2017 Neuroscience Graduate Program Symposium

Abstracts

A 01
VOLUNTARY WHEEL RUNNING CAN IMPROVE INCREASED UROGENITAL SENSITIVITY AND FUNCTION RESULTING FROM NEONATAL MATERNAL SEPARATION IN MALE MICE

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Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) shares many symptoms with interstitial cystitis/painful bladder syndrome (IC/PBS), and the two are commonly co-diagnosed. Almost half of those diagnosed with these functional pain disorders also suffer from mood disorders, particularly depression and/or anxiety. Additionally, many of these patients have a reported history of early life stress. Experience of such stress has been associated with dysfunctional stress response in adulthood, attributed to altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Comorbidity of these disorders and unknown etiology has complicated or hindered the development of effective treatment options. However, regular exercise has been shown to improve pain perception and attenuate many symptoms ascribed to irregular HPA axis function in clinical and preclinical studies. Here we investigated the therapeutic potential of voluntary wheel running to mitigate perigenital hypersensitivity and mood behaviors associated with a male mouse model of neonatal maternal separation (NMS), as well as molecular changes observed. Mice were born in house and were either unhandled, aside from normal husbandry procedures, or subjected to NMS from postnatal day 1 to 21. Mice were then placed in new cages with unlimited running wheel access beginning at 4 (-Eex) or 8 (-Lex) weeks of age. Sedentary controls (-Esed/-Lsed) remained in home cages. NMS-exposed mice displayed significant perigenital mechanical sensitivity, increased micturition patterning, and increased mast cell degranulation in urogenital tissues. These alterations were prevented or reversed by voluntary wheel running. Differences in concentrations of serum corticosterone and central gene expression changes suggest improper functioning of the HPA axis. However, our data does not indicate NMS-induced anhedonic- or anxiety-like behaviors.

Conclusion: Together, these data suggest NMS in male mice can be a useful model for comorbid CP/CPPS and IC/PBS, and voluntary exercise may be a viable therapeutic option to improve symptom severity.

A02 Poster presentation winner

EVALUATION OF TAURINE NEUROPROTECTION IN AGED RATS WITH TRAUMATIC BRAIN INJURY

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Despite higher rates of hospitalization and mortality following traumatic brain injury (TBI) in patients over 65 years old, this age group is highly underrepresented in drug development studies. Worse outcomes in elderly individuals compared to younger adults could be attributed to exacerbated injury mechanisms including oxidative stress, inflammation, blood-brain barrier disruption, and bioenergetic dysfunction. Taurine, an endogenous amino acid and nutritional supplement that functions as an anti-oxidant, anti-inflammatory, cellular osmolyte, and neuromodulator is neuroprotective in adult rats with TBI. However, its effect in aged rats is unknown. The objective of our study was to determine whether taurine is neuroprotective in an aged rat model of TBI. Aged (20-21 month old) male F344 rats were anesthetized and subjected to unilateral controlled cortical impact injury to the sensorimotor cortex (5 m/sec, 2.5 mm depth). Experimenters were blinded to the treatment group and rats were randomized into four groups and administered taurine (200mg/kg, 50mg/kg, or 25mg/kg i.p.) or saline 20 minutes post-injury and then daily for 7 days. Sensorimotor functional outcomes were determined using beam walk and bilateral adhesive removal test at 1, 3, 7, 10 and 14 days post-TBI. Magnetic resonance images were obtained at day 14 post-TBI and quantified using ImageJ to estimate lesion volumes. MRI analysis showed a dose-dependent decrease in TBI lesion volume in taurine-treated groups compared to saline. However, taurine did not significantly improve sensorimotor outcomes.

Conclusion: These results suggest that while taurine promotes brain tissue sparing in aged rats following TBI, this may not be sufficient to improve gross function and plasticity of the remaining tissue.

A03

INDEPENDENT COMPONENT ANALYSIS RECOVERS DISCRETE CELLULAR SOURCES CONTRIBUTING TO THE ELECTRICAL POTENTIAL RECORDED AT THE ROUND WINDOW

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As medical and technological treatments for cochlear hearing loss become more available and sophisticated, there is a growing need for diagnostic techniques that can identify anatomical damage or dysfunction underlying a patient's hearing loss. The round-window cochlear response (CR) is an electrophysiologic signal comprised primarily of current flow through outer hair cells and an auditory nerve response. A study using near-infrasonic stimuli (45 Hz) yielded an atypical response waveform – uncharacteristic of outer hair cells yet highly consistent across animals –suggesting (an) additional cellular source(s) in addition to outer hair cell and auditory nerve.

This study used a blind source separation technique, independent component analysis (ICA), to separate the low-frequency CR into its distinct cellular sources. The CR was recorded from an electrode on the round window of Mongolian gerbils to a 45 Hz tone burst embedded in 18 high-pass filtered noise conditions. Multiple trials were recorded for each animal at each noise condition and in two different stimulus phase conditions, targeting apical to basal regions along the cochlear partition. The animals' CR waveform at each noise condition and in each stimulus phase condition served as a mixture, or linear combination of signals from multiple cellular contributors, which ICA used to recover statistically independent source signals.

