



MDI Biological Laboratory

Abstracts

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MDI Biological Laboratory
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Cover image credit: Losick Lab (2018), Polyploidy, Drosophila

Abstracts Session I

Opioid Systems in Pain and Reward

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The brain's opioid system regulates both pain and reward. This presentation will examine the distinct and overlapping pathways through which opioids can inhibit pain and produce reward. Much of the overlap involves the negative emotional consequences of pain and the ability of pain relief to act as a reward. The opioid system includes mu, delta, and kappa receptors. The opposing actions of mu-opioid receptors and kappa-opioid receptors in pain and addiction will be discussed, along with changes that occur after chronic exposure to morphine. The intertwining of pain and reward systems creates particular challenges when trying to develop new medications for chronic pain that do not have the potential for addiction.

Genetics of addiction and the opioid crisis

Chesler, E.¹

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Individual differences in addiction vulnerability are driven by life history, sex differences, genetics and drug exposure. These differences play a role in all stages of addiction and can influence when and how individuals initiate drug use, the amount of drug use necessary to develop an addiction and even the preference for particular classes of drugs. These biological differences in basic behavioral characteristics, drug response and the effects of lasting drug use are evident across species. Genetic studies of addiction provide insights into the biological basis of addiction risk, the molecular mechanisms of addiction and may one day lead to advances in the treatment of addiction. Genetic studies in laboratory mice provide precise and powerful discovery of biological mechanisms of addiction vulnerability, and in the study of effects of addictive substances under carefully controlled conditions. Using new, advanced mouse populations and bioinformatics tools, it is now possible to connect findings within and across species to understand the interplay of biological variation, drug seeking, drug exposure and biobehavioral effects of drugs.

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Abstracts Session I

Actin regulation in drug addiction

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Drugs of abuse induce neuroadaptations, including synaptic plasticity, that are critical for transition to addiction, and genes and pathways that regulate these neuroadaptations are potential therapeutic targets. Using forward genetic approaches we have found members of the Rac and Rho signaling cascade regulate acute, sensitized, and self-administration of cocaine. These include WAVE Regulatory Complex member Cyfip2. Recently we discovered that actin capping protein TMOD2 regulates cocaine responses. In this talk I will present our data demonstrating with Tmod2. Tropomodulin 2 (Tmod2) is an actin-regulating gene that plays an important role in synapse maturation and dendritic arborization and has been implicated in substance-abuse in humans. Here we mine the KOMP2 data and find that Tmod2 mice show emotionality phenotypes that are predictive of addiction. Detailed addiction phenotyping showed that Tmod2 deletion does not affect the acute locomotor response to cocaine administration. However, sensitized locomotor responses are highly attenuated in these knockouts, indicating a lack of necessary drug-induced plasticity. In addition, in IVSA assays paradigm the Tmod2 mutant animals do not acquire indicating lack of hedonic responses to cocaine. Whole brain MRI imaging shows differences in brain volume across multiple regions although RNAseq analysis did not reveal perturbations in gene co-expression networks. Detailed electrophysiological characterization of Tmod2 KO neurons, showed increased spontaneous firing rate of early postnatal and adult cortical and striatal neurons. Cocaine-induced synaptic changes that are critical for sensitization were either missing or reciprocal in Tmod2 KO nucleus accumbens shell medium spiny neurons, providing a mechanistic explanation of the cocaine response phenotypes. Combined, these data provide compelling evidence that Tmod2 is a major regulator of plasticity in the mesolimbic system and regulates the reinforcing and addictive properties of cocaine.

Abstracts Session I

Understanding the Opioid Crisis: Getting to the Heart of the Matter

Ochs, L.¹

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Opioid use and overuse are a source of great concern for families and communities in Maine and throughout the United States. Together with the increasing use of illicit opioids, overuse and misuse of prescription opioids represents an epidemic that contributes to dependency, addiction, overdose and death. Much data has been reported on opioid use and misuse, including the volume of opioid prescriptions, overuse of opioid prescriptions, and prescribing trends for opioid medications in concert with other medications of potential abuse. Research will be shared regarding the unnecessary use and over use of prescription opioids and prescribing practices of these medications from a pharmacist perspective.

