including a marked reduction in truncal ataxia.

Discussion:

OPCA was first described in 1900 by Dejerine & Thomas. It has since been subclassified as Multisystem Atrophy-Cerebellar type. Multisystem Atrophy (MSA) includes three unique syndromes which all possess variable degrees of autonomic failure/ dysregulation, cerebellar dysfunction, parkinsonism, and corticospinal degeneration. The proposed mechanism of disease is alpha-synuclein deposits spreading from neurons to glia in prion-like fashion, ultimately causing secondary neurodegeneration from the inflammatory cascade and myelin degeneration. Neuroimaging may reveal atrophy in the putamen, pons, and/or middle cerebellar peduncles.

The estimated incidence and prevalence of OPCA in the population > 50 years old are 3 cases per 100,000 and 2-5 cases per 100,000 respectively. OPCA is a purely clinical diagnosis and is frequently misdiagnosed as one of the other synucleinopathies, namely Lewy body dementia, Parkinson's disease, and progressive supranuclear palsy. Unique to OPCA are the following: its earlier average age of onset (mean age of 54 years), unresponsiveness to levodopa therapy, and presence of autonomic dysfunction that spares the peripheral autonomic nervous system (interestingly, one of the first reported cases of OPCA presented as orthostatic hypotension from autonomic failure). The ability to distinguish between Parkinson's disease and OPCA dramatically changes the treatment plan (i.e. levodopa is ineffective in OPCA) and as such is crucial.

Conclusion:

This case is significant in that it represents an initial misdiagnosis of Parkinson's disease that, upon further imaging studies, was determined to be OPCA. Additionally, it represents the novel use of 4-aminopyridine, which was used historically for the treatment of Lambert-Eaton Syndrome and Multiple Sclerosis, to treat OPCA. Further study is indicated to have validated Quantitative Cerebellar Functional Severity Score testing on this individual to follow longitudinally of his progress.

NeuroRehabilitation

Impact of corticospinal integrity on the effects of tDCS

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Staff

Neurorehabilitation

Research Objective(s): To determine if cathodal and anodal tDCS would yield differential effects depending on presence or absence of motor evoked potentials (MEP +, MEP-), measured with transcranial magnetic stimulation at baseline.
Design: Double-blind feasibility study
Setting: Outpatient Neurorehabilitation Research Clinic
Participants: A convenience sample of 10 chronic stroke participants with severe motor deficit. Chronic was defined as occurring more than12 months from stroke onset. Severe motor deficit was defined as Fugl-Meyer Assessment (FMA) score between 7 and 34 as well as the inability to extend the affected metacarpophalangeal joints at least 10° and the wrist 20°.
Interventions: Each participant received 10 days of either cathodal or anodal tDCS (2.0 mA, 20 minutes) paired with 2 hours of motor therapy. Motor therapy focused on skill acquisition via activities to improve functional use of the impaired UE.
Main Outcome Measure(s): Action Research Arm Test (ARAT) and Fugl-Meyer Assessment (FMA) were collected at baseline, after completion of intervention, and 1-month follow-up.
• Results: Mean changes in ARAT were 7.0, 2.5, 2.0, and 1.7 for Cathodal/MEP -, Cathodal/MEP +, Anodal/MEP -, and Anodal/MEP +, respectively. By follow-up, MEP + participants maintained their improvement, regardless of receiving anodal or cathodal tDCS. However, MEP - participants receiving anodal tDCS lost all improvement, while those who received cathodal tDCS maintained their improvements. Mean changes in FMA were 10, 1.5, 4, and 6 for Cathodal/MEP -, Cathodal/MEP +, Anodal/MEP -, and Anodal/MEP +, respectively. By follow-up, improvements were decreased but still observed for all groups except Cathodal/MEP+.
• Conclusions: Cathodal tDCS appears to be particularly beneficial in participants without MEP at baseline. Larger prospective and randomized studies are required to substantiate these preliminary findings.

