43rd MAINE BIOLOGICAL AND MEDICAL SCIENCES SYMPOSIUM
hosted by
MDI Biological Laboratory
with support from the
Maine IDeA Network of Biomedical Research Excellence (INBRE)

MDI Biological Laboratory Conference Center
Salisbury Cove, Maine
April 29-30, 2016

Poster session B

Eden Parish Hall: Neuroscience/Physiology

Listed alphabetically by presenting author

Cell-derived Exosome Targeted Delivery of Therapeutics in Autologous Cancer Cells
Yang T[1], LaForge B[1], Nandoe K[1], Cabili A[1], and Bai S[1]
[1] Department of Basic Pharmaceutical Sciences, School of Pharmacy, Husson University, Bangor, ME
bais@husson.edu

Small interfering RNA (SiRNA) inhibiting targeted tumor markers appears to improve effectiveness and safety in the cancer treatment. We investigated that cancer cell-derived exosomes as carriers of siRNA to knockdown a tumor marker, intercellular adhesion molecule 1 (ICAM-1), in autologous lung cancer cells. Exosomes were isolated from human adenocarcinomic A549 and brain bEND.3 cell cultures using centrifugation. Assessed by flow cytometry and fluorescence microscopy, fluorescence intensity in lung cancer cells treated by exosome delivered markers was significantly improved. From western blotting analysis of ICAM-1 levels, it was shown that both exosomes-delivered siRNAs significantly enhanced inhibition efficacy compared to siRNA alone. ICAM-1 siRNA formulated in autologous lung cell-derived exosomes with the assistance of transfection agent showed the best knockdown efficacy compared to other treatments. Cancer cell-derived exosomes can be used as effective carriers of siRNA, bringing the agent into the cells and increasing its therapeutic efficacy to their parental cells.

Measuring effects of electrostatic charge on bumble bee behavior
Belanger, K., Dobrin, S.E.
University of Maine at Presque Isle, Presque Isle, Maine
kaitlyn.belanger@maine.edu

Greggers, et. al. (2013) observed that honey bees respond to biologically relevant electrical stimuli, such as the electrical charge produced by another bee while dancing, differently than to random electrical fields. We hypothesize that bees rely on their ability to detect electric charge for several biological functions, and electrical stimulation of varying polarity and voltage may

differently affect behavior. The purpose of this experiment was to observe the effects of electric fields of varying polarity and voltage on bumble bee behavior in a laboratory setting. Individual bumble bees were randomly exposed to voltages ranging between positive and negative 400 - 1200 volts. The behavior of each bee was recorded before, during, and after exposure to electrical stimuli. Time spent walking, flying, upside-down, grooming, stopped, or fanning were recorded. We analyzed changes in behavior between baseline (before the bees were exposed to an electrical field) and during presentation of charge.

Do depressive symptoms predict performance on a behavioral measure of functional impairment: Findings from the Maine-Syracuse Longitudinal Study (MSLS)

Bogucki, O. E.[1], Dearborn, P. J. [1], Goetze, R. E. [1], Haigh, E. A. P. [1], & Robbins, M. A. [1]
[1]University of Maine, Department of Psychology, Orono, ME
peter.dearborn@umit.maine.edu

Depressive symptoms are associated with self-reported functional impairment. This study examined the impact of depressive symptoms on *actual* functional impairment on a behavioral task.

Data from the MSLS (N = 893) was used to assess whether depressive symptoms predicted functional impairment on timed performance tasks five years later.

Multivariate logistic regression was used. Model 1 adjusted for demographics, obesity, chronic illnesses, and cognitive impairment. Wave 6 depression status predicted impairment in one (OR = 2.56, CI: 1.74 – 3.77), two (OR = 3.02, CI: 2.03 – 4.49), and three tasks (OR = 3.09, CI 1.95 – 4.92) at wave 7. Model 2 also adjusted for functional impairment. Wave 6 depression status predicted impairment in one (OR = 1.76, CI: 1.09 – 2.84), two (OR = 1.87, CI: 1.17 – 2.99), or three tasks (OR = 1.93, CI: 1.21 – 3.08) at wave 7.

Results suggest that depressive symptoms increase *actual* functional impairment on a behavioral task.

