NeuroRegeneration Collaborative Symposium:

Repairing the Nervous System

September 1-2, 2015
BioScience Research Collaborative

Organizers:
Charles Cox, University of Texas Health Science Center at Houston
Jane Grande-Allen, Rice University
Laura Smith Callahan, University of Texas Health Science Center at Houston
Ben Deneen, Baylor College of Medicine
Behnaam Aazhang, Rice University
Supinder Bedi, University of Texas Health Science Center at Houston
Melissa S. Thompson, Gulf Coast Consortia

Sponsored by:

Gulf Coast Consortia for Quantitative Biomedical Sciences

&

a 2014 John S. Dunn Collaborative Event Award

CONFERENCE SPONSORS

Gulf Coast Consortia for Quantitative Biomedical Sciences

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The Gulf Coast Consortia (GCC) brings together the strengths of its six member institutions to build interdisciplinary collaborative research teams and training programs in the biological sciences at their intersection with the computational, chemical, mathematical, and physical sciences. Comprised of six prominent and geographically proximate Gulf Coast institutions, Baylor College of Medicine, Rice University, University of Houston, University of Texas Health Science Center at Houston, University of Texas Medical Branch at Galveston, University of Texas M. D. Anderson Cancer Center and Texas A&M Health Science Center's Institute of Biosciences and Technology, the GCC's goal is to provide a cutting edge collaborative training environment and research infrastructure, one beyond the capability of any single institution. The GCC's mission is to train the next generation of bioscientists and to enable scientists to ask and answer questions that cross scientific disciplines to address the challenging biological issues of our time and, ultimately, to apply the resulting expertise and knowledge to the treatment and prevention of disease.

John S. Dunn Collaborative Research Award Program

The John S Dunn Collaborative Research Program began in 2009 to foster new, exemplary interdisciplinary and inter-institutional engagement in the quantitative life sciences by providing two types of seed grants:

1. Research: Up to $98,000 total to support research/preliminary work for 2 years that is essential to be competitive for future funding.
2. Event: Up to $8,000 for 1 year to support events/activities designed to bring together new interdisciplinary communities.

Funds are awarded yearly to new collaborative teams in which one member of each team is required to be part of the BRC-Associated Faculty. A list of these faculty is updated each cycle and available on the website.

The RFA for the next cycle of the program will be announced in early 2016 and is usually due around May or June. For further information, see the GCC website for previous announcements and a list of previously awarded teams.

This event is co-sponsored by the GCC and a John S. Dunn Collaborative Event Award, entitled "TMC-GCC Collaborative Workshop: Regenerative Medicine in Neuroscience and NeuroEngineering," awarded to Charles Cox, UTHSC, Jane Grande-Allen, Rice University, Behnaam Aazhang, Rice University and Ben Deneen, Baylor College of Medicine.

Wifi Access: Rice Visitor

CONFERENCE ORGANIZERS

Charles S. Cox, Jr., M.D.
George and Cynthia Mitchell Distinguished Chair in Neurosciences and Director, Program in Children's Regenerative Medicine, Department of Pediatric Surgery
Co-Director, Texas Trauma Institute
University of Texas Health Science Center at Houston

A Texas native, Dr. Cox received his undergraduate degree from the University of Texas at Austin in the Plan II Liberal Arts Honors Program. Upon graduating from the University of Texas Medical Branch, he completed his Surgery residency at the University of Texas Medical School at Houston. Further post-graduate fellowships were completed in Pediatric Surgery at the University of Michigan, an NIH T32 sponsored clinical and research fellowship in cardiopulmonary support/circulatory support devices/bio-hybrid organs at the Shriner's Burns Institute, and Surgical Critical Care/Trauma at UTHealth Medical School. He is certified by the American Board of Surgery in Surgery, with added qualifications in Pediatric Surgery and Surgical Critical Care.

The Pediatric Translational Laboratories and Pediatric Program in Regenerative Medicine is a multi-disciplinary effort that addresses problems that originate with traumatic injury and the consequences of resuscitation and critical care. The Program focuses on progenitor cell based therapy (stem cells) for traumatic brain injury, and related neurological injuries (hypoxic-ischemic encephalopathy, stroke, spinal cord injury), recently completing the first acute, autologous cell therapy treatment Phase I study for traumatic brain injury in children.

The program also develops novel bio-hybrid organs using cell-based and tissue engineering approaches to trauma and injury related problems. These efforts have recently resulted in two IND based cell therapeutic studies, and three patents in the past two years. The program is funded through the National Institutes of Health, Texas Higher Education Coordinating Board, Industry Collaboration, and philanthropic contributions.

Dr. Cox has served on scientific study sections/review groups for the National Institutes of Health, American Heart Association, Veterans Affairs MERIT Awards, Department of Defense, Congressionally Directed Medical Research Programs, as well as National Research Programs in Canada, Singapore, and the Czech Republic. He is the author of over 100 scientific publications, 20 book chapters, and is the editor of a text in press entitled, Progenitor Cell Therapy for Neurological Injury.

Jane Grande-Allen, Ph.D.
Isabel C. Cameron Professor of Bioengineering
Rice University

Jane Grande-Allen's research applies engineering analysis to understand and fight heart valve disease. This involves mechanical testing, biochemical measurements, and microstructural analysis of critical components found in the extracellular matrix (ECM) that makes up cardiac tissue. Her studies into the basic and applied physiology of heart valve tissue have shown that the ECM – collagen, elastin, glycosaminoglycans and proteoglycans – forms an intricate network of connective tissue that is influenced by valvular function, growth, and abnormalities.

Her investigations of the chemical and mechanical conditions in both diseased and healthy valves are designed to reveal why structural defects occur and how to develop alternatives to conventional open-heart surgery to repair/replace diseased heart valves. These alternatives include drug therapies and engineered heart valves for patients of different age groups.

Grande-Allen's research has been supported by the American Heart Association; March of Dimes; National Heart, Lung, and Blood Institute; National Institute of Biomedical Imaging and Bioengineering; the National Science Foundation; Pfizer; and the Whitaker Foundation.
The overall focus of Integrative Matrix Mechanics Laboratory is to characterize and eventually manipulate the structure-function-environment relationship of cardiac valves in an integrative fashion (cellular, tissue, organ, and clinical). The matrix composition and material properties of heart valves are believed to be determined by the load patterns imposed during valve function. Consequently, alterations to the normal tissue loading patterns will affect the cellular phenotypic production of extracellular matrix and transform the valve morphology, mechanics, and function.

This research includes using cells, organ cultures, and tissue engineering approaches to investigate valve disease from a more mechanistic angle. The main diseases studied include myxomatous ("floppy") mitral valve disease and secondary remodeling of the heart valves in patients with heart failure, but this research may have implications and applications for other soft tissues. Ultimately the group hopes to use the characterization of normal and pathological mechanisms of valvular remodeling to derive novel surgical and medical therapies that can be used to treat patients earlier in the disease process. Ongoing projects include:

- Understanding the extracellular matrix basis for the tissue mechanics of valves and other soft tissues, particularly with respect to proteoglycans;
- Identification, characterization, and mechanical/humoral/pharmaceutical control of valvular glycosaminoglycans and proteoglycans;
- Biochemical, proteomic, biomechanical, and echocardiographic quantification of valvular remodeling in clinical congestive heart failure (a secondary valve disease) and myxomatous mitral valves (a primary valve disease); and
- Development of in vitro surrogates (via cell/organ culture and tissue engineering) and bioreactor systems to study disease and remodeling in valves and other soft tissues.