Neural Correlates of Grade Movement for Brain-Computer Interfaces

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Student

Neurorehabilitation

Brain-computer interfaces (BCIs) are designed to collect, process, and translate brain signals into commands for an external device. Today, BCIs are primarily limited to research settings due to operational challenges, specifically the need to generate and discriminate between brain signals associated with distinct mental tasks to control an external device. Here we propose to model graded event-related potentials (GERPs) from the electroencephalogram, i.e., signals that reflect the level of effort associated with a movement task. Predicting gradations in motor effort associated with a single isometric force production task from the electroencephalogram (EEG) would multiply the number of available command signals. Our results found spatio-temporal differences related to exertion of different levels of motor effort. These features were modeled and classified using linear discriminant analysis to yield F1 scores between 63-85% and 65-80% in the dominant and non-dominant handed runs, respectively. This offline exercise from an ongoing study shows signals from EEG during graded motor movement are discriminable and hold promise as command signals for BCIs aimed at improving rehabilitative efforts and the quality of life for those afflicted with neuromuscular disorders.

Neurophysiology

Ruling out PGRP-LC and PGRP-LE peptidoglycans receptors in the response to bacterial endotoxin LPS in larval Drosophila

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Student

Neurophysiology

Gram-negative bacteria produce and release endotoxins in the form of lipopolysaccharides (LPS). The different forms of LPS produce varying secondary immune responses; however, the direct effect of LPS itself has not been well studied. Peptidoglycan recognition proteins (PGRPs) PGRP-LC and PGRP-LE in body wall muscle, heart and neurons are potential receptors for LPS in Drosophila. Exposing the heart of larval Drosophila to LPS (500 µg/ml) from Serratia marcescens causes the heart rate to initially increase and then slow down; whereas, exposing the body wall muscle, while stimulating the motor nerve, results in hyperpolarization. Evoked as well as spontaneous excitatory junction potentials become depressed in the presence of LPS. PGRP-LC and PGRP-LE were individually knocked-down using RNA interference (RNAi) specifically targeted to body wall muscle, heart and motor neurons. RNAi impairment of these genes did not affect the LPS responses in any of these tissues as compared to wildtype Canton S or parental lines. We conclude that these LPS responses are either mediated by other signaling pathways or that these PGRPs are redundant. The decrease in synaptic transmission is likely due to the postsynaptic glutamate receptors being blocked by LPS. However, the mechanism to explain the hyperpolarization of the body wall muscle and alterations in heart rate has yet to be determined. Thus, no mechanism has been described to account for LPS causing these rapid cellular changes within seconds. Uncovering the underlying mechanism will be critical to understanding effects in human and other animals exposed to gram negative bacterial infections.

Fibroblast growth factor 19 in the dorsal vagal complex alters excitability of dorsal vagal motor neurons and lowers blood glucose concentration

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Student

Neurophysiology

According to the CDC, there are more than 30 million Americans living with diabetes. Although most diabetes research focuses on defects in insulin and glucose metabolism, emerging evidence suggests that the brain plays an underappreciated role in systemic glucose regulation. One such homeostatic regulatory center is the brainstem dorsal vagal complex (DVC), which monitors metabolic status through both vagal afferent neural and humoral signals including glucose, insulin, and leptin. Parasympathetic motor neurons in the DVC respond to this information by altering vagal output to regulate pancreatic hormone release and hepatic glucose production. Fibroblast growth factor 19 (FGF19) has potent, insulin-independent antidiabetic effects when administered to the brain, though the mechanisms of action are unknown. This information, together with the fact that FGF19's receptor/co-receptor combination is present in the DVC, suggests that this area is a candidate region mediating the observed antidiabetic effects. Here, FGF19 (137μM) was shown to significantly decrease blood glucose concentration for up to 12 hours when administered to the DVC via fourth ventricular microinjection in type 1 diabetic mice (STZ). To understand the effects of FGF19 on DMV neuron excitability, patch-clamp electrophysiology was used in acute brainstem slices. FGF19 (230pM) altered action potential frequency, post-synaptic current frequency, and voltage-gated potassium current amplitude, all trending towards reduced excitability. These cellular effects are consistent with the hypothesis that FGF19 modifies central vagal circuitry controlling parasympathetic output to the viscera and could contribute to the peptide's effects on metabolism. Further studies are underway exploring the effects of FGF19 on DMV neuron excitability and peripheral glucose metabolism in diabetic mice.