Quantification of Multiple Drug Arrests Reported to the Maine Diversion Alert Program in 2015

Fisher H.[1], Abdirahman, Z.[1], Desrosiers, C.[2], Piper B.[1]
[1]Bowdoin College, Brunswick, ME, [2]Diversion Alert, Houlton, ME
hefisher@bowdoin.edu

Prescription drug use and abuse in Maine has reached an epidemic scale. The Trust for America's Health reported that between 1999 and 2010, Maine had a 96 percent increase in drug overdose deaths, mostly from prescription drugs. The objective of this study was to analyze drug arrests which are reported to the Diversion Alert Program by law enforcement. From 2015, 16.7% of drug arrests involved multiple drugs. Of this subset (n=317), 40.0% involved heroin, 39.8% cocaine (including crack cocaine and cocaine base), 20.8% buprenorphine, and 12.3%

gabapentin. Almost two-thirds (65.2%) of individuals involved in multiple-drug arrests were male. These data shed light on Maine's heroin epidemic and suggest that drug misuse should not be considered in isolation but rather understood in the context of other prescription and non-prescription drugs.

Long-term consequences of chronic-intermittent ethanol vapor exposure on affective behavior in selectively-bred Withdrawal Seizure Prone and Withdrawal Seizure Resistant mice

Hartmann, M.[1], McCulley III, W.[2], Holbrook, S.[3], Crabbe, J.[4], Rosenwasser, A.[1,2,3]
[1]Graduate School of Biomedical Science and Engineering, [2]Department of Psychology, [3]School of Biology and Ecology, University of Maine, Orono, ME, USA, [4]Portland Alcohol Research Center and Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR, USA
matthew.hartmann@maine.edu

Ethanol withdrawal is associated with both short- and long-term physiological and behavioral consequences. While much is known regarding the expression of acute withdrawal symptoms, less is known concerning long-term consequences in post-dependent individuals. Since relapse commonly occurs long after initial detoxification, it is critical to understand the affective disruptions that sometimes persist during protracted abstinence, and that may be linked to increased risk of relapse. In the present study, we examined the long-term consequences of chronic-intermittent exposure to ethanol vapor (CIE) in Withdrawal Seizure Prone (WSP) and Withdrawal Seizure Resistant (WSR) mice. Male and female WSP and WSR mice from the second selection replicate (WSP2, WSR2) were exposed to CIE and then subjected to behavioral assays for depressive- and anxiety-like behavior at weekly intervals for six weeks. Initial results indicate that CIE mice display increased sucrose preference and increased marble-burying for about 2 weeks post-CIE, relative to air-exposed controls.

Establishing a mouse model of ongoing and breakthrough cancer induced bone pain

Havelin, J.[1, 2], King, T.[1,2,3]
[1]Univeristy of Maine Orono, Graduate School of Biomedical Sciences and Engineering, Orono, ME, [2]Center for Excellence in the Neurosciences, University of New England, Biddeford, ME, [3]Department of Biological Sciences, College of Osteopathic Medicine, University of New England, Biddeford, ME
Joshua.havelin@maine.edu

Cancer pain is inadequately treated in many patients. Development of better therapies is dependent on gaining better understanding of the biological mechanisms underlying the multiple components of cancer bone pain, ongoing and breakthrough pain. We are adapting novel measures for cancer-induced ongoing and breakthrough pain that we developed in rats to mice.

Surgical implantation of LLC cells into the femur results in bone remodeling and tactile hypersensitivity in C57BL/6 mice. Breakthrough pain is measured by determining aversion to movement induced pain. Ongoing pain is measured as conditioned place preference to pain relief. Developing these measures are a critical step for our future work that will use transgenic and optogenetic techniques to examine the roles of specific subpopulations of sensory neurons in ongoing and breakthrough cancer bone pain.

