Fall Research Symposium
2008

List of Abstracts

Session 1a (NHS 105)

1:30 **Temporal judgment as a performance-based measure of auditory streaming**

Nathan Heggem

Studies of how the auditory system makes sense of complex environments have established general characteristics of auditory streaming and investigated the perceptual differences in hearing sounds as arising from one source (stream integration) or two sources (stream segregation). More current research indicates that performance on rhythmic and temporal judgment tasks is less accurate when stream segregation is perceived. In this study, objective tasks of temporal judgment were used to measure performance within parameters that also direct stream perception. When measured against frequency separation, subjects? performance followed expected directions in both integration and segregation objective tasks. The effects of sequence length on performance were inconclusive. Data suggest these stimuli follow at least some of the characteristic rules of auditory streaming and could be used in nonhuman subjects to test for neural correlates.

1:45 **Time to spatial coding**

Mike Ferragamo

The challenge to reformat a temporal representation of a sensory stimulus into a spatial code as information ascends neural pathways will be discussed. Parallels will be drawn to human processing of speech.

2:00 **Lin28 Subcellular Localization and Function in Embryonic Stem Cells**

Heather Rusk

MicroRNAs (miRNAs) are small regulatory RNAs that suppress the expression of their target genes. The let-7 family of miRNAs regulates ?stemness? by repressing stem cell self-renewal and promoting differentiation during normal development, and has been implicated in breast and lung cancer. The RNA-binding protein Lin28 promotes pluripotency and negatively regulates let-7 expression by inhibition of Microprocessor-mediated processing of let-7 precursor miRNAs. Paradoxically, unlike the nuclear Microprocessor complex, Lin28 is reportedly localized to the cell cytoplasm. We analyzed Lin28 subcellular localization in ES cells and found Lin28 in nucleoli, leading to an investigation of the mechanism of Lin28 function.

2:15 **Quantitative allometric description and genetic analysis of a novel skeletal mutant, rhizomelia (rzm)**

Matt Chroust

Rhizomelia (rzm) is a murine recessive skeletal mutant, characterized by disproportionate dwarfism in the craniofacial and axial skeleton, but most noticeably in the limbs. Rhizomelia was induced by the integration of a chimeric transgene (Sry:Sox3)77Ei between D7Mit58

and D7Mit281, a region of Chromosome 7 a region that includes the important skeletal regulatory gene, Sox6. Our analysis of the humerus, radius, ulna, femur and the tibiofibula of homozygous (rzm/rzm) mice shows these limb bones to be approximately 50 to 75% the size of those from unaffected (FVB/NJ) mice. Carriers (rzm/+) are mildly affected. RT-PCR and 3? RLM-RACE analysis of total testis RNA suggest aberrant splicing of Sox6 transcripts, with undetectable amplification occurring between exons one and two, and across exons three through five. This suggests an integration of the Sry:Sox3 transgene within the first few introns of Sox6.

2:30 **Cryptochromes and the Circadian Clock**

Karla Marz

Many biological functions occur on an approximately 24-hour, or circadian, cycle. These rhythms and their adjustment by light and other cues enable organisms to anticipate daily and seasonal environmental changes. At their base, circadian rhythms are controlled by a clock mechanism contained within cells. In mammals, two proteins called Cryptochromes play critical roles in this mechanism. Current projects in my laboratory include characterizing Cryptochrome interactions with other clock components and the regulation of cryptochrome gene expression. Student involvement in these projects will begin in January or next summer. There will also be opportunities in January to aid in preparing methods and materials for the new advanced cell biology course that will be taught in the spring.

2:45 **Role of glucocorticoid receptor binding site sequences on transcriptional regulation by the glucocorticoid receptor**

Leah Hogdal, Keith Yamamoto, Ph.D.,[*] Sebastiaan Meijsing, Ph.D.[*]

The glucocorticoid receptor (GR) binds to glucocorticoid receptor binding sites (GBS) to mediate transcriptional regulation of its target genes. In this study, the role of the precise GBS sequence on transcriptional regulation was assessed by examining the function of GBS sequence on cofactor requirement and cooperation with transcription factors (TF). I found that changing the GBS sequences of the GR target gene GILZ, resulted in increased activation which may depend on the cofactor Brm, but not CARM1 as assayed by shRNAi knockdown of these factors in A549 lung epithelial cells. Additionally, I found that the GBS sequences GILZ, sgk and Fkbp5 do not cooperate with the TF NF1. On the other hand, the TF SP1 did cooperate with sgk, but not with the TFs GILZ or Fkbp5. These results suggest that specific GBS sequences may play a role in determining the cofactors required as well as specific synergistic relationships with TFs.

