First Annual Maine Research Symposium on Biomedical Science and Engineering

Faculty Poster Abstracts

October 13-15, 2022

Friday October 14; 11:30 am – 1:30 pm Session
MDIBL and The Jackson Laboratory

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Biology
Frederic Bonnet, Ph.D.
MDI Biological Laboratory
Microscopist

The MDIBL Light Microscopy Facility: an open-science research resource dedicated to increasing biomedical research excellence in Maine

Biomedical scientists benefit from access to complex microscopes and sophisticated imaging technologies. Due to the accelerating rate of imaging technology development, turnover and high costs, it is difficult for individual research labs to afford, master and maintain them. Another challenge to researchers in Maine is the geographic distance between institutions. For an external user who would like to use microscopes at a core facility, getting access can be difficult due to the several hours of driving. The Maine INBRE and MDI Biological Laboratory (MDIBL) have invested in developing a state-of-the-art Light Microscopy Facility (LMF) to provide access to cutting-edge microscopes, professional scientific expertise, and up to date training and education in quantitative light microscopy. Our facility is available 24/7 to researchers throughout Maine. The LMF can be accessed remotely through the "remote microscopy imaging services" program, allowing distant users to obtain data while staying at their home institution. The MDIBL LMF is a unique open-science microscopy facility dedicated to increasing biomedical research excellence in Maine.

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Biology
Physiology/pathophysiology
Molecular biology
James Coffman, Ph.D.

MDI Biological Laboratory
Associate Professor

Gene regulatory circuitry controlling developmental programming of stress responsivity

Early life stress, or maternal stress during pregnancy, perturbs development of the immune and neuroendocrine stress systems, with persistent effects on health and susceptibility to inflammatory and metabolic disease. We have used zebrafish as a model system to examine the hypothesis that these effects stem in part from chronic exposure to the stress hormone cortisol, which perturbs glucocorticoid receptor (GR)-dependent gene expression. We discovered that the GR-responsive regulatory gene that is most consistently upregulated by cortisol treatment is klf9, which encodes a ubiquitously expressed Krüppel-like transcription factor important for macrophage regulation, neurogenesis, and metabolic regulation in the liver. We have carried out multiple bulk RNA-seq experiments that have established that Klf9 mediates much of the transcriptomic response to chronic cortisol treatment downstream of the GR and plays a key role in regulating metabolic genes. Furthermore, we have shown that Klf9 regulates glucocorticoid responsivity as a GR-activated feedforward repressor of the biomedically important GR antagonist fkbp5, a proinflammatory gene that contributes to unhealthy aging and mental health problems. Although klf9 is ubiquitously expressed, it likely has context-specific functions that cannot be studied at the whole organism level. Macrophages are a cell type of particular interest, given their central roles in the regulation of inflammation, metabolism, tissue regeneration, and responsivity to stress. We are therefore using single cell approaches to delineate the functions of klf9 and its downstream targets in macrophages, focusing on the regulation of macrophage plasticity. We are also addressing the question of how environmental arsenic exposure dysregulates the inflammatory response to viral infection, testing the hypothesis that it does so by impeding klf9 activation. The latest results from these projects will be presented.

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Friday October 14; 11:30 am – 1:30 pm
Health and Social Sciences
Rural Health
Jane Disney, Ph.D.

MDI Biological Laboratory
Associate Professor of Environmental Health

The public health impact of a school-based citizen science effort to assess well water for arsenic in Maine and New Hampshire

"Ashley Taylor1, Karen Bieluch2, Bill Zoellick3, Kate Buckman2, Hannah Lust1, Alexis Garretson1, Cait Bailey1, Anna Farrell1, Brian Jackson2, Rebecca Lincoln4, Erin Arneson4, Bruce Stanton2, Jane Disney1

1 MDI Biological Laboratory
2 Dartmouth College
3 Schoodic Institute

4 Maine Center for Disease Control

Abstract

Exposure to arsenic in well water is a well-documented public health issue for Maine and New Hampshire as well as other New England states. Arsenic contamination of well water in these locations may be attributed to historic use of arsenical pesticides in agricultural areas or to metasedimentary bedrock that leaches arsenic into groundwater. These groundwater reserves often exceed the EPA limit of 10 ppb. Arsenic exposure is known to cause cardiovascular disease, reduced resistance to infections, bladder cancer, and reduced IQ in children. Despite these known health impacts, many people still do not test and treat their wells. We approached this problem by developing the All About Arsenic project, that involves engaging secondary-school teachers and students in collecting well water samples for analysis and providing support for outreach to their communities about their findings. We have assessed the public health impact of this project by analyzing the contribution of the student data relative to the existing well water quality data in both states. Students have collected nearly 3,000 water samples; the additional data more than doubles the amount of information available to the public about well water quality in multiple municipalities across both states. In addition, we have surveyed private well owners who contributed well water samples to the project to determine the actions taken to mitigate arsenic in well water. Preliminary results indicate that participation in the project is a significant factor in well owner decisions to mitigate arsenic in their drinking water."

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Friday October 14; 11:30 am – 1:30 pm
Computational Biology & Medicine
Other
Heath Fuqua

MDI Biological Laboratory
Bioinformatician

Empowerment, Support, Efficiency: Structure and Function of the MDIBL Computational Biology Core

J. Heath Fuqua1, Joel H. Graber1
1MDI Biological Laboratory, 159 Old Bar Harbor Road, Bar Harbor, Maine 04609, USA

The MDI Biological Laboratory (MDIBL) Computational Biology/Bioinformatics Core provides research and educational support to a broad community, which includes MDIBL faculty and staff, visiting scientists, and faculty/students at our INBRE partner institutions. Biological and biomedical research is increasingly data-intensive and accordingly requires skills and resources that facilitate rigorous, reproducible, and efficient analysis of genome-scale data sets. As a small core, our goal is to educate and empower all levels of the research hierarchy, supporting MDIBL and partner institutions' broader efforts in pioneering new approaches in biomedical science. Our work is organized in two distinct, but mutually reinforcing, lines of effort: Research/Analysis and Education/Training. Here, we present an overview of these efforts, showing examples of each. Our research support efforts include the use of cloud-computing infrastructure, with a focus on running standardized workflow pipelines on Amazon Web Services and Google Cloud. Our

educational efforts include formal courses, such as those sponsored through Maine INBRE, as well as in-house training activities for research assistants and students. These opportunities are of value to researchers at all levels, as they provide the most up-to-date tools and knowledge necessary to explore and analyze data, leading to the generation of novel hypotheses, and ultimately discoveries. MDIBL Computational Biology Core efforts are supported by two Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P20GM103423 and P20GM104318, with additional funds from the National Institute of Allergy and Infectious Diseases under grant number P01AI152337."

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Molecular biology

Zhengxin Ma, Ph.D.
MDI Biological Laboratory
Postdoctoral Associate

Canonical ISR is not required for lowering and redirecting translation associated with lifespan extension under DR in C. elegans

Dietary restriction (DR) extends lifespan, in part, by lowering and redirecting mRNA translation. Initiation is the rate-limiting step of protein synthesis and is controlled by two translation complexes. One comprises the cap-binding complex downstream of TOR, which circularizes mRNA and helps recruit additional translation factors. The other is the ternary complex, which supplies methionyl-tRNA to initiate translation. Increased concentration of uncharged tRNAs associated with nutrient scarcity activates the kinase GCN-2, which phosphorylates the EIF-2ALPHA subunit of the ternary complex. This activates the integrated stress response (ISR), which lowers and redirects translation. In this study, we investigated the role of the ISR in differential translation and lifespan extension associated with DR in C. elegans. Changes in translation under DR were measured by polysome profiling and surface sensing of translation (SUnSET). We confirmed that GCN-2, but not the other EIF-2ALPHA kinase PEK-1, is required to phosphorylate EIF-2ALPHA (S49) in the ternary complex and activate the ISR under DR conditions. However, an eif-2alpha S49A phosphorylation mutant was still responsive to DR, showing rapid downregulation of translation and lifespan extension. Similar results were obtained using gcn-2 or pek-1 mutants, suggesting that the ISR is dispensable for lifespan extension under DR. Work in mammalian tissue culture has shown that certain forms of cellular stress downregulate the nonsense-mediated decay (NMD) pathway via the ISR so that normally unstable stress-responsive mRNA can be translated. We find that, while NMD is still downregulated under DR conditions, the ISR is not required for this response. Together, results suggest that the ISR is not required to drive differential translation associated with longevity benefits of DR in C. elegans.

