2014 MBL Undergraduate Research Symposium

Wednesday, August 20, 2014

Program Schedule and Abstracts

Sponsored by:

NSF-REU “Biological Discovery in Woods Hole” program
The Marine Biological Laboratory Office of Research & Programs and Education Office
William D. Cohen Endowed Undergraduate Research Fund
Jane C. Kaltenbach-Townsend Endowment for Undergraduate Research

Organizers:

Robert Paul Malchow-University of Illinois at Chicago
Allen Mensinger-University of Minnesota-Duluth
Emily Cardinal- University of Minnesota-Duluth

Special Thanks to

Ann Woolford, Jane MacNeil and Molly Kelleher for program support

2014 MBL Undergraduate Research Symposium
August 20, 2014, Lillie Auditorium

9:00 Opening Remarks –

Jonathan Gitlin, Deputy Director for the Office of Research & Programs, MBL

Dr. William Reznikoff, Director of Education, MBL

Session One – Robyn Crook, University of Texas Medical School at Houston

9:05 Advancing mussel farming with research in the shellfish hatchery. Patricia N. Claudio Vázquez[1], David Bailey[2] and Scott Lindell[2]. *[1]Department of Biology, University of Puerto Rico, Cayey, PR; [2]Scientific Aquaculture Program, Marine Biological Laboratory, Woods Hole, MA.*

9:20 Grounding the invasive flying carp; development of an early warning and fish detection system. C. Lee Austin[1,2] and Allen F. Mensinger[1,2]. *[1]University of Minnesota Duluth, Duluth, MN; [2]Marine Biological Laboratory, Woods Hole, MA.*

9:35 Effects of injury on *Dorytheuthis* (*Loligo) pealeii's* response to predation threats. Megumi C. Oshima[1,4], Theodor Di Pauli von Treuheim[2,4], Robyn J. Crook[3,4], Roger T. Hanlon[4] and Edgar T. Walters[3]. *[1]Coastal Carolina University, Conway, SC; [2]Union College, Schenectady, NY; [3]University of Texas Medical School at Houston, Houston, TX; [4]Marine Biological Laboratory, Woods Hole, MA.*

9:50 Serotonin evokes nociceptor hyperexcitability in *Doryteuthis (Loligo) pealeii*'s peripheral nervous system. Theodor Di Pauli von Treuheim[1,4], Megumi C. Oshima[2,4], Robyn J. Crook[3,4], Roger T. Hanlon[4] and Edgar T. Walters[3]. *[1]Union College, Schenectady, NY; [2]Coastal Carolina University, Conway, SC; [3]University of Texas Medical School at Houston, Houston, TX; [4]Marine Biological Laboratory, Woods Hole, MA.*

10:05 The effect of diet on fatty acid content and assimilation efficiencies in *Fundulus heteroclitus*. Alison Hall[1,2], James Nelson[2] and Amanda Spivak[3]. *[1]Biology Department, Carleton College, Northfield, MN; [2]Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA; [3]Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institute, Woods Hole, MA.*

10:20 Break

Session Two – Beth Giuffrida, Wareham Middle School and Marine Biological Laboratory

10:45 Behavioral analysis of nicotine-conditioned place preference in squid. Medha Biswas[1,2] and William Green[1,2]. *[1]The University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA.*

11:00 PolyQ-Htt Proline binding motif in exon 1 inhibits fast axonal transport. Daniel T. MacVeigh-Fierro[1,2], Gerado A. Morfini[2,3] and Scott T. Brady[2,3]. *[1]Worcester Polytechnic Institute, Worcester, MA; [2]Marine Biological Laboratory, Woods Hole, MA; [3]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL.*

11:15 Using squid axons to elucidate the pathogenic mechanisms of Huntington's Disease. **Nicola Kriefall[1]**, Minsu Kang[2], Scott Brady[2] and Gerardo Morfini[2]. *[1]Department of Biology, CUNY Hunter College, New York, NY; [2]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL.*

11:30 Investigating the contribution of SH3 Binding Domain interactions for toxicity in Huntington's Disease. Jennifer Purks[1,3], Gerardo Morfini[2,3] and Scott T. Brady[2,3]. *[1]Department of Biology, Georgetown University, Washington, D.C.; [2]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL; [3]Marine Biological Laboratory, Woods Hole, MA.*

11:45 Tau isoform toxicity on fast axonal transport in squid. Brenda Abdelmesih[1,4], Kristine Cox [2,4] and Scott Brady [3,4]. *[1]Hunter College, New York, NY; [2]Michigan State University, Grand Rapids, MI; [3]University of Illinois at Chicago, Chicago, IL; [4]Marine Biological Laboratory, Woods Hole, MA.*

12:00 Distribution of Tau isoforms along axonal microtubules. Meghan Pantalia[1,3], Gregory Hoeprich[2,3] and Christopher Berger[2,3]. *[1]University of Texas at Dallas, Dallas, TX; [2]University of Vermont, Burlington, VT; [3]Marine Biological Laboratory, Woods Hole, MA.*

12:15-1:15 Lunch

Session Three – Maria Gomez, Universidad Nacional, de Colombia and Marine Biological Laboratory

1:15 Doxorubicin mediates toxicity in INS-1 832/13 cells via activation of Poly (ADP-Ribose) Polymerase (PARP). **Tiffany M. Richardson[1]**, Delaine M. Zayas-Bazán Burgos[2], Joshua P. Gray[3,4] and Emma Heart[4]. *[1]Princeton University, Princeton, NJ; [2]University of Puerto Rico at Cayey, Cayey, PR; [3]US Coast Guard Academy, New London, CT; [4]Marine Biological Laboratory, Woods Hole, MA.*

1:30 Shark and zebrafish retinal cells as model systems for studying regulation of extracellular pH in the vertebrate retina. **Travis Bautista[1]**, Mark Messerli[2] and Robert Paul Malchow[3]. *[1]University of South Florida, Tampa, FL; [2]Marine Biological Laboratory, Woods Hole, MA; [3]University of Illinois at Chicago, Chicago, IL.*

1:45 Does a multiple protein binding domain regulate the G-protein cascade in phototransduction? **Gretchen A. Walch[1,2]**, Jason S. Sloan [1,3], Enrico Nasi [1,4] and Maria Gomez [1,4]. *[1]Marine Biological Laboratory, Woods Hole, MA; [2]Smith College, Northampton, MA; [3]Hunter College, New York, NY; [4]Universidad Nacional, de Colombia.*

2:00 Localizing G_q and TRP channels to elucidate the phototransduction pathway in *Loligo pealei* retina cells. **Jason S. Sloan[1,4]**, Gretchen A. Walch[2,4], Enrico Nasi[3,4] and Maria Gomez[3,4]. *[1]Hunter College, New York, NY; [2]Smith College, Northampton, MA; [3]Universidad Nacional, de Colombia; [4]Marine Biological Laboratory, Woods Hole, MA.*