[*]Department of Cellular and Molecular Pharmacology University of California San Francisco

3:30 **The Influence of Age and 5-HT4 Receptors on Mouse Colonic Paracellular Permeability**

Nicholas M. Murray, David R. Linden, Ph. D.,[*] Joseph H. Szurszewski, Ph. D.[*]

In Crohn's disease, ulcerative colitis, and other autoimmune gastrointestinal diseases the barrier function of the epithelial layer, accomplished physically through tight junctions, is severely dysfunctional. Paracellular permeability through tight junctions is highly regulated and can be increased by 5-hydroxytryptamine (5-HT) 4 receptor activation. Control of ion and solute selectivity of these paracelluar pores may also be altered with the age-dependent loss of enteric neurons. The aims of this study were to develop a permeability assay for use in mouse colonic mucosal tissue, determine whether differences in permeability exist between mice lacking 5-HT4 receptors and their wild type littermates, and examine age-related changes in paracellular permeability. A high throughput and reproducible permeability assay was developed using tissue mounted in an Ussing-type chamber that is perfused on the mucosal side with 12.5 mM each of fluorescently labeled 4000 and 10000 mw dextran molecules. For a morphological correlate of tight junction integrity immunohistochemistry for tight junction protein zona occludens-1 and confocal microscopy were used. Permeability rates of both the lower and higher molecular weight molecule were significantly lower in the 5-HT4 receptor knockout mice compared to wild type littermates (4,000 mw: KO: 2.10 ± 0.58 nmol/cm2/h N= 4; WT: 7.67 ± 1.94 nmol/cm2/h N= 5; T-test, P = 0.04; 10,000 mw: KO: 0.14 ± 0.06 nmol/cm2/h N= 4; WT: 0.57 ± 0.12 nmol/cm2/h N= 5; T-test, P = 0.02). A decrease in the number of myenteric neurons as a function of age in both wild type and knock out mice was found, as well as a significant inverse correlation between permeability and age (4,000 mw: Pearson correlation test, P = 0.03; 10,000 mw: Pearson correlation test, P = 0.04). Collectively, these findings indicate that 5-HT4 receptors contribute to paracellular permeability and that neurodegeneration in the mouse colon is associated with an increase in intestinal barrier function. Thus, this study suggests that intestinal barrier function may increase with age in humans, which is likely to be protective, and further that 5-HT4 receptors may be a viable therapeutic target for altering paracellular permeability.

[*]Mayo Clinic Enteric Neuroscience Program, Department of Biomedical Engineering and Physiology

3:45 **Earlier first flowering dates as a result of climate change in the Northern Rocky MountainsPresenter**

Pamela Kittelson and Peter Lesica[*]

Abstract: Climate change is predicted to have effects on phenology, which is the timing of seasonal events such as flowering. Changes in phenology could decouple ecological relationships thus it is important to know how climate and phenology interact in different communities. We collected first-flowering dates (FFD) for 34 broad-leaved plant species from 1995 through 2008 in one location in the semi-arid grasslands of Mount Jumbo, Montana. Species showed a strong tendency for earlier flowering during the 14-year study (t = 3.94, P<0.001). Twenty-six of the 34 species (76%) showed a trend toward earlier flowering and this trend was significant for ten of species (slope >1.0, P<0.10). Only one species showed a significant trend toward later flowering. Mean slope for the early flowering species was -1.6 days per year suggesting that these ten species flowered 22 days earlier in 2008 than in1995, which is a more rapid advance than similar studies in the eastern or central U.S (0.08-0.12 d/yr). Average March temperature and combined January plus

December precipitation best explained the change in FFD (R2=0.65, P=0.003). During our study, winter precipitation declined an average of 4.8 mm per year and average March temperatures increased 0.2oC per year. Our results suggest that a decrease in winter precipitation is as important as rising spring temperatures to flowering. Edaphic factors and photoperiod are important cues for initiating flowering suggesting that some species will be able to respond to a changing climate while others will be constrained by photoperiod, but both scenarios could result in a failure to produce offspring.