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Friday October 14; 11:30 am – 1:30 pm

Basic and Applied Research in Biological Sciences
Biology
Romain Madelaine, Ph.D. and Romain Menard
MDI Biological Laboratory
Assistant Professor

Sarcofish: A new genetic model of muscle aging

Sarcopenia, the degenerative loss of muscle associated with aging, has been recently recognized as a disease by the World Health Organization1. Over the last century, progress in medicine and biomedical research has significantly increased the human lifespan. Unfortunately, with increasing age, the number of people suffering from age-related diseases has also increased drastically. Age associated degenerative loss of muscle leading to muscle atrophy (or sarcopenia) is characterized by a reduction of muscle mass, strength, and function. This age-associated muscle disease affects more than 60% of people over 80 years of age and results in mobility disorders and significantly increased risk of mortality. Currently, the only treatment to delay the onset of sarcopenia is physical exercise2. Despite this recommendation, with increasing age, elderly people are often not capable of performing regular exercise, highlighting the need for the development of therapeutic strategies. The overall goal of this research is to establish an innovative genetic tool in zebrafish to model the sarcopenia disease. This animal model of muscle aging will accelerate the identification of dysregulated biological mechanisms that lead/cause sarcopenia and will allow to test molecules with a therapeutic potential to treat or limit the symptoms of sarcopenia.

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Biology
Molecular biology
Dilawar Ahmad Mir, Ph.D.
MDI Biological Laboratory
Post Doc-researcher

Development of an analysis platform to identify chromatin dynamics in C. elegans body muscle cells

Under low nutrient signaling/translation conditions. Dilawar Ah Mir 1, Jordan Horrocks1, Matthew Cox1, Zhengxin Ma1 and Aric N. Rogers1* 1.
Mount Desert Island Biological Laboratory, Davis Center for Regenerative Biology and Medicine, Bar Harbor, ME, United States"
Aging results in functional decline and increases susceptibility to chronic diseases. Dietary restriction (DR) improves age related health and protects against age-related diseases across species. DR lowers mTOR signaling and translation, with nutrient limitation or pathway suppression improving age-related resilience by redirecting resources to preserve the soma. Caenorhabditis elegans helped spearhead ageing research with the discovery of numerous genetic pathways controlling its lifespan. Here, we restricted

translation downstream of mTOR separately in major tissue-types in C. elegans to better understand their roles in systemic adaptation. Only attenuating translation (via translation initiation factor IFG-1) in germline or neurons increases survival, heat shock response, muscle maintenance gene expression and stress resistance in adult animals. To further investigate how these tissues regulate somatic resilience, we adopted an approach aimed at understanding the role of active regulatory elements and chromatin modifications in C. elegans muscle cells. Here, we describe an adapted protocol for dissociation and preparation of single cell suspensions from developmentally synchronized aged populations of C. elegans. The protocol is optimized for efficient FACS-based purification of single cells from body muscle cells. This approach will be used to identify cell-specific chromatin accessibility changes (ATAC-seq) and quantification of differential gene expression between age-synchronized C. elegans with or without attenuated translation. This approach will help address questions regarding chromatin remodeling and increased muscle maintenance-related gene expression under these conditions and helps to establish a foundation for a comprehensive C. elegans single-cell epigenetic remodeling and gene expression atlas.

Key words Caenorhabditis elegans, Translational reduction, Cell dissociation, Fluorescence-activated cell sorting, ATAC-seq, epigenetic modifications"

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Biology
Prayag Murawala, Ph.D.
MDI Biological Laboratory
Assistant Professor

Two tissue – Two modes of regeneration in axolotl

"The major difference between embryonic development and tissue regeneration is the scale and starting material. While embryonic progenitors drive the development of an organism, tissue regeneration begins with a stump of adult tissue. How does adult tissue transform itself into the progenitor pool of blastema cells (an equivalent of embryonic progenitors), remains a central question in the tissue regeneration field? We have previously shown that axolotl limb regeneration proceeds via dedifferentiation of fibroblasts (Gerber et al, Science, 2018). We have shown that dedifferentiated fibroblasts of the axolotl limb acquire blastema status which is very similar to the embryonic limb bud progenitors. Furthermore, we have shown that during the redifferentiation phase, blastema cells recapitulate limb developmental program and reconstruct the entire connective tissue lineage (fibroblasts, tendon, periskeletal, and skeletal cells) of the limb. While this is one mechanism of tissue regeneration, a broader question in the field is, do all body parts of axolotl regenerate via dedifferentiation?
To test this, we combined tissue-specific inducible Cre-loxP mediated lineage tracing and single-cell transcriptomics experiments to study axolotl tail regeneration with an aim to identify the cellular source of the tail blastema. Our results suggest that contrary to the limb, the axolotl tail harbors embryonic progenitors in the intermyotomal space. These progenitors carry dual signatures of 1) postsomitic embryonic tail progenitors and 2) differentiated tendon cells and persist throughout axolotl life. Further, we show that, upon tail amputation, these progenitors populate tail blastema and can form three distinct cellular lineages (dermatome, myotome, and sclerotome) of the primary body axis. The differences

between limb and tail regeneration within the same species emphasize that there is more than one way to regenerate tissue and each case should be studied on its own."

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Biochemistry
Jarod Rollins, Ph.D.
MDI Biological Laboratory
Assistant Professor

Role of ribosomal protein 6 (RPS-6) in regulating responses to dietary restriction..

Forms of dietary restriction like intermittent fasting (IF) and caloric restriction (CR) promote health and longevity through changes in gene expression. While the transcriptional changes that occur in response to DR have been well described across several species, the role of translational regulation has lagged. Using polysome profiling and mRNA-seq, we quantified changes in actively translated mRNAs that occur in C. elegans under CR compared to well-fed conditions. The analysis revealed hundreds of transcripts regulated on the translational level that would have been missed using conventual transcriptomics. Among the translationally down-regulated genes that where pro-longevity when knocked down were regulators of the cell-cycle. In search of the mechanisms regulating selective translation under CR we investigated a role for ribosomal protein 6 (RPS-6) as its phosphorylation status is thought to regulate cell cycle and selective translation of mRNA transcripts. Using RPS-6 phospho-null and phospho-mimetic mutants, we show that phosphorylation and de-phosphorylation of RPS-6 is necessary for the pro-longevity effects of CR and IF. Translatome analysis of the phospho-mutants suggested a role for RPS-6 in regulation of p38 mitogen-activated protein kinases and autophagy. Accordingly, autophagy and mobilization of lipid stores fail to be activated in the RPS-6 phospho-mimetic mutant in response to fasting. Furthermore, the deactivation of p38 MAPK in response to fasting also failed to occur in the RPS-6 phospho-mutant. Therefore, the phosphorylation status of RPS-6 is important for regulating innate immunity and autophagy in response to nutrition stress. Future studies will determine if this regulation is due to RPS-6 mediated selective translation of mRNA or by direct interactions between RPS-6 and p38.

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Biology
Heiko Schenk, MD
MDI Biological Laboratory
Research Fellow

Evidence for NF-kB / inflammatory cytokine signaling in new nephron formation after AKI in adult zebrafish

Introduction: Adult progenitor cells in the mesonephric kidneys are required during neo-nephrogenesis replacing injured tubules by forming new nephrons. Single-cell RNA transcriptomes of adult kidney progenitor cells point to components of NF-kB and inflammatory cytokine receptors that may initiate stem cell-based nephrogenesis. Here, we present evidence that gentamicin induces inflammation-associated injury which potentially stimulates stem cell-based nephrogenesis, while the stimulatory effect on the progenitor cells to form new nephrons can be recapitulated by LPS injection.
Methods: Adult zebrafish were injected i.p. with gentamicin or LPS at day 0. NF-kB signaling was determined 4 days post-injection (dpi) by NF-kB:GFP detection of the NF-kB reporter line Tg(NF-kB:EGFP) and NF-kB-associated gene expression using qRTPCR. Requirement of NF-kB signaling during regeneration was evaluated by pharmacological NF-kB inhibition. Bulk RNAseq from positive selected GFP+ and mcherry+ single cells by FACS was performed from kidneys 7 dpi by gentamicin injection using Tg(lhx1a:EGFP;cdh17:mCherry) fish.
Results: Gentamicin-induced kidney injury leads to increased tubular NF-kB nuclear translocation at 4 dpi and is associated with an upregulation of NF-kB downstream target gene expression detected by qRTPCR. Gentamicin also causes GH receptors mRNA upregulation at 7 dpi along with the kidney progenitor markers osr1 and eya4, while the formation of new nephron aggregates as marked by Tg(lhx1a:GFP) expression is increased. NF-kB pharmacological inhibition reduces mRNA expression of kidney progenitor markers, while LPS injection induces mRNA upregulation of kidney progenitor markers. Bulk RNAseq from positive selected GFP+ Lhx1a+ cells 7 dpi with gentamicin confirmed the induction of cytokine receptors in the kidney progenitor cells.
Conclusion: Multiple pathways may converge on adult kidney stem cells to activate new nephron formation. We conclude that DAMP and NF-kB signaling is required and sufficient to induce neo-nephrogenesis. Further experiments are required to determine whether cytokine stimulation of neo-nephrogenesis is a direct or indirect effect."