Abstracts Session II

Molecules and the Microbiome

O'Loughlin, C.¹

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The human microbiome is a vast, understudied community of bacteria, fungi, and viruses. Over the course of evolution we outsourced essential chemistry to these life-long companions. They carry out a multitude of tasks that keep us alive, including, but not limited to, synthesizing vitamins, breaking down food, educating our immune systems, and potentially other processes we have yet to discover. Recent work cataloging the abundance of bacterial genomes present in and our bodies has revealed that we know very little about the chemistry these bacteria carry out. Work by The Human Microbiome Project and the NIH have demonstrated that there are over 3,000 common, prevalent gene clusters in the genomes of the bacteria that reside on and in our bodies. Using bioinformatic tools to identify motifs in these gene clusters, it has become clear that a wide-range of molecules are synthesized on and in the human body. My laboratory looks to elucidate the chemical structure of these molecules, and to determine the biological role of these molecules in microbe:microbe and microbe:host interactions. By studying the gene clusters that are present on healthy adults, we hope to identify a core set of molecules and metabolites that are essential for maintaining the symbiotic relationships between these bacteria and us, their hosts. Once we understand the native chemical repertoire of these microbes, we hope to use genetic tools to expand the range of molecules that these microbes can make for a variety of applications.

Abstracts Session II

Chemical photoswitches for precise protein control

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Understanding the activity and regulation of enzymes and receptors is a major objective in molecular biology. To accomplish this, new tools that can precisely modulate the spatial and temporal function of specific proteins are in high demand. New techniques that generate detailed structural information about the shape and function of proteins are providing insights into the binding of small molecules, enabling chemists working at the biology interface to create a variety of novel molecular tools. One emerging field is photopharmacology, which combines synthetic light-responsive small molecules called photoswitches with pharmacology, the development of designer drugs targeting proteins of interest. The resulting light-dependent activity of synthetic photoswitches allows for exceptional spatiotemporal control of a wide array of native and engineered proteins. The Kienzler lab specializes in the application of photoswitches to make light-modulated tools, capitalizing on azobenzene: a synthetically tractable, photochemically resilient, and biologically tolerated chemical photoswitch. Our current projects focus on applying azobenzene containing ligands and pore blockers to cell membrane-bound protein receptors and ion channels.

Abstracts Session II

Tempering positive feedback in population models of mutualism

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Ecology is the study of the abundance and distributions of organisms. Among the multitude of factors that affect the abundance and distributions of organisms is the interactions between species. One type of interaction, mutualism, describes where two species increase each other's survival or reproduction such that fitness is increased in each other's presence. Despite mutualism being omnipresent in ecological communities, it's been ignored by theoreticians because the standard dynamic model of mutualism (known as the Lotka-Volterra mutualism model) inconveniently results in positive feedback where populations grow exponentially without bound. I will present one of several ways that I have added realistic aspects of biology into the Lotka-Volterra mutualism model. Accounting for the realism of how mutualistic benefits are received when explicitly considering the receiving species' density was driven by dissecting results from empirical studies. My meta-analysis found that most mutualistic benefits received by a species only increases fitness when the receiving species is at low densities. I subsequently developed a model to incorporate species benefitting from mutualism at low densities, high densities, and independent of density. When species receive benefits at low densities, mutualistic interactions never result in positive feedback. By adding realistic biology into the Lotka-Volterra mutualism model of mutualism, I've described mechanisms that can temper positive feedback, and I hope to have have opened up some provocative lines of investigation about implicit assumptions of commonly-used population models and the nature of modeling species interactions.

Abstracts Session II

Utilizing primary astrocytes to understand early events of a deadly and untreatable viral infection in the brain

