SESSION 2F

BRAIN DEVELOPMENT, INJURY, REGENERATION AND RESTORATION OF FUNCTION

Session Moderator: Gwenn Garden, Neurology
242 MGH
3:30 PM to 5:00 PM

Developing a Brain-Machine-Spinal Interface (BMSI) to Reanimate Forelimb After Spinal Cord Injury
Ryan James (Ryan) Carlson, Senior, Neurobiology, Biochemistry
Mary Gates Scholar
Mentor: Chet Moritz, Physiology & Biophysics

Of the many types of devastating spinal cord injuries, incomplete injuries of the cervical spinal cord are the most common among patients. For these individuals, the highest treatment priority is restoration of hand and arm function; much more important than any other symptom of paralysis. The goal of our project is to restore hand and arm function by recording movement intention in the brain and using it in real time to control spinal cord stimulation. We use single unit activity encoding movement intention in the rat motor cortex, and intra-spinal microstimulation (ISMS) for movement induction. ISMS may be superior to direct muscle stimulation (FES), since it causes little fatigue and a more natural recruitment of motor pools. ISMS in the cervical and lumbar regions of the spinal cord can also be utilized to produce a wide array of functional limb movements. By using rats that I have trained to perform a lever-pressing task, I can quantify the functional relevance of movements evoked by varying ISMS parameters. By combining both recorded cortical data and ISMS, we have created a brain-machine spinal interface (BMSI). This BMSI can deliver brain-controlled, functionally useful stimulation directly to the spinal cord and caudal to a contusion injury in order to restore some movements to the animal’s injured forelimb. Our long-term goal is to translate this device to a clinical setting, where it could be used to restore hand and arm function to patients with complete spinal cord injuries.

SESSION 2R

IMPLEMENTING PRECISION MEDICINE

Session Moderator: Edward Fox, Pathology
111 JHN
3:30 PM to 5:00 PM

Induced Pluripotent Stem Cells as a Tool to Personalize Medicine
Gregory Allen (Greg) Shintani, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine

The current approach for FDA drug approval requires an exorbitant amount of time and money, often spent in pre-clinical animal studies, and has a high late-stage failure rate. This is partly due to the fact that pharmaceutical companies develop drugs to treat the average patient with any given disease. A personalized medicine approach would increase the effectiveness of a new drug for each patient and decrease the need for animal testing. Induced pluripotent stem cells (iPSCs) are becoming the next-generation tool to study disease and discover new drugs. Patient-specific somatic cells are reprogrammed to a stem-like state, and then differentiated in culture into the cell type most likely to manifest features of a particular disease. High-throughput drug screens can be designed to find new compounds that can correct the disease defect in the culture dish. Using this technology, we extracted somatic cells from the urine of patients with autistic syndrome disorder (ASD). In addition to their cognitive deficiencies, these young patients suffer from chronic and debilitating constipation, which profoundly impacts their quality of life. This lack of gut motility is suspected to be caused by a defect in enteric neuron function. Our hypothesis is that the same synaptic malfunction causing cognitive deficits in ASD children also causes the enteric neuron defect. Preliminary data also suggests that neural crest cells - an intermediate cell type in the enteric neuron lineage - can be generated using a combination of growth factors. Therefore, I am conducting growth factor dose-response experiments on iPSCs to optimize neural crest cell differentiation. I will phenotype the resultant cells via...
immunocytochemistry and qPCR analysis. If successful, this project will lead to the discovery of new compounds able to relieve ASD patients of their constipation and further validate iPSCs as a tool for disease modeling and drug discovery.

**SESSION 2S**

**IMPROVING HEART FUNCTION WITH BIOENGINEERING**

*Session Moderator: Pierre Mourad, Neurological Surgery*

175 JHN

3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

**Effects of Mechanical Stretch on AAV Mediated Gene Therapy in Cultured Skeletal Muscle**

_Hannah Maricia (Hannah) Wear, Senior, Aquatic & Fishery Sciences_

_Mentor: Robynne Braun, Rehabilitation Medicine, Institute for Stem Cell and Regenerative Medicine_

_Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine_

Gene therapy for monogenic diseases is undergoing an exciting resurgence in the last few years. Patients with hereditary blindness, blood cancers and hemophilia have been cured with minimal to non-existent long-term side effects. Our lab has been studying X-linked myotubular myopathy—a rare disease of young boys that causes profound weakness in all skeletal muscles including the diaphragm. An adeno-associated viral (AAV) vector gene therapy has been proposed to treat the X-linked muscle degenerative disease. However, the viral dose required is in excess of $10^{13}$ viral particles per kilogram. The goal of this proposed project is to find ways to manipulate muscle cells to increase virus uptake and enhance activity of the muscle protein expression. Artificially induced mechanical stretch and contraction have been shown to stimulate intracellular signaling and gene expression. Thus, we hypothesize that preconditioning muscle cells by in vitro stretching will increase both virus uptake and transgene expression. Increases in both will enable future attempts of gene therapy in animals or humans to use lower doses, which should be accompanied with lower toxicity and fewer and less severe adverse side effects.