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Biology
Physiology/pathophysiology
Biochemistry
Aric Rogers, Ph.D.
MDI Biological Laboratory
Associate Professor

Canonical ISR is not required for lowering and redirecting translation associated with lifespan extension under DR in C. elegans

"Background: Dietary restriction (DR) extends lifespan, in part, by lowering and redirecting mRNA translation. Initiation is the rate-limiting step of protein synthesis and is controlled by two translation complexes. One comprises the cap-binding complex downstream of TOR, which circularizes mRNA and helps recruit additional translation factors. The other is the ternary complex, which supplies methionyl-

tRNA to initiate translation. Increased concentration of uncharged tRNAs associated with nutrient scarcity activates the kinase GCN-2, which phosphorylates the EIF-2ALPHA subunit of the ternary complex. This activates the integrated stress response (ISR), which lowers and redirects translation.
In this study: We investigated the role of the ISR in differential translation and lifespan extension associated with DR in C. elegans. We confirmed that GCN-2, but not the other EIF-2ALPHA kinase PEK-1, is required to phosphorylate EIF-2ALPHA (S49) in the ternary complex and activate the ISR under DR conditions. However, an eif-2alpha S49A phosphorylation mutant was still responsive to DR, showing rapid downregulation of translation and lifespan extension. Similar results were obtained using gcn-2 or pek-1 mutants, suggesting that the ISR is dispensable for lifespan extension under DR. Work in mammalian tissue culture has shown that certain forms of cellular stress downregulate the nonsense-mediated decay (NMD) pathway via the ISR so that normally unstable stress-responsive mRNA can be translated. We find that, while NMD is still downregulated under DR conditions, the ISR is not required for this response. Together, results suggest that the ISR is not required to drive differential translation associated with longevity benefits of DR in C. elegans."

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Biology
Molecular biology
Dustin Updike, Ph.D.
MDI Biological Laboratory
Associate Professor

Germ Granules to Nucleoli: Decoding Biomolecular Condensates in Development and Disease

Germ granules are a defining feature of germ cells. These biomolecular condensates facilitate post-transcriptional expression, transgenerational epigenetic inheritance, and help confer the germline's stem-cell-like properties and immortal potential. The ability of germ granules to phase separate from the rest of the cytoplasm is mediated through intrinsically disordered protein motifs, which include glycine-rich domains regularly interspersed with phenylalanine (FG-) and arginine (RG-) repeats. These disordered repeats also promote phase-separation within nucleoli and impact biomolecular condensation in neurons. Here we examine the contribution of FG- and RG- repeats to biomolecular condensation and their function in germ-cell specification, development, and disease.

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213 n/a

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Friday October 14; 11:30 am – 1:30 pm
Basic and Applied Research in Biological Sciences
Genetics and Genomics
Courtney Willey
The Jackson Laboratory, Bar Harbor, ME
Research Assistant

Investigating Tdrd5 and Its Potential Role in the Kidney's Resistance to Damage

Gene expression profiles from American black bear kidneys have shown a significant increase in Tdrd5 expression after hibernation and may be involved in kidney recovery. Tdrd5 has only been described in the testes, but antibody staining shows expression in the parietal epithelial cells of the kidney. It is known to be involved in the maturation of small RNAs called piwi-interacting RNAs (piRNAs). We hypothesize that the upregulation of Tdrd5 may cause parietal cells in the glomerulus to increase the expression of a specific subset of piRNAs. This may signal the parietal cells to differentiate into podocytes or proximal tubule cells, increasing the kidneys resistance to damage. To investigate the role Tdrd5 has in this process, we are developing tools to characterize the relationship between Tdrd5 and piRNA in the kidney. We have isolated, sequenced, and analyzed piRNA from the kidneys of three adult mice and three pups to reveal highly expressed piRNA sequences, as Tdrd5 expression in parietal cells declines with age. We have been developing two mouse models, a Tdrd5 knockout model to characterize changes in the kidney in the absence of Tdrd5 and a Tdrd5 overexpressing model which will be inducible in the parietal cells. The Tdrd5 knockout model is in development, however, our overexpressing model had failed. We are currently redesigning this model using a different promoter. Through these efforts we hope to better understand the black bear's ability to preserve kidney function during hibernation and develop a new treatment option for chronic kidney disease patients.

Friday October 14; 4:30 pm – 6:00 pm Session
UMaine and UNE

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Microbiology/Virology
Suzanne Ishaq, Ph.D.
University of Maine
Assistant Professor

Biogeography may be key to microbial anti-inflammatory production using dietary precursors.

Inflammatory bowel disease (IBD) is a chronic condition of the gastrointestinal (GI) tract characterized by aberrant immune responses to gut microbiota. Immature broccoli sprouts contain inactive precursors, such as glucoraphanin (GLR), which can be converted to bioactive anti-inflammatory components like sulforaphane (SFN). Plant enzymes create some SFN, but more often create non-functional end-products. Humans lack the necessary enzymes, but some gut microbes robustly create SFN.

We have demonstrated that: different broccoli sprout preparations alter how much SFN can be produced and the fecal bacterial composition, gut bacteria convert GLR to SFN, SFN reduces colitis and colon tumorigenesis in mice. We also demonstrated anatomical specificity: SFN was only present in colon tissues, implying localized conversion there, and in high enough concentration to reduce inflammation at the site of IBD symptoms. Further, some SFN was observed in plasma and urine, indicating it can be absorbed from the colon and have a systemic effect. We are investigating colon microbial communities, isolating bacteria involved in biotransformation, and investigating the specific mechanisms behind this diet-microbe-host interaction.

In the past year, we collected >200 community samples from mice on sprout diets under DSS challenges to fully map the gut microbiome along the GI tract using 16S rRNA sequencing. We also isolated gut bacteria responsible for converting GLR to SFN using selective media and anaerobic culturing. Currently, >800 bacterial isolates are being screened for their β-thioglucosidase activity and their capacity to convert SFN from precursor compounds."

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Microbiology/Virology
Melissa Maginnis, Ph.D.
University of Maine
Associate Professor of Microbiology

Cell-type Dependent Differences in JC Polyomavirus Signaling Pathway Regulation

JC polyomavirus (JCPyV) infects the majority of the population and causes an incurable persistent infection in the kidneys. In immunocompromised individuals, JCPyV can become reactivated in the central nervous system and infect glial cells, oligodendrocytes and astrocytes, which are critical for myelin production. The molecular mechanisms of JCPyV infection of astrocytes are poorly understood, in part due to most studies being limited to an immortalized cell model. To better understand the cellular and molecular basis of JCPyV infection in astrocytes, we developed and characterized a new infection model using normal human astrocytes (NHAs). We determined that viral infection was regulated differently in primary and immortalized cell types, due to viral factors, such as T antigen expression, and cellular factors, including activation of cellular signaling pathways. Using RNA Sequencing analysis and complementary molecular approaches, we defined cell-type specific differences in the regulation of the MAPK pathway through dual-specificity phosphatases (DUSPs) and also determined the importance of the PI3K/AKT signaling pathway

in NHAs. Through this comparative genomic analysis, we elucidated how JCPyV orchestrates differential gene expression and regulation of cellular signaling pathways to mediate infection in primary astrocytes. Outcomes of this research provide an enhanced understanding of cell-type dependent differences in viral infection and can be applied to our broader understanding of cell signaling pathways and future development of antiviral therapies.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Biochemistry
Molecular Biology
Computer Modeling & Data Acquisition/Analysis
Josh Kelley, Ph.D.
University of Maine
Associate Professor

Gα spatial control of septin organization during the pheromone response is mediated through the Gα Ubiquitination Domain and endocytic machinery.