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JC polyomavirus (JCPyV) is the causative agent of a fatal, demyelinating disease of the central nervous system (CNS). Epidemiological studies indicate that the virus is present in 50-80% of the adult population, causing a persistent infection in the kidneys of healthy people. In immunocompromised individuals, JCPyV reactivates in the CNS and infects glial cells, astrocytes and oligodendrocytes, causing the disease progressive multifocal leukoencephalopathy (PML). Currently, there is no treatment for PML, and it is fatal in 1-2 years. Until recently, the role of astrocytes in PML pathogenesis have been obscured by the noticeable histology caused from the lytic destruction of oligodendrocytes. Furthermore, JCPyV can only establish infection in human cells and thus studies of PML pathogenesis is limited to cell culture models. The in vitro studies of JCPyV infection has been mostly limited to SVG-A cells, a mixed glial cell line. However, SVG-A cells present several limitations for studying astrocyte-specific infection due to their immortalized characteristics and heterogenous population. Thus, we have established an innovative in vitro model to study JCPyV infection using primary normal human astrocytes (NHAs). Using infectivity assays, qPCR, and immunofluorescence quantification, we have demonstrated that JCPyV replication is delayed in NHAs compared to SVG-A cells. JCPyV-infected astrocytes effectively produce early viral gene products, but the production of late viral gene products is significantly delayed. These findings suggest that differential host cell factors initiate viral replication in astrocytes and SVG-A cells. Defining these differences will provide a better understanding of the role of astrocytes in PML pathogenesis.

Abstracts Session II

KEYNOTE

The Quest to Understand Lyme Disease

Bockenstedt, L.¹

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Harold W. Jockers
Professor of Medicine,
Yale University
*The Quest to
Understand Lyme
Disease*

Lyme disease, due to the Ixodes tick-transmitted spirochete *Borrelia burgdorferi*, is the most common vector-borne disease in North America. Despite public health measures, the geographic range of *Borrelia burgdorferi*-infected ticks is expanding and the incidence of Lyme disease is increasing. Humans infected with *Borrelia burgdorferi* can exhibit a variety of clinical signs and symptoms, the pathogenesis of which are incompletely understood. This presentation will describe recent advances in the pathogenesis of Lyme disease in animal models and humans.

Abstracts Session III

Origin and function of animal specific gene regulatory networks: From eyes to immunity

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This talk will present data about the evolution of genes and gene regulation from an early branching animal lineage, the sponges. We will discuss the role of some highly conserved genetic pathways deployed over the course of animal evolution leading to the diversity of animal forms and functions. For example, the Wnt signaling pathway, as well as the Pax/Six gene network are involved in numerous functions in establishing animal body axes as well as specifying body part like eyes, kidneys, and nervous systems in complex animals. We have found that these gene pathways evolved at the earliest stages of animal evolution and are involved in patterning of the sponge aquiferous system and body plan. We also found that the gene regulatory connections between the Wnt signaling pathway and the Pax/Six gene network that are known in vertebrate models were likely established before nervous and organ systems evolved. We present evidence that components of the Wnt signaling system are present in sponge stem cell populations and likely play a conserved role in initiating morphogenetic processes.

Abstracts Session III

Inhibition of TOR Pathway Activates Myogene Transcription and Enhances Health of Striated Body Muscle in *C. elegans*

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Previous studies reported that DR or low nutrient signaling reduces translation and extends lifespan, while attenuating growth and reproduction. In this study using *C. elegans* as a model organism, we found that reduced translation in neurons or germ tissues increases survival and primes the HSR against unfolded protein stress in adult animals. Surprisingly, lowering translation in these tissues significantly upregulates transcription of several key muscle maintenance genes, including those encoding the myogenic response factor HLH-1 and heavy myosin chain factor UNC-54 and MYO-3. It also improves muscle maintenance according to motility assays. However, reduced translation in muscle tissue does not activate the same level of myogenic gene expression. Instead, lowering translation in striated body muscle results in increased reproduction and accelerated development. To further investigate, we examined maintenance of muscle health via deconvolution fluorescent microscopy and immunostaining. Using computational analyses involving Fiji ImageJ and MATLAB program, we discovered that lowering translation delays decrepitude in body muscle structure, resulting in improved mitochondrial morphology and increased thermotolerance. Here we report that, combining physiological, molecular, and microscopic technique, muscle is protected during low translation, low TOR signaling, or DR. We hypothesize that results reflect evolutionary responses that preserve function required for foraging during nutrient scarcity and accelerate development during times low energy usage and high nutrient abundance.