*The University of Montana

4:00 **Gene Therapy in Therapeutic Angiogenesis: The Option for No Option Patients**

David Pearson

Critical limb ischemia (CLI) is associated with the formation of ulcers and rest pain in the lower limbs. Surgical methods are often ineffective at repairing occluded vessels and amputation is frequently the only solution in severe cases. Gene transfer is a promising alternative to traditional approaches, and has been linked to the growth of new collateral vessels. VM202, a DNA plasmid vector containing a hybrid human hepatocyte growth factor (HGF) coding sequence (HGF-X7), can be injected intramuscularly to treat CLI. The goal of this study is to determine if assay levels of endothelial progenitor cells (EPCs) are a viable quantitative analytical technique for evaluating the efficacy of VM202 gene transfer in CLI patients.

4:15 **Characterization of HIV T-cell Invasion and Drosophila melanogaster Sperm Storage Pathways and Switches Using Gene Ontology and Transcription Factor Binding Site Profiling**

Jason Pitt

Both HIV infection and sperm storage in Drosophila melanogaster similarly require the immune system's acceptance of a foreign object. Understanding how HIV and sperm manipulate and alter an organism's biological mechanisms to evade destruction provides vital information on novel methods that could impede infectious disease. Using mRNA microarray gene expression profiling data for host T-cell HIV infection and a sperm storage knockout screen of the second chromosome of Drosophila we calculated the gene ontologies with GenMAPP, a web-based program that statistically determines the relative associations of genes based on user-established criteria. Furthermore, transcription factor binding site signatures up to 10 kilobase upstream were analyzed for all significant genes using the web-based program MAPPER. The transcription factor binding site signatures of both datasets were compared to their respective controls, as well as one another. Similar ontologies or signatures between HIV infected T-cells and Drosophila sperm storage mutants would present a novel use for Drosophila as a model system for HIV infection.

4:30 **Tregs Licensed to Kill**

Kathleen Coughlin, Dr. Tobias Peikert, Virginia Van Keulen,* Dr. Larry Pease.**

Effective cancer immunotherapy remains elusive. One of the major barriers to anti-tumor immune responses is the immunosuppressive activity of the regulatory T cells (Tregs, CD4+CD25+FOXP3+). Bone marrow derived DC treated with B7-DC XAb (or a control IgM) and tumor cell lysate (B16) were co-cultured with various T cell types for 24 hours. These T cells were then isolated and used in a cytotoxic T lymphocyte assay (CTL) where

chromium release from mouse melanoma (B16) target cells was measured. In the CTL assay Tregs were shown to acquire cytotoxic activity against B16 melanoma cells when co-cultured with DC treated with B7-DC XAb. The extent of cytotoxicity was similar to the level observed when CD8+ killer T cells were treated in the same manner. No cytotoxicity was seen when Tregs and CD8+ T cells were co-cultured with DC treated with a control IgM or when non-Treg T helper cells were used in the CTL assay. We verified these results by using Tregs and non-Treg T helper cells isolated from CD8 knockout mice.

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4:45 **Agrochemicals and Aquatic Organisms**

Kendra Kesty

The objective of this research is to develop protocols to systematically observe and document effects of an agrochemical and its breakdown products on aquatic organisms. We studied the compound dimethenamid, found in the herbicide Frontier®, because it has a relatively high toxicity and concentration in Midwest streams. We exposed dimethenamid to two small vertebrates, fathead minnows (Pimephales promelas) and zebra fish (Danio rerio), using different methods and analyzed the tissue to look for gross anatomical changes after each exposure.