**POSTER SESSION 4**

**Commons East, Easel 76**

4:00 PM to 6:00 PM

**Gene Therapy in a Canine Model of Duchenne Muscular Dystrophy: Analysis of a Preclinical Measurement**

_Mac Kenzie (MacKenzie) Rinaldi, Senior, Biochemistry_

_Mentor: Martin Childers, Rehabilitation Medicine_

_Mentor: Melissa Goddard_

_Mentor: Zejing Wang, Medicine_

Duchenne muscular dystrophy (DMD), is a degenerative disease caused by a recessive X-chromosome mutation, resulting in lack of the protein dystrophin. Affected males experience profound muscular weakness in childhood and by adolescence the heart and respiratory muscles are affected. The Golden Retriever muscular dystrophy (GRMD) dog model displays phenotypes analogous to patients and is considered the premier preclinical model. We evaluated changes in limb function by assessing gait in GRMD (n=3), wildtype (n=2), and in GRMD dogs injected with an adeno-associated virus carrying a dystrophin replacement gene in a single limb (n=3). Dogs were walked at a self-selected pace along an instrumented carpet to measure gait parameters. The average velocity over time of non-injected GRMD dogs was 60.39% of wildtype. Treated dogs displayed slightly increased velocities (67.17% of normal). By the final timepoint, velocity in all groups was comparable. The average paw pressure of the non-injected GRMD group increased 8.05%, while the average paw pressure for the wildtype decreased by 12.75%. The ratio of mean paw pressure for untreated GRMD: wildtype increases from 0.819 to 1.014 over time. We therefore determined that paw pressure might be a sensitive indicator of a treatment effect. To estimate the minimum sample size required for future studies, a power of analysis was performed. Results indicate that n=14 is required to detect differences between groups with a confidence level of 5% and power of 80%. Given that human patients suffer multiple symptoms of muscular weakness analogous to the dogs, including reduced limb function, changes in treated GRMD dogs as measured through gait analysis may evaluate the efficacy of the gene therapy as treatment for DMD. Human patients suffer multiple symptoms from muscular weakness (non-ambulatory, cardiac impairment, etc). Future studies hope to transition treatment to human patients to improve ambulation and increase quality of life.
Evidence for Sustained Anatomical and Functional Forelimb Deficits in a Model of Cervical Spinal Cord Contusion Injury

Chloe Alexandra (Chloe) Stiggelbout, Senior, Biology (Physiology)
Mentor: Chet Moritz, Physiology & Biophysics

Spinal cord injury (SCI) is a devastating condition that impacts the lives of people around the world. In order to develop cures for SCI, it is necessary that researchers are able to produce sustained and repeatable injuries in experimental animals. We induced a cervical contusion injury with a 4th generation Ohio State injury device that utilizes tissue displacement as the controlled variable during impact. We then quantified the resulting in behavioral and morphological changes in adult rats from 2 weeks to 12 weeks after injury. Quantitative behavioral assessment employed Irvine, Beatties and Bresnahan’s (IBB) rating scale, a forelimb-reaching task (FRT), and a cylinder exploration task. Morphological assessment involved observation of bruise evolution and quantitative evaluation of histology from the spinal cord. All animals showed significant loss of forelimb function after impact to the cervical spinal cord, followed by prolonged and relatively stable functional deficits. Histological evidence revealed significant loss of both white matter and gray matter near the injury epicenter. This device therefore provides a tool for SCI research by delivering a displacement-defined impact that results in a relatively repeatable and sustained injury.

Intra-Observer and Inter-Observer Reliability in a Neurological Assessment Scoring System for Canine X-linked Myotubular Myopathy

Ian Coulter, Junior, Biology (Molecular, Cellular & Developmental)
Mentor: Martin Childers, Rehabilitation Medicine
Mentor: Jessica Snyder

X-linked Myotubular myopathy (XLMTM) is a congenital myopathy caused by mutations in the myotubularin (MTM1) gene. Clinical symptoms of the disease including muscle weakness, respiratory failure, and hypotonia. XLMTM is caused by deficiency of the protein myotubularin necessary for normal muscle excitation-contraction. Dogs harboring a canine MTM1 gene model the disease and provide valuable insight due to phenotype similarities with patients. Affected dogs exhibit muscular atrophy, weakness, and stilted gait. A neuromuscular assessment score was developed to measure neurologic impairment in affected dogs. My project involved validation of this previously tested neurological assay by an independent observer. I was the independent observer who reviewed and scored neurological examination forms. I also reviewed and scored videotaped examinations and conducted statistical analyses looking at the correlation of the results within groups of dogs and between groups of dogs. Intra- and inter-reliability were performed by another observer and myself and wild type (n=6) and affected dogs (n=4) were scored. Prior to beginning the study, I received training on the assessment scoring system, including how to perform and analyze clinical tests. The intra-reliability test involved repeated examinations of wild type dogs and generated a consistent score in 88% (46/52) of exams based on interpretation of written neurological examinations and 100% (9/9) of exams based on scoring videotaped examinations at two time-points. The inter-reliability tests generated a consistent score in 54/71 examinations (76%) based on scoring of pre-recorded neurological exams and generated a consistent score in 12/15 cases (80%) based on scoring video-taped exams. The two observers scored dogs within 1 point in 68/71 (96%) of cases based on written notes of the neurological examination and within 1 point in 15/15 (100%) of videotaped examinations. Together, these data support the reliability of the neurological assessment score in XLMTM dogs.