Laura Smith Callahan, Ph.D.
Assistant Professor, Department of Neurosurgery
University of Texas Health Science Center at Houston

The Smith Callahan Laboratory focuses on the developing tissue engineering approaches (FIGURE 1) toward clinical treatments for spinal cord injury, traumatic brain injury and cartilage defects using an interdisciplinary approach involving techniques from cell, molecular, and stem cell biology, chemistry, and material science. Utilizing engineering approaches, the laboratory seeks to optimize scaffold design and the expansion of clinically relevant cell sources.

Current Projects

- Development of multi-component scaffolds to facilitate tissue regeneration through better replication of the native extracellular matrix.
- Optimization of culture surfaces for the differentiation of human induced pluripotent stem cells to neural stem cells and oligodendrocyte progenitor cells.
- Identification of optimal artificial matrix properties such as bioactive signaling moiety concentration or mechanical properties using combinatorial approaches.
- Synthesis of novel biomaterials for spinal cord, brain, and vertebral disk repair.

An Assistant Professor in the Department of Neurosurgery, Dr. Smith Callahan earned her doctorate in Biomedical Engineering from the University of Michigan, where her work under focused on the effects of nanofibrous scaffolding on the osteogenic differentiation of embryonic stem cells. Upon completion of her thesis, she was awarded a post-doctoral fellowship on the Regenerative Science T90 training grant

which allowed her to further study the effects of nanofibrous scaffolding on the neural differentiation of embryonic stem cells. To obtain additional training in peptide and polymer chemistry and soft material characterization, Dr. Smith Callahan transitioned to a post-doctoral position at the Institute of Polymer Science at the University of Akron with Matthew L. Becker. At the University of Akron, her work focused on the effects of bioactive peptides and gradient hydrogels on stem cell differentiation to mesenchymal and neuronal lineages.

Benjamin Deneen, Ph.D.
Associate Professor
Center for Stem Cells and Regenerative Medicine
Baylor College of Medicine

Dr. Deneen's laboratory studies the molecular and cellular mechanisms that control the generation and differentiation of glial cells. While glia constitute roughly 90% of the central nervous system (CNS) and are associated with numerous neurological disorders and malignancies, the transcriptional mechanisms that control their development and diversity remain shrouded in mystery. Using prospective isolation of stem cell populations from different stages of embryonic spinal cord, coupled with microarray analysis, the lab has identified a family of transcription factors (the Nuclear Factor I family or NFI) that control the specification of glial cell identity. One line of investigation in the laboratory involves using similar methods of temporal profiling of spinal cord stem cell populations from knockout embryos to identify target genes of NFI family members that are required for the initiation of gliogenesis. Another, related line of investigation includes the identification of the mechanisms that control NFI gene induction during CNS development.

Many of the markers that are normally expressed in glial cells are also expressed in gliomas, glial based malignancies of the CNS and the most common and deadly form of adult brain cancer. Consistent with this, NFI genes are also expressed in gliomas and manipulation of NFI gene expression in established glioma cell lines impacts tumor formation. Currently, the lab is validating and extending these studies in more contemporary, stem cell models of glioma. Lastly, given that NFI genes are expressed in gliomas and may be important for tumorigenesis, the biology surrounding their normal function during gliogenesis is therefore also implicated in glioma biology. Thus, any of the NFI target genes or mechanisms that control their induction identified in the developmental studies, may also be pertinent to glioma biology and will be examined in this context.

Dr. Deneen earned his PhD from the University of California, Los Angeles and pursued a postdoctoral fellowship at the California Institute of Technology.

Behnaam Aazhang, Ph.D.
J.S. Abercrombie Professor, Electrical and Computer Engineering
Rice University

Behnaam Aazhang received his B.S. (with highest honors), M.S., and Ph.D. degrees in Electrical and Computer Engineering from University of Illinois at Urbana-Champaign in 1981, 1983, and 1986, respectively.

From 1981 to 1985, he was a Research Assistant in the Coordinated Science Laboratory, University of Illinois. In August 1985, he joined the faculty of Rice University, Houston, Texas, where he is now the J.S. Abercrombie Professor and Director of the Cluster on Neuroengineering within the Gulf Coast Consortium, a multi-university research center in Houston, Texas. In addition, Dr. Aazhang holds an Academy of Finland Distinguished Professor Program (FiDiPro) working in the Center for Wireless Communication (CWC) at the University of Oulu, Oulu, Finland. He served as the Chair of the Department of Electrical and Computer Engineering from 2004-2014, and served as the founding director of Rice's Center for Multimedia Communications from 1998-2006.

His research interests are in the areas of communication theory, information theory, signal processing, and their applications to wireless communication, wireless networks, and neuroengineering with emphasis on closed-loop neuro-modulation and modeling of neuronal circuits connectivity and the impact of learning on connections in the circuits.

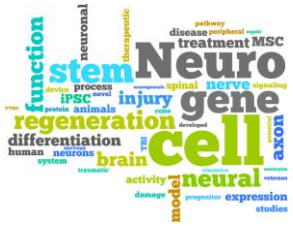
Supinder Bedi, Ph.D.
Instructor, Department of Pediatric Surgery
University of Texas Health Science Center at Houston

Central nervous system (CNS) injuries such as stroke, traumatic brain injury (TBI) and spinal cord injury (SCI) cause significant morbidity and mortality. Dr. Bedi's research focus has been on injury and regeneration of the CNS, and recently, the attenuation of the inflammatory response after a CNS injury. He utilizes rodent models to investigate the deleterious effects of injury. Some of these finding have been published in Journal of Neuroscience (2001 and 2010), Journal of Neurotrauma (2012). We are interested in the cellular changes that contribute to detrimental behavioral outcomes after a CNS injury. Specifically, he uses progenitor stem cells therapy to systemically attenuate the inflammatory effects of TBI via modulation of the spleen. These findings have been recently published in Stem Cells Translational Medicine (2013) and Journal of Trauma and Acute Care (2013). Stem cell treatments attenuate the inflammatory response and improved cognitive behavior. His focus is to delineate the role and mechanism(s) by which stem cells attenuate the inflammatory response of microglia/macrophages after TBI, and how that affects cognitive behavior. Dr. Bedi has a demonstrated record of successful and novel research projects and publications in an area of high relevance, and my expertise and experience have prepared me to be an independent investigator.

Dr. Bedi received his Ph.D. from the University of California Los Angeles and completed postdoctoral fellowships at New York University, University of California San Francisco, and the University of Texas Medical School at Houston.

Melissa S. Thompson, Ph.D.
Program Director, Research Consortia
Gulf Coast Consortia

After obtaining a doctorate in Chemical and Biomolecular Engineering at Johns Hopkins University, Dr. Thompson completed a postdoctoral fellowship at UT MD Anderson Cancer Center in the Department of Gynecologic Oncology and Reproductive Medicine. Her thesis and postdoctoral work focused on how external cues, including structural and signaling inputs, affected ovarian cancer progression. Dr. Thompson has joined the Gulf Coast consortia as Program Director in 2012 and has worked to foster and grow multidisciplinary research at the GCC's 7 member institutions, including research in the areas of NeuroEngineering and Regenerative Medicine.



NeuroRegeneration Symposium

September 1-2, 2015
BioScience Research Collaborative (BRC)

The symposium will be in the auditorium, unless otherwise noted.