Conclusion –ICA shows promise as a technique to recover distinct cellular source signals buried within the CR. An accompanying model combined with our previous data and existing literature, provide evidence that inner hair cell, as well as outer hair cell and phase-locked auditory nerve potentials, are the primary sources of the low-frequency CR, and their relative contributions change as a function of location along the cochlear partition.

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A04

ADDRESSING STRUCTURAL FLEXIBILITY AT THE A-RING ON SALVINORIN A: DISCOVERY OF A POTENT KAPPA OPIOID AGONIST WITH ENHANCED METABOLIC STABILITY

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Previous structure-activity studies on the neoclerodane diterpene salvinorin A have demonstrated the importance of the acetoxy functionality on the A-ring in its activity as a kappa opioid receptor agonist. Few studies have focused on understanding the role of conformation in these interactions. Herein we describe the synthesis and evaluation of both flexible and conformationally restricted compounds derived from salvinorin A. One such compound with spirobutyrolactone functionality was synthesized in a single step from salvinorin B and has similar potency and selectivity to salvinorin A (EC_{50} = 0.6 ± 0.2 nM at κ and >10,000 nM at μ and δ). Microsomal stability studies demonstrated that this rigid spirobutyrolactone was more resistant to metabolism than salvinorin A. Evaluation of analgesic and anti-inflammatory properties revealed similar *in vivo* effects for both the rigid derivative and salvinorin A.

Conclusion: To our knowledge, this study represents the first example of bioisosteric replacement of an acetate group by a spirobutyrolactone to produce a metabolically resistant derivative compound.

A05 Junior Student Lecture Presentation 1

TARGETING CYCLOPHILIN D AS A NEW THERAPEUTIC APPROACH FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the sixth leading cause of death in the United States, killing more people than breast and prostate cancer combined. Despite this, there are no available drugs to prevent, slow, or cure AD. Only five drugs have been approved to treat the symptoms of AD. Genetic data from our animal models with neuronal Cyclophilin D (CypD) overexpression and P301S tau overexpression show more intense tauopathies, reduced mitochondrial respiration, and anxiety-like behaviors when compared to a P301S tau model of AD; this data points to CypD overexpression leading to a more aggressive AD phenotype. Data from animal models with CypD knockout and P301S tau overexpression show striking protection with less hyperphosphorylated tau, better mitochondrial respiration rates, and no anxiety-like phenotype. These results suggest targeting CypD as a promising novel therapeutic approach for AD by reducing AD tau pathology and improving mitochondrial and synaptic function. Using a novel drug screening platform, we have identified several small molecules showing promising activity in suppressing CypD activity. We are evaluating whether a pharmacokinetic CypD inhibitor can create the same protective effects seen in the CypD-deficient tauopathy mouse model, which is a widely used human mutant tau model of AD.

Conclusion- Cylcophiln D is an important effector in the progression of AD, and inhibition of CypD appears to be a good target for the therapuetic intervention in AD.

A06 Senior Student Lecture Presentation 2

MODULATING MOLECULAR CHAPERONES IMPROVES DEMYELINATING NEUROPATHY IN THE MPZ-RAF MOUSE MODEL

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Demyelinating neuropathies result from Schwann cell (SC) dedifferentiation upon loss of axonal contact or injury. Recent evidence suggests that c-jun is critical in promoting Schwann cell dedifferentiation. Elevated c-jun levels have been detected in a variety of human neuropathies suggesting that it may be a potential target for preventing or slowing the demyelination process. We previously demonstrated that modulation of heat shock protein 90 (Hsp90) with a small molecule Hsp90 modulator called KU-32 decreased c-jun expression and prevented demyelination in SC-neuronal co-cultures in a heat shock protein 70 (Hsp70)-dependent manner. In the current study, we utilized a transgenic mouse model (MPZ-Raf) in which injection of tamoxifen (TMX) leads to activation of the Raf-MAPK kinase pathway specifically in SCs. Elevated SC MAPK activity increased c-jun expression, demyelination and subsequent motor dysfunction. With this model, we sought to determine whether modulating heat shock proteins with KU-596, a third generation Hsp90 modulator, is sufficient to ameliorate the motor neuropathy that develops in these mice. Treating MPZ-Raf mice with KU-596 reduced the induction of c-jun but had no effect on the extent of MAPK activity. Drug treatment improved motor performance, delayed the onset of rear-limb paresis and ameliorated the extent of peripheral nerve demyelination in both prevention and intervention studies. Hsp70 was necessary for the neuroprotective efficacy of KU-596 since MPZ-Raf×Hsp70KO mice did not respond to KU-596 treatment.