Yeast utilize a GPCR signaling pathway to from a polarized mating projection that requires septin structures at the base, peripheral to the site of polarity. Septin organization to the periphery is disrupted when desensitization of the pathway by the RGS Sst2 is defective. The Gα signaling that direct septins to organize proximal to the site of polarity is not understood. We set out to identify the proteins that mediate Gα control of septin localization by rescuing septin organization in cells expressing the hyperactive Gα mutant, gpa1G302S. We found that deletion of the septin chaperone GIC1 rescued septin organization, while deletion of GIC2 did not. Septin organization is known to be controlled by Cdc42 GAP activity. We found that deletion of BEM3 rescued the ability of cells to place septins proximal to the polarity site. The endocytic adapter proteins called epsins are known to both bind Cdc42 GAPs and to influence septin organization. We found that deletion of either epsin was able to rescue septin organization. We hypothesized that hyperactive Gα signaling may enhance the rate of endocytosis of some cargo leading to an increased rate of septin structure assembly. Our mathematical modeling indicates that the rate of becoming competent for endocytosis can change endocytosis from happening at the center of the polar cap to the periphery. We disrupted endocytosis of the GPCR and of the Gα, and found that Gα endocytosis was required for altered septin organization. Deletion of the UD from the hyperactive Gα (gpa1G302S/ΔUD) lead to a rescue of septin localization. Thus Gα regulates septin localization through its UD and a process that includes epsins, Gic1, and Bem3. These data suggest that the location of Gα endocytosis serves as a spatial mark for septin structure assembly and that activation state of the Gα may influence its endocytosis.

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Friday October 14; 4:30 pm – 6:00 pm
Computational Biology & Medicine
Managing large data sets

Data acquisition/analysis
Benjamin King, Ph.D.
University of Maine
Associate Professor of Bioinformatics

Maine INBRE Bioinformatics Core: Reusable Training Materials on Cloud-Based Computing Environments to Enhance Data Science Research and Training

Modern biomedical research is increasingly data-driven, and accordingly is dependent upon state-of-the-art data management and analysis methods that facilitate rigorous, robust, and reproducible research. The Maine Institutional Development Award Network of Biomedical Research Excellence (ME-INBRE) Bioinformatics Core supports biomedical research and research training in Comparative Functional Genomics by providing training and expertise in experimental design, data management, analysis, and access to computational resources. We recently completed a pilot project to facilitate the application of bioinformatics and data science in biomedical research. Cloud-based computing and data storage resources provide opportunities to broaden the application of bioinformatics and data science in biomedical research. Two major obstacles for many researchers, such as small primarily undergraduate institutions, is having: 1) access to bioinformatics analysis environments tailored to their research; and 2) training in how to use cloud-based computing resources. We developed reusable training materials on how to build cloud-based bioinformatics analysis environments to enhance bioinformatics research and training to address these two obstacles. We worked collaboratively with the Google Cloud team to leverage resources made available through the NIH, and create reusable cloud-based bioinformatics analysis environments for RNA sequencing analysis workflows, and provide reusable training materials on how to use the workflow. These resources became the foundation for the development of additional training materials that are currently being created by INBRE Bioinformatics and Data Science Cores in Maine and across the nation. ME-INBRE is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103423.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Microbiology/Virology
Benjamin King, Ph.D.
University of Maine
Associate Professor

Inhibition of NADPH Oxidase 2 Improves Survival in Zebrafish Infected with Influenza A Virus

Influenza A virus (IAV) is a major health concern since it can cause severe lung infections. The innate immune system is the host's first defense against pathogens, including IAV. The innate immune system consists of multiple cells including neutrophils and macrophages. Neutrophils are phagocytes that engulf and destroy pathogens through the production of reactive oxygen species (ROS). The production and

release of ROS is a process called the respiratory burst response that begins with NADPH oxidase (NOX). Because ROS is highly reactive, levels must be tightly controlled to limit host tissue damage. The long-term goal of our research is to learn how to balance the respiratory burst response following IAV infection. Using a zebrafish model of IAV infection, our preliminary studies show that limiting ROS production improves survival. We hypothesize that reducing the respiratory burst response will limit tissue damage and improve survival. To test this hypothesis, a respiratory burst assay is used to measure the respiratory burst capacity. First, ROS is induced using phorbol myristate acetate and then ROS levels are measured. In these assays, we measure the amount of a fluorescent product, dichlorofluorescein, that is generated when ROS oxidases 2,7-dihydrochlorofluorscein diacetate. Ongoing studies have shown that the respiratory burst response decreases by 48 hours post infection and gradually rebounds over the course of infection. We are currently measuring changes in the respiratory burst response with and without the NOX inhibitor, GSK205739. These studies will help identify the molecular mechanisms that regulate the respiratory burst response.

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Friday October 14; 4:30 pm – 6:00 pm
Health and Social Sciences
Nursing
Kathryn Robinson, Ph.D.
University of Maine
Associate Director, Assistant Professor of Nursing

Orientation and preceptors may not translate into job satisfaction among nurses in Maine and Massachusetts: A secondary analysis of the National Sample Survey of RNs

Background: The intention that orientation and preceptor programs transition nurses into satisfying clinical roles may be mitigated by high turnover/low retention creating a culture of low job satisfaction.

Purpose: To explore job satisfaction among nurses who had orientation or a preceptor at their most recent employer.

Methods: Using the 2018 National Sample Survey of Registered Nurses (NSSRN), limited to Maine and Massachusetts nurses at their current employer <5 years, the association of orientation or preceptor with job satisfaction was explored using weighted multivariable logistic regression stratified by state.

Results: We found n=368 (representing 8012) nurses from Maine and n=492 (representing 41204) nurses from Massachusetts. The sample was mostly female (weighted % ME:87%; MA:88%), White (ME:74%; MA:74%), partnered (ME:53%; MA:60%), and ≥$75,000 annual household income (ME: 59%; MA:78%). Having only orientation meant lower odds of being satisfied (OR 0.94, 95% CI [0.82, 1.07]) and those who had both orientation and preceptor had the lowest odds of being satisfied (OR 0.36, 95% CI [0.30, 0.42]) at their current job, compared to those that had none. New nurses – within 5 years of their first nursing degree – had 1.50 (95% CI 1.20, 1.88) times the odds of being satisfied compared to nurses that were more experienced. Males had higher odds of being satisfied (OR 2.54, 95% CI [1.75, 3.70]).

Conclusion: Given orientation programs' intention to promote satisfaction by reducing turnover/increasing retention, these findings suggest a need for evaluation of program effectiveness and a potential mediating effect of new nurse graduates on job satisfaction."

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Biology
Physiology/pathophysiology
Molecular biology
Timothy Breton, Ph.D.
University of Maine at Farmington
Associate Professor of Biology

Using fish to better understand the phoenixin/SREB systems

Some novel hormone and receptor systems exhibit potential in medical applications but may be yet unrealized due to a lack of functional understanding. One such system involves the hormone phoenixin (PNX) and its possible receptor SREB3. PNX is associated with inflammatory roles, appetite modulation, and hypothalamic control of reproduction, while the SREBs (Super-conserved Receptors Expressed in Brain) are a family of receptors independently associated with schizophrenia, autism spectrum disorder, diabetes, and reproductive dysfunction. Both hormone and receptors are highly conserved across vertebrates, but relatively little is known about their functions. The purpose of this work was to provide more information on PNX/SREB using comparative genomic, transcriptomic, and steroid analyses in fish. Using comparative genomics of over 75 fishes, we identified divergence from the mammalian receptor system, including the addition of a possible duplicated PNX receptor (sreb3b) in some species, while others have lost SREB genes. We also identified that the novel sreb3b was dominant in the brain, and patterns diverged from sreb3a. One species that exhibited both possible receptors was pufferfish (Dichotomyctere nigroviridis), which was exposed to PNX using intramuscular injections. Hypothalami and ovaries were removed to assess transcriptome changes, while livers and blood plasma were collected for gene expression assays and steroid quantification, respectively. Using pathway analysis, we identified pro-inflammation signals and broad suppression of cell proliferation in the hypothalamus, which may be associated with gut-brain axis functions. Ovaries largely exhibited anti-inflammatory signals and transcriptional downregulation. Livers were unresponsive, but blood plasma exhibited elevated 17-hydroxyprogesterone, which may promote oocyte maturation. Overall, PNX exhibits pleiotropic effects and may modulate immune responses and cell growth across vertebrates.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Neuroscience
Michael Burman, Ph.D.
University of New England
Professor

CeA-CRF cells mediate the effects of NICU-like medical trauma on juvenile fear conditioning and pain sensitivity in a sex-specific manner.