Utilizing Chromatin Immunoprecipitation (ChIP) to Investigate Transcriptional Regulation in Zebrafish (*Danio rerio*) during Early Development

Henderson, J.[1], Williams, L.[1]
[1] Department of Biology, Bates College, Lewiston, Maine
jhenders@bates.edu

Chromatin Immunoprecipitation (ChIP) is a molecular biological technique that allows for the detection and analysis of protein-DNA interactions. Previous research has identified that the aryl hydrocarbon receptor (Ahr) is a transcriptional regulator of the nuclear factor erythroid 2-related factor (nrf) gene family. Ahr is hypothesized to bind to xenobiotic response elements (XREs) within the cis-promoters of *nrf* genes in order to regulate their transcription. This study utilized ChIP on 24 hours post fertilization zebrafish (Danio rerio) embryos in order to explore the protein-DNA interactions occurring between Ahr1b and *nrf*-XREs. This study also aimed to further develop the ChIP protocol in order to utilize quantitative polymerase chain reaction for DNA analysis following ChIP. Initial PCR results indicate preferential binding of Ahr1b to *nrf1a* XRE 3, *nrf2a* XRE 3&4, and *nrf2b* XRE 2, though nonspecific antibody binding that occurs during the immunoprecipitation step needs to be reduced to generate accurate results.

A protein kinase C theta mutation causes early-onset exudative retinal detachment

Ji, X[1, 2], Liu, Y[1], Hurd, R[1], Wang, J[1], FitzMaurice, B[1], Naggert, JK[1], Nishina, PM[1,] and Chang, B[1]
[1]The Jackson Laboratory, Bar Harbor, ME 04609, [2]Graduate School of Biomedical Science and Engineering, University of Maine, Orono, ME 04469
xiaojie.ji@jax.org

The mechanisms underlying retinal detachment (RD) remain largely unknown. To study RD, we screened for novel murine models of early-onset exudative RD. Here we identified a model, *rpea1*, with a mutation in the *Prkcq* gene, which encodes protein kinase C, theta (PKCθ). Previous studies have shown that PKCθ and the actin cytoskeleton, especially the perijunctional actin ring, may play an important role in maintaining barrier function. Our results demonstrate that PKCθ deficiency-induced aberrant F-actin perijunctional rings in RPE cells may be in part due to the change of ezrin/radixin/moesin (ERM) protein phosphorylation, and the consequent weakening of the barrier function and defective fluid homeostasis in the posterior retina.

Aberrant RPE barrier function that leads to suboptimal regulation of subretinal fluid homeostasis may be a common theme for RD.

Quantification of Undisclosed Conflicts of Interest in Biomedical Education

Keefe, R.[1A], Eckstein, L.[1A], Selemon, N.[1A], Lappas, C. [1A], Duperry, Z. [1], Bryant, M. [1], Smuckler, P. [1], Lambert, D.[2], Piper, B.[1]
[1]Bowdoin College, Brunswick, ME, [2]Husson University School of Pharmacy, Bangor, ME
[A]designated authors contributed equally
rkeefe@bowdoin.edu

Conflicts of interest (CoI) occur when an individual's personal interests, often economic, compete with their professional responsibilities. Biomedical textbooks currently do not disclose their authors' CoI. The objective of this study was to analyze CoI among authors of four textbooks commonly used in the teaching of medicine, pharmacy, and pharmacology. Potential CoI were determined by the number of patents listing an author as an inventor as listed by Google Scholar and the compensation ($) received from pharmaceutical companies as reported by ProPublica's Dollars for Docs (https://projects.propublica.org/docdollars/). The books evaluated were Harrison's *Principles of Internal Medicine*, (N=475 authors, 79.8% MDs) and Koda-Kimble and Young's *Applied Therapeutics* 10th Ed. (N=193 authors, 92.2% PharmDs) and others. The top ten highest compensated Harrison's authors received 3.1 million (Max=$560,021/author). The most undisclosed patents held by a single author was 27. These findings indicate a need for greater transparency in biomedical educational materials.