September 1, 2015

3:45 PM	Welcome, Introductions, Charge to Participants
4:00 PM	Keynote Address ***Combined Electrical Stimulation and Cell Engineering to Formulate New Circuits in the Chronically Injured Spinal Cord*** Philip Horner, Houston Methodist Research Institute
5:00 PM	Posters and Networking Reception (Event Space)

September 2, 2015

9:00 AM	Overview
9:10 AM	**Panel Discussion: Neuro Training Programs** Moderator: Ben Deneen, Baylor College Medicine Sean Savitz, PI, University of Texas Houston Stroke Training Program (T32 NINDS) Rob Raphael, PI, NeuroEngineering IGERT (NSF) Charles Cox, Representative, Potential NeuroRegeneration Training Program
	Short Talks, Session I Chair: Ben Deneen, Baylor College of Medicine
10:00 AM	***Stem Cell Therapy for Stroke: From Bench to Clinical Trials*** Sean Savitz, University of Texas Health Science Center at Houston
10:35 AM	***Cell Therapies for Neurotrauma*** Charles Cox, University of Texas Health Science Center at Houston
11:10 AM	***Long Term Striatal Changes in Functional Connectivity in Veterans with Mild Traumatic Brain Injury (mTBI)*** Mary Newsome, Michael E. DeBakey VA Medical Center/Baylor College of Medicine
11:45 AM	Session Discussion
12:00 PM	**Breakout Session** *with Lunch (Event Space)*
	Short Talks, Session II Chair: Laura Smith Callahan, University of Texas Health Science Center at Houston
1:30 PM	***Scalable Neurotechnologies*** Jacob Robinson, Rice University
2:05 PM	***The Challenges of Mammalian Hair Cell Regeneration*** Andy Groves, Baylor College of Medicine
2:40 PM	***Localized Inhibition of P2X7 Receptors Using a Nanocomposite Hydrogel Improves Locomotion and Bladder Function after Spinal Cord Injury in Rats*** Alvaro Munoz, Houston Methodist Research Institute
3:15 PM	***Daam2-PIP5K is a Regulatory Pathway for Wnt Signaling and Therapeutic Target for Remyelination in the CNS*** Hyun-Kyoung Lee, Baylor College of Medicine
3:40 PM	Session Discussion
4:00 PM	*Break*
4:10 PM	**Break-In Discussion**
5:00 PM	Conclusion, Closing Remarks, and Awards
5:10 PM	Reception (Event Space)

KEYNOTE SPEAKER

Philip Horner, Ph.D.
Scientific Director, Center for Neuroregenerative Medicine
Co-Director, Center for Regenerative and Restorative Neurosurgery
Vice-Chairman, Research, Department of Neurological Surgery
Houston Methodist Research Institute

Horner comes to Houston Methodist from the University of Washington School of Medicine, where he was a professor of stem cell biology and neural repair in the Department of Neurological Surgery. He was also affiliated with UW's Institute for Stem Cell & Regenerative Medicine. His research focuses on the manipulation of a patient's own stem cells to regenerate cells damaged or lost following traumatic injuries.

Horner received his M.S. and Ph.D. in physiology in 1992 and 1995, respectively, from Ohio State University. Before joining the University of Washington faculty in 2001, he held research associate and staff scientist positions at the Salk Institute for Biological Studies in La Jolla, Calif. He brings several active research projects to Houston, including one funded by a National Institutes of Health R21 grant to investigate the diverse properties of astrocytes, specialized cells in the brain that play roles in everything from feeding neurons to repairing damaged brain and spinal cord cells. Horner is the co-principal investigator, with University of Washington Professor of Bioengineering and PI Suzie Pun, Ph.D., of an NIH R01 project to study the use of ultrasound to guide helpful genes into special cells that can replace damaged brain tissue. Horner is also the PI on a Department of Defense grant to treat spinal cord injury with bio-responsive gels that will protect a patient's own stem cells to support regeneration.
(via newswise.com, July 2015, "Brain Scientist Horner Joins Houston Methodist Neurological Institute")

Abstract:

Combined Electrical Stimulation and Cell Engineering to Formulate New Circuits in the Chronically Injured Spinal Cord

Mondello SE[1], Sunshine MD[1], Emerson, S[2], Fischedick AE[2], Moritz CT[1], Horner, P.J.[3]

1. Department of Rehabilitation Medicine, University of Washington, Seattle, WA, USA
2. Department of Neurological Surgery, University of Washington, Seattle, WA, USA
3. Center for Neuroregenerative Medicine, Houston Methodist Research Institute, Houston, Tx

Corresponding author: Philip J. Horner, Houston Methodist Research Institute, 6670 Bertner, Ave., MSR10-112, pjhorner@houstonmethodist.org

Spinal cord injury (SCI) results in the destruction of local spinal circuitry as well as the connections of descending motor fibers. The goal of this research is to replace neuronal circuitry using targeted activity and cell reprogramming methodologies. Human induced pluripotent stem cells (hiPSCs) can be differentiated into neural cells including spinal neurons and glial. In previous work we have shown that hiPSC-derived neural progenitor cells (hiPSC-NPCs) can be delivered to a chronic, cervical model of SCI. Human neurons extensively elongate processes within the chronic scar matrix at the lesion epicenter but functional recovery is limited. In addition, there is limited data indicating functional connectivity between transplanted neurons and the spared host circuitry. In the current work we explored the role of activity dependent plasticity as a method to drive neuronal connections. Electrical stimulation using wires implanted into the brain and spinal cord were used to activate circuitry involved in forelimb movement. Optogenetic approaches were also applied to hiPSC-NPCs. These studies reside at the interface between functional electrical stimulation and stem cell biology and suggest a pathway for targeted repair of circuitry in a variety of conditions including injury, stroke and degenerative disease. This work is supported by the NIH and a grant from the Craig H. Neilsen Foundation.

SPEAKER ABSTRACTS

(in order of appearance)

Sean Savitz, M.D.
Professor, Department of Neurology
Frank M. Yatsu, M.D. Chair in Neurology
Director, Vascular Neurology Program and Fellowship
University of Texas Health Science Center

Abstract:

Stem Cell Therapy for Stroke: From Bench to Clinical Trials

Savitz S

Dr. Savitz is a neurologist specializing in cerebrovascular disease (stroke and brain hemorrhage). He completed his neurology residency at the Beth Israel Deaconess Medical Center/Harvard Medical School Neurology Program in Boston. Recruited from the faculty at Harvard Medical School, he is an Associate Professor in the Department of Neurology and directs the stroke program at the University of Texas Health Science in Houston. He has built a Stroke Stem Cell/Regenerative Medicine division involving a bi-directional laboratory and clinical research program testing stem cells and other types of cell therapies for stroke. He has over 80 peer reviewed papers in the biomedical literature. Dr. Savitz completed the first NIH funded phase I, single arm study in the world on a patient's own bone marrow-derived stem cells given intravenously within 3 days of the onset of a stroke. In his laboratory funded by the NIH. Dr. Savitz's research team is finding that bone marrow cells dampen inflammation and repair damage after stroke in animal models. Dr. Savitz is also leading a randomized controlled trial testing the intra-arterial delivery of autologous stem cells in patients 11 to 19 days after stroke. He also has led a double-blind clinical trial examining the safety and efficacy of umbilical cord tissue-derived cells for acute ischemic stroke. Dr. Savitz believes cellular therapy, including the use of stem cells, is a promising new investigational approach for the treatment of stroke. Extensive animal studies have shown that various stem cells and other types of cells enhance recovery from stroke.