2:15 Quantitative imaging of suspended animation in the zebrafish embryo. **Clara Kao[1]**, Jonathan Gitlin[2] and Shalin Mehta[2]. *[1]University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA.*

2:30 Polarized glow for microflow: Investigating shear flow in a microfluidic channel using polarized light microscopy. **Nguyen Le[1]** and Rudolf Oldenbourg[2,3]. *[1]Department of Chemical Engineering, Brown University, Providence, RI; [2]Marine Biological Laboratory, Woods Hole, MA; Department of Physics, Brown University, Providence, RI.*

2:45 Break

Session Four – Joel Smith, University of Chicago

3:00 Phosphorus partitioning and phase association in sinking marine particulates from the deep Sargasso Sea. **Shaunae Alex[1]**, Maureen Conte[2] and J.C. Weber[2]. *[1]University of Chicago, Chicago, IL; [2]Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.*

3:15 Influence of beavers on streamside vegetation in coastal New England. **Lia Tosiello[1]** and Christopher Neill[2]. *[1]Brown University, Providence, RI; [2]Marine Biological Laboratory, Woods Hole, MA.*

3:30 Plant protein content as a predictor of palatability to cattle on Naushon Island. **Emma R. Sheffield[1,2]** and Christopher Neill[2]. *[1]Eckerd College, St. Petersburg, FL; [2]Marine Biological Laboratory, Woods Hole, MA.*

3:45 Nitrogen loads to Waquoit Bay, 1990-2013: have they changed? **Caroline Owens[1]**, Ivan Valiela[2] and Anne Reynolds[3]. *[1]University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA; [3]Cape Cod Commission, Barnstable, MA.*

4:00 Patterns of nitrogen loss from agriculture in East Africa: the role of soil type. **Madeline McKenna[1]**, Katherine L. Tully[2,3] and Christopher Neill[1]. *[1]Marine Biological Laboratory, Woods Hole, MA; [2]Earth Institute at Columbia University, New York, NY; [3]University of Maryland, College Park, MD.*

4:15 The effects of eutrophication and sea level rise on root profiles in the Great Sippewissett Salt Marsh. **Rachel Folz[1]** and Ivan Valiela[2]. *[1]University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA.*

4:30 Closing Remarks – Robert Paul Malchow, University of Illinois at Chicago

Tau isoform toxicity on fast axonal transport in squid

Brenda Abdelmesih[1,4], Kristine Cox [2,4] and Scott Brady [3,4]
[1]Hunter College, New York, NY; [2]Michigan State University, Grand Rapids, MI; [3]University of Illinois at Chicago, Chicago, IL; [4]Marine Biological Laboratory, Woods Hole, MA

Tauopathies are a subset of neurodegenerative diseases where the microtubule-associated protein tau (MAPT) becomes abnormally phosphorylated and forms pathogenic filamentous inclusions in neurons and glial cells. Pathogenic forms of tau have been previously shown to disrupt fast axonal transport in neurons. Recently, we identified a regulatory region on the N-terminus of tau, specifically amino acids 2-18 called the phosphatase-activating domain (PAD). Under normal conditions, PAD is masked by a "paperclip" conformation where the C-terminus interacts with the microtubule binding repeats (MTBRs) on tau and the N-terminus with the C-terminus. One mechanism by which PAD becomes exposed is through the disruption of the paperclip conformation in the generation of tau filaments. When PAD becomes exposed, it activates a PP1-GSK3 signaling cascade that impairs kinesin-based anterograde fast axonal transport (FAT). Compromises in FAT lead to synaptic dysfunction, ultimately causing a dying back neuropathy where the distal portion of the axon degenerates toward the cell body. In the normal adult brain, there are six naturally occurring isoforms that are generated through alternative splicing equally divided into two subtypes: isoforms containing three (3R) and four MTBRs repeats (4R). In tauopathies, there is a shift in the ratio of tau isoforms and little is known about the pathological mechanisms underlying the inhibition of axonal transport among different tau isoforms. To address this question, we used isolated axoplasms from squid to measure effects on FAT comparing the two shortest tau isoforms, hTau23 (0N3R) and hTau24 (0N4R) in different conformational states (monomer vs. filament). hTau23 and hTau24 were chosen based on the largest differential in TNT1 immunoreactivity to filaments, an antibody specific to the PAD domain. Our data shows that 2μM concentrations of filamentous forms of hTau23 and hTau24 inhibit anterograde FAT similarly while equimolar monomeric forms of hTau23 and hTau24 do not inhibit FAT. These results highlight the importance of tau's conformation on PAD exposure implicating tau as an important regulator of kinesin based anterograde FAT.

Funding by NIH (STB), HHMI REU (Hunter College)

Phosphorus partitioning and phase association in sinking marine particulates from the deep Sargasso Sea

Shaunae Alex[1], Maureen Conte[2] and J.C. Weber[2]
[1]University of Chicago, Chicago IL; [2]Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA

Phosphorus is an important nutrient that often limits photosynthesis and plays several critical roles within organisms, including as an important constituent of DNA and RNA, phospholipids in cell membranes, and ATP (adenosine triphosphate). Understanding the retention of phosphorus in marine systems, phosphorus diagenesis and the removal of phosphorus through burial in marine sediments can give further insight into biogeochemical cycling of phosphorus. This research focused on the phase associations of phosphorus in deep ocean sinking particulate material at 500, 1500 and 3200m in the Sargasso Sea collected by the Oceanic Flux Program (OFP) sediment traps. We analyzed the easily-dissolvable fraction of the sinking flux and then used a sequential extraction method (SEDEX) to further determine the phase associations of the phosphorus remaining in the particulate material. The partitioning between dissolved and particulate phosphorous was quantified in the OFP samples. Six distinct (operationally-defined) reservoirs of phosphorus were quantified: (1) rapidly-dissolved phosphorus in the sample cup supernatant, (2) loosely sorbed or exchangeable phosphorus, (3) ferric (Fe3+) bound phosphorus, (4) authigenic carbonate fluorapatite (CFA) + biogenic apatite + CaCO3 bound phosphorus, (5) detrital apatite and other inorganic phosphorus, and (6) organic phosphorus. Sediments from diverse marine and estuarine settings were analyzed for comparison. We found that the easily dissolvable fraction of the P flux decreases on average from 100 to 10 µg/m2/day between 500m and 3200m depth. Preliminary SEDEX results indicate that most of the P remaining in the particulate phase is associated with the "organic phosphorus" reservoir. Results of this study will help elucidate the marine phosphorus cycle and geochemical behavior of phosphorus in the water column.