5:00 **"Paste Only": An In-Vitro Study of *Tol2* and *Hermes* Transposase**

Vwaire Orhurhu, Jun Ni,[*] Nancy Craig,[*] Stephen C. Ekker[*]

Transposons are mobile genetic elements with an inverted terminal repeat (ITR) at each end. Transposons encode a transposase protein which acts on specific DNA sequence within the ITRs. Transposase excises transposons from DNA, then inserts them into a new DNA site. This mobilization, "transposition," is an effective tool for gene discovery and permanent delivery of foreign DNA into the vertebrate genome, including germline transmission in zebrafish. However, the biochemistry of transposition is largely unknown.
We have studied the biochemistry of transposase-mediated integration for two members of the hAT transposon family, *Tol2* from the medaka fish, and *Hermes* from the housefly. Previous studies in our lab (unpublished) suggest that *Tol2* transposase can integrate DNA fragments with limited or absent ITR sequences into a target plasmid. To further understand the transposition activity of hAT transposases, we compared the transposition frequency of *Hermes* with *Tol2*. We hypothesized that *Hermes* would similarly catalyze the insertion of kanamycin DNA fragments without ITRs into target plasmids. We used purified *Hermes* transposase protein and generated pre-cut DNA fragments by PCR. We used a cell-free *in vitro* integration assay to test for transposase-catalyzed integration of kanamycin fragments into target plasmids containing the ampicillin gene. Plasmid DNA isolated from kanamycin-resistant cells was sequenced to verify kanamycin integration, and to assess whether the gene integration reactions were due to *Hermes*-catalyzed transposition.
Tol2, our reference system, integrated kanamycin fragments without ITRs at a frequency of 0.302% whereas *Hermes* integrated kanamycin fragments at a frequency of 0.294%. DNA sequencing showed an eight base pair duplication at the integration site, characteristic of hAT-mediated integration. Our results suggest that (1) *Hermes* transposase can integrate pre-cut kanamycin fragments without ITRs into target plasmids *in vitro*; and (2) there were no statistical differences in the transposition frequency of *Hermes* and *Tol2* transposase.

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Session 2A

1:30 **Synthesis of Shaped Nanoparticles for Two-Dimensional Assemblies**

Molly Beernink

Hybrid molecular orbitals with specific bonding directions provide a useful model to correlate bonding of atomic building blocks with molecular structure. The concept of directional bonding can, in principle, be translated to larger, shaped nanoparticles when these are assembled into extended arrays, which are of interest for nanotechnology applications. Directional interactions established by chemically controlled reactive sites may promote the association of particles similar to sigma bonding of atoms in molecules. We synthesized particles that model the trigonal planar shape of sp2 hybrid orbitals with 120º angles by a two-dimensional template methodology. A two-dimensional silica film was fabricated on a substrate coated with physically and chemically adhered polymer spheres. Removal of the spheres resulted in pores of uniform size and shape in the film. The optimized conditions for polymer sphere deposition and reactive-ion etching to promote ordered sphere packing onto a substrate were determined. The porous silica film was removed from the substrate and separated into smaller structures through a series of etching and mechanical stress techniques. These silica fragments were characterized via high-resolution scanning electron microscopy. The nanoparticles were functionalized by multiple surface modification steps for use in assembly processes.

1:45 **Incorporation of Myohemerythrin Protein with Iron(II) in the Presence of Small Molecules**

Laura Groskreutz

Myohemerythrin (myoHR) is an oxygen transport protein in *Phascolopsis gouldii* which contains a diiron active site. MyoHR shares sequence identity to a Cd(II) binding protein in *Nereis diversicolo,* leading our research team to attempt binding Cd(II) to myoHr. The incorporation of Fe(II) into the metal-free form of myoHR (apomyoHR) has been published (Zhang, 1991), but this method is incompatible with Cd(II) incorporations. This led us to further study the necessary conditions for the incorporation of the metal free form of myoHR (apomyoHR) with Fe(II). Eventually, incorporations of myoHR with other metals including Cd(II) should be feasible. The published protocol for incorporations of Fe(II) into apomyoHR uses 2-mercaptoethanol, which may contribute to the stability of Fe(II) in solution iron stability and/or prevent disulfide bond formation. Unfortunately, 2-mercaptoethanol also causes Cd(II) to precipitate when in the presence of Fe(II), so other small molecules were investigated. Small molecules tested in incorporations include: 2-mercaptoethanol, tris(2-carboxyethyl) phosphine hydrochloride, citric acid, imidazole, and potassium thiocyanate. UV-vis spectra of the resulting incorporations showed that using 2-mercaptoethanol produced the most successful incorporation, which may suggest that disulfide bond reduction is a key part of metal incorporation in myoHr.