Charles S. Cox, Jr., M.D.
George and Cynthia Mitchell Distinguished Chair in Neurosciences and Director, Program in Children's Regenerative Medicine, Department of Pediatric Surgery
Co-Director, Texas Trauma Institute
University of Texas Health Science Center at Houston

Abstract:

Cell Therapies for Neurotrauma

Charles Cox, Supinder Bedi, Jennifer Juranek, Ryan Kitagawa, Linda Ewing-Cobbs, Robert Hetz,, George Lia, Ben Aertker, Fabio Triolo
University of Texas Health Science Center at Houston

TBI remains challenging in both the understanding of the pathophysiology and the development of therapeutics. As the injured brain progresses from neuroinflammatory degradation to regeneration, numerous molecular and cellular processes occur that can influence outcome. While many pharmaceutical agents have been developed and tested in preclinical trials, their contribution to the complex and evolving inflammasome following TBI has not materialized in any successful clinical trials. The advancement of preclinical studies for TBI using cell therapy has been slow due to the heterogeneity of injury models, outcome measures and assays. Thus, the International Society for Cellular Therapy (ISCT) recently has recently advocated validating and strengthening standardized assays to improve the reproducibility and consistency of such data.

Cellular therapy offers a pharmaceutical bioreactor that can sense and interact with the inflammasome. Studies have demonstrated that inflammatory M1 to regenerative M2 phenotypic shifts can be initiated by progenitor cells.. Off the shelf heterologous cells have been proposed for TBI therapy. Exogenous cells introduced either locally or systemically have not shown to reliably engraft or survive, and thus the optimal dosing time and regimen remains unanswered. Finally, any therapy for TBI can only be deemed successful if clinical trials can demonstrate improvements in outcome. Neuroimaging has demonstrated promising volume preservation with cell therapy, but the impact of these findings to cognitive and functional outcomes are unknown and require further understanding about the role of cell therapy in the regenerative process following TBI.

Our group has pursued the use of both autologous and allogeneic bone marrow derived cells and cord blood derived cells in pre-clinical models and early phase clinical trials. As the putative target is the neuroinflammatory response via microglial activation, it has become imperative that microglial activation can be measured in patients. To that end, we have pursued TSPO radioligand imaging via PET MRI/DT MRI to understand patients who may be most likely to respond to a cellular therapy.

In the short term, a multimodal approach that includes drugs such as neurostimulants, neurorehabilitation and immunomodulatory cell therapy likely offers the best strategy to maximize recovery potential.

Mary Newsome, Ph.D.
Associate Professor, Physical Medicine and Rehabilitation
Baylor College of Medicine

Abstract

Long term Striatal Changes in Functional Connectivity in Veterans with Mild Traumatic Brain Injury (mTBI)

Mary R. Newsome[1,2*], Xiaodi Lin[1,2], Maya Troyanskaya[1,2], Andrew R. Mayer[3,4], Randall S. Scheibel[1,2], Elisabeth A. Wilde[1,2], Joel L. Steinberg[5], Brian A. Taylor[1], Rajan Agarwal[1], and Harvey S. Levin[1,2]
[1]Michael E. DeBakey VA Medical Center, Houston, TX
[2] Department of Physical Medicine & Rehabilitation, Baylor College of Medicine, Houston, TX
[3]The Mind Research Network, Albuquerque, New Mexico
[4]Neurology Department, University of New Mexico School of Medicine, Albuquerque, New Mexico
[5] Institute for Drug and Alcohol Studies, Department of Psychiatry, VCU, Richmond VA

Objectives: Blast explosions are a common occurrence in the Iraq and Afghanistan wars; however, little is known about their long term effects on the brain. Animal and *in silico* models of blast traumatic brain injury (TBI) suggest there may be impact on structures involved in dopamine transmission. This study investigated the functional connectivity (FC) of subcortical regions implicated in dopamine transmission and hypothesized that veterans exposed to blast an average of five and a half years earlier would demonstrate altered FC in these regions in comparison to veterans who had not been exposed to blast.

Methods: Functional connectivity magnetic resonance imaging (fcMRI) was acquired from 17 veterans who had experienced a blast explosion an average of five and a half years after their most serious blast exposure, as well as from 15 veterans with similar demographic characteristics. FC was measured from bilateral caudate, putamen, and globus pallidus were used as seeds.

Results: The Functional Connectivity Toolbox (Conn) (Whitfield-Gabrieli and Nieto-Castanon, 2012) within SPM8 (Wellcome Department of Cognitive Neurology, University College, London, UK) implemented in Matlab (Mathworks Inc. Sherborn MA, USA) was used to process and analyze data. Between groups T-Tests revealed altered FC from the right putamen and right globus pallidus. However, covarying for depression and posttraumatic stress disorder revealed significant findings only in the globus pallidus. Covarying for depression revealed greater FC in the blast TBI group than the control group between right globus pallidus and right temporal occipital fusiform cortex, occipital fusiform gyrus, lingual gyrus, and cerebellum, while covarying for PTSD revealed greater FC in the same regions bilaterally. Accounting for PCL-C also revealed increased FC between the right caudate and left inferior frontal gyrus, opercular cortex, precentral gyrus, and postcentral gyrus.

Conclusions: Altered FC with the globus pallidus is evident 5.5 years after mild TBI. Altered FC between the globus pallidus and temporo-occipital regions may be related to impaired temporo-occipital projections to the striatum, which projects to the globus pallidus. Future studies are necessary to further explore the long term impact of blast on subcortical structures and development of related neurodegenerative diseases.

Funding Sources: This study was supported by the Department of Veterans Affairs, Veterans Health Administration, Office of Rehabilitation Research and Development, Project #B6812C Traumatic Brain Injury Center of Excellence and Project # I21RX001608.

Jacob Robinson, Ph.D.
Assistant Professor, Electrical and Computer Engineering and Bioengineering
Rice University

Abstract:

Scalable Neurotechnologies

Daniel L. Gonzales[1,2], Krishna N. Badhiwala[3], Daniel G. Vercosa[1,2], Martin Bell1,2, Ben W. Avants[2], Charles Sebesta 3, Zheng Liu[4], Weiwei Zhong[4], Arun Mahadivan3, Amina Qutub3, Jacob T. Robinson[1-3,5*]

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[3] Department of Bioengineering, Rice University, 6100 Main St., Houston, TX 77005.
[4] Department of BioSciences, Rice University, 6100 Main St., Houston, TX 77005
[5] Department of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Scalable technologies to read and write activity in individual neurons will help reveal how the brain works and how well treatments restore neural function. Our lab explores how nanotechnology will help create scalable technologies for studying the brain. One example is nanowire electrodes that can measure and manipulate the electrical potential across the cell membrane. Unlike traditional glass pipettes, nanowire electrodes can be fabricated over large areas providing a route toward scalable high-throughput electrophysiology that will open to the door to electrophysiological phenotyping and electrophysiology-based cell sorting. To realize the potential of this high-throughput nanowire-based electrophysiology we have developed a suite of integrated microfluidic devices that feature nanowire electrodes that can manipulate and measure the membrane potential as part of a larger lab-on-a-chip concept. With these integrated electrophysiology chips we can rapidly measure and sort cells based on the kinetics of ion channels or the kinetics of voltage sensitive proteins. In addition to these single measurements we show that integrated nanowire devices can perform electrophysiology in intact whole organisms like the nematode C. elegans. The high-throughput capability of our nanowire electrophysiology device allows us to identify mutant strains that show differences in firing rates and action potential waveforms, providing a platform for studying neurological development, disease and disease treatment. Overall, by integrating microfluidics with nanowire electrophysiology we believe electrophysiology will stand beside gene sequencing and fluorescence imaging as a complementary high-throughput assay for single cells and whole organisms.