Conclusion- KU-596 is currently in Phase 1 clinical trials and our data indicate that modulating heat shock proteins may provide a novel therapeutic approach to attenuate c-jun induced demyelinating neuropathies in humans.

A07
HOW IS MUSIC PROCESSED? TENTATIVE ANSWERS FROM COGNITIVE NEUROSCIENCE

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Music therapy is a profession that uses 'organized sound and silence in time' as its basic mechanism for behavioral change. Several music therapists (Hunt & Legge, 2015; Stegemoller, 2014, 2015) have turned to neuroscience in search of a mechanistic understanding of the therapeutic effect of music. This integrative review, which will be shared through images, explores and summarizes a small amount of existing literature as an introduction to the neural correlates of music. The review shows that music is a complex, generative, and recursive phenomenon that uses similar networks for acoustic processing as other sounds and speech (e.g. STG). On the other hand, some recent findings with MPVA show distinct regions for music processing when compared to speech. As a complex acoustic signal, music generates emotional responses that are processed by the reward system (NAc, and caudate nucleus), but also cortical and subcortical regions normally associated with emotion processing (the vmPFC, insula, amygdala, thalamus, hippocampus and parahippocampus, and hypothalamus). Motoric responses to music, sometimes in response to music imagery only, generate activations in motor areas (SMA, premotor, primary motor, basal ganglia, and cerebellum). As a cognitively complex stimulus, with significant relationships with other associative knowledge, it also engages higher-order processing in areas such as the dlPFC, OFC, ventromedial PFC, IFG, ACC, and STG. Cognitive control to monitor and resolve syntactic violations might be a critical mechanism in music processing, as shown by the activation of these areas. Preliminary conclusions from this emerging research include a) everyday perception of music never stops with the processing of a single musical element; its therapeutic effect is probably the result of the Gestalt of processing at all levels, b) recommendations for music therapy practice should parallel those of other areas of neuroscience: applications should not be based on simple, linear interpretations of activations; equating activation of a few areas with cognitive processing is a gross underestimation of the complexity of the multilevel analysis of music, and of the limitations of our current technology, c) extraneous variables, such as expertise, attitude, mood, and environment, also impact the results of neuroscientific studies, limiting straightforward recommendations.

Conclusion- Careful understanding of neuroscience methods and principles, continued collaborations between neuroscientists and music therapists, and contrasts with behavioral paradigms will support mechanistic explanations of the therapeutic effects of music.

A08

KU596 IMPROVES MITOCHONDRIAL BIOENERGETICS BY DECREASING OXIDATIVE STRESS IN DIABETIC SENSORY NEURONS VIA HSP70.

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Neuronal mitochondrial dysfunction is a key pathophysiologic mechanism of diabetic peripheral neuropathy (DPN). *In vivo* treatment with the Hsp90 modulator KU596 can reverse DPN development via Hsp70 and this correlates with improving mitochondrial bioenergetics (mtBE) in diabetic sensory neurons. The goal of this study is to determine if KU596 improves mtBE by decreasing glucose-induced oxidative stress. In WT sensory neurons, KU596 significantly improved mtBE in diabetic neurons under both normal and hyperglycemic conditions. Surprisingly, although KU596 increased mtBE in diabetic Hsp70 KO neurons maintained under normal glucose conditions, the drug was not able to improve mtBE under hyperglycemic condition. These results suggest that induction of hyperglycemia requires Hsp70 for KU596 to improve mtBE. This effect was related to mitochondrial oxidative stress. In WT and Hsp70 KO neurons, superoxide levels were significantly increased in diabetic neurons incubated with high glucose. Although KU596 decreased hyperglycemia induced superoxide levels in WT neurons, this effect was lost in the hyperglycemically stressed Hsp70 KO neurons. These data means that the ability of KU596 to increase mtBE in diabetic sensory neurons is linked to an Hsp70-dependent inhibition of glucose-induced superoxide production. Since MnSOD is the main mechanism to detoxify mitochondrial superoxide radicals, we determined the cause and effect relationship between improved respiration and decreased oxidative stress by knocking down MnSOD to increase mitochondrial oxidative stress in sensory neurons. MnSOD downregulation blocked KU596 effect on enhancing mtBE in diabetic sensory neurons. This means the ability of KU596 to improve mitochondrial bioenergetics is through reducing mitochondrial oxidative stress.

Conclusion- These results demonstrate that Hsp90 inhibitor, KU596 is efficient improving mitochondrial bioenergetics in diabetic sensory neurons in an Hsp70 dependent manner, and one mechanism is through decreasing mitochondrial oxidative stress.