Over the past several decades infant admission to the neonatal intensive care unit (NICU) has been on the rise. However, NICU infants are predisposed to later-life mental health challenges, including alterations of fear, anxiety, depression, and sensory thresholds. These studies utilize a rat model of NICU procedures, to examine the role of Corticotropin Releasing Factor (CRF)-expressing cells in the Central Nucleus of the Amygdala (CeA) in these outcomes. Newborn offspring from hemizygous transgenic CRF-Cre X SAS-SD crossings were taken from their mother 4 times daily and received either a brief paw needle prick (neonatal pain group) or nonpainful tactile handling (neonatal handled group) over postnatal days (PND) 1-7. Control rats were left undisturbed. On PND 8 rats (Cre+ and Cre-) received an intracranial injection of pAAV-hSyn-DIO-hM4D(Gi)-mCherry targeted at the CeA allowing the Cre+ rats to express an inhibitory DREADD receptor. The Cre- served as the non-active controls. As the pain of AAV injection caused a neonatal-pain phenotype, additional control rats were left non-injected. On PND 24, all subjects received an injection of clozapine N-oxide (CNO), prior to fear conditioning. Conditioning consisted of 10 tone-shock pairings. Subsequent days consisted of contextual fear testing, auditory fear testing and sensory withdrawal threshold testing. Consistent with our previous work, neonatal trauma caused only a modest decrease in measures of conditioned freezing at this age. Moreover, we once again observed a fear conditioning-induced tactile hypersensitivity on the Von Frey test. Silencing CeA CRF cells caused a further reduction in conditioned freezing that was more prevalent in females compared to males. In contrast, silencing the CRF-cells reversed the tactile hypersensitivity more strongly in males, compared to females. These data are further evidence of a strong sexual dimorphism in the role of CeA CRF-expressing cells in pain and anxiety.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Neuroscience
Geoff Ganter, Ph.D.
University of New England
Professor

Determinants of Nociceptor Sensitivity in D. melanogaster

The Drosophila model system has afforded effective characterization of multiple pathways that control nociceptive sensitivity, leading to discovery of well-conserved novel mechanisms that may be exploited for pain drug discovery efforts. Expression of candidate genes may be efficiently manipulated specifically in the primary nociceptor, allowing knockdown and/or overexpression of normal or mutant alleles. Any resulting effects of these manipulations on the evoked behavioral responses to noxious stimulation are then easily ascertained. Using this approach, we have identified 15 determinants of nociceptor sensitivity, including those affecting baseline sensitivity and injury-induced nociceptive sensitization. In particular, several components of the Bone Morphogenetic Protein pathway have been implicated. Implication of the Wnt/Wingless pathway includes Armadillo, the fly homolog of Beta-catenin (Beta-cat). When Beta-cat/Armadillo levels are increased specifically in the nociceptor, behavioral nociceptive sensitivity is also significantly increased. When Beta-cat/Armadillo levels are reduced specifically in the nociceptor, behavioral nociceptive sensitivity is also significantly reduced. Neither manipulation results in any detectable change in the nociceptor's pattern of arborization. Since Beta-cat/Armadillo is known to have at least two distinct cellular roles including transcriptional control and cell adhesion, future work seeks to determine the details of its possible transcriptional mechanism, and implicate any potential cell adhesion partners, such as epidermal cells, that may play a role in determining nociceptive sensitivity.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Biochemistry
Neuroscience
Ramaz Geguchadze, Ph.D.
University of New England
Research Assistant Professor

Activity-dependent activation of eukaryotic elongation factor 2 kinase contributes to nociceptor sensitization and pain

Eukaryotic elongation factor 2 (eEF2) is part of the ribosomal machinery that mediates translation of mRNA into protein by catalyzing translocation of the ribosome along the mRNA transcript. eEF2 function is inhibited by phosphorylation by eEF2 kinase (eEF2K), which is activated by increases in intracellular calcium and by AMP-activated kinase (AMP kinase), a sensor of ATP depletion. We used an antibody selective for phosphorylated eEF2 to examine eEF2 regulation in peripheral sensory neurons of the mouse dorsal root ganglion (DRG). In DRG tissue sections from naïve mice, immunohistochemistry for peEF2 revealed tonically high levels of peEF2 in most neurons identified as nociceptors (pain-sensing neurons) by staining for either the heat-gated channel TRPV1 or the lectin IB4. Depolarization of cultured DRG neurons with buffer containing 50 mM K+ for 1 or 10 minutes induced intense peEF2 staining that remained elevated for at least 60 minutes and was prevented by a selective eEF2 kinase inhibitor, A484954 (30 μM). A484954 bioavailability and pharmacokinetics were evaluated by mass spectrometry in plasma and brain 30, 60 and 90 minutes after oral administration of 1 or 7.5 mg/kg by gavage. Peak plasma and brain concentrations were observed 60 minutes after administration. In a model of inflammatory pain (injection of carrageenan into the hindpaw), EEF2 phosphorylation was increased during the period of hypersensitivity to noxious heat. However, daily oral administration of A484954 significantly reduced hypersensitivity throughout the behavioral response to carrageenan, suggesting that

suppression of eEF2 phosphorylation is anti-nociceptive. These results indicate that peEF2 is rapidly induced in response to sensory neuron activation and sustained during inflammatory hyperalgesia. Suppression of eEF2 phosphorylation is anti-nociceptive, possibly through a direct action on DRG nociceptors.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Neuroscience
Josh Havelin, Ph.D.
University of New England
Visiting Assistant Teaching Professor

Contribution of parabrachial projecting corneal afferents to ocular pain

Primary afferent neurons innervating the cornea maintain ocular homeostasis through the regulation of tearing and blinking, and protect the eye from injury by evoking behavioral and sensory responses to noxious stimuli. Previous studies have demonstrated projections from corneal afferents to two distinct regions within the trigeminal brainstem nucleus, one located at the transition between Vi and Vc (Vi/Vc) and the other located further caudally at the transition between Vc and the first cervical vertebra (Vc/C1), that regulate tearing, blinking, and nociceptive responses, respectively. While a direct projection from the trigeminal ganglion (TG) to lateral parabrachial nucleus (lPBN) has been described, it is currently unknown whether trigeminal afferents from the cornea contribute to this projection. This study identified lPBN projecting corneal afferents and determined their role in corneal pain-evoked responses in male and female C57B/6 mice. Fluorogold was applied to the corneal surface and DiI or the retrograde AAV pAAV-CAG-tdTomato injected into the lPBN. Dual labeled cell bodies within the TG were identified, indicating the presence of corneal innervating neurons that project directly to the lPBN. The contribution of lPBN projecting corneal afferents to corneal hypertonic saline evoked eye wipe behavior was examined using double heterozygous Nav1.8-Cre;ArchT and Nav1.8-Cre;tdTomato (genotype control) mice. A wireless LED probe directed above the lPBN was implanted to allow for photic stimulation with ArchT activating light. Eye wipe behaviors were evoked with corneal application of hypertonic saline 5 and 7 days after LED implantation. ArchT activating light reduced eye wipe behaviors and palpebral opening compared to the light off control. The direct projection from TG corneal afferents to the lPBN may contribute to the heightened pain and anxiety experienced by patients with ocular pain.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Pharmacology
Karen L. Houseknecht, Ph.D.
University of New England
Professor and Associate Provost for Research

Looking for drugs in all the wrong places: Exploring drug exposure in tissue niches empowers mechanistic pharmacology/toxicology and drug discovery

Pharmacokinetic and pharmacodynamic (PK/PD) modeling lies at the heart of drug discovery and development, as ensuring efficacy and safety at a given drug exposure is required for clinical development and proof of concept testing. PK/PD is most often determined by evaluating plasma drug and biomarker exposure under a range of dosing and defined formulation paradigms, however determining drug (and in some cases drug metabolite) exposure at the target tissue is powerful, and often necessary, to definitively establish efficacy and toxicology effects relating to drug mechanisms of action. The Houseknecht laboratory employs sensitive LC/MSMS methodology coupled with therapeutically relevant drug dosing paradigms to explore mechanisms of drug efficacy and toxicity in tissue niches/microcompartments such as bone marrow, dorsal root ganglia, heart and liver collected from preclinical models including mouse, rat and zebrafish, as well as in clinical samples. These analyses are conducted as part of NIH-funded projects exploring discovery and development of novel therapeutics and projects exploring pharmacological effects of FDA approved medications (antipsychotics, antidepressants, opioids, beta blockers) on tissues of interest with the goal of elucidating novel mechanisms of drug target action. This approach and methodology allows us to answer questions such as: Do CNS medications increase fracture risk due to direct effects on the bone marrow compartment? Specific examples from our laboratories include evaluation of antipsychotic (AA), antidepressant (SSRI) and opioid distribution to the bone marrow niche as part of studies focused on elucidating the mechanistic toxicology of medications known to increase clinical fracture risk, as well as quantifying AA distribution to heart and liver as part of studies elucidating the metabolic side effects of psychiatric medications. Additionally, quantification of signaling molecules, specifically catecholamines, in target tissue niches further empowers evaluation of pharmacological effects of focused drug distribution as part of target validation studies.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Neuroscience