Abstracts Session III

The promise of genetic profiling for late blight resistance in potato

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Potato is the most important agricultural crop in Maine, with a majority of the acreage in Aroostook County where the climate is conducive to the disease late blight, the source of the Irish Potato Famine. Breeding late blight resistance genes from wild species into cultivated potato is important but requires many years of introgression within the potato breeding and improvement selection process. Fortunately, DNA-based technologies have made it easier for potato breeders to quickly identify disease resistance genes in their potential new varieties. The technology is only effective if breeders know what genes are present in the parental material. Therefore, my work is focused on establishing late blight genetic profiles for popular potato breeding material which have been used across the country to deliver late blight resistance to new potato varieties. Six genes were screened in 40 varieties and clones. The resulting profiles will be of interest to both biotech and traditional breeders, and to commercial, organic, and home-gardener growers alike.

Abstracts Session III

***In vivo* translational profiling of motor neurons reveals activation of the integrated stress response through GCN2 kinase in tRNA synthetase-mediated peripheral neuropathy**

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Mutations in five tRNA synthetases cause axonal forms of Charcot-Marie-Tooth (CMT) peripheral neuropathy. Dominant mutations in glycyl-tRNA synthetase (GARS) cause CMT type 2D (CMT2D). How mutations in GARS cause neuropathy is unclear, but impaired translation has emerged as a potential toxic gain-of-function mechanism based on work with *Drosophila*. To test this mechanism in mice, we are profiling translation in motor neurons of validated CMT2D models. *In vivo*, cell type-specific, fluorescent non-canonical amino acid-tagging (FUNCAT) reveals impaired translation in mutant *Gars* motor neurons. Ribosome-tagging reveals an upregulation of transcripts associated with the integrated stress response (ISR), including *Atf4* and several of its gene targets. Using RNAScope *in situ* hybridization, we show that activation of the stress response occurs in 70% of motor neurons and does not occur in any other cell types of the spinal cord. We have also sequenced RNA from the spinal cords of mice with mutations in tyrosyl-tRNA synthetase (YARS), a model of dominant intermediate CMT type C. Among the upregulated transcripts are some of the same targets of *Atf4* that are most highly upregulated in mutant *Gars* motor neurons, suggesting that ISR activation is involved in both GARS and YARS mutations. Genetic experiments reveal that removing the ISR-activator, GCN2 kinase, from mutant *Gars* mice decreases expression of *Atf4* targets and significantly alleviates neuropathy. Because GCN2 senses translational imbalance through the presence of stalled ribosomes, we hypothesize that its activation signals impairments in translation as the primary disease mechanism in GARS and YARS peripheral neuropathy.

Abstracts Session III

Primary cilia of the cardiac neural crest orchestrate critical aspects of ventricular myocardial maturation and postnatal cardiac function

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Primary cilia are tiny, plasma-membrane bound organelles that function to modulate intra- and extracellular signaling implicated in both normal development and various disease pathologies, including congenital heart disease (CHD). Cardiac neural crest cells (CNCC) display primary cilia and function as a major progenitor cell population contributing to the developing heart. Using a *Wnt1:Cre-2*, *Ift88*-targeted conditional elimination of primary cilia and a *Td-tomato* reporter to track CNCC, we observed loss of primary cilia in CNCC at embryonic day E9.5, with notable CNCC contribution to the ventricular myocardium and disorganization of the endocardium at E10.5. The phenotype resulting from loss of cilia in CNCC was characterized by a variety of predicted CHDs in addition to disorganization of the ventricular endocardium, pronounced noncompaction of the ventricular myocardium, and perinatal lethality. To investigate potential Hedgehog signaling aspects resulting from cilia loss, we implemented an *in vivo* conditional knockout in which *Wnt1:Cre-2* drives expression of a mutant, constitutively-active Hedgehog receptor. The noncompacted ventricular myocardial phenotype observed in all *Wnt1:Cre-2/Ift88* mutants became exacerbated with the introduction of forced Hedgehog signaling in CNCC. These results support a critical role for both the primary cilium and its associated Hedgehog signaling in the formation and maturation of the ventricular myocardium. Further electrophysiological assessments of *Wnt1:Cre-2/Ift88* newborn mutant mice revealed bradycardia, conduction defects, and ECG tracings consistent with ventricular hypertrophy. Taken together, these data collectively support a role for Hedgehog signaling via primary cilia of CNCC in the pathogenesis of outflow tract defects, VSD, and most notably, ventricular maturation CHDs.