Andy Groves, Ph.D.
Professor and Co-Director, Program in Developmental Biology
Baylor College of Medicine

Abstract:

The Challenges of Mammalian Hair Cell Regeneration

Maass, J.C.[1], Gu, R.[1], Jen, H.-I[3], Cai, T.[3]., Klisch, T.J.[2,5]., Zoghbi, H.Y.[2,5] and Groves, A.K.[1,2,3]
[1]Department of Neuroscience, [2]Department of Molecular and Human Genetics [3]Program in Developmental Biology and [4]Howard Hughes Medical Institute, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030
[5]The Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX 77030

Sensory hair cells are specialized vertebrate mechanoreceptors that detect sound, gravity and angular acceleration. Most vertebrates are capable of robustly regenerating hair cells after damage – for example, birds are able to recover at least 90% of their hearing after deafening in a matter of months. However, mammals have lost this ability and as a result, hair cell loss and its associated hearing impairments are permanent and progressive. Non-mammalian vertebrates mobilize supporting cells resident in the inner ear to divide and generate new hair cells. Although supporting cells exist in the hearing and balance organs of mammals, they are only capable of producing new hair cells for a very short period prior to the onset of hearing. We have been investigating the mechanisms that underlie this rapid loss of regenerative ability in collaboration with the Zoghbi lab at Baylor. We have identified a number of signaling pathways and molecular mechanisms that are modulated to prevent hair cell regeneration as mammals mature. For example, cell cycle re-entry of neonatal mouse supporting cells is mediated through EGF receptor signaling, and this leads to a rapid down-regulation of the cyclin-dependent kinase $p27^{kip1}$. However, p27 levels rise 40-fold in mouse supporting cells in the 14 days between birth and the onset of hearing. Second, inhibition of the Notch signaling pathway is capable of generating 50% more hair cells in the neonatal mouse cochlea, but is completely ineffective in one week old animals. Finally, activation of the hair cell-specific Atoh1 transcription factor can generate new hair cells in postnatal mice, but not after the onset of hearing at two weeks of age. In all these cases, the age-dependent failure of regeneration are due to maturational changes in the supporting cells of the ear, as no new supporting cells are added or lost after birth. We are now using deep sequencing approaches to understand the transcriptional and epigenetic changes that occur in the cochlea during this period. One of the challenges we face in this endeavor is the small amount of tissue present in the mouse cochlea. We have been working to refine methods for Chip-seq and RNA-seq with small numbers of hair cells and supporting cells in an attempt to understand the maturational changes that prevent hair cell regeneration in mammals.

Alvaro Munoz, Ph.D.
Assistant Research Professor of Urology, Institue of Academic Medicine
Assistant Research Member, Houston Methodist Research Institute
Regenerative Medicine Research Program, Center for Cardiovascular Regeneration

Abstract:

Localized Inhibition of P2X7 Receptors Using a Nanocomposite Hydrogel Improves Locomotion and Bladder Function after Spinal Cord Injury in Rats

Munoz A[1], Yazdi IK[1], Taraballi F[1], Tang X[1], Rivera C[1], Boone TB[1,2], and Tasciotti E[1]

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Objectives: Secondary expansion of tissue scar after spinal cord injury (SCI) leads to a decreased plasticity for neuronal regeneration. This condition is reflected on the failure for an efficient sensory control of different organs, including the urinary bladder, where neurogenic detrusor overactivity (NDO) significantly affects the quality of life in patients with SCI. The goals of our study were to develop a nano-formulation for decreasing microgliosis and scar formation using a localized and time-controlled delivery of a P2X7-type purinergic receptor antagonist at the injury site. The main objective was to decrease NDO and improve bladder sensory function.

Materials and Methods: PLGA-Si nanocomposite particles loaded with the P2X7R antagonist brilliant blue-G (BBG) were synthesized using a water-in oil-in suspension technique. Particles were further embedded into Pluronic® F-127. The release properties of BBG from the nanohydrogel were measured *in vitro* at 37°C using UV Spectroscopy at predetermined time points, and the degradation was characterized through SEM. The effect of the P2X7R antagonist-loaded nanohydrogel was evaluated in a partial-SCI rat model. Briefly, animals received a transection of the dorso-sensory aspect of the spinal cord at the thoracic T8/T9 level, and were immediately treated with either an empty, or an antagonist loaded nanohydrogel. Changes in locomotion using the Basso, Beattie, and Bresnahan scoring system were evaluated over a period of one month. At the end of the four weeks, an open cystometric assessment was performed in animals under urethane anesthesia; rats were euthanized after cystometry, perfused with 4% paraformaldehyde and immunohistochemistry of the spinal cord was performed to determine the effects of BBG on the spinal cord expression of the neuronal marker MAP2, and the degree microglia activation using CD11b as a microglia marker.

Results and Discussion: We have optimized a fabrication protocol for P2X7R antagonist-loaded nanohydrogels. The formulation has been characterized for the long-term release of the antagonist *in vitro,* demonstrating sustained and constant release for more than two weeks. The functional evaluation of locomotive capacities confirmed an improvement throughout the 4 weeks evaluation period. The cystometric evaluation showed a trend towards better recovery of bladder function and reduced frequency of non-voiding contractions (i.e. less damaging NDO conditions) in the BBG treated group at one month post-SCI. The expression of MAP2 and CD11b were evaluated at a cord region as close as possible to the center of the dorsal transection. Neuronal immunostaining suggest that the dorsal horns from rats receiving the empty preparation have a more severe injury than rats treated with the P2X7R antagonist.

Conclusions: Our injectable formulation has suitable characteristics for a quick and effective SCI-localized as well as long-term inhibition of P2X7R. Data indicated a trend towards better locomotive recovery, and bladder function in the SCI rats treated with the P2X7R antagonist. Decreasing microgliosis may improve neural plasticity after SCI.

Acknowledgements: This work was supported by the Methodist Hospital Foundation, the Brown Foundation, and the Cullen Trust Fund for Healthcare grants.

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Abstract:

Daam2-PIP5K Is a Regulatory Pathway for Wnt Signaling and Therapeutic Target for Remyelination in the CNS

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Wnt signaling plays an essential role in develop- mental and regenerative myelination of the CNS; however, contributions of proximal regulators of the Wnt receptor complex to these processes remain un- defined. To identify components of the Wnt pathway that regulate these processes, we applied a multifac- eted discovery platform and found that Daam2- PIP5K comprise a novel pathway regulating Wnt signaling and myelination. Using dorsal patterning of the chick spinal cord we found that Daam2 pro- motes Wnt signaling and receptor complex forma- tion through PIP5K-PIP_2. Analysis of Daam2 function in oligodendrocytes (OLs) revealed that it sup- presses OL differentiation during development, after white matter injury (WMI), and is expressed in human white matter lesions. These findings suggest a phar- macological strategy to inhibit Daam2-PIP5K func- tion, application of which stimulates remyelination after WMI. Put together, our studies integrate infor- mation from multiple systems to identify a novel regulatory pathway for Wnt signaling and potential therapeutic target for WMI.

Funding source

This work was also supported in part by the Baylor College of Medicine IDDRC (HD024064) and by the Integrated Microscopy Core at Baylor College of Medicine, with funding from the NIH (HD007495, DK56338, and CA125123), the Dan L. Duncan Cancer Center, and the John S. Dunn Gulf Coast Consortium for Chemical Genomics.