Funding by University of Chicago Jeff Metcalf Internship Program

Grounding the invasive flying carp; development of an early warning and fish detection system

C. Lee Austin[1,2] and Allen F. Mensinger[1,2]
[1]University of Minnesota Duluth, Duluth, MN; [2]Marine Biological Laboratory, Woods Hole, MA

The silver carp, one of four invasive carp species in the United States, is currently migrating north via the Mississippi river threatening native fish in Minnesota waters by outcompeting them for food supplies. Known for its jumping ability when startled, silver carp place recreational boaters in danger of being injured during collisions with airborne fish. However, early detection or accurate census of existing populations is difficult as the fish will avoid traps or nets, and reside in murky waters. By using sound and or vibration to induce jumping, an accurate assessment of the population may be possible. A small surface buoy has been developed that contains acoustic and vibrational stimuli and video recording equipment. The CB-150 data buoy is equipped with an mp3 player, amplifier, hydrophone, underwater loudspeaker, vibration apparatus, and two surface video cameras. The instrumentation is operated remotely via a 433 MHz long-range UHF radio receiver that interfaces with the onboard controller. The system is operated remotely from a boat or shore and will intermittently induce jumping through sound and/or vibrational stimuli. We anticipate this buoy will provide early warning for the vanguard species of the carp invasion as well as an accurate census of the population. In addition, we are using this technology to develop safety devices for boaters, to reduce collisions and increase boater safety in carp infested waters.

Funding by NSF-REU Biological Discovery in Woods Hole grant #1359230, Legislative-Citizen Commission on Minnesota Resources (LCCMR), and Environment and Natural Resources Trust Fund - M.L. 2014, Chp. 226, Sec. 2, Subd. 04b

Shark and zebrafish retinal cells as model systems for studying regulation of extracellular pH in the vertebrate retina

Travis Bautista[1], Mark Messerli[2] and Robert Paul Malchow[3]
[1]University of South Florida, Tampa, FL; [2]Marine Biological Laboratory, Woods Hole, MA; [3]University of Illinois at Chicago, Chicago, IL

Changes in extracellular pH in the outer synaptic layer of the retina have been suggested to underlie the establishment of receptive fields of retinal neurons. Four distinct cell types converge at the level of the outer plexiform layer (OPL): photoreceptors, horizontal cells, bipolar cells and Müller cells - all of which may contribute to the overall regulation of extracellular pH in the OPL. The goal of this study was to begin to explore pH regulatory mechanisms of horizontal cells and Müller cells from shark (*Mustelus canis*) and zebrafish (*Danio rerio*) retinas. Shark was chosen because of the large size of their horizontal cells, while zebrafish were chosen because of their previous use in studying pH regulation in the intact retina. pH-selective micro-electrodes were used in a self-referencing format to examine fluxes of extracellular pH from cells isolated using a papain dissociation protocol. Recordings were made in Ringer's solutions containing 1 mM of the pH buffer HEPES. Isolated shark horizontal cells typically displayed a standing acidic pH flux; this flux was reduced when cells were bathed in Ringer's solution in which choline replaced all extracellular sodium. Stimulation of horizontal cells with 200 µM glutamate, the likely neurotransmitter released by photoreceptors, led to a reduction in extracellular pH flux. Shark Müller cells also displayed a standing acidic pH flux, and the replacement of extracellular sodium with choline also led to a small reduction in the standing signal. Horizontal cells isolated from zebrafish were significantly smaller in size than shark horizontal cells and displayed significantly smaller standing pH fluxes. Moreover, zebrafish horizontal cells tended to display spontaneous oscillations in extracellular pH that were reduced with application of 5 mM cobalt. Müller cells of zebrafish displayed small standing pH fluxes, and removal of extracellular sodium did not significantly alter the standing pH flux. These results are the first to examine pH regulatory mechanisms from retinal cells isolated from shark and zebrafish. Future experiments examining pH fluxes from photoreceptors and bipolar cells will be needed to help further clarify the mechanisms regulating pH in the outer retina.

Funding by NSF grants 1359230 ("REU Site: Biological Discovery in Woods Hole"), 0924372, & 0924383

Behavioral analysis of nicotine-conditioned place preference in squid

Medha Biswas[1,2] and William Green[1,2]
[1]The University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA

Mice and zebrafish have previously been used to study nicotine effects through molecular analysis or conditioned place preference assays. Squid hatchlings may provide further insight into the complexity of addiction due to their simplistic neural circuitry compared to the other organisms studied. In order to determine whether squid could serve as a viable model for drug abuse, we employed a nicotine induced conditioned place preference assay. It was hypothesized that nicotine addiction would be quantifiable by the number of animals in proximity to a nicotine plug over time. For conditioning, stage 30 squid hatchlings (n=45) were placed in solutions of filtered sea water and nicotine: 0uM (control), 5uM (low-dose), and 10uM (high-dose) for 2 hour. Squid behavior was observed (t=60 min) in large petri dishes (p1000) with a partial barrier dividing one half containing a nicotine plug from the other half with a control plug thus allowing the animals to move freely in both areas. From the general movement of the animals taken at 5 minute time points, it appears the animals show a preference for nicotine regardless of initial exposure to the drug.

Funding by Metcalf grant and the University of Chicago

Advancing mussel farming with research in the shellfish hatchery

Patricia N. Claudio Vázquez[1], David Bailey[2] and Scott Lindell[2]
[1]Department of Biology, University of Puerto Rico, Cayey, PR; [2]Scientific Aquaculture Program, Marine Biological Laboratory, Woods Hole, MA

Aquaculture, the rearing of aquatic organisms for food, yields about half of the seafood consumed globally by humans each year. As the demand for aquacultured goods increases, it becomes essential that the cultivation techniques be refined in order to increase sustainability and reduce environmental impact. The cultivation of mussels is a highly productive sector of the shellfish culture industry internationally, and while it is an expanding opportunity in the northeastern U.S., imports still outpace local production by 10 to 1. The focus of this study is to research ways to improve mussel aquaculture in New England, both environmentally and financially.

Traditionally, blue mussel (*Mytilus edulis*) seed is gathered by dragging from wild mussel beds but recently, concerns have a risen in many countries about the over-exploitation of beds that serve other ecological functions. In many regions, mussel seed may no longer be collected from wild mussel beds and must rely on natural spat settlement on collecting ropes. The efficiency of collecting spat on ropes is seasonally dependent on the spawning cycle of the mussels, and on occasion there may be a shortage of mussel seed. At the MBL, hatchery techniques are being developed in order to supplement or even replace the use of seed from wild origin. Hatcheries ensure a consistent and high-quality seed supply and may soon be more cost-effective than wild-caught seed.

Aquaculture research on mussels at the MBL is focused on spawning, feeding and settlement biology. By means of feeding and water temperature control, we have been able to induce mussels to spawn out of season. Larvae were fed live microalgae until settlement on two types of ropes. Feed and growth studies of post-settled spat were conducted to determine whether alternative diets such as algal paste or freeze-dried algae are more cost-effective than the standard live microalgal diet. Ultimately, these studies are all part of a greater goal to support the expansion and diversification of the northeastern shellfish culture industry.