Tamara King, Ph.D.
University of New England
Professor

Analysis of mid-stage and advanced OA pain states in chemical and surgical murine osteoarthritis models

Osteoarthritis (OA) is one of the most prevalent causes of chronic pain in US adults suffering with OA. OA pain can be characterized into three stages; early stage, mid-stage, and advanced- stage with advanced-stage OA often accompanied by constant dull, aching pain as well as intermittent bouts of intense pain. Although NSAIDs are commonly prescribed for OA pain and help mitigate the predictable episodes of pain associated with early and mid-stage OA, evidence has shown that NSAIDs are not sufficient in treating chronic advanced OA pain. It is imperative to develop new and improved treatments for advanced OA pain. To achieve this, a better understanding of how the mechanisms driving advanced OA pain differ from

mid-stage OA pain is required. Here we compared behavioral readouts of mid-stage and advanced OA pain between the monosodium iodoacetate -induced (MIA) murine OA model and a surgical partial meniscal excision (PMX) murine OA model. The MIA model allows for phenotypic and mechanistic study of different stages of OA 14 days post-induction, a major benefit of the model, while the PMX surgical models trauma-induced OA, with behaviors indicating mid-stage OA joint pain developing over a 12-week time course. However, whether advanced OA develops in the PMX model of OA pain is unknown. Our data demonstrate development of mid-stage and advanced OA pain in both models. This work has been supported by the NIH through a National Institute of General Medical Sciences COBRE grant P20-GM-103643 at UNE and a National Institute for Arthritis and Musculoskeletal and Skin Disease grant, P30-AR-079206).

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Physiology/pathophysiology
Neuroscience
Derek Molliver, Ph.D.
University of New England
Professor

Mitochondrial regulation of sensory neuron function and pain

Most pathological pain conditions are maintained by hyper-excitability of peripheral sensory neurons that transmit information about noxious stimuli (nociceptors) to the brain. We recently found that painful hypersensitivity caused by the inflammatory mediator prostaglandin E2 (PGE2) increases mitochondrial respiration in sensory neurons. Reducing mitochondrial function with a mitochondrial membrane potential (MMP) uncoupling drug (2,4-dinitrophenol (DNP)) decreased hypersensitivity, but had no effect on baseline sensitivity, suggesting that mitochondrial function is linked to nociceptor sensitization. To explore this phenomenon, we tested 2 uncoupling drugs, DNP and BAM15, in diverse pain models. In mouse, systemic DNP or BAM15 (1mg/kg) reduced hypersensitivity caused by hindpaw inflammation and in the sciatic nerve crush model of neuropathic pain. In a rat model of uveitis, systemic DNP reversed sensitization of the capsaicin-evoked eye wipe response by ultraviolet light exposure. We next tested BAM15 in rat models of opioid-induced hyperalgesia, a form of persistent pain that can occur in patients treated with opioids. A low dose (0.03mg/kg) of morphine causes hypersensitivity that was prevented by local injection of BAM15. This morphine regimen also causes a prolonged priming effect: as a result, hindpaw injection of PGE2 4 days after morphine causes greatly prolonged hypersensitivity. Local injection of BAM15 reduced the prolonged hypersensitivity in morphine-primed rats. However, priming reappeared 1 week later, indicating that BAM15 does not prevent maintenance of morphine-induced priming. To confirm a direct action of these drugs on nociceptors, we recorded from isolated mouse sensory neurons. DNP (20μM) or BAM15 (2μM) suppressed firing in nociceptors through an apparent activation of K+ channels. Together, these results indicate that mitochondrial uncoupling drugs have robust analgesic effects in diverse rodent pain models.

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Friday October 14; 4:30 pm – 6:00 pm

Health and Social Sciences
Public Health/ Nutrition Security
Michele Polacsek, Ph.D.
University of New England
Professor, Director of the University of New England Center for Excellence in Public Health

A university-low-income-housing partnership to support food security, healthy shopping, eating, health and wellness among seniors in rural Maine: Preliminary findings

"The University of New England Centers for Excellence in Public Health and Aging and Health, in partnership with Westbrook Housing Authority and Southern Maine Agency on Aging, are implementing and evaluating the impact of an innovative, pandemic-responsive nutrition education program, Enhanced-10 Tips for Adults (e-TTA), on food security, socialization, and perceived health and wellbeing of residents in a rural low-income senior housing setting in Westbrook, Maine.
The project aims are to: 1) deliver e-TTA to residents of low-income senior housing; 2) assess implementation of the intervention using a "Re-Aim" framework; 3) measure effectiveness of e-TTA on meal planning knowledge, attitudes, beliefs and skills (KABS), food security, diet, physical activity, socialization, health, and depression; and 4) disseminate findings to local, state, and national stakeholders. The e-TTA series is a direct education intervention that reinforces messages related to increasing fruit and vegetable consumption, increasing physical activity, and providing skills to purchase healthy foods on a budget, adapted during the COVID-19 pandemic and tailored to include health professions student support, virtual education, congregate meals, and class cohorts based on personal preference. Anticipated outcomes include improved: 1) KABS related to meal planning, food purchasing, and physical activity; 2) diet, food security, and physical activity; and 3) socialization and reduced loneliness and depression.
Preliminary findings on implementation lessons learned along the way to help improve the program for maximum effectiveness, and outcomes on healthy eating and physical activity knowledge, attitudes, beliefs and skills and effects on socialization, loneliness, and depression will also be shared. At the time of abstract submission, the first of five cohorts had completed the intervention."

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Neuroscience
Scott Stackhouse, Ph.D.
University of New England
Associate Professor of Physical Therapy

Harnessing the Conditioned Pain Modulation Effect for Therapeutic Use in Knee Osteoarthritis

Knee osteoarthritis (OA) is the most common lower extremity joint pain condition in the US. The nervous system contains networks that naturally inhibit the pain experience, which can be invoked using noxious electrical stimulation (NxES) delivered at a painful, but tolerable intensity. The purpose of this pilot study

was to assess treatment acceptability and pain modulation of a single NxES treatment in people with knee OA.

Ten volunteers (70.3 ± 5.9 yr; 3 females) with knee OA participated. Using a repeated measures design, participants attended 4 study visits (baseline/familiarization, NxES treatment, 24-hr post-NxES, and 72-hr post-NxES). Movement-related pain and quantitative sensory testing was assessed at each session. Electrical stimulation (400 μs phase duration; 50 pps; 10s on:10s off with 2s on-ramp, intensity to highest tolerable level) was applied across the medial/lateral knee joint line for 20 minutes. Treatment acceptability was measured at the 24-hr post-NxES visit. One-way repeated measure ANOVAs ($\alpha = 0.05$) were used to assess differences from baseline over time, followed by post-hoc paired t-tests with Bonferroni correction.

Participants rated their acceptability of NxES in response to the prompt, “NxES meets my approval” as 4.3/5, where 1 = “completely disagree” and 5 = “completely agree”. A decrease in movement-related pain during the 5-times Sit-to-Stand test (5xSTS) was found across time ($F=4.55;p=0.005$), with significant pain reduction during the 5xSTS immediately post-NxES ($p=0.001$), that persisted during the 5xSTS performed 1hr ($p=0.005$), and 72-hrs ($p=0.002$) post-NXES. Mechanical pain sensitivity was reduced across time at the knee ($F=5.37;p=0.002$), with less sensitivity found immediately post-NxES ($p<0.001$) that persisted 1hr ($p=0.011$), and 72-hr ($p=0.006$) post-NXES.

As a non-pharmacological treatment for pain in people with knee OA, NXES appears to be an effective and acceptable treatment and warrants further investigation."

319 n/a

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Neuroscience
Jared Zuke
University of New England
Lab Manager

The effect of early life trauma on hypothalamic corticotropin releasing factor (CRF) expression in the juvenile rat

Increases in neonatal intensive care unit (NICU) admissions over the past several decades are likely responsible for the concomitant drop in infant mortality. However, the experiences these infants endure in the NICU leave them vulnerable to later life psychological disorders such as anxiety and depression. Utilizing a rodent model, our lab explores how common NICU procedures (maternal separation, handling, and needle pricks) four times daily for the first seven days of life, alter CRF expression in neonatal and juvenile rats. We’ve previously demonstrated that neonatal pain increases amygdala corticotropin releasing factor (CRF) expression during our neonatal manipulations (PND 6). Developmentally, we’ve observed that neonatally manipulated rats display reduced amygdala CRF expression during the juvenile stage (PND 24). Outside the amygdala, we hypothesize that neonatal pain is also altering hypothalamic CRF. The current study examines how neonatal pain, followed by a juvenile psychological stressor (foot

shocks), influences juvenile hypothalamic CRF within two sub regions of the hypothalamus (paraventricular nucleus [PVN] and ventral medial hypothalamus [VMH]). All neurological tissue was processed using RNAscope® florescent in situ hybridization (FISH) targeting expression of CRF and the immediate early gene c-fos. Results were quantified and compared to our previous FISH work quantifying CRF expression in the basolateral and central nucleus of the amygdala in juvenile rodents. Current juvenile findings suggest that neonatal pain alters hypothalamic CRF expression in both a sex and region-specific manner. These alterations are most apparent in subjects who also received a secondary stressor as juveniles. Males indicate a decrease in PVN CRF expression while females' show an increase in VMH CRF expression following our neonatal pain and juvenile stress procedures. This study provides evidence that neonatal trauma alters CRF expression in a sex dependent manner.