2:00 **Developing an electrocyclization reaction for the synthesis of pyrrolizidine alkaloids**

David Guptill

This project focuses on the synthesis of a pyrrolizidine alkaloid (a nitrogen containing fused bicycle). Compounds in this class tend to display tend to display biological activity and are thus have the potential for use in medicine. New methods for constructing the core are

therefore valuable, allowing naturally occurring molecules to be more easily synthesized in a lab. This particular alkaloid was derived from an amine and a carboxylic acid. The synthesis of the amine begins with the amino acid methionine while the carboxylic acid was synthesized from methyl acrylate and nitromethane. The amine and the carboxylic acid were coupled with DCC and D-MAP to form an intermediary amide in good yield (78%). We are currently working on the formation of the bicyclical alkaloid using a lewis acid as a catalyst.

2:15 **Degradation of Endocrine Disrupting Chemical Nonylphenol Using UV Photolysis and Hydrogen Peroxide**

Jeremy Bedard

Several experiments were conducted in order to study the UV/H_2O_2 oxidative degradation of the endocrine disrupting chemical nonylphenol using photolysis of hydrogen peroxide. Previous studies have been successful in degrading similar water contaminants with this type of oxidation as well as others. The effect of H_2O_2 concentration as well as UV exposure time was investigated. Lamp photolysis showed no discernable difference in the ability to degrade nonylphenol at three H_2O_2 concentrations. Rooftop sunlight photolysis showed a positive relation with respect to the amount of H_2O_2 in the system. At a H_2O_2 concentration of 100 mM, 24 hours of lamp photolysis resulted in 70.2% NP degradation. At a H_2O_2 concentration of 200 mM, 87.5% NP degradation was achieved with 8 hours of sunlight photolysis. A close, but not purely linear trend was found between UV exposure time and nonylphenol degradation. The trend did suggest that all nonylphenol should degrade if ample time is supplied. With the threat of this and other water contaminants rendering wastewater useless and hazardous, it is imperative to discover processes to destroy these chemicals.

2:30 **Role of Silica Surface Chemistry on the Stability of Au Nanoparticles**

*Amanda Staker, P.I. Abhaya Datye**

A catalyst is a substance that causes or accelerates a chemical reaction without itself being affected. Gold is active as a catalyst when it is less than 5 nm in size. The chemistry of gold as a catalyst was investigated by using a model substrate, such as, a flat silica wafer. Also, a system was developed to deposit stable gold nanoparticles < 5 nm in size on a functionalized model silica substrate. Gold was deposited on the model support after being functionalized with N-Hydroxysuccinimide (NHS) and the gold particle size was determined with scanning electron microscopy (SEM). The surface was also hydroxylated before being functionalized with NHS, to observe how additional hydroxyls affect gold particle size. Silica wafers functionalized with NHS maintained the size of gold nanoparticles after aging the sample at various temperatures and times. The samples with additional hydroxyls did not produce stable gold nanoparticles that remained under 5 nm in size after ageing. NHS is effective at maintaining gold nanoparticles size and distribution.

* University of New Mexico

2:45 **Synthesis of Substituted Phenytoin for use in Affinity Chromatography**

Nick Hefty

Phenytoin (diphenylhydantoin) is an anticonvulsant drug widely used in the treatment of Epilepsy and other medical conditions, so an understanding of the molecular mechanism of action of phenytoin is highly desirable. One approach to this problem is to attempt to isolate a phenytoin-binding receptor.

Here we describe our progress on the synthesis of a phenytoin derivative that bears a tethering chain capped by a primary amino group for binding to an affinity gel. The sequence begins with a Mitsunobu reaction of an N-protected aminoalcohol with p-iodophenol; the remainder of the sequence consists of Sonogashira coupling of the resulting iodo derivative to phenylacetylene, conversion of the alkyne to a benzil, and reaction of the benzil under basic conditions to form the substituted phenytoin.