Funding by NSF-REU Biological Discovery in Woods Hole grant 1359230 and National Institute of Health grant R25 GM059429 through the Research Initiative for Scientific Enhancement Program

Serotonin evokes nociceptor hyperexcitability in *Doryteuthis (Loligo) pealeii*'s peripheral nervous system

Theodor Di Pauli von Treuheim[1,4], Megumi C. Oshima[2,4], Robyn J. Crook[3,4], Roger T. Hanlon[4] and Edgar T. Walters[3]

[1]Union College, Schenectady, NY; [2]Coastal Carolina University, Conway, SC; [3]University of Texas Medical School at Houston, Houston, TX; [4]Marine Biological Laboratory, Woods Hole, MA

Peripheral injury imposes physical costs that can negatively affect an animal's survival. It would be advantageous therefore, if recognition of injury could take place on a cellular level at afferent neurons, which would then influence behavioral change accordingly. Through previous studies with the model organism of this study, *Loligo pealeii*, it had been shown that these animals possess nociceptors; yet mechanisms of nociceptor neural plasticity in squid and their role in memory of injury remain unknown. Response to injury should be evident on a cellular level through sensitization of high threshold nociceptors and mechanoreceptors alike. Similar studies in the gastropod *Aplysia* have already identified 5-HT (serotonin) as being an endogenous signal of injury and inflammation that has successfully evoked increased nociceptor responsiveness to noxious stimuli. Here we investigate the role of 5-HT in mediating nociceptor hyperexcitability in squid. Suction electrode recordings were made from an excised fin nerve branch, while the fin was superfused by seawater externally and exposed to an acute 5-HT perfusion internally through the vascular system. Applications of calibrated mechanical stimuli before and after 5-HT application produced a significant change in neural activity. We show that acute perfusion of 5-HT to the site of stimulation (a) elicits hyperexcitability of high-threshold nociceptors, (b) decreases the threshold at which the nociceptors fire, and (c) evokes sensitization of low-threshold mechanoreceptors. Combining results from previous studies of *Aplysia* and our new findings with *Loligo*, this ubiquitous peripheral inflammatory signal can be concluded to be well-preserved in the signaling system among these two somewhat dissimilar invertebrates. Furthermore this finding stimulates discussion of 5-HT's role in the invertebrate nervous system with respect to neural plasticity and short-term memory of injury or inflammation.

Funding by NSF-REU Biological Discovery in Woods Hole grant #1359230, NSF IOS-1145478

The effects of eutrophication and sea level rise on root profiles in the Great Sippewissett Salt Marsh

Rachel Folz[1] and Ivan Valiela[2]
[1]University of Chicago, Chicago, IL; Marine Biological Laboratory, Woods Hole, MA

In recent decades, eutrophication and sea level rise have exposed coastal ecosystems to a new suite of challenges. Salt marshes provide a number of important ecological functions including coastal protection and carbon sequestration, but there have been recent significant global reductions in salt marsh area. In this study we assess the relative effect of sea level rise and increased eutrophication on salt marshes by taking measurements in salt marsh plots that have been experimentally subjected to increased nutrient inputs for four decades in the Great Sippewissett Marsh. We sampled sediment cores from a gradient of elevations from creek bank up the marsh platform in a control plot and fertilized plot. Below-ground biomass of both live and dead roots and rhizomes of the dominant salt marsh grass Spartina alterniflora decreased with below-ground depth in control and fertilized plots, but the decrease was greater where nutrients were added. Percentage of sediment in cores decreased with elevation, and increased with eutrophication level. Dead biomass was found throughout the core but generally decreased with depth below-ground in the control samples and increased with depth in the nutrient enriched samples. Live roots and rhizomes of S. alterniflora maintain sediment coherence in the ecosystem. As sea level rise takes place, marsh surfaces will be increasingly submerged. The results reported here indicate that sediment lower in the intertidal range, and in enriched marsh areas will be weakened and susceptible to creek-bank slumping and erosion of the marsh platform. Sea level rise and eutrophication may both contribute to the decline of salt marsh habitats.

Funding by Jeff Metcalf Internship Program

The effect of diet on fatty acid content and assimilation efficiencies in *Fundulus heteroclitus*

Alison Hall[1,2], James Nelson[2] and Amanda Spivak[3]
[1]Biology Department, Carleton College, Northfield, MN; [2]Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA; [3]Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institute, Woods Hole, MA

Fundulus heteroclitus is a species of killifish that is abundant in Atlantic tidal marshes. Mummichogs play an important role in the ecosystem as both a secondary consumer and a prey species. Mummichogs are considered omnivores. However, a comparison of assimilation efficiency values between diets of algae, protein, and detritus has not been done before. We examined the fatty acid and assimilation efficiencies of mummichogs given four different diets: a protein diet consisting of crickets, marsh detritus, naturally growing algae, and a diet consisting of mix of the three. Mummichogs that consumed cricket appear to be both longer and weigh more than mummichogs that consumed algae, detritus, or a mixed diet. Quantifying and comparing the assimilation values will allow us to better understand the trophic dynamics at play in salt marsh ecosystems.

Funding: NSF-REU Biological Discovery in Woods Hole grant #1359230

Quantitative imaging of suspended animation in the zebrafish embryo

Clara Kao[1], Jonathan Gitlin[2] and Shalin Mehta[2]
[1]University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA

Confronted with limited oxygen or nutrient availability, numerous organisms demonstrate evolutionarily conserved responses, arresting fundamental biological processes to achieve a physiological state termed suspended animation. Although this unique defense strategy that permits adaptation and survival by "turning down to the pilot light" has been extensively studied for more than a century, the mechanisms determining this extraordinary biological process remain largely unknown. A striking example of suspended animation of relevance to human biology and disease is observed in the vertebrate zebrafish *Danio rerio*, where developing embryos with established cardiac function, blood flow and central nervous system activity can completely arrest development for an extended period in anoxia and then subsequently rapidly recover, complete development and achieve normal reproductive lifespans. Importantly, this suspended animation can be achieved in normoxic embryos utilizing inhibitors of oxidative phosphorylation, revealing that mitochondrial energy production rather than molecular oxygen serves as the proximate signal for this process. In this study, we observe and quantify the changes in molecular structure during this reversible anoxic arrest. Through the use of KCN, a cytochrome c oxidase inhibitor, we are able to chemically induce suspended animation, paralleling the shutdown of mitochondrial oxidative phosphorylation and ATP production in an anoxic environment. Using advanced imaging techniques, we explore the structures in play as the developing zebrafish embryo senses anoxia, enters a state of suspended animation, and recovers with an entirely normal developmental program. We quantified architectural changes in embryos by imaging transmittance, optical anisotropy of tissue, and actin fluorescence. While untreated embryos display a consistent rate of change across all channels, embryos treated with KCN show changes in actin concentration and anisotropy at a molecular level. Imaging of somite regions display little to no growth during arrest; however, resumption of growth quantitatively similar to that of a normal embryo, in recovery. This pattern suggests muscle growth as an indicator of developmental staging. Molecular changes within the somite and brain regions demonstrate the adaptive abilities of zebrafish to maintain viable biological pathways despite environmental stresses. Understanding how such perturbations in energy homeostasis are tolerated and how these unique signals coordinate the sensing of energy status and adaptation to oxygen availability will provide new insights into the role of these pathways in vertebrate embryogenesis and may permit the development of novel therapeutic approaches in disorders of altered energy homeostasis including mitochondrial failure, cancer and neurodegenerative disease.