A09

CONTINUOUS AND ON-LINE MONITORING OF L-DOPA METABOLISM IN RAT BRAIN USING MICROCHIP ELECTROPHORESIS COUPLED WITH MICRODIALYSIS SAMPLING

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Catecholamines, in particular L-DOPA and dopamine, are important neurotransmitters involved in many neurological processes and disease states, including Parkinson's disease. L-3,4-dihydroxyphenylalanine (L-DOPA), dopamine (DA), 3,5-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) are all analytes involved in the L-DOPA metabolic pathway. In order to study the transport of L-DOPA across the blood brain barrier, its conversion into dopamine, and the subsequent degradation processes, an analytical method must be developed. Microdialysis (MD) is a widely used *in vivo* sampling technique used to monitor extracellular concentration changes of analytes in the brain. Off-line analysis with conventional methods is most commonly employed for the analysis of microdialysis samples. However, this can lead to a loss of valuable temporal information concerning dynamic processes due to the large sample volumes necessary for more traditional analysis methods. In order to preserve temporal information the ideal analysis system is one that can be employed on-line, is fast, and has the ability to analyze very small sample volumes. Microchip electrophoresis (ME) is best suited to this type of analysis and incorporates the possibility of integrating electrochemical detection (EC) directly on-chip. In this study, a simple approach for coupling microdialysis to microchip electrophoresis with electrochemical detection for continuous and on-line monitoring of L-DOPA metabolism is described.

Conclusion- Using the developed MD-ME-EC method, L-DOPA and its metabolites can be separation in less than 100 s with baseline resolution. Further, the on-line *in vitro* analysis performed using this device confirmed the timely increase in dopamine concentration as a result of L-DOPA metabolism in homogenized rat brain.

A10 Senior Student Lecture Presentation 1

EVALUATING THE IMPACT OF EARLY LIFE STRESS AND EXERCISE ON ELICITED MIGRAINE IN MICE

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Millions of Americans currently suffer from chronic pain disorders including chronic pelvic pain, irritable bowel syndrome, migraine, and fibromyalgia. Although the underlying mechanism of chronic pain disorders remains to be elucidated, it is hypothesized that dysfunction of the hypothalamic pituitary adrenal (HPA) axis plays a role in pathogenesis. The HPA axis regulates stress responses and is involved in pain perception. We have developed a mouse model of early life stress (neonatal maternal separation (NMS)) that displays evidence of dysfunctional HPA axis regulation and chronic pelvic pain disorders. To expand our studies, we are now investigating if our model also displays evidence of migraine, fibromyalgia, and metabolic disease. Additionally, we are exploring the therapeutic potential of voluntary exercise to attenuate the changes brought on by NMS. Mice either underwent NMS from postnatal day 1 (P1) to P21 or remained in their home cage until weaning on P22. Mice were then further divided into exercised (Ex) or sedentary (Sed) groups. At four weeks of age, Ex mice were pair-housed and received access to a running wheel. We then utilized two ways to elicit migraine in mice: dural injection of inflammatory soup (IS) or intraperitoneal injection of nitroglycerin (NTG). At eight weeks of age, a modified cannula was transiently inserted through the lambdoidal suture to apply IS or saline onto the dura. Painful behaviors, including rearing episodes, mouse grimace scoring (MGS), and widespread mechanical allodynia were then evaluated. Results indicate a significant impact of IS application on forepaw allodynia and that exercise impacts painful behaviors displayed by our mice. At 10 months of age, mice received an IP injection of NTG or saline at a 10mg/kg dose and painful behaviors were evaluated. NMS significantly impacted hindpaw withdrawal threshold and NTG injection elicited a significantly greater MGS in NMS mice that was attenuated by exercise. Finally, to evaluate potential metabolic disease, 10-month-old mice were weighed and placed in an EchoMRI to quantify body fat percentage and fat free mass. We found that NMS significantly increased body fat percentage in aged sedentary female mice compared to naïve or exercised mice.

Conclusion- These results suggest that widespread allodynia is stimulated during migraine episodes, that NMS mice are more susceptible to NTG induced migraine than naïve mice, and that voluntary wheel running influences the painful behaviors mice display during elicited migraine attacks. Finally, NMS shows potential in causing metabolic disease in mice.

A11

ASSESSING THE ROLE OF MITOPHAGY IN CONTRIBUTING TO DIABETIC PERIPHERAL NEUROPATHY USING A NOVEL TRANSGENIC MOUSE MODEL: MITO-QC MICE

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Diabetic peripheral neuropathy (DPN) affects about 70% of diabetic patients, leading to infection, inflammation, amputation, and even death. Neuronal mitochondrial dysfunction is a key pathophysiologic mechanism of DPN. Studies in animal models of DPN have found an accumulation of fragmented mitochondria, which may indicate diminished mitochondrial homeostasis is associated with impaired mitochondrial quality control through altered clearance mechanisms. Mitophagy is a quality-control mechanism that selectively targets damaged mitochondria to autophagosomes for degradation through the autophagy pathway. Recruitment of the cytosolic E3-ubiquitin ligase, parkin, has been described as a critical pathway in controlling mitophagy by regulating recruitment to the autophagosomes. In vivo treatment with the heat shock protein (Hsp) 90 modulator, KU596, can reverse symptoms of DPN and this correlates with improving mitochondrial bioenergetics (mtBE) in diabetic sensory neurons in an Hsp70 dependent manner. However, we have not determined the mechanism by which KU596 improves mtBE. The long term goal of my project is to determine if KU-596 therapy improves DPN by affecting the rate of mitophagy. Toward this goal, we are validating the ability of MITO-QC mice to provide a direct in vivo assessment of the effect of diabetes and KU-596 on mitophagy.