Disruption of circadian rhythm through misexpression of a frontotemporal dementia gene in *Drosophila* circadian pacemaker neurons

Krasniak, C. S., Kavaler, P. J., and Ahmad, S. T.
Colby College Department of Biology, Waterville ME
cskrasni@colby.edu

Frontotemporal dementia (FTD) is the second most common early-onset neurodegenerative disease. One subtype of FTD causes the production of CHMP2B^{Intorn5}, a mutant isoform of an ESCRT-III subunit. This causes disruptions in the autosomal-lysosomal and autophagy pathways. FTD patients show a variety of neurological symptoms, including disinhibition, apathy, aggressive behavior, and circadian rhythm deficits. To investigate these circadian rhythm deficits we ectopically expressed human CHMP2B^{Intron5} using the GAL4-UAS system with the driver lines Cry-GAL4 and Pdf-GAL4 in *Drosophila melanogaster*. These drivers are specific to an important subset of circadian pacemaker neurons in the brain. Using activity monitoring, we observed moderately disrupted circadian behavior. We did not observe any cellular death phenotype through whole brain imaging. To investigate the circadian deficits we are currently examining *timeless* and *period* transcript levels to investigate possible disruption of the molecular clock. This will allow us to further describe the circadian deficits caused by CHMP2B^{Intorn5} misexpression.

Induction of gene expression in the CA1 cell fields of the hippocampus by behavioral exploration in DISC1-knockout rats

Larrabee, M., & Glenn, M.
Department of Psychology, Colby College, Waterville, ME
mjlarrab@colby.edu

Schizophrenia is a debilitating disorder characterized by a wide range of symptoms, including major deficits in cognition. We sought to study these cognitive symptoms in the DISC1-knockout rat model of schizophrenia and focused on memory and hippocampal function. We hypothesized that the DISC1-knockout rats would have impaired spatial memory abilities on a water maze task and our results confirmed this. In addition, we sought to compare DISC1-knockout and wildtype rats' hippocampal engagement when they explored novel and familiar environments. To do this, rats explored different environments and were sacrificed 90 min later; neuronal activation to the experience was gauged using immunohistochemistry for the immediate early gene, c-fos. This analysis is ongoing but our hypothesis is that wildtype rats will differentially respond to the novel and familiar conditions, whereas DISC1-knockout rats will react to both as if they were novel. These results will span our understanding of hippocampal functioning in schizophrenia.

Altered Addiction Potential? Behavioral Sensitization to Amphetamine and Cross-Sensitization to Cocaine in Prenatal Choline Supplemented Rats

Mathieu, L. P.[1], Glenn, M. J.[2]
[1]Department of Biology, Colby College, Waterville, ME, [2]Department of Psychology. Colby College, Waterville ME
lmathieu@colby.edu

The essential dietary nutrient choline produces long-term enrichment in neural function. In our lab, we have found that prenatal choline supplementation increases neuroplasticity and is protective against neuropsychiatric disorders, like schizophrenia and depression. One way it may exert these effects is through changes to dopaminergic circuits. This hypothesis was tested by comparing prenatal standard-fed and choline-supplemented adult male rats in the extent of their behavioral sensitization to amphetamine and cross-sensitization to cocaine. Behavioral sensitization is a phenomenon whereby repeated intermittent drug administration increases responses to it; cross-sensitization reflects an increased sensitivity to a drug of a similar class following sensitization. Rats received an injection of amphetamine once per week for 3 weeks, followed by a cocaine injection in the 4th week. The main dependent measure was their locomotor response to the drugs. We are finding that choline-supplemented rats exhibit significantly less sensitization. Thus, choline may modify risk for drug addiction.

The α-1 adrenergic receptor antagonist prazosin and α-2 adrenergic receptor antagonist yohimbine modulate the alcohol deprivation effect in alcohol withdrawal sensitive *C3H/HeJ* mice.

McCulley III, W.[1], Fixaris, M.[1], Rosenwasser, A.[1,2,3]
[1]Department of Psychology, [2]School of Biology and Ecology, [3]Graduate School of Biomedical Science and Engineering, University of Maine, Orono, ME, USA
walter.mcculley@maine.edu

Relapse to high levels of alcohol consumption is a common feature of alcoholism after brief and long periods of abstinence (DSM-5). During alcohol withdrawal and moments of stress, the level of norepinephrine is increased in the periphery and central nervous system (CNS) contributing to the voluntary consumption of alcohol to alleviate a hyper-aroused state. Drugs targeting adrenergic receptors have produced promising results in reducing alcohol consumption and hyper-aroused states in both humans and rodents. The current study used the alcohol deprivation effect to mimic moments of relapse and increases alcohol intake in C3H/HeJ mice. Mice were treated with the α-1 adrenergic receptor antagonist prazosin, the α-2 adrenergic receptor antagonist yohimbine or saline injections. Prazosin reduced alcohol consumption after but not before the ADE, while alcohol intake persisted in yohimbine treated mice. This suggests that pharmacological treatments targeting the pre-synaptic actions of adrenergic receptors may be more beneficial in reducing relapse.