Funding by NIH DK 44464 (JDG), HFSP LT000096/2011-C (SM)

Using squid axons to elucidate the pathogenic mechanisms of Huntington's Disease

Nicola Kriefall[1], Minsu Kang[2], Scott Brady[2] and Gerardo Morfini[2]
[1]Department of Biology, CUNY Hunter College, New York, NY;
[2]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL

Huntington's Disease (HD) is an adult-onset autosomal dominant neurodegenerative disease that leads to the loss of motor neurons and cognitive decline. HD originates from a CAG-repeat mutation in the Huntingtin (Htt) gene, resulting in an expansion of poly-glutamine region in the Htt protein (polyQ-Htt). While it is known that those with over 35 glutamines in this region show symptoms of HD, the connection between the CAG-repeat and the symptoms of HD is unclear. A significant pathogenic event of HD is loss of axonal connectivity and deficits in fast axonal transport (FAT). Previous work from our lab has shown that the perfusion of mutant Htt constructs with over 35 glutamine residues within the polyQ region in the axoplasms from squid giant axons inhibits both anterograde and retrograde FAT, while perfusion of wild-type Htt does not have an effect. Here, we present biochemical evidence suggesting that introduction of polyQ-Htt leads to the abnormal activation of a mitogen-activated protein kinase (MAPK) cascade within axons. Immunoblots of squid axoplasms perfused with polyQ-Htt showed increased activation of c-Jun N-terminal kinase 3 (JNK3) and its upstream kinase MAP kinase kinase 7 (MKK7), compared to sister axons perfused with Htt constructs with less than 35 glutamine residues within the polyQ region. This provides a potential mechanism of the evident disruption of FAT, since activated JNK3 phosphorylates kinesin heavy chains (KHC), leading to dissociation of conventional kinesin, the molecular motor protein that drives anterograde FAT, from microtubules.

In summary, our data show that the polyQ-Htt leads to dysregulation of MAPK pathway, resulting in inhibition of kinesin-based anterograde FAT. Further experiments aim to elucidate the protein-protein interaction between Htt protein and mixed linage kinases (MLKs), upstream kinases of MKK7, and their role in FAT deficits in HD pathology.

Funding by the Howard Hughes Medical Institute

Polarized glow for microflow: Investigating shear flow in a microfluidic channel using polarized light microscopy

Nguyen Le[1] and Rudolf Oldenbourg[2,3]

[1]Department of Chemical Engineering, Brown University, Providence, RI; [2]Marine Biological Laboratory, Woods Hole, MA; [3]Department of Physics, Brown University, Providence, RI

Earlier research on flow birefringence has shown the orientation effect on molecules in shear flow between concentric cylinders. In this project, we aimed to establish some experimental and theoretical approaches for modeling the relation between birefringence and shear flow observed in microfluidic devices. Potential applications include investigating molecular dynamics in blood flow and polymerization/ depolymerization inside a microreactor.

In this project, a microfluidic channel of PDMS on cover glass was fabricated with a height of 100µm and a width of 80µm. Loaded inside the channel was 1 mg/ml solution of filamentous bacteriophage (fd) suspended in 8.0 pH Tris-Sodium Hydroxide buffer. A syringe pump was used to induce flow inside the channel at flow rates ranging from 0.1ml/hour to 15ml/hour. The sample was observed with a polarizing microscope equipped with LC-PolScope components[1] to map the retardance and slow axis orientation of the flow birefringence in the channel at the resolution of the imaging optics (~1µm, 4x/0.13NA objective). Static birefringence in the PDMS material was subtracted from the sample image to reveal flow birefringence only. The flow birefringence of fd solutions, as recorded in LC-PolScope images, was measured as a retardance profile across the channel width for several flow rates (Fig.1). Using Matlab, we also simulated a shear profile by averaging the shear along the channel height, using well established analytic expressions for the shear flow in a circular channel cross section[2].

The data acquired show that near the middle of the channel, the retardance is lowest and increases towards the wall. At the wall, the measured retardance is unreliable because optical diffraction and geometric distortions near the walls compromised the image data.

We also simulated the flow birefringence, using the simplifying assumption that the birefringence is proportional to the shear at each positon in the channel cross section. We then estimated the retardance profile by averaging the shear along the height for each position along the diameter of the channel. The average shear profile, confirms the overall shape of the retardance profile, including the minimum in the center of the channel and increasing average shear towards the channel walls.

These data allows us to recognize a correspondence between the birefringence distribution and the shear profile across the channel. Specifically, the birefringence dropped as we examined area closer to the midline of the channel. In the future, we plan to observe retardance profiles behind partial channel obstructions (stenosis) which will trigger distrotions in the fluid flow. We also aim to model the impact of flow on the orientation of the fd particles, which causes the flow birefringence.

Funding by Brown-MBL Partnership, Brown LINK Award

PolyQ-Htt Proline binding motif in exon 1 inhibits fast axonal transport

Daniel T. MacVeigh-Fierro[1,2], Gerado A. Morfini[2,3] and Scott T. Brady[2,3]
[1]Worcester Polytechnic Institute, Worcester, MA; [2]Marine Biological Laboratory, Woods Hole, MA;
[3]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL

Adult Onset Neurodegenerative Diseases have long been a great topic for research as there is no effective treatment for them to this day. As the name suggests the diseases damage the connections between neurons but these symptoms only appear in mid to late life. Huntington's Disease (HD) is no different; symptoms appear between the ages of 30-50 and progress for 10-25 years before death. HD was found to be a familial disease that results from a genetic mutation in the huntingtin (HTT). The mutation occurs in expansions in a polyglutamine (polyQ) region of the gene; any more than 35 repeats and HD is developed. Studies using polyQ-Htt in axon motility assays showed that the toxic protein inhibited fast axonal transport (FAT). Questions arose concerning how the toxic protein inhibited FAT. Pharmacological and biochemical experiments showed polyQ-Htt activated a kinase pathway involving MAP and JNK kinases, specifically ending with JNK3, an isoform of JNK that is expressed in the neurons of the CNS. When the JNK3 kinase was activated it would phosphorylate Ser176 from kinesin-1 motor domain. This prevented kinesin from binding to microtubules thereby inhibiting FAT. Attention then shifted to what part of the polyQ-Htt gene causes this cascade. Experiments revealed that the region responsible for the toxicity was a proline rich region adjacent to the polyQ region. These studies implicated a common motif of polyprolines as a potential toxic element. However, further research using a combination of biochemistry and a squid axon motility assay on variants of the polyprolines in polyQ-Htt determined that the polyproline were not the toxic element. The studies lead to implications that the toxic element may be a proline related binding domain close to the polyproline region.