POSTER SESSION

Characterizing the Variability of Sequential Neural Activity in the Hippocampus under Various Models of Dementia

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Several neurodegenerative diseases and/or dementias are characterized—at least in part—by a decline in memory performance. As a consequence, it is important to understand how these diseases affect the hippocampus (HP), which plays a critical role in learning and memory. Place cells in the HP fire in a sequential order as animals move around in an environment, and these sequences have been known to "replay" during sharp-wave ripples (SWRs)—this reactivation is believed to play an important role in memory consolidation.

A number of previous studies have reported a remarkable increase in SWR activity in the HP—including in calcineurin knockout, as well as dominant negative DISC1 (DN-DISC1) models of schizophrenia (SCZ). In addition, the HP has been reported to be "hyperexcitable" in other dementia models including at least a mouse model of tauopathy, where the hyperexcitability has been hypothesized to be a result of the reduced function of parvalbumin-containing interneurons in the HP.

However, the nature of the activity in the HP under these different models of dementia is still not well understood. Cheng & Ji (2013) have reported a nearly-complete dissociation between place cells in the HP and external cues or stimuli in the environment in rTg4510 mice—a model of tauopathy—but remarkably, they still observed robust firing sequences as animals ran in both familiar and novel environments. In most of the other studies mentioned before, no sequential activity was found during SWRs in the diseased animals, whereas sequential activity was preserved in the healthy controls. These findings might at first seem contradictory, but it is important to note that Cheng & Ji did not consider HP activity during SWRs, but only during active behavior. As a consequence, it is perhaps possible that the dissociation and transient nature of sequential activity observed by Cheng and Ji was caused by a disruption in the consolidation process, as suggested by the lack of sequential activity during SWRs reported by other authors.

It seems important to better characterize and understand the neural activity in the HP—both during SWRs and during normal behavior—to facilitate a deeper understanding of the effects of these diseases or dementias on the HP and memory. To this end, we propose to use a latent variable modeling approach to characterize the variability in HP activity. Latent variable models seem like a natural choice, because for both replay-like activity during SWRs as well as for dissociated sequential activity during normal (diseased) behavior, the driving stimuli are *unobserved* (or latent). In this way, we hope to gain some insight into the disrupted nature of sequential activity, as well as to answer more explicit questions like "how variable (disorganized) is the activity in the HP during dissociated place cell sequences?" or "is all sequential organization lost during SWRs under SCZ, or is there previously unseen sequential activity, driven by some (latent or unobservable) factor?"

In this poster, we will describe some of the ways in which latent variable models can help us to answer questions like these, and the efficacy of this approach will be demonstrated on synthetic data.

Funding sources: This work was funded by an NSF CAREER award (CBET-1351692) and a HFSP Young Investigator's award (RGY0088).

Effect of Pre-Injury Depletion of Peripheral Macrophages on Blood Brain Barrier Permeability at 72 Hours after Controlled Cortical Impact

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Traumatic brain injury remains without a proven therapeutic agent for improving long-term outcomes and reducing secondary injury despite numerous phase III clinical trials. Systemically administered stem cells are currently being investigated as a potential therapeutic agent due to their immunomodulatory effects which appear to be due to interaction with immune cells outside the area of injury. This experiment was performed to help determine the effect of macrophage depletion on the treatment with human mesenchymal stromal cells and whether this interaction is necessary for restoring blood brain barrier permeability following a model of traumatic brain injury.

To test this interaction 60 male Sprague-Dawley rats were divided equally into 6 different groups: CCI pretreated with PBS control liposomes, sham-depleted, CCI-depleted, CCI-depleted + hMSC, CCI + MSC, and sham-non depleted. Animals undergoing macrophage/monocyte depletion received 4 uL/g of clodronate liposomes IV 24 and 48 hours prior to injury. The CCI injury was performed over the site of a right sided craniotomy to a depth of 3.1 mm at 5.6 m/s. The groups receiving hMSC were then treated at 24 hours with 10 x 10^{6} cells/kg. At 72 hours following CCI or sham injury, animals were injected with Alexa Fluor 680 dye via the tail vein. The dye was allowed to circulate for 30 minutes prior to euthanasia and tissue extraction. Six 2 mm coronal slices from each brain encompassing the area of injury were then imaged using a LI-COR Odyssey CLx scanner at 700 and 800 nm. Image analysis was performed with ImageJ 1.48p (http://imagej.nih.gov/ij), an open source image processing program. Group data were analyzed with one-way analysis of variance with Dunnett's multiple comparison test.

A significant increase in blood brain barrier permeability was observed at the 72 hour time point in macrophage depleted animals when compared to animals pretreated with PBS control liposomes. There was no statistically significant difference in this experiment between animals pretreated with PBS control liposomes, macrophage depleted animals receiving hMSC and non-depleted animals receiving hMSC.

Depletion of peripheral macrophages has been shown in previous studies to have both beneficial and detrimental effects on CNS injuries, possibly dependent on the timing of depletion. This experiment further confirms the benefits of an intact macrophage response on restoring blood brain barrier permeability. Unfortunately, due to a lack of positive control in this experiment between hMSC treated animals and those undergoing pre-treatment with PBS control liposomes we are unable to determine whether hMSC administration provided any benefits in macrophage depleted animals. It may be necessary to perform subsequent investigations without performing pretreatment with PBS control liposomes.

In vivo Axonal Branching Inside Microfluidic Channels

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Axon branching is a complex morphological process, which numerous axonal branches begin to emerge from the peripheral nerve injury. The remarkable capability of a single axon to extend multiple branches and form terminal arbors enables vertebrate neurons to integrate information from different regions of the nervous system. Axons select appropriate pathways during development, but it is the branches that extend from the axon shaft and grow toward specific targets that are responsible for virtually all of the synaptic connectivity in the vertebrate. Many factors known to be important for axon growth and guidance have emerged as key regulators of axon branching, the regulation of which we are just beginning to understand.

Further studies are needed to clarify the cooperation of the signaling cascades of axonal growth, guidance and branching that together generate the characteristic trajectories of a particular axon type. Branching could profoundly improve our understanding of the malfunction of neuronal circuits that cause neurological and mental disorders. Therefore, more detailed information on diverse and complex molecular processes of axonal branching will not only promote our understanding of the development of nervous system circuitry but also lead to new therapeutic strategies in the treatment of disease improved techniques to stimulate growing and collateral formation might also lead to a better functional recovery of neuronal circuits after axonal injury.

We developed a method to observe axon branches by using multichannel PDMS stacks. In order to fabricate PDMS nerve scaffolds, multiple straight pattern with 120 µm microchannel was designed by using AutoCAD software, and printed on the transparent film. By using MEMS technology, straight structure was fabricated on top of silicon wafer by using SU-8 photoresist. Then PDMS (Sylgard® 184, Dow Corning) was spin coated on the silicon wafer and cured in oven at 90°C for 30 minutes. PDMS layer was cut into eight individual straight structure and stacked on top of each other, then wrapped with thin PDMS film for suture guide and hold PDMS layers. PDMS was applied as a glue between PDMS film and PDMS layers and cured in oven at 90°C for 30 minutes to hold structures together. The device was successfully implanted by suturing both the distal and proximal ends of the nerves to the guides of the device in the animal facility at UTPA.

All harvest tissues have been stained with anti neurofilament 160 antibodies in order to visualize axons inside the microfluidic channels. Entire axon lengths from the lesion site to their distal ends could be followed using a laser-scanning confocal microscope. On average, we observed six axon branches in each microchannel layer. Axon branches were formed either by splitting of the growth cone that is the highly motile sensorimotor structure at the tip of an extending neurite or by outgrowth of a collateral from the rigid axonal shaft.