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Neuroscience
Kathleen Becker, Ph.D.
University of New England College of Osteopathic Medicine
Assistant Professor, Department of Biomedical Sciences

Saphenous Nerve Transection Results in Sensory and Sympathetic Denervation of the Mouse Tibia

The saphenous nerve is primarily a sensory nerve that is thought to innervate the tibia. Injury to this nerve is a common result of ACL repair, varicose vein surgery, and other procedures resulting in numbness and/or pain from denervation. While sensory and sympathetic input to bone impacts bone homeostasis, little is known about the specific consequences of saphenous nerve injury on tibial innervation and bone mineral density. We hypothesize that saphenous nerve transection will result in a decrease in sensory nerve fibers in the tibia. A greater understanding of factors regulating tibial innervation will help identify risk factors influencing tibial bone mineral density.

To demonstrate that the saphenous nerve innervates the tibia, fast blue dye was injected into the tibia of saphenous nerve transected or sham control mice. Labeling was analyzed in the L2-L5 dorsal root ganglia (DRG). The highest level of retrograde labeling was observed in the L2 and L3 DRGS. Furthermore, retrograde labeling to the L2 DRG was reduced by 75% in mice with saphenous nerve transection, consistent with the paradigm that the saphenous nerve is associated with the L2 DRG. Tibial innervation was also quantified in the proximal, lateral-most periosteum of the ipsilateral tibia from mice with unilateral saphenous nerve transection and compared to the contralateral control tibiae. Calcitonin gene-related peptide (CGRP, sensory fiber marker), tyrosine hydroxylase (TH, sympathetic fiber marker) and βIII-tubulin (β3T, pan-neuronal marker) positive fibers were assessed by immunohistochemistry in cryo-embedded tibiae. Fiber length was quantified and normalized to periosteal volume. CGRP, TH, and β3T positive fibers were reduced by 40-60%. Our findings demonstrate that saphenous nerve denervation reduces innervation of the tibia. Further studies are necessary to determine the impact of tibial denervation on bone mineral density."

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Friday October 14; 4:30 pm – 6:00 pm
Basic and Applied Research in Biological Sciences
Molecular Biology
Harilaos Filippakis, Ph.D.
University of New England, College of Osteopathic Medicine
Assistant Professor

Tryptophan catabolism is a metabolic vulnerability in mTORC1-hyperactive cells

"Lymphangioleiomyomatosis (LAM) is a rare destructive lung disease affecting primarily women and is the primary lung manifestation of Tuberous Sclerosis Complex (TSC). In LAM and TSC, biallelic loss of TSC1/2 leads to mTORC1 hyperactivation, with a profound impact on cellular metabolism via several inter-connected mechanisms, including glucose and glutamine utilization, nucleic acid and lipid synthesis and autophagy. In the clinic, treatment with mTORC1 inhibitors stop lung function decline in LAM and TSC patients, however life-long treatment is required. Therapeutic targeting of metabolic pathways represents a novel approach, which may yield more durable clinical responses.
We hypothesized that essential amino acids play a key role on the metabolism and survival of TSC2-deficient cells. Many studies have focused on the impact of non-essential amino acids on mTORC1-activation, however little is known on the role of essential amino acids in TSC and LAM disease progression. We found that tryptophan supplementation increased the proliferation of TSC2-deficient cells four-fold, compared to TSC2-expressing cells. Interestingly, treatment with inhibitors that target TDO2 and IDO1, two key enzymes in the tryptophan-kynurenine pathway, selectively inhibit the growth of TSC2-deficient cells (~50%). Using Lyso-IP we found that the metabolism of TSC2- deficient cells is reprogrammed in a way that promotes tryptophan degradation and utilization of kynurenine in the lysosome, potentially utilized for redox reactions and enhanced protein synthesis.
Collectively, our data indicate that TSC2-deficient cells upregulate kynurenine pathway enzymes to metabolize tryptophan and maintain metabolic homeostasis and proliferation. Inhibition of this metabolic vulnerability by pharmacological or nutrient deprivation approaches leads to growth inhibition of TSC2-deficient cells, but not TSC2-expressing cells. Finally, therapeutic targeting of tryptophan catabolism in TSC2-deficient cells represents an entirely new therapeutic approach for TSC and LAM."

Saturday October 15; 8:30 am – 10:00 am Session
Bates, Colby, MaineHealth, MaineHealth Institute for Research

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Saturday October 15; 8:30 am – 10:00 am
Basic and Applied Research in Biological Sciences
Molecular biology
Neuroscience
Levi Adams, Ph.D.
Bates College
Visiting Assistant Professor

Aging and the Parkinson's Brain: Using single-cell multiome analysis to explore how aging may predispose us to disease

Parkinson's disease (PD) is characterized by the selective death of dopamine-producing neurons in a small midbrain region called the substantia nigra. Age is the primary risk factor for Parkinson's disease, but how the aging process affects the brain and how that might predispose us to developing a neurological condition isn't clear. To help address this gap, we used samples of the human midbrain from Young and Aged neurologically healthy donors, and compared them to PD. We explored the RNA expression and DNA accessibility simultaneously in nearly 70,000 individual cells using a single-nuclei multiomic approach. Our analysis suggests that all types of cells in the midbrain are somewhat altered by age. However, we found two cell types that were further affected by PD: microglia and oligodendrocytes. We present evidence for a new disease-associated oligodendrocyte subtype and identify genes lost over the aging and disease process, including CARNS1, that may predispose healthy cells to develop a disease-associated phenotype. We also used peak-gene association to link gene expression with specific DNA locations and found altered connections in 89 locations previously linked to PD (known as SNP loci) that are associated with disease-associated oligodendrocytes. These results suggest a previously undescribed role for oligodendrocytes in the aging and PD processes.

401 n/a

402

Saturday October 15; 8:30 am – 10:00 am
Basic and Applied Research in Biological Sciences
Biology
Biochemistry
Anyonya Guntur, Ph.D.
Maine Health Institute for Research
Faculty Scientist I

Impaired Mitochondrial stress signaling in osteoblasts mediates bone loss in male mice in the absence of BNIP3.

Osteoblasts generate bone by secreting collagen and mineralizing it in response to various signaling cues. We have previously shown that a majority of ATP generated by differentiated osteoblasts is through glycolysis in contrast to undifferentiated cells that are more dependent on oxidative phosphorylation. To understand the mechanisms involved in this shift, we focused on mitophagy (mitochondrial autophagy). We hypothesized that an increase in mitophagy shifts ATP generation towards glycolysis. To test this hypothesis, we first confirmed an increase in mitophagy with osteoblast differentiation from primary calvarial osteoblasts isolated from a mitophagy reporter mouse (tandem mCherry-GFP transgenic mouse, MitoQC). Next, we identified a mitophagy receptor, Bnip3, whose expression coincided with increased mitochondrial mitophagy and osteoblast differentiation in vitro. Knockdown of Bnip3 using adenoviral-mediated shRNAi delayed osteoblast differentiation with decreases in proteins involved in mitochondrial dynamics, translation, and protein folding. We utilized a BNIP3 global knockout mouse model to study

bone mass in vivo and identified a significant decrease in both trabecular and cortical bone parameters in male mice. Histomorphometry analysis identified decreased osteoblast numbers as a cause of the low bone mass. Mechanistically, we identified increased mitochondrial dysfunction and cell apoptosis and decreased ATF4 (Activating transcription factor 4) expression in the absence of Bnip3. We discovered that Bnip3 acts as a sensor for mitochondrial stress, and in its absence, mitochondria are unable to transduce stress signals to ATF4. These sets of data for the first time demonstrate that Bnip3, along with its role in mitophagy, is necessary for communicating mitochondrial stress to ATF4 to maintain optimal osteoblast differentiation and bone mass.