Funding by NSF-REU Biological Discover in Woods Hole grant #1359230

Patterns of nitrogen loss from agriculture in East Africa: the role of soil type

Madeline McKenna[1], Katherine L. Tully[2,3] and Christopher Neill[1]
[1]Marine Biological Laboratory, Woods Hole, MA; [2]Earth Institute at Columbia University, New York, NY;
[3]University of Maryland, College Park, MD

Fertilizer use is increasing in sub-Saharan Africa (SSA) in response to interventions at the national and international level. The Alliance for a Green Revolution in Africa (AGRA) has called for a six-fold increase in fertilizer application by 2015 in order to reduce poverty and malnutrition across SSA. Although fertilizer application will surely improve yields, associated environmental consequences (such as nitrogen loading in surface and groundwater) is difficult to predict as (1) soils in this region have been cultivated for centuries with little to no amendments, (2) are highly degraded, and (3) because very few field studies have investigated the fate of nitrogen losses from SSA agricultural systems. We found that in the absence of fertilizers, high clay soils in Kenya showed a decline in extractable nitrates (NO3) and ammonium (NH4) with increasing depth in the soil profile. Fields receiving high rates of fertilizer showed lower extractable NO3 concentrations (2M KCl) than the untreated soils. However, extractable NO3 concentrations were highest at 250 to 300 centimeters below the soil surface. In contrast, we found that adding high amounts of fertilizer (200 kg N/ha/yr) to low-clay Tanzania soils led to high extractable NO3 concentrations in the top 150 cm of soil. Other less intense treatments made little difference in the overall concentrations of extractable NO3. Extractable NH4 only made up a small percentage of total nitrogen in both Kenya and Tanzania, and the concentration of NH4 was similar in fertilized and unfertilized soils. Our data provides much-needed information on soil biogeochemistry and will help inform improved farm management practices in these understudied regions.

Funding by NSF PIRE - Land Use, Ecosystem Services and Human Wellbeing: Responses to Millennium Village Interventions; IIA 0968211

Effects of injury on *Dorytheuthis* (*Loligo) pealeii's* response to predation threats

Megumi C. Oshima[1,4], Theodor Di Pauli von Treuheim[2,4], Robyn J. Crook[3,4], Roger T. Hanlon[4] and Edgar T. Walters[3]

[1]Coastal Carolina University, Conway, SC; [2]Union College, Schenectady, NY; [3]University of Texas Medical School at Houston, Houston, TX; [4]Marine Biological Laboratory, Woods Hole, MA

Animals often experience sublethal injuries in the wild that make them more vulnerable to predation. Previous studies have shown that injured squid are more likely to be targeted by predators but this risk can be offset partially by quicker flight response in the presence of a threat. However, how injury can affect one of the most common behavioral responses to predation threats, schooling or shoaling, has not been examined. The objective of this study was to determine the effect of minor, peripheral injury on squid's schooling behavior. In order to test this, we looked first to see if an injury changed the likelihood that the squid would school with others without injury in a safe environment and then in the presence of a predation threat. Firstly, one fin was injured by applying a brief pinch with grooved forceps and the squid's schooling behavior was monitored over a 24-hour period. Next, low-level and high-level predation threats were introduced to the tank and changes in the behavior of the individual (injured) squid and the larger school were monitored over a 24-hour period. An olfactory cue, water taken from a tank heavily stocked with predatory fish, was used as the low-level threat and a visual cue, a fish model, was used for the high-level threat. Thus far, there is evidence of certain schooling trends emerging. In the presence of a low-level threat, the squid were less likely to form a school. In the presence of both low-level and high-level threats, injured squid were less likely to be in the school for the entire observational period. When it was in the school, the injured squid was less likely to be on the edge of the school 24 hours after injury, with or without a threat present. Lastly, if the squid was on the edge of the school, when a threat was introduced it was less likely to have its injured fin orientated away from the school. The tendency of the injured squid to spend more time in the middle of the school than on the edge when a predator is present suggests that squid are cognitively aware of injuries and adjust their behavior accordingly. This claim is also supported by the injured squid being more likely to keep the injured fin towards the school, minimizing the exposure of its weakness to the threat. Evolutionarily, these changes would be advantageous in helping injured animals survive during the period after an injury when their predation risk is highest. Understanding a squid's ability to make such cognitive decisions can provide further insight into how squids perceive and deal with injury.

Funding by NSF-REU Biological Discovery in Woods Hole grant #1359230, NSF IOS-1145478

Nitrogen loads to Waquoit Bay, 1990-2013: have they changed?

Caroline Owens[1], Ivan Valiela[2] and Anne Reynolds[3]
[1]University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA; [3]Cape Cod Commission, Barnstable, MA

Nitrogen (N) is the limiting nutrient for most coastal ecosystems. Excess nitrogen leads to eutrophication and anoxic conditions, a major environmental concern in estuarine systems. Nitrogen loads to estuaries depend not only on anthropogenic changes in land covers and uses within the watershed (which affect contributions from fertilizers and wastewater), but also on changes in the delivery of atmospheric nitrogen deposition. In this study, we examined whether the changes in land covers and in atmospheric deposition of nitrogen that took place between 1990 and 2013 altered N loads to the Waquoit Bay estuarine system. Data on land covers and on nutrients in atmospheric deposition for 1990 and 2013, input to a well-validated nitrogen loading model, showed that over the study period N contributions from wastewater and lawn fertilizer increased significantly. These increases were offset by a 50% reduction in atmospheric N deposition recorded across the Northeast, resulting in total N loads to the bay that did not change significantly between 1990 and 2013.

Funding by Ecosystems Center and Jeff Metcalf Internship Program

Distribution of Tau isoforms along axonal microtubules

Meghan Pantalia[1,3], Gregory Hoeprich[2,3] and Christopher Berger[2,3]
[1]University of Texas at Dallas, Dallas, TX; [2]University of Vermont, Burlington, VT; [3]Marine Biological Laboratory, Woods Hole, MA

The neuron is a highly polarized cell with long extensions known as axons that extend far distances from the cell body and interact with other cells. Intracellular cargo synthesized in the cell body must therefore be transported long distances to their destinations within the axon along microtubule tracks using motor proteins such as kinesin. Our lab is interested in studying how this process of axonal transport works, as it is essential to the survival of neurons. Tau is a microtubule-associated protein known to be vital in the process of axonal transport, and dysfunction in Tau can result in a number disease states including Alzheimer's, Parkinson's, and Huntington's. Tau is known to stabilize the microtubule lattice and has been implicated in a number of other axonal functions including the regulation of kinesin motor movement, but the mechanisms by which Tau carries out its varied functions are not well understood.