Supplementing the type 1 interferon response in morphine-potentiated LP-BM5 murine AIDS

Virginia D. McLane[1,2], Colin L. Willis[2], and Ling Cao[2]
[1]Graduate School of Biomedical Science and Engineering, University of Maine, Orono, ME 04473, [2]College of Osteopathic Medicine, University of New England, Biddeford, ME 04005
virginia.mclane@maine.edu

Opioid abuse increases the severity of HIV-associated neurocognitive deficits (HAND). Morphine attenuates the inflammatory response of glia, which are critical in CNS immune defense. Using the LP-BM5 murine AIDS model, we investigated the effect of morphine on the glial immune response. In our model, morphine treatment increased viral RNA expression ('viral load') in hippocampus while decreasing viral load in striatum. These changes correlated to regional differences in expression of type 1 interferons (IFNs). Using quantitative RT-PCR, we found that morphine increased striatal expression of IFN-α and IFN-β. Multiple genes in the IFN signaling pathway were downregulated in hippocampus. We hypothesize that an insufficient type 1 IFN response in combination with morphine's immunosuppressive effects leaves the hippocampus vulnerable to LP-BM5 infection. To test this, we conducted preliminary studies administering intranasal interferon-beta to boost the type 1 IFN response. With this work, we aim to provide fresh insight into morphine-potentiated HAND.

Can dietary choline supplementation prevent postpartum depression in a rat model?

Mitchell, V. R.[1], Glenn, M. J.[2]
[1]Department of Biology, Colby College, Waterville, ME, [2]Department of Psychology. Colby College, Waterville ME
vrmitche@colby.edu

Choline is an essential dietary nutrient that induces neuroprotective effects. We have found that dietary choline supplementation has antidepressant effects in rats, suggesting it may also protect against the behavioral and neurological symptoms of postpartum depression. This hypothesis was tested using a hormone withdrawal model of postpartum depression in which rats received either choline-supplemented or standard diets during a 23-day simulated pregnancy. The postpartum symptoms were induced by withdrawing rats from ovarian hormones over a 5-day period. During this period, sucrose preference, open field, elevated plus maze, and forced swim tests were conducted. We expect that animals experiencing hormone withdrawal will exhibit depressive symptoms that are rescued by choline supplementation. Such findings influence our understanding and treatment of postpartum depression.

Severity of demyelinating and axonal neuropathies are modified by genetic mutations affecting sodium channels at nodes of Ranvier

Morelli, KH.[1,2], Seburn, KS.[2], Schroeder, DG.[2], Cox, GA.[1,2], Burgess, RW.[1,2]
[1]Graduate School of Biomedical Sciences & Engineering, The University of Maine, Orono, ME, 04469, USA, [2] The Jackson Laboratory, Bar Harbor, ME, 04609, USA
kathryn.morelli@maine.edu

Charcot-Marie-Tooth disease (CMT) is a collection of inherited neuropathies caused by either nerve demyelination or axonal dysfunction. CMT patients experience progressive muscle weakness, although the severity of each case varies. Recently, we identified a mouse strain with a double null mutation in *Sh3tc2*, a gene linked to a demyelinating CMT (CMT4C) and *Nrcam* which encodes a protein involved in sodium channel localization at nodes. The *Sh3tc2* mutant mice resemble reported mouse models of CMT4C, whereas mice lacking NRCAM do not have an overt phenotype but, combined, these mutations cause paralysis. Considering the loss of NRCAM also exacerbates mouse models of axonal CMT, we hypothesized that subclinical deficits in sodium channel function at nodes synergize with mutations affecting the propagation of depolarization to cause a severe phenotype. We validated this hypothesis by showing *Scn8a* heterozygotes phenocopy *Nrcam* null mice. These data demonstrate that genes encoding node proteins are potential modifier loci for CMT.