Session 2B (NHS 222)

3:30 **Synthesis and Diels-Alder Reactions of Siloxyfurans**

Amanda Franey

Furans are often used as the diene in Diels-Alder reactions. Oxygen-substituted furans are predicted to be good dienes, but little is known about their reactivity. This research focused on the rate of the reaction between trimethylsiloxyfuran and both maleic anhydride (and its derivatives) and fumarate (and its derivatives). The maleic anhydride proved to be an intriguing start to this project. The reaction yielded a kinetic and a thermodynamic product mixture. As expected, with the other maleate derivatives the rate of the reaction got increasingly slow as the steric hinderance increased. The fumarate derivatives reacted to give complex mixtures. It has been determined that the rate of the reaction was directly correlated to the size of the dienophile. Further research will determine the significance of the cis versus trans nature of the dienophile and the corresponding stereochemistry of the products.

3:45 **New approach to discovering drug to protein interactions**

Kristen Jahr

Drug research typically requires synthesizing a whole library of compounds to screen against one known defective receptor or protein in the human body; however, by instead allowing the cells to make a library of proteins to screen against one known biologically active compound, we can eliminate time-consuming organic synthesis and therefore speed up the process of drug research. This specific project focuses on the interactions between proteins and the drug nitrofurazone, an anti-bacterial. By attaching a derivative of this drug to an affinity column and running proteins from lysed cells through, we can discover how this drug interacts with proteins and find other possible uses for this drug.

4:00 **Preparation of Fluorinated Phthaloylphosphines and Primary Phosphines as Precursors for New Fluorinated Anions**

Jason Schultz

A straightforward method for the preparation an array of fluorinated organophosphorus compounds by P-alkylation and P-arylation of phthaloylphosphide ion is described. The procedure for using the resulting phthaloylphosphines for preparation of primary phosphines and fluorinated organic derivatives of hexafluorophosphate will be illustrated. This is done by a process of hydrazinolysis of the phthaloylphosphines, followed by the reaction of the primary phosphines with Selectfluor® to convert them to tetrafluorophosphoranes. Our goal is to use these tetrafluorophosphoranes to prepare organofluorine analogs of hexafluorophosphate ion by reaction with cesium fluoride.

4:15 **The Hygroscopic Study of Dicarboxylic Acids**

Joel Rindelaub

Aerosols are prevalent in the atmosphere and can affect air quality, visibility, and atmospheric processes. Global climate is also influenced by aerosols through the direct and indirect effect. The direct effect results from aerosol absorption/scattering of solar and

terrestrial radiation while the indirect effect results from aerosol particles taking up water to form cloud condensation nuclei (CCN). One type of aerosol particle that has both direct and indirect influence on climate is water-soluble organic compounds, specifically low-molecular-weight dicarboxylic acids. Low-molecular-weight dicarboxylic acids are abundant in the atmosphere and are emitted from both anthropogenic (eg. motor vehicle exhaust) and natural sources (eg. biomass burning). They are also reported products of photochemical oxidation. We concentrated on the indirect effect by investigating the hygroscopic properties of C2-C6 dicarboxylic acids as a function of relative humidity (RH) using a scanning mobility particle sizer (SMPS). Results show that low-molecular-weight dicarboxylic acids can affect climate as they exhibit hygroscopic growth based on the odd-even effect.

4:30 **The effects of Amylin on Sympathetic Nerve Activity in Mice**

*Asitha Jayawardena, Kamal Rahmouni, PhD**

Amylin, a 37-amino acid peptide, has been shown to reduce body weight by reducing food intake. Studies also reveal increased energy expenditure related to amylin. Some have speculated that this increased energy expenditure is due to the altered body composition of amylin-injected mice (more lean mass vs. body fat). However our study suggests amylin plays a more direct role in altering energy expenditure. Amylin was injected via intracerebroventricular (ICV) cannulation while simultaneously recording the nerve activity of brown adipose tissue (BAT). Here, we show that amylin activates the sympathetic nervous system. It was also found that the injection of amylin-receptor antagonist (AC 187) prohibited inhibited an increase in sympathetic nerve activity (SNA) suggesting that the effect of amylin is receptor-mediated. Increased SNA in BAT suggests that amylin causes increase energy expenditure via thermogenesis. Our data provide an explanation for increased energy expenditure following amylin-injection in mice.