Conclusion- MITO-QC mice show early signs of developing DPN allowing detection of mitophagy in tissues sensitive to diabetic stress, which preliminary examination indicated that diabetes may decrease the rate of mitophagy in kidney, but possibly increase the rate of mitophagy in peripheral nerve.

A12

MUTANT HUNTINGTIN-CALMODULIN INTERACTION: POTENTIAL THERAPEUTIC TARGET FOR HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative disorder caused by an autosomal dominant mutation in the huntingtin (htt) gene. Previous studies in our lab demonstrated that disrupting the binding of mutant huntingtin (mhtt) to calmodulin (CaM) had beneficial effects in cell culture and the R6/2 transgenic animal model. The goal of the current study is to identify and develop small chemical compounds that are non-toxic and can selectively disrupt the binding of mhtt to CaM. To this end, we screened ~ 199,270 compounds from various chemical libraries using a high throughput AlphaScreen assay. The primary AlphaScreen assay along with counter-screening assays have identified 481 hit compounds that disrupt the interaction between (His)htt-CaM(GST). The structures of the hits obtained were analyzed and 8 structurally diverse representative compounds were chosen. These compounds were re-screened in the primary AlphaScreen assay and also counter-screened in the alpha screen His-GST assay to eliminate compounds that disrupt the His-GST interaction without the presence of protein. Three out of the eight compounds have shown preferential activity in disrupting the (His)htt-CaM(GST) interaction. Currently, secondary assays are being employed to determine if the compounds identified in the primary screen can selectively disrupt the mhtt-CaM interaction without affecting other functions of CaM. The compound selectivity is being determined using two CaM dependent enzymes which are abundantly expressed in neuronal cells and play an important role in neuronal function; Ca^{+2}/CaM dependent protein kinase kinase alpha and Ca^{+2}/CaM dependent protein kinase 2 gamma (CAMK2Y). To date, kinase inhibition assay results indicate that one compound has ~ 75 fold selectivity and another compound has a 8 fold selectivity for inhibition of the CAMK2Y enzyme when compared to the IC50 value obtained in the primary screen.

Conclusion- Thus far, we have identified compounds that selectively inhibit the binding of mhtt to CaM and do not have off-target effects on other functions of CaM. The selective compounds will further be tested for cytotoxicity and neuroprotective effects in cells expressing mhtt. Overall, these studies will aid in identifying compounds that will serve as novel and promising biological probes for drug development in HD.

A13 Poster presentation winner

STRESS-INDUCED MECHANICAL ALLODYNIA, BLADDER HYPERSENSITIVITY, AND ANHEDONIA IN AN ANXIETY-PRONE MOUSE STRAIN

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80% of Americans suffer from at least one stress-related symptom in the past month according to American Psychology Society (2017). Co-morbidities linking pain and mood disorders are associated with altered limbic regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis. Stress activates the HPA axis and subsequently initiates and/or exacerbates symptoms related to both chronic pain and mood disorders. Previous work from our lab has revealed that early life stress induces visceral and perigenital hypersensitivity and behavioral evidence of mood disorders that occur later in life. Currently, our hypothesis is that chronic stress exposure in adulthood can increase somatic and visceral sensitivity and anhedonic behaviors in a mouse strain with a genetic predisposition to anxiety. Adult, A/J female mice were exposed to repeated foot shock stress for 10 continuous days and mechanical sensitivity, sucrose preference, visceromotor response (VMR) during urinary bladder distension, mast cell degranulation, and serum corticosterone levels was test at day 11. The shock group had a significantly decreased mechanical withdrawal threshold in the hind paw compared to their baseline and sham group measurements. Sucrose was measured prior to shock exposure and throughout the 10-day shock paradigm. In comparison to mice in the sham group and their baseline measurements, the shock group displayed a trend towards decreased sucrose preference, indicating anhedonia. Mice that underwent shock stress displayed significant increases in VMR during bladder distension compared to sham mice. Histological identification of mast cells in the bladder revealed that both sham and shock groups had very high rates of mast cell degranulation (84% and 89%, respectively). Finally, significantly higher serum corticosterone levels in the shock group indicated a stress-induced increase in HPA axis output. Together, these data suggest that chronic stress exposure can induce mechanical allodynia, visceral hypersensitivity, and depression-like behaviors in an anxiety-prone mouse strain. Future studies include examination of gene expression changes in the hypothalamus, amygdala, and hippocampus, as well as investigation of the chronic effects of foot-shock stress on the HPA axis and pain.