In humans there are six different isoforms of Tau alternatively expressed from a single gene on Chromosome 17. Tau can have either three (3R-Tau) or four (4R-Tau) microtubule binding repeats in the C-terminal half of the molecule and zero, one, or two acidic inserts in the N-terminal half. The isoforms of Tau are likely to be functionally different, and imbalances of 3R- and 4R-Tau expression are well documented to play a direct role in the onset of a number of dementias. In addition to differential expression of 3R- and 4R-Tau, localization may also give insight as to the function of these different isoforms. In this study, we are using immunofluorescence to stain neurons from primary rat hippocampal cell culture for 3R- and 4R-Tau isoforms and high resolution images will be obtained using confocal microscopy. This method allows us to examine the distributions of 3R- and 4R-Tau along the axon, and provides important clues as to their respective functions within the neuron. Preliminary data show that both 3R- and 4R- Tau appear punctate along the axon of rat hippocampal neurons, suggesting non-uniform localization and specialized functions of both isoform classes.

Funding by NSF-REU Biological Discovery in Woods Hole (Grant #1359230) and NIH R01 (GM 101066) to C.B

Investigating the contribution of SH3 Binding Domain interactions for toxicity in Huntington's Disease

Jennifer Purks[1,3], Gerardo Morfini[2,3] and Scott T. Brady[2,3]
[1]Department of Biology, Georgetown University, Washington, D.C.; [2]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL; [3]Marine Biological Laboratory, Woods Hole, MA

Huntington Disease (HD) is an autosomal dominant genetic neurodegenerative disease with advancing cognitive, motor, functional, and psychiatric symptoms eventually leading to death. Huntingtin, the protein implicated in HD, shows expansion of a poly-glutamine (polyQ) tract in the N-terminus. When the number of glutamines in the poly-Q region exceeds 36, HD pathology is observed, but the exact mechanism of neuronal toxicity is not understood and there is no effective treatment. Previous experiments in isolated axoplasm indicate that pathogenic Htt inhibits anterograde and retrograde transport and exerts its toxic effect on axonal transport by activating JNK3 via activation of a mitogen-activating protein kinase kinase kinase (MAP3K). JNK3, when activated, phosphorylates kinesin heavy chain (KHC) at Ser 176, which causes kinesin motors to detach from microtubules thereby inhibiting kinesin-based anterograde axonal transport. Activation of some MAP3K pathways requires release of an autoinhibitory conformation. In their inactive conformation, MAP3Ks are autoinhibited due to sequestering of the kinase domain through intramolecular interactions between SH3 domain and SH3-binding domain. Proteins with SH3-binding domains may be able to activate these MAP3Ks by competing with the intramolecular SH3-binding domain and releasing the autoinhibition of MAP3Ks. Htt, when mutated, exposes its proline rich domains (PRDs). HD's toxic element has been narrowed down to two of these PRD subdomains within exon 1 as PRD1 and PRD3, but not PRD2 inhibit fast axonal transport. The PRDs contain both poly-P and SH3-binding domains whose consensus motif is PxxP. We propose that exposure of one or more of these P-containing motifs may play a role in activation of the JNK3 MAP kinase pathway in HD. Perfusing squid giant axons with recombinant Htt with 55 glutamines in polyQ region (Htt-Q55) results in inhibition of both anterograde and retrograde axonal transport. Interestingly, addition of polypeptides corresponding to an SH3 domain along with Htt-Q55 rescued both directions of transport, while addition of a mutant SH3 (Y52A) failed to rescue axonal transport. These findings strongly suggest that an SH3-binding domain within Htt is responsible for its toxic effect on axonal transport. Future experiments with constructs that eliminate specific PxxP motifs in PRD1 and PRD3 may elucidate if PxxP is necessary and sufficient for toxicity in HD.

Funding by The CHDI Foundation

Doxorubicin mediates toxicity in INS-1 832/13 cells via activation of Poly (ADP-Ribose) Polymerase (PARP)

Tiffany M. Richardson[1], Delaine M. Zayas-Bazán Burgos[2], Joshua P. Gray[3,4] and Emma Heart[4]
[1]Princeton University, Princeton, NJ; [2]University of Puerto Rico at Cayey, Cayey, PR; [3]US Coast Guard Academy, New London, CT; [4]Marine Biological Laboratory, Woods Hole, MA

Exposure to chemotherapeutic agents has been linked to the increased risk of type 2 diabetes (T2D), a metabolic disease characterized by both the peripheral insulin resistance and impaired glucose-stimulated insulin secretion (GSIS) from pancreatic beta cells. Using clonal rat pancreatic beta cell line, INS-1 832/13 cells, we investigated the effects of the chemotherapeutic drug doxorubicin (adriamycin) on pancreatic beta cell survival and function. Exposure of INS-1 832/13 cells to doxorubicin caused impairment of GSIS as early as 1 hour post-exposure to this drug. In order to investigate the underlying mechanism, we analyzed the effects of time- and dose-dependency of doxorubicin exposure on beta cell redox status, cellular viability and toxicity. Toxicity (measured as leakage of an intracellular protease and cell titer blue reduction) and apoptosis (measured as caspase 3/7 enzymatic activity) were both significantly increased after 6 h of doxorubicin exposure; glucose utilization was also impaired. Oxidative stress did not play a major role in the induction of toxicity and apoptosis, as doxorubicin failed to undergo redox cycling and appreciably increase H_2O_2 levels in INS-1 832/13 cells. Doxorubicin was reduced in vitro by cytoplasmic fractions in a NADPH-dependent manner, indicative of bioreductive activation of this compound. In live INS-1 832/13 cells, doxorubicin caused a significant decrease in the total NADH/NAD^+ pool , consistent with the activation of the poly-ADP ribose polymerase (PARP) pathway, which consumes NAD^+ to repair DNA damage and leads to programmed cell death if activated in excess. Treatment of INS-1 832/13 cells with the PARP inhibitor MK-4827 offered protection of these cells from doxorubicin, ameliorating doxorubicin-dependent reductions in cell viability and total NADH/NAD^+ . These data suggest that PARP activation rather than oxidative stress induction is the major mechanism of toxicity of doxorubicin in INS-1 832/13 cells.