*University of Iowa

4:45 **Expression of ErbB Receptors in Phrenic Motoneuron Development**

*Brandon Baartman, Dr. Carlos Mantilla,**

Motor units comprising a motoneuron and the muscle fiber it innervates show remarkable heterogeneity in their properties. This heterogeneity becomes apparent during postnatal development and is important for the wide range of behaviors that a muscle must accomplish. Importantly, the functional properties of motor units are associated with size differences in motoneurons and muscle fibers. The mechanisms underlying postnatal growth of motor units are poorly understood. Neuregulin (NRG), a known ligand to the ErbB family of tyrosine kinase receptors, may influence growth of motor units. ErbB homo- and heterodimers activate the Phosphoinositide 3-Kinase/Akt intracellular signaling pathway which is known to influence cellular growth and differentiation. The possibility of a NRG/ErbB influence on phrenic motoneuron growth is supported by recent experiments showing expression of ErbB receptor mRNA in spinal motoneurons as well as the presence of NRG-containing synaptic boutons surrounding phrenic motoneurons. We hypothesize that 1) NRG may exert a growth-promoting effect on phrenic motoneurons depending on their expression of ErbB receptors and 2) NRG may exert a growth-promoting effect on diaphragm muscle fibers depending on NRG expression by phrenic motoneurons. We examined mRNA expression levels for ErbB2 and ErbB3 receptors as well as NRG in phrenic motoneurons, and stratified their mRNA expression based on motoneuron size.

Using laser capture microdissection, we isolated retrogradely-labeled phrenic motoneurons from both juvenile (postnatal-day 21) and adult rat spinal cord. Using mRNA extracted from these isolated phenic motoneurons, real-time RT-PCR analyses of ErbB2, ErbB3, and NRG mRNA expression were completed. Preliminary results indicate that ErbB2, ErbB3 and NRG are expressed in phrenic motoneurons during this developmental period. Based on a limited number of motoneurons, there was no evidence of differences in ErbB receptor or NRG mRNA expression across motoneurons of different size. Continuing experiments seek to investigate whether significant differences exist in ErbB receptor or NRG mRNA expression between large and small motoneurons. These studies should provide fundamental understanding regarding the trophic regulation of motor unit growth and diversity.

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5:00 **Synthesis with 2-Silyoxyfurans**

Mallory Richards

Biologically active natural products have been recognized as valuable guides in leading pharmaceutical expansion. The efficiency of production of these molecules is of increasing importance due to cost and conservation of preparatory materials. 2-Silyoxyfurans provide a way to create a core structure of several natural products. Limited success of the synthesis of the core structure has been achieved. This is due to the difficulty in handling the source aldehyde. Recent research has shown a successful addition to the 2-silyoxy furan, while current studies are being preformed to complete the cyclization of the molecule to form the core structure.

5:15 **Optimizing the Purification of Myohemerythrin and Metalloprotein II from *Nereis diversicolor***

Jordan Makela

Metalloprotein II and Myohemerythrin are both metal binding proteins, 119 amino acids in length, found in the marine invertebrate *N. diversicolor*. Their exact physiological function is unknown, however Myohemerythrin is known to bind iron while Metalloprotein II is known to bind cadmium. The purpose of my project was to find a method to purify these two proteins using *E. coli* carriers. The gene coding each protein is known, however the bacteria need a T-7 promoter region on a plasmid in order to produce the protein when stimulated by IPTG. The known gene was cut from its original plasmid using HindIII and NdeI restriction enzymes. The purpose was to use T4 DNA ligase to connect the gene with the T-7 promoter sequence on a plasmid vector, however, complications arose in the ligation step. *E. coli* that had been transformed with the new hybrid plasmid using electrophoresis did not survive on LB plates containing ampicillin, indicating that the entire plasmid was not intact. Further lab work can be done to make this ligation step a success.