403 n/a

404

Saturday October 15; 8:30 am – 10:00 am
Basic and Applied Research in Biological Sciences
Pharmacology
Richard Riker, MD

Maine Health Institute for Research

Clinical Investigator

Intensive Care Analgesic Review and Opioid Use (ICARUS)

"PURPOSE: The association between opioid therapy during critical illness and persistent opioid use after discharge is understudied relative to ICU opioid exposure and modifiable risk factors. Our objectives were to compare persistent opioid use after discharge among patients with and without chronic opioid use prior to admission (OPTA) and identify risk factors associated with persistent use.

DESIGN: Retrospective cohort study in a medical, surgical, or neurologic ICU at an academic hospital.

MEASUREMENTS AND MAIN RESULTS: The primary outcome was persistent opioid use accounting for greater than 70% of days 4–6 months after discharge. Among 2,975 included patients, 257 (8.6%) were classified as OPTA, and 305 (10.2%) persistently filled opioid prescriptions, including 186/257 (72%) OPTA and 119/2,718 (4.4%) with no chronic opioid fills prior to admission. Among all patients, OPTA was strongly associated with persistent opioid use (odds ratio, 57.2 [95% CI, 41.4–80.0]). Multivariable logistic regression revealed that male sex, surgical procedure, and ICU opioid-free days were associated with reduced persistent opioid use for OPTA patients. Age and ICU opioid-free days were associated with reduced persistent opioid use for non-OPTA patients. Total ICU opioid dose and dose per day of ICU exposure were not associated with persistent use for either group.

CONCLUSIONS: In this mixed cohort of ICU patients, 10.2% persistently filled opioid prescriptions 4–6 months after discharge. Although ICU opioid doses were not associated with persistent use, duration of ICU opioid administration is a modifiable risk factor that may reduce persistent opioid use after critical illness."

405

Saturday October 15; 8:30 am – 10:00 am

Health and Social Sciences
Acute Care and Rural Disparities
Liz Scharnetzki, Ph.D.
Maine Medical Center Research Institute
Staff Scientist

Assessing readiness: Understanding community knowledge of, attitudes towards, and experiences with health research in Southern Maine

Community engaged research (CER) is a process in which researchers work collaboratively with communities to develop evidence-based solutions for local health issues. By centering community voices and priorities, CER is theorized to optimize the "bench to bedside" translation, ultimately producing effective and sustainable strategies for improvement. Importantly, CER offers a framework for addressing a primary factor that contributes to health disparities: access to research participation for members of underrepresented or systemically disadvantaged populations. Relative to other populations, there has been little empirical focus on rural communities in the Northeastern United States. For example, we have little information about whether persons believe research participation will lead to programs that improve health and wellness. For CER efforts to be successful, it is important that we understand the attitudes of persons living in rural communities toward health research and determine a community's readiness to engage in collaborative work. With this goal in mind, we piloted a newly-developed "Community Readiness Assessment" in Southern Maine. Using a survey-based approach, we assessed community members' familiarity, attitudes and experience with health research, and their willingness to be involved in research conducted by MaineHealth. To ensure that we heard diverse and representative voices, surveys were disseminated via social media to key stakeholders and general community members. Data collection is ongoing; however, our assessment will be completed prior to the statewide research symposium. MaineHealth has two CER infrastructure grants that aim to improve the health of Maine communities impacted by disparities (COBRE in Acute Care Research and Rural Disparities; Northern New England Clinical Translational Research). The Community Readiness Assessment will facilitate the longer term goal of developing researcher and community stakeholder partnerships by taking the necessary first step of understanding local beliefs about, interest in, and readiness for collaboration, as well as our communities' past experiences with health research.

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Saturday October 15; 8:30 am – 10:00 am
Health and Social Sciences
"Other" Clinical and Translational Centers
Thomas Gridley, Ph.D.
MaineHealth Institute for Research
Faculty Scientist

Northern New England Clinical and Translational Research Network

Northern New England Clinical and Translational Research Network
Clifford Rosen MD and Thomas Gridley PhD

The goal of the Northern New England Clinical and Translational Research Network (NNE-CTR), a collaboration of MaineHealth, the University of Vermont, the University of Southern Maine, and the Dartmouth Co-Op/Northern New England Practice-Based Research Network, is to build and sustain a clinical and translational research infrastructure that supports improved community health for the inhabitants of Maine, New Hampshire, and Vermont. The northern New England states have the oldest populations in the U.S., and also have a growing underserved immigrant population, as well as the Wabanaki Native American Confederacy. Age, coupled with rurality, predisposes northern New England residents to disorders ranging from cancer, obesity, diabetes, and cardiovascular disease, to environmental toxin exposure, food insecurity, and substance abuse disorders. NNE-CTR provides a variety of resources for clinical and translational research, including the Biostatistics, Epidemiology and Research Design Core, which provides research design support, large data analysis and research navigation services; Professional Development Core, which offers an array of educational and mentorship opportunities to new investigators; Pilot Projects Program, which provides flexible pilot funding mechanisms, including support for community-based projects; Translational Research Technologies Core, offering access to state-of-the-art laboratory-based technologies; and the Community Engagement and Outreach Core, which is building bridges to rural and underserved communities. Funding for the NNE-CTR was recently renewed for another five years. We will strengthen our network of clinical and translational research through a learning infrastructure, supported by the principles of diversity, equity, and inclusion, for the benefit of all who reside in northern New England."

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Saturday October 15; 8:30 am – 10:00 am
Basic and Applied Research in Biological Sciences
Pharmacology
Neuroscience
Richard Riker, MD
MaineHealth Institute for Research
Clinical Investigator

Evaluation of Free Valproate Concentration in Critically Ill Patients

"Purpose: Protein binding of valproate is variable in ICU patients, and the total valproate concentration does not predict the free concentration, even when correcting for albumin. We sought to quantify valproate free concentration among ICU patients, identify risk factors associated with an increasing valproate free concentration, and evaluate the association between valproate free concentration with potential adverse drug effect.

DESIGN: Retrospective multicenter cohort study at two academic medical centers.

MEASUREMENTS AND MAIN RESULTS: 256 patients were included in the study, with a median age of 56 years (42–70 yr), and 65% of patients were male. The median total valproate concentration was 53 μg/mL (38–70 μg/mL), the free valproate concentration was 12 μg/mL (7–20 μg/mL), and the free fraction was 23.6% (17.0–33.9%). Therapeutic discordance between the free and total valproate concentration occurred in 70% of patients. On multivariable analysis, increased valproate free concentration was associated with higher total valproate concentration (per 5 μg/mL increase, increase 1.72 μg/mL,

95% CI, 1.48–1.96) and lower serum albumin (per 1 g/dL decrease, increase 4.60 µg/mL, 95% CI, 2.71–6.49). There was no association between valproate free concentration and adverse effects.

CONCLUSIONS: The valproate total and free concentration was discordant in the majority of patients (70%). Increased valproate free concentration was associated with hypoalbuminemia and total valproate concentration. Clinical decisions based on total valproate concentration may be incorrect for many ICU patients. Prospective, controlled studies are needed to confirm these findings and their clinical relevance."

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Saturday October 15; 8:30 am – 10:00 am

Douglas Sawyer, MD

Mane Medical Center, MaineHealth

Chief Academic Officer

Center of Biomedical Research Excellence in Acute Care Research and Rural Disparities

Acute emergency care across rural states like Maine varies, with disadvantages and poorer outcomes for emergency events occurring in rural areas. Maine is the most rural state in the US: more than 61% of Maine's population lives in areas designated as rural, with a rural land area of almost 99%. In rural states, the need for improvements in acute care through acute care research are pressing, as medical advances have increased the health disparities between urban and rural areas. These disparities are due in large part to reduced access in rural areas to specialty-trained clinicians, resources and facilities, as well as clinical research studies. The goal of the Center of Biomedical Research Excellence in Acute Care Research and Rural Disparities (Acute Care COBRE) at Maine Medical Center/MaineHealth is to mentor acute care clinician-scientists performing research projects addressing significant clinical and translational areas of need, while developing a foundation and infrastructure for these studies to impact communities and patients in all regions of Maine and eastern New Hampshire. Three of the current research projects address clinical and translational aspects of cardiac arrest post-resuscitation care, while the fourth project involves research utilizing telemedicine to improve survival and neurological outcomes for newborns born at risk for encephalopathy in rural hospitals. The Acute Care COBRE also funds a pilot projects program to attract and support a pipeline of clinician-scientists with interests in acute care research. This cohesive and interactive group of clinician-researchers is supported by a robust mentorship and advisory network, and a Community Engagement, Bioethics, and Outreach Core that is developing state-wide health professional and community partnerships to enhance understanding of and increase inclusion in human subjects research, and to synergize with existing NIH-funded programs across our region.