Lower visual acuity is associated with lower cognitive performance across multiple cognitive domains: The Maine Syracuse Longitudinal Study

Sullivan, C.[1], Robbins, M.[1,2], Dearborn, P.[2], Sullivan, K.[1,2], & Elias, M.[1,2]
[1] Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME,
[2]Department of Psychology, University of Maine, Orono, ME

cara.sullivan@maine.edu

There is evidence for associations between lowered visual acuity (VA) and lowered cognitive functioning. Most studies have used only a few tests of cognitive ability and have not controlled for cardiovascular risk factors which confound relations between VA and cognitive functioning. This study used data from the Maine-Syracuse Study (N=852; mean=62, range=23-98 years). VA was measured by the Snellen Eye Chart with participants best corrected vision prior to cognitive testing. Lower VA was significantly associated with lower cognitive performance in visual/spatial organization ($p<.02$), scanning/tracking ($p<.0001$), working memory ($p<.005$), similarities ($p<.03$) and global composite ($p<.0001$) domains. This was true with statistical adjustment for demographics, cardiovascular disease, BMI, chronic kidney disease, homocysteine levels, hypertension, diabetes and diabetes treatment. However, examination of the raw beta weights indicated that the magnitude of the associations between VA and cognitive functioning measures were modestly attenuated with adjustment for demographic and cardiovascular covariates.

Integrating single-cell and whole-brain transcriptomes to study the progression of Alzheimer's disease

Xulong Wang[1], Robyn L. Ball[1,2], Harriet Jackson[1], Gareth Howell[1], and Gregory W. Carter[1]
[1]The Jackson Laboratory, 600 Main St., Bar Harbor, ME, 04609, USA, [2]Quantitative Sciences Unit, Stanford University, 1070 Arastradero Rd., Palo Alto, CA, 94304, USA
xulong.wang@jax.org

Alzheimer's disease (AD) affects one in nine people of age 65 or older, and one in three above the age of 85. However, therapeutic development has been limited by an incomplete understanding of AD progression. To detect early markers of AD development, we measured gene expression profiles of whole brains from wild type (C57BL/6J) or AD-prone mouse models at multiple ages between two and six months. The AD-prone transgenic mouse model carries the human mutant APP and PSEN1 genes, and show evidence of Aβ deposits at four months of age. A total of 108 age-specific, transgene-specific, or age-transgene-interacting genes were identified, which were potential gene markers for the relevant sample groups. While meaningful in inferring biological dysfunction related to AD development, marker genes provide little information of their cell type origins. Meanwhile, single cell transcriptome measurements have advanced rapidly, which can facilitate identifying cell types that characterize AD development. To infer these cell-specific signals in our bulk RNA-seq samples, we developed a novel method, permutation-based maximum covariance analysis (PMCA). PMCA uses the covariance of gene expression profiles from bulk and single-cell samples to detect the bulk-cell pairs that significantly covary. By integrating gene expression profiles of 48 major cell types from mouse brain data, we found that oligodendrocyte and microglia transcript signatures are absent in the AD mice relative to the wild type. This suggests a loss-of-function of oligodendrocytes and microglia during AD progression.

Ketamine as a neuroprotective agent against a novel model of mild traumatic brain injury in male and female Sprague Dawley rats

Wirth, P.[1] and Glenn, M.J.[2]
1. Department of Biology, Colby College, Waterville, ME 2. Department of Psychology, Colby College, Waterville, ME
pjwirth@colby.edu

Mild traumatic brain injuries (mTBI) have significantly increased in the last decade and there is mounting evidence of their adverse cognitive and emotional effects. Many animal models apply force through projectiles or blasts to a stationary animal. These mechanical forces do not adequately induce rotational acceleration in the animal's head, which is thought to be a key component of human sports-related injuries. Thus, we designed a device in which the animal is accelerated toward a stationary impact zone to produce rapid rotational movement of the head. The present study aimed to characterize the neuroprotective effects of ketamine, an NMDA antagonist, on post-injury behavioral outcomes. Following the mTBI, male and female rats were given three subanesthetic doses of ketamine when glutamate levels are expected to be highest. Preliminary analysis of behavioral data is underway, and we hypothesize that ketamine will offer neuroprotection in tests of memory and locomotion.