Funding by NSF-REU Biological Discover in Woods Hole grant #1359230

Plant protein content as a predictor of palatability to cattle on Naushon Island

Emma R. Sheffield[1,2] and Christopher Neill[2]
[1]Eckerd College, St. Petersburg, FL; [2]Marine Biological Laboratory, Woods Hole, MA

The grasslands on Naushon Island were historically maintained by disturbance from a large herd of free-roaming sheep. With the arrival of coyotes in the 1980s and the subsequent loss of the herd, shrubland consisting largely of *Smilax rotundifolia* and *Gaylussacia baccata* (black huckleberry) has expanded upon much of the historical grasslands. The introduction of grazing cattle as well as mowing are currently being studied as potential methods of restoring and maintaining grasslands on Naushon. Using percent nitrogen as a proxy, we analyzed the protein content of five plant species common on Naushon in order to predict their palatability to cattle. Samples were taken in June and July to detect differences in protein content between early and mid-growing season (late season samples have not yet been collected), and the two shrub species were also collected from areas that had been mowed the prior year in order to examine the effect of mowing. The two grasses, *Carex pensylvanica* and *Schizachyrium scoparium* were found to be similar in protein content to each other and to huckleberry, while *Smilax* was higher in protein ($p < 0.001$) and the forb *Euthamia caroliniana* was higher still ($p < 0.01$). This suggests that these two shrub species should be palatable to cattle based on protein content. However, we have observed that the cattle avoid eating *Euthamia*, and as this species is fragrant when crushed it is likely that it contains unpalatable secondary compounds. Huckleberry decreased in protein content in mid- compared to early season ($p = 0.015$), and was the only species to show significant change. Mowed *Smilax* appears to be higher in protein than unmowed ($p = 0.07$). This may indicate that a combination of mowing and cattle grazing may have more potential as a method of managing this shrub species than either treatment alone.

Funding by the Beech Tree Trust

Localizing G_q and TRP channels to elucidate the phototransduction pathway in *Loligo pealei* retina cells

Jason S. Sloan[1,4], Gretchen A. Walch[2,4], Enrico Nasi[3,4] and Maria Gomez[3,4]
[1]Hunter College, New York, NY; [2]Smith College, Northampton, MA; [3]Universidad Nacional, de Colombia;
[4]Marine Biological Laboratory, Woods Hole, MA

Photoreceptors have proven invaluable for the study of signal transduction pathways in animal cells. While the pathways involved in the phototransduction cascade have been mapped out in detail in a number of species, the specific molecular arrangements and interactions are less well known. In invertebrates, photons induce a conformational change in rhodopsin, which causes the dissociation of $G_{q\alpha}$ from the heterotrimeric G-protein G_q, which continues through a complex phototransduction cascade until the cation-permeable light-sensitive transient receptor potential (TRP) channel is activated, causing the cation influx that depolarizes the photoreceptor. A significant amount of amplification occurs during each step in this process, which is the fastest known instance of signal transduction in an organism (20 ms). InaD, a protein with multiple PDZ binding sites, comprises the scaffold that affords this process its unprecedented speed, although not much is known about the 'transducisome' formed via the scaffold. TRP is known to bind to PDZ sites and is thought to anchor InaD to its location near the cell membrane, while G_q is the first signaling molecule of the phototransduction pathway. Using the recently uncovered *Loligo pealei* retina transcriptome, we were able to repurpose an antibody to bind the putative *L. pealei* TRP channel. Through immunostaining and western blotting, we can determine the locations of $G_{q\alpha}$ and TRP channels in *L. pealei* retina cells and confirm whether their functions are conserved throughout invertebrates. Furthermore, by defining the intracellular locations of $G_{q\alpha}$ and TRP, it is possible to identify the InaD homolog in *L. pealei* and assess its role in the signal transduction pathway.

Funding by Hunter College HHMI Summer Program

Influence of beavers on streamside vegetation in coastal New England

Lia Tosiello[1] and Christopher Neill[2]
[1]Brown University, Providence, RI; [2]Marine Biological Laboratory, Woods Hole, MA

Beavers have a long and relevant history in New England. Once abundant in the past, beavers were decimated by European settlers for the fur trade. Post-World War II beavers began to recolonize New England. That recolonization was accelerated in Massachusetts when leghold traps were banned in 1996. Beaver activity has important impacts on the landscape as they impound water, remove trees and create wetlands. Increased beaver population has become an important issue because beaver dams may cause flooding in human developed areas. The increase of beavers may have important influences on forest structure, particularly near their preferred stream habitats. It is also important to understand the effects of beaver re-colonization so humans can coexist with beavers and determine the most effective management strategies. This project studied the ways in which beavers affect streamside vegetation. On Cart Creek in Newbury, MA, ten 20 x 20 m plots were set up in the forest along the stream. Six plots were located in a beaver-active area, a stretch of stream approximately 700 m long that had two lodges and seven beaver dams. Four plots were in a beaver-absent area, a separate reach of stream about 300 m long with no dams or lodges. In each plot all the trees were identified and measured for diameter at breast height. Stumps and trees chewed by beavers were noted. The beaver-absent plots averaged 8.5 tree species per plot, and the beaver-active plots averaged 7.7 species per plot. There were significantly more stumps and chewed trees in the beaver-active plots. The plots showed differences in the abundances of species present. The beaver-absent plots averaged 81.5 total trees and a tree basal area of 34.1 m^2/ha, while the beaver active plots averaged 50.8 trees per plot and a basal area 36.2 m^2/ha. This information can help us understand how beavers might alter forest structure as they continue to increase in New England.

Funding by Brown University Undergraduate Teaching and Research Award

Does a multiple protein binding domain regulate the G-protein cascade in phototransduction?

Gretchen A. Walch[1,2], Jason S. Sloan [1,3], Enrico Nasi [1,4] and Maria Gomez [1,4]
[1]Marine Biological Laboratory, Woods Hole, MA; [2]Smith College, Northampton, MA; [3]Hunter College, New York, NY; [4]Universidad Nacional, de Colombia

Light absorption by rhodopsin initiates phototransduction and triggers a G-protein-coupled cascade, which amplifies and translates chemical signals into an electrical response. This entire process occurs over an unexpectedly short period of time on the order of milliseconds. The discovery of a scaffolding protein, InaD, in Drosophila, seemed to elucidate this phenomenon. Its multiple protein binding domains (PDZ Domains) are thought to bind the molecules involved in the G-protein cascade, lessening the time otherwise spent relying on diffusional encounters. Though this offers an intriguing model, the function of the signaling complex remains to be definitively proven.

Further investigation of these protein-to-protein interactions requires large amounts of retinal tissue. Additionally, the evolutionary inception and conservation of this signaling complex may be elucidated through investigation in other organisms. Coming from the same class of photoreceptors and being a significantly divergent species from Drosophila, *Loligo pealei* offers a fitting model organism for this investigation.

One facet of this project is identifying a protein with multiple binding domains in the *Loligo* retina. Two putative orthologues of InaD, from Drosophila and giant squid, were searched against the assembled transcriptome of the *Loligo* retina. Multiple candidate isotigs were identified, two of which were used to design four sets of primers. One primer set from each isotig successfully amplified sequences in cDNA amplified from mRNA extracted from *Loligo pea lei* retinal tissue. Through vector cloning these sequences were amplified and sequenced via Sanger sequencing. The sequences from both primer sets show promising similarity to PDZ domains through BLAST alignment, and work still continues to obtain their full sequences.

Funding by NSF-REU Biological Discovery in Woods Hole grant #1359230