Conclusion- These results reveal that 10-day repeated foot shock stress will induce bladder hypersensitivity, allodynia as well as depression-like behaviors in anxiety-prone mouse and alternation of HPA axis might involve since the significant serum corticosterone increase in shock group was found.

A14 Poster presentation winner

DYSFUNCTION OF PRIMARY CILIA IN THE ARCUATE NUCLEUS ALTERS MOLECULAR RESPONSE TO FEEDING SIGNALS AND CAUSES OBESITY AND METABOLIC SYNDROME

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Primary cilia are antenna-like signaling organelles present on most vertebrate cells, including neurons of the central nervous system. Ciliary dysfunction underlies ciliopathies, which are genetic syndromes that manifest multiple clinical features. The ciliary gene, *THM1,* mediates retrograde transport in cilia and is mutated in 5% of individuals with ciliopathies, such as Bardet Biedl Syndrome, which manifests obesity as a cardinal clinical feature. Recently, we reported that global deletion of murine *Thm1* during adulthood causes hyperphagia (over-eating) and obesity, with gender differences in severity of obesity and susceptibility to diabetes. *Thm1* ablation causes shortened primary cilia with bulbous distal tips on neurons of the hypothalamic arcuate nucleus, an integrative center for signals that regulate feeding and activity. Prior to weight gain, expression of *pro-opiomelanocortin* (*POMC*), which encodes an anorexogenic neuropeptide, was decreased by 50% in the arcuate nucleus of *Thm1* conditional knock-out mice, which likely caused the hyperphagia. Fasting of *Thm1* conditional knock-out mice did not alter *POMC* nor orexogenic *agouti-related peptide* (*AgRP*) expression, suggesting impaired sensing of changes in peripheral signals. These data indicate that the *Thm1* ciliary defect perturbs neuronal signaling at the level of the arcuate nucleus. Moreover, *Thm1* conditional knock-out mice represent the first cilia mutant of obesity of the intraflagellar transport (IFT)-A class. This has led to several directions. To determine which signaling pathways are misregulated and lead to hyperphagia in *Thm1* cko mice, we have examined response to peripheral leptin. Here we report that pre-obese *Thm1* cko mice have an impaired molecular response to peripheral leptin, suggesting that ciliary dysfunction directly affects leptin signaling, which has been controversial. To identify neuronal populations responsible for *Thm1*-deficient obesity, we deleted *Thm1* in neurons expressing Cre-recombinase under the promoters of *POMC, AgRP,* or RIP (Rat insulin promotor). POMC- and RIP-Cre-specific *Thm1* conditional knock-out mice caused weight gain, indicating a role for THM1 in appetite regulation and thermogenesis, respectively. Interestingly, AgRP-Cre-specific *Thm1* conditional knock-out mice showed similar body weights to control littermates, yet adipose depots were 3x heavier. This suggests a role for THM1, and in turn, cilia, in AgRP control of nutrient partitioning, the coordinated regulation of carbohydrate versus lipid utilization or storage.

Conclusion: Our results show that deficiency of *Thm1* disrupts hypothalamic signaling pathways that regulates appetite, thermogenesis and nutrient partitioning, thus causing obesity and metabolic disease.

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A15
NEURAL PROGENITOR CELL SURVIVAL AND DEVELOPMENT AFTER TRANSPLANTATION INTO JAUNDICED AND NON-JAUNDICED RAT BRAIN

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Neurotoxicity caused by neonatal hyperbilirubinemia targets specific brain nuclei, including globus pallidus (GP), inferior colliculus, and cerebellum, and can lead to kernicterus. The most debilitating symptom of kernicterus is dystonia associated with damage to the GP. Stem cell transplantation has been shown to be an effective treatment for motor deficits in basal ganglia-related diseases such as Parkinson's disease and Huntington's disease. Targeting affected brain regions with neuronal stem cells is a promising therapeutic approach for treating dystonia in kernicterus. It is unknown, however, how elevated bilirubin levels in the brain will affect neural progenitor cell (NPC) survival and development. In this study, we compared the survival and functional development of different subtypes of NPCs resembling either excitatory spinal cord interneurons (SCI-like) or inhibitory basal ganglia neurons (BGN-like) transplanted into the basal ganglia of jaundiced (jj) Gunn rats and their non-jaundiced (Nj) littermates. We injected NPCs unilaterally into 21-day-old jj and Nj rats. In the SCI group, 10K cells were injected into the striatum; in the BGN group, 20K cells were injected into the GP. Three weeks later, injected rats were perfused with 4% paraformaldehyde. 30 µm brain sections encompassing the injection site were collected for immunohistochemical analyses (IHC). Stem121 (human cytoplasmic specific marker), Ku80 (human cell nucleus marker), GAD-6, ChAT, parvalbumin (PV), and proenkephalin (PENK) were used to identify cell survival and cell phenotypes. Our results showed that 1) both types of NPCs survived and formed abundant neurites in the basal ganglia of both jj and Nj rats. 2) SCI NPCs had higher survivability than BGN NPCs after transplantation in both jj and Nj groups. 3) Transplanted cell survival was greater in the jj brain compared to the Nj brain for both types of grafts.