Funding Sources: DoD Army Research Office DURIP

FGF14 is Required for Neurogenesis of Granule Neurons in the Adult Dentate Gyrus

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Adult neurogenesis, the production of mature neurons from progenitor cells in the adult mammalian brain, is linked to the etiology of neurodegenerative and psychiatric disorders. However, a thorough understanding of the molecular elements at the base of adult neurogenesis remains elusive. Here, we provide evidence for a previously undescribed function of fibroblast growth factor 14 (FGF14), a brain disease-associated factor that controls neuronal excitability and synaptic plasticity, in regulating adult neurogenesis in the dentate gyrus (DG). We found that FGF14 is dynamically expressed in restricted subtypes of Sex Determining Region Y-Box 2 (Sox2) positive and doublecortin (DCX) positive neural progenitors in the DG. BrdU incorporation studies and confocal imaging revealed that genetic deletion of *Fgf14* in *Fgf14*$^{-/-}$ mice leads to a significant change in the proportion of proliferating, and immature and mature newly born adult granule cells. This results in an increase in the late immature and early mature population of DCX and calretinin (CR) positive neurons. Electrophysiological extracellular field recordings showed reduced minimal threshold response and impaired paired-pulse facilitation at the perforant path to DG inputs in *Fgf14*$^{-/-}$ compared to *Fgf14*$^{+/+}$ mice, supporting disrupted synaptic connectivity as a correlative read-out to impaired neurogenesis. These new insights into the biology of FGF14 in neurogenesis shed light into the signaling pathways associated with disrupted functions in complex brain diseases.

Funding sources: This work was supported by NIH Grant R01MH095995 (F.L.). M.A.A. and T.K.A. are sponsored by King Saud University, Saudi Arabia, PhD scholarship (M.A.A.), and PhD scholarship (T.K.A.).

Spinal Cord Regeneration with Autoregenic Stem Cells – a Case Study*

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The scientific literature reports that about 180,000 cases of spinal-cord injuries (SCI) occur yearly in the world. Recent publications show neurological benefit in selected quadriplegics undergoing intra-lesion transplantation of autologous cultured bone-marrow mesenchymal stem cells. While these studies are promising, we were interested in a possible advance based on use of quasi-autologous SCNT cell-therapy improve extremity motor and sensory impairment in chronic quadriplegia (Objectives). We call such cells 'autoregenic' stem cells – ARSCs - produced with regulatory approval in our affiliate laboratory in South Africa.

Also with regulatory approval, our surgical team obtained level-III objective evidence and partial neurological clinical recovery in a 32-year old-male with chronic complete quadriplegia who underwent ARSC (cell) therapy for traumatic spinal-cord injury (SCI) sustained 6-years previously.

Methods: Cell-transplantation was by neuro-surgical implantation into the damaged cervical cord 6-years after SCI that rendered the patient a complete quadriplegic confirmed on neurological examination and magnetic resonance imaging (MRI). Neurologic assessment, restoration of dermatomes and myotomes were evaluated post-procedurally for 12-months together with MRI, and American Spinal Injury Association grading (ASIA).

Results: Neurological improvement was asymmetrically improved in the shoulder girdle, upper extremity bilaterally and trunk without dramatic change in leg-function at 12-months. ASIA-scales increased from 29/112 to 64/112 at 6-months after treatment and at least one ASIA-level was gained.

Conclusion: Compared to baseline findings, measured neurological improvement was documented in the shoulder-girdle and upper-extremities, 6-12 months after intra-lesion autologous SCNT cell transplantation in a chronic-quadriplegic.

*Interim surgical results herein were previously reported in the literature by our surgical team (Rev Arg de Anat Clin; 2014, 6 (1): 35-42; DuToit, D.F., Liebenberg, W.A.).

Arhgef4 Influences Reactive Astrocyte Formation and Blood-Brain Barrier Re-Establishment in a White Matter Injury Model

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Astrocytes are emerging as vital for the physiologic and cognitive functions of the central nervous system (CNS) and their deregulation is associated with several neurological (ALS) and inflammatory disorders (multiple sclerosis). Not only are astrocytes involved in numerous physiological functions including blood-brain barrier formation/maintenance and metabolic regulation, but they also play essential roles during CNS injury. Upon injury, astrocytes become "reactive" and form the glial scar, a structure necessary for repair, especially for the re-establishment of the blood-brain barrier (BBB). Despite these vital functions, little is known about the formation, regulation and activity of reactive astrocytes. Understanding mechanisms that regulate reactive astrocytes will have important implications for multiple disorders.

In a screen to identify regulators of astrocytes formation, we recently characterized Arhgef4 as a novel gene expressed in both glial precursor cells and mature astrocytes. Arhgef4 is a guanine exchange factor for the Rho GTPase family of protein and has been shown to impact cell cytoskeleton in other systems. While not essential for CNS development, Arhgef4 is expressed in Human Multiple Sclerosis lesions, suggesting that Arhgef4 might play a significant role during CNS injuries. We used lysolecithin injection into the ventral white matter region of the spinal cord as a model for White Matter Injury, and multiple sclerosis in particular. Lesions performed on Arhgef4 germline knock-out (KO) animals appear to have delayed remyelination when compared to heterozygous littermate animals. Since Arhgef4 is expressed in OPCs, it was necessary to assessed whether loss of Arhgef4 KO affected oligodendrocyte differentiation. In vitro cultures of OPCs from Arhgef4 KO and HET animals showed no difference in differentiation potential, implying that defective remyelination is probably due to astrocytes, and reactive astrocytes especially. We looked into BBB re-establishment after lysolecithin injection and found that blood was still present in lesions from Arhgef4 KO animals only. Overall, lesions from KO animals are bigger and take longer to repair.

Ongoing studies are focusing on assessing whether Arhgef4 is essential in other CNS injuries, such as photothrombotic stroke. Further work will also center on better understanding how Arhgef KO astrocytes differ from Arhgef4 HET. Guanine exchange factors, such as Arhgef4, are potentially good target for therapies since they affect critical cellular pathways while being tissue specific. This study specifically identified Arhgef4 as a critical gene in reactive astrocyte formation during white matter injuries.

Funding source provided by CPRIT RP101499

A microchannel array for *in vivo* Growth Cone Analysis of the Peripheral Nervous System

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The complex process of axon guidance is largely driven by the growth cone. During development of the nervous system, neurons extend axons through a dynamic environment to reach its final destination with growth cones leading the way at the tip of each axon. In this way, the growth cone can be called an ever-changing cytoskeletal vehicle that serves to define and guide, and its distinctive cytoskeletal structure offers an intriguing platform to study how extracellular cues can be translated into mechanical outgrowth and turning behaviors. In order to propel the growth cone forward and accurately navigate to find its specific targets, the growth cone must integrate multiple sources of guidance cue information to modulate its cytoskeleton during axon outgrowth. The neural wiring that occurs during this process happens through a combination of initial neuronal activity-independent guidance events. These early formed connections are subsequently refined through electrical signaling among neurons. The cues that direct axons and dendrites initially can perform at variety of ranges, and are capable of controlling the bundling of axons together into nerves or fascicles and also of mediating interactions between nerves and the substrates on which they extend.

Despite notable advances over decades of research, our current understanding of how the growth cone achieves its impressive outgrowth is far from complete. Here, we introduce a microchannel scaffold capable of facilitating the growth cone's cytoskeletal vehicle. The microchannel scaffolds have been developed for growth cone analysis of *in vivo* peripheral nerve and will provide us a clear view of the growth cone. Because of the linear nature of the array, we have more control over the path the growth cones are able to take as opposed to other nerve conduits.