Pharmacological profiling of stretch activated channels in proprioceptive neurons

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Student

Neurophysiology

Proprioception in mammals and invertebrates occurs through stretch activated ion channels (SACs) localized in sensory endings. In mammals, the primary organ for proprioception are the intrafusal muscle spindles embedded within extrafusal muscle. In invertebrates there are varied types of sensory organs from chordotonal organs embedded within muscle to muscle receptor organs (MRO), which are analogous to the mammalian muscle spindle that monitor stretch of muscle fibers. The classification of the SACs in the sensory neurons associated with mammal muscle spindles have recently been described as a type of PIEZO 2 subtype, and these receptors also have a role in sensing mechanical stimulation on skin. The PIEZO channels are comprised of a distinct type of protein sequence and are similar among species from mammals to invertebrates. Relatively new agents have been identified to have action on SAC of the PIEZO 1 subtype. Yoda1 and Jedi2 will activate PIEZO 1 and the action by Yoda1 can be reversed by dooku. Additionally, OB-1 can modulate activity of PIEZO 1 channels. To date, the SACs of the crustacean proprioceptors have not been satisfactorily pharmacologically classified, nor has their molecular makeup been identified. We are screening the pharmacological profile of these sensory organs in crustaceans with these various listed compounds to determine if they may serve as a model for mammals.

Other Topics

OPTIMIZING METHODS FOR LIGHT-BASED PRODUCTION OF BDNF AFTER TRANSFECTION

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Neuroimmunology/MS

Objective: Neuronal networks require significant neurotrophic support for recovery after stroke[1,2], but the delivery of neurotrophins (NTs) failed thus far in clinical trials[3-5]. B cells possess the ability to produce NTs[6,7], including brain-derived neurotrophic factor (BDNF), and infiltrate the post-stroke brain. Studies in our lab show that systemic B cell depletion impedes neurogenesis, increases anxiety, and exacerbates memory deficits in mice after stroke. Thus, we hypothesize that stimulating the therapeutic production of BDNF from B cells could enhance neuroplasticity after stroke.

Methods:
We are currently working with HEK 293 cells to optimize a dose-dependent BDNF production strategy we will use in B cells. We are performing a dual transfection with constitutive expression of light-sensitive protein (VEL) and c120-controlled BDNF expression plasmid (LightSwitch). Blue light (465nm) dimerizes the protein that translocates into the nucleus and transcribes BDNF. We replaced the light source with co-transfected luciferase as an internal bioluminescent light-source. NanoLuc Luciferase catalyzes the novel analog, Furimazine. The reaction produces blue light (465nm), is ATP independent, and only requires oxygen as a co-factor. This would allow the cell to be its own light source for VEL dimerization, and therefore BDNF expression. We use a Lipofectamine transfection protocol that contains about 500ng of DNA per well. Our controls cells are transfected with CMV-BDNF-p2A-mCh and CMV-p2A-mCh as positive and negative controls We have successfully performed a co-transfection with c120-BDNF-p2A-mCh and pVEL plasmids, forming “OptoLuc” Cells. We also went further with a triple transfection by also adding CMV-NLuc to the OptoLuc Cells.

Preliminary Results:
After running BDNF ELISAs on our co-transfected OptoLuc Cells, we were able to record BDNF production in the cells treated with light, versus no BDNF in supernatant from cells that were not exposed to light. In our triple plasmid system, we recorded bioluminescence and are currently optimizing Furimazine dosages to produce the largest BDNF quantities over 24-48 hours.

References:
1.) PMID: 15717063
2.) PMID: 25018717
3.) PMID: 10227630
4.) PMID: 11464953
5.) PMID: 18790057
6.) PMID: 12892403
7.) PMID: 26399960

In vivo monitoring of intracellular pO2 in response to CAR T cell immunotherapy against glioma

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Faculty

Neuro-oncology

Introduction
We explore the temporal dynamics of tumor intracellular partial pressure of oxygen (pO2) in a murine glioma model receiving chimeric antigen receptor (CAR) T cell immunotherapy. Tumor hypoxia is associated with increased tumor angiogenesis, recurrence, and malignant progression. Noninvasive monitoring of tumor pO2 levels during treatment can provide a preclinical surrogate biomarker for the effectiveness of emerging immunotherapeutic strategies and can be used to optimize therapeutic course and dosage. We tested the hypothesis that a measurable increase in pO2 is commensurate with CD8+ T cell apoptotic processes in a mouse model of sub-cutaneous glioblastoma (U87-EGFRvIII-Luc) treated with human CAR T cells. Perfluoro-crown-ether (PCE) nanoemulsion dissolves oxygen, resulting in a linear increase in the 19F spin-lattice relaxation rate (R1) with increasing pO2. Using 19F MRI/MRS, we measured the pO2 time-course in flank glioma cells that were intracellularly labeled with PCE nanoemulsions and treated with intravenously-infused CAR T cells.