Conclusions: These results suggest that elevated bilirubin levels enhance the survivability of the grafted cells. This may due to the antioxidant and immunosuppressant properties of bilirubin. These results support the feasibility of stem cell therapy in kernicterus.

A16
HUMAN APOE2/3/4-EXPRESSING STABLE CELL LINES AND APOE PROTEIN SIALYLATION

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Continued clinical failures in the search for a successful treatment of Alzheimer's disease (AD) raise questions about the validity of current therapeutic targets, underscoring the need of a novel approach that focuses less on the pathological mechanism of the disease but more on neuroprotective mechanism that would promote brain resilience against the onset of AD. We have previously demonstrated that human ApoE isoforms (ApoE2, ApoE3, ApoE4) differentially modulate brain energy metabolism with the ApoE2 brain being the most robust while ApoE4 brain displays the most deficient profile. This ApoE2-mediated bioenergetic robustness provides a mechanistic rationale for its protective role against AD. We are now conducting a proof-of-concept study to test the hypothesis that the introduction of ApoE2 gene/protein into ApoE4-expressing cells/brain would reduce ApoE4-induced negative effects thereby increasing brain resistance against the impact of AD risk. To test this hypothesis, we first established N2a cell lines that stably express human ApoE2, ApoE3, or ApoE4 gene, and characterization of these cell lines provides evidence that they could serve as a translational cell model for the in vitro studies. Moreover, we observed that, in addition to the native form of ApoE protein (34-kDa), a significant amount of the sialylated form of ApoE (37-kDa) was also expressed in all three cell lines. However, the relative ratio of the sialylated form versus the native form was markedly different, with the sialylated form as the predominant form in ApoE2-expressing cells, while the native form being the primary form in ApoE4-expressing cells, and ApoE3-expressing cells demonstrated a nearly equal amount of two forms. It has been previously reported that the sialylated form of ApoE protein is more preferentially associated with neurons and correlates with the time course of nerve development and regeneration. Taken together, these findings lead to the new research question, i.e., would increased ApoE sialylation contribute to the neuroprotective properties of ApoE2 in the brain? This novel line of research will be explored in future studies.

A17 Poster presentation winner

APPROACHES TO STUDYING THE EFFECTS OF *IN VIVO* BRAIN BIOENERGETIC MANIPULATIONS IN DISTINCT CELL POPULATIONS.

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The ketogenic diet largely emphasizes fats and minimizes carbohydrates as a calorie source and leads to the production of ketone bodies. This diet has been in use as a therapy for intractable epilepsy for nearly a century and has been increasingly investigated for its potential to treat neurodegeneration and neuropathic pain. While its benefits to the central nervous system are widely accepted, the precise molecular mechanism of benefit remains a mystery. Historically, attempts to study the influence of various compounds on specific cell types in the nervous system have relied either on immunohistochemistry or primary cell cultures. While useful, these techniques either lack high throughput for molecular studies or fail to accurately recreate *in vivo* conditions cells experience. Presently, we have adapted the use of fluorescence assisted cell sorting technology to perform single cell separation of distinct nervous system cell types from whole brain. In doing so, we have created a method to study the effects of extended dietary interventions in distinct CNS compartments. This will better allow us to understand the multi-faceted relationships between neurons and glia and will further not only our understanding of neurochemistry but also lead to the development of new bioenergetic therapies for the treatment of neurologic disease.

Conclusion- Fluorescence assisted cell sorting provides a useful technique to rapidly dissociate and segregate distinct nervous system cell types for the study of downstream molecular signaling events.

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A18 Junior Student Lecture Presentation 2

CHANGES IN MITOCHONDRIAL RESPIRATORY FLUX ALTER TAU SPLICING *IN VITRO*

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Microtubule associated protein tau aggregates into neurofibrillary tangles in numerous neurodegenerative diseases, collectively referred to as tauopathies. Mutations in MAPT, the gene encoding tau, cause certain familial tauopathies and alter tau splicing. Sporadic tauopathies, such as progressive supranuclear palsy and Pick's disease, also display altered tau splicing. Based on this knowledge, numerous groups hypothesize changes in tau splicing contribute to neurofibrillary tangle formation and pathogenesis of sporadic tauopathies. The mechanisms mediating altered tau splicing in sporadic tauopathies remain unclear. Mitochondrial dysfunction is common among sporadic tauopathies and represents one potential mediator of altered tau splicing. Inhibiting complex I of the mitochondrial respiratory chain triggers changes in tau splicing and expression of relevant splicing factors. The goal of these studies is to determine how different states of mitochondrial function affect both tau splicing and factors known to control tau splicing. Initial data suggests loss of mitochondrial respiratory function via mitochondrial DNA depletion leads to alterations in tau splicing. Future studies aim to learn whether mitochondrial DNA from patients with sporadic tauopathies is sufficient to alter tau splicing in vitro.