The surgical procedures for implanting the microchannel scaffold device involved first placing a *Lewis* rat into an induction chamber and put under gas anesthesia (Isoflrane) until unconscious. The locations to be operated on were shaved and cleaned with isopropyl alchohol and a betadine scrub. Throughout the surgery, the *Lewis* rat was kept under anesthesia by hooking its maxillary central incisors into a gas mask and continuously providing it with small doses of the gas. Incisions were made along the right thigh to expose the sciatic nerve, tibialis anterior (TA), and soleus (SOL) muscles once it was securely placed on the surgery table and its body temperature regulated with a hot pad. The nerve was severed, proximal to the tibial and fiular nerves, and the microchannel scaffold was implanted by suturing both the distal and proximal ends of the nerves to the guides of the device.

With the device implanted, the nerve is free to regrow through the channels. Once regrown, the device can be removed, and because the scaffolds are arranged in layers, each layer can be separated and looked at individually. Neurofilaments play a big role in helping us view the growth cones. They are intermediate filaments and are found in large numbers in axons, where they are vital for the maintenance of axon caliber, the radial growth of axons during development and the transmission of electrical impulses along axons. By staining the neurofilaments, the axons and the growth cones would be highlighted and with the help of an electron microscope, we were able to observe the growth cone inside the channels. The growth cone appeared as a bulbous shape alongside the neurons confirming a successful growth inside the microchannels.

Funding Sources: DoD Army Research Office

Novel Role of RNA/DNA Binding Protein TDP-43 in DNA Damage Response in Motor Neurons: Implications to Amyotropic Lateral Sclerosis.

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TAR DNA-binding protein 43 (TDP-43),is an RNA/DNA-binding protein involved primarily in RNA processing, but its cytoplasmic aggregates have been found to be a pathological hallmark in motor neurons affected with Amyotrophic Lateral Sclerosis (ALS). In normal neurons, TDP-43 shuttles between nucleus and cytoplasm due to its function in RNA processing; but TDP-43 also binds to DNA, although its DNA-binding functions have not been explored. A significant accumulation of genomic damage is observed in TDP-43-linked diseases and previous studies identified a DNA repair protein "Ku" in a TDP-43 immunocomplex from human cells. In this study we investigated the possibility of TDP-43's involvement in DNA damage repair; a previously unexplored area. We used *in situ* Proximity Ligation Assay (PLA) to examine *in cell* interaction of TDP-43 with double strand breaks repair (DSBR) proteins in human neural stem cell (hNSC) line differentiated into spinal motor neurons. The PLA showed a strong interaction between TDP-43 with DSBR proteins Ku70, DNA-PKcs, XRCC4 and DNA ligase IV. The TDP-43's association with DSBR proteins was significantly enhanced in cells exposed to DSB-inducing ionizing radiation (IR). The *in cell* association was confirmed by immuno-precipitation (IP) analysis with endogenous TDP-43 and FLAG-TDP-43 ectopically expressing cells, including enhancement of interaction after IR. Neutral and Alkaline comet assay analysis in TDP-43 depleted cells showed a persistent accumulation of DSBs in the absence of any genotoxic agent which is highly significant. Loss of TDP-43 affected recruitment of 53BP1 and XRCC4/DNA LigaseIV complex (two key components of non-homologous end joining mediated DSB repair pathway) at DSB sites. Together these data strongly suggest a role of TDP-43 in efficient DSB repair in neuronal genomes and its pathological linkage to neurodegeneration.
(Supported by NIH/NINDS and MDA grants to MLH).

A Microfluidic Channel for Monitoring *in vivo* Peripheral Nerve Regeneration

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Peripheral nerve regeneration offers a significant clinical challenge and the current state of the art using autografts to repair peripheral nerve gaps is unsatisfactory because of the sacrifice of sensory sural nerves and associated dimensional mismatching. Commercial nerve conduits for nerve repair are usually composed of type I collagen or biodegradable polymers such that the conduit will degrade once the nerve has healed. In this manuscript, Multilayer microfluidic channels as many as 80 (ten channels by eight layers) were developed for a 3 mm gap peripheral nerve regeneration and successfully implanted within the sciatic nerve of eight Lewis rats and harvested post implantation of two and four weeks to give detailed information regarding the nerve regeneration process. Commercially available microwires were efficiently used and no special micromachining equipment was required to implement the microfluidic channels. PDMS has been chosen for the base material of the scaffolds due to its biocompatibility, flexibility, transparency, and well-developed fabrication techniques and the process of observing the axon outgrowth across the nerve gaps with PDMS scaffolds has been challenging due to the limited number and fineness of longitudinal sections that can be extracted from harvested nerve tissue samples after implantation. The separable layer by layer structure is treated as an individual layer during the histology process, provides the details of biological events during axonal regeneration. The process of sectioning PDMS scaffolds often results in the destruction of the transversal sample sections. To overcome this issue, the presented multilayer microfluidic channels have been developed in such a way that can be disassembled after nerve regeneration and tissue harvesting which can maintain the structural integrity of the device while still allowing separation of individual layers and sample extraction. Multilayer PDMS microfluidic channels allow an in-depth observation of the regenerative process, especially when demonstrating the distribution of axon regrowth through all individual microchannels.

Confocal microscopic imaging showed the details of peripheral nerve regeneration within the microfluidic channels. Immunohistochemistry analysis revealed that the microfluidic channels facilitated the robust nerve regeneration of transected sciatic nerves. Regenerated nerves occupied all 80 microchannels and there are two and four week's nerve regeneration patterns to compare axonal growth at two different time period. The numbers of axons were counted by scanning the 3D confocal images. Observation of the z-stack images of layer sections post removal showed an average of 15 axons after the second week and 38 axons after the fourth week per 120 μm diameter channel out of four animals per group. Though some motor and sensory axon types are indistinguishable by size alone, some sensory axon types are considerably thinner than most motor axons.

Since the microfluidic channels can be easily modified in the fabrication process, scaffolds for use in a variety of nerve gap injuries can be quickly fabricated on an individual case basis with a minimum of expense and the unique microwire molding technique could be easily combined with high temperature polymer melting and reforming methods. In the future, this device may be used in conjunction with electrode arrays and signal processing techniques for prosthetic control applications or be fabricated from biodegradable polymers for solely nerve regeneration purposes.

Funding Sources: DoD Army Research Office DURIP

Misregulation of LIN28 Affects Gliogenesis and Neuronal Maturation in Human iPSC Models of Rett Syndrome

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Rett Syndrome (RTT) is an autism spectrum disorder (ASD) caused by mutations in MECP2 primarily affecting girls. Complete loss of MECP2 function in boys causes congenital encephalopathy, characterized by neurodevelopmental arrest and early lethality. Patient-derived human induced pluripotent stem cell (iPSCs) have been successfully used to model various cellular aspects of the disorder, such as deficits in synaptic numbers and neuronal connectivity (Marchetto, Carromeu et al., 2010, Cell). Novel iPSC lines from male patients harboring mutations in MECP2, along with control lines from their unaffected fathers, give us an unprecendented opportunity to identify the earliest molecular changes directly in human cells. We differentiated iPSC-derived neural progenitor cells (NPCs) using retinoic acid and used Stable Isotope Labeling with Amino acids in Cell culture (SILAC) followed by mass spectrometry to compare their proteomes. We found that the differentiation of astrocytes following retinoic acid treatment is perturbed in two different patient iPSC lines. We then discovered that LIN28, a gene important for pluripotency, is upregulated in RTT NPCs. Overexpression of LIN28 