Methods
U87 glioma cells expressing EGFRvIII and luciferase (U87-EGFRvIII-Luc) were labeled ex vivo with laboratory-prepared PCE nanoemulsion overnight in conventional medium followed by wash. Cell uptake was measured by 19F NMR yielding the mean 19F/cell. Human peripheral blood mononuclear cell-isolated T cells were transduced with a CAR lentiviral vector to express a surface antibody against EGFRvIII, a common receptor in glioblastoma multiforme. Transduction efficacy and phenotype of the T cells was confirmed by flow cytometry; a population of >70% CAR T cells was used for intravenous injection. Female SCID mice (N=15) received unilateral sub-cutaneous injections of 5×10^6 PCE-labeled U87-EGFRvIII-Luc cells. All mice were subjected to MRI and bioluminescence imaging (BLI) four days post tumor implantation, then received intravenous cell treatments (day 0). Groups 1-3 (N=5 per group) received 20×106 CAR T cells, 20×10^6 untransduced T cells, and no T cells, respectively. Longitudinal MRI and BLI scans were acquired using a Bruker 11.7T BioSpec and an IVIS Spectrum, respectively, on days 1, 3, 7, and 10 while anesthetized with 1.5% isoflurane in 100% O2 and maintained at 37C. Proton images were acquired using RARE (TR/TE=1400/7.8 ms, RARE factor 2, NA=2, FOV=3×3 cm^2, matrix = 256×256). Co-registered 19F images were also acquired with RARE (TR/TE=400/23 ms, NA=32, matrix = 64×64). The 19F R1 was measured over entire tumor volume using PRESS (15 TR values, 0.1-6 s, single exponential recovery fit) to yield pO2 values, calculated with a calibration curve. At the experimental endpoint, tumors and spleens were harvested and fixed for histology to qualitatively assess (CAR) T cell homing.

Results
Prior to implantation, U87-EGFRvIII-Luc cells were labeled ex vivo with PCE to (average) level ~7×10^12 atoms/cell measured via 19F NMR. Following sub-cutaneous injection, labeled glioma cells appear as an MRI 19F hotspot with SNR~10 at day 1 post-injection (Fig. 1A). PRESS voxel encompassing the hotspots was used to measure R1 values to calculate pO2 (Fig. 1B). Longitudinal in vivo measurements show a transient spike in tumor pO2 approximately three days after CAR T cell infusion (R1=0.990.12 s-1, pO2=134 mmHg) compared to untransduced T cells (pO2=61 mmHg) and control (pO2=40 mmHg, p = 0.026, Fig. 1B). There is no significant pO2 change in the untransduced T cell Group at day 3 (p=0.35). These data suggest specific CAR T cell homing to the tumor tissue, presumably initiating a target killing cascade, and altering intracellular pO2. By day 7, tumor oxygenation returns to baseline in the CAR T cell Group (Fig. 1B). Longitudinal bioluminescence measurements show significant tumor regression 7 days post CAR treatment with an average radiance of 4*10^10 photons/sec, which is half the amount

measured for both naïve T-cell treated and untreated groups (p=0.012, Fig. 1C). Histopathological staining confirmed the presence of CAR T cells in greater amounts than untransduced T cells in the tumors at day 3 post-infusion (p=0.001, data not shown), consistent with the MRS results.

Conclusions

In this study, we show that 19F MRI enables temporal measurements of tumor cell oxygen tension in response to CAR T cell therapy. Peak pO2 was observed at 3 days post-infusion and suggests significant CAR T cell infiltration and targeted tumor cell killing, compared to untransduced T cells. Preliminary quantitation of tumor infiltrating T cells in the same glioma model via 19F detection is reported elsewhere. Overall, these data support the view that 19F pO2 MRI and MRS can serve as a biomarker for cell-mediated apoptosis and provide insight into the modes of action of engineered T cell immunotherapy against cancer.