Conclusion- Decreased mitochondrial respiratory flux via mitochondrial DNA depletion alters tau splicing.

A19

CORRELATIVE FRET: A NEW METHOD IN DETERMINGING DISTANCES WITHIN THE NANOSCALE ARCHITECTURE OF A SYNAPSE

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Studies of epilepsy, one of the most prevalent neurological disorders worldwide, have suggested that gap junctions (GJ) play a critical role in mediation of epileptic activity. Human connexin 36 (Cx36) gene has been mapped to chromosome 15q14 that is linked to juvenile myoclonic epilepsy. Data on expression levels of Cx36 in epileptic models is conflicting, with some studies showing an increase, some a decrease, and others showing no change; Laura et al ([2015], ***J. Biomed. Sci.*** 22[69]:1-12) suggest, "that the function of GJs formed by Cx is altered even if the expression of the Cx does not change". Taken together, these data suggest that the role of Cx in epilepsy could be more related to structure and orientation than to expression levels. Determining structure and orientation of proteins within a synapse has always faced the challenge of overcoming resolution limitations. Förster Resonance Energy Transfer (FRET) by acceptor photobleaching has long been used as a method to increase fluorescence resolution, the transfer of energy from a donor to an acceptor that generally occurs between 10-100Å, the relative distance between the donor molecule and the acceptor molecule. To establish that FRET by acceptor photobleaching is possible with the addition of a Nanogold® particle for correlative microscopy work, we immuno-labeled GFP-tagged Cx35- (a homologue to the human Cx36) expressing cells with anti-GFP and with anti-Cx35 antibodies, and photobleached the Cx. Based on the results, we used FRET by acceptor photobleaching with FluoroNanogold™ conjugates and were able to determine the spatial distance of Cx-containing-GJ within a stimulated synapse. **Conclusion**-This strategy (a) allowed us to determine the spatial distance of Cx-containing-GJs within a stimulated synapse, (b) assisted in the development of a stimulated synapse model that allows precise testing of multiple parameters, and (c) increased throughput. Future work will include use of different sizes of FluoroNanogold™ conjugated antibodies for correlative microscopy to determine accurate distances between Cx. Funding provided by NIGM-115042; NIMH-106245; NSF-1002410.

A20

The Synapses between Renshaw Cells: Rethinking the Recurrent Inhibitory Circuit of the Spinal Cord

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Our laboratory has shown that motor neurons associated with a teleost fast spinal microcircuit, are dye-coupled by an abundance of gap junctions at glutamatergic mixed synapses (Serrano-Velez, et al., [2014]; ***Front. Neural Circuits*** 8[66]1-16). These gap junctions are "homotypic"; that is, their pre-and post-synaptic connexin (Cx) proteins, i.e., Cx35/36, are the same (Serrano-Velez, et al., [2014]***; op. cit***.). Recent work from our laboratory, as described in this poster, demonstrates that Renshaw cells, a, Ia inhibitory interneuron (IaIN), ventrally-derived from lamina 1 (V1) in the spinal cord, are coated with Cx-immunoreactive-gap junction-puncta. These gap junctions are "heterotypic"; that is, their pre- (*i.e.,* Cx35/36) and post- (*i.e.,* Cx34.7) synaptic Cx proteins differ. As in gap junctions at glutamatergic mixed synapses, homotypic Cx35/36 immunoreactive gap junction puncta were found on Renshaw cells to be spatially segregated on the base of the proximal dendrites and all along the full extent of their dendritic projects. In contrast, heterotypic Cx35/36-Cx34.7 immunoreactive gap junction puncta were found on Renshaw cells to be mainly localized on the soma where they were co-localized with glycine-like immunoreactivity.
Conclusion-The results lead us to speculate that the heterotypic Cx35/36-Cx34.7 glycinergic Renshaw cells are inhibitory. The connexins could contribute to this inhibition if current from the presynaptic neuron (i.e., spinal motor neuron) is able to flow out of the neuron but not enter the post-synaptic neuron (i.e., Renshaw cell). Dye-coupling experiments support this hypothesis, showing that spinal motor neurons and Renshaw cells do not dye-couple. Taken together these data begin to establish a picture of the functional circuitry of a recurrent inhibitory loop, a rare form of synaptic transmission (Bennett and Zukin [2004]; ***Neuron*** 41:495–511) that remains unresolved.
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