Identifying Neural Correlates of Nicotine Withdrawal in Mice: The Neuregulin Signaling Pathway

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Background. Addiction to nicotine and the ability to quit smoking are influenced by genetic factors. Therefore, it is important to understand how genes and drugs of abuse mechanistically impact each other. Previous work from our lab has shown that cAMP response element-binding protein (CREB) activity in the ventral hippocampus (Vhipp) mediates anxiety-like responses in mice during nicotine withdrawal (WD) (Fisher et al, 2017). High throughput chromatin immunoprecipitation sequencing (ChIP-seq) studies determined that WD from nicotine differentially modulates CREB binding to a gene called Neuregulin-3 (nrg3). Interestingly, the Neuregulin Signaling Pathway, which has been widely implicated in schizophrenia, has also been recently linked to nicotine dependence in humans. For example, work from our lab (Turner et al, 2014) and that of our colleagues (Loukola et al, 2014) has shown that polymorphisms within two genes in the Neuregulin Signaling Pathway, NRG3 and its cognate receptor ERBB4, have been linked to failed smoking cessation. Nrg3, a neural-enriched epidermal growth factor-like protein located on pyramidal cells binds to and activates ErbB4 receptors located on GABAergic interneurons. With high hippocampal expression, the interaction between these two synaptic proteins play an important role in synaptogenesis and overall plasticity at these excitatory/inhibitory synapses within the Vhipp. This work aims to evaluate how Neuregulin signaling in the Vhipp may impact nicotine WD-induced affective phenotypes in mice and the underlying physiological and biochemical changes that occur.

Methods. Quantitative PCR (qPCR), Western blotting and fluorescent in situ hybridization experiments were conducted on Vhipp tissue from male and female 8-10 week old B6129-F1 mice implanted with pulsatile osmotic minipumps and separated into treatment groups (saline, nicotine (12mg/kg/day), 24h WD) (N=10-12). ErbB4-floxed animals were generated for behavioral testing and functional experimentation using whole cell patch clamp electrophysiology and Ca2+ imaging techniques. Both male and female animals were stereotactically injected with AAV-CRE or AAV-GFP (control) into the Vhipp at 6 weeks of age to induce temporal and spatial knockout of erbb4 (N=12-15).

Results. qPCR and Western blotting experiments established that Nrg3 and ErbB4 are upregulated at the 24h WD time point in the Vhipp, with expression returning to baseline by 1-week post WD. Furthermore, conditional Vhipp deletion of ErbB4 blocks WD-induced anxiety-like behaviors as measured by the Novelty-induced Hypophagia test and the Open Field Exploratory test, two well-validated behavioral models of anxiety-like behaviors in mice. This phenotype is accompanied by decreased levels of inhibitory GABAergic release and altered network clustering of excitatory pyramidal cells within the ventral CA1, an area enriched in Nrg3 and ErbB4 mRNAs sensitive to nicotine WD.

Conclusion. These data support a model of aberrant Nrg3-ErbB4-induced plasticity underlying nicotine withdrawal-induced behaviors. We found that disruption of Vhipp Nrg3-ErbB4 signaling attenuates WD-induced anxiety-like phenotypes through altering GABAergic modulation of CA1 pyramidal cell activity. This suggests blocking Nrg3 activation of ErbB4 at GABAergic synapses weakens inhibitory inputs and regulation of excitatory pyramidal cell activity and prevents WD-induced synaptic remodeling. Further examination of downstream signals of ErbB4 activation may lead to the identification of potential targets for treating nicotine withdrawal symptomology.

Use of Marijuana and Narcotics on Emotion Recognition

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Behaving appropriately in social situation requires accurate discrimination of emotional expression. Inability to recognize facial expressions is closely associated with psychiatric disorders. In this study we examined the effects of drug use on discrimination of emotions, focusing primarily on marijuana and narcotics. Narcotics or opiates are known to suppress brain function through mu-receptors. Marijuana is mildly analgesic and produces hallucinogenic effects through cannabinoid receptors. Subjects were college students at Morehead State University. Drug effects on accuracy of emotion recognition were examined using the DANVA2 task, which consists of four subsets of emotion-related stimuli, including visual (48 trials, faces of adults or children) or auditory (48 trials, voices of adults or children). A drug survey measured frequency and duration of drug use. Overall, college students could more readily discriminate facial expressions conveying positive emotions rather than negative ones, with no overall sex differences in accuracy. Such pattern depended on the type of emotion and the stimulus subjects: students could discriminate ‘sad’ faces of children with a high accuracy. Compared to controls, frequent drug users (>3 times/week) of either opiates or marijuana made more errors. A pattern of error also depended on emotion category, with a greater accuracy for happy expressions than for angry or fearful ones. Our data suggest that the ability to discriminate emotions vary with the nature of the emotional stimuli--stimulus type (adult or child), sensory modality (face or voice), or emotional category (positive or negative). The present findings provide evidence that among college students, frequent drug use impairs the ability to process emotions, particularly negative emotions, thereby reducing accuracy in discrimination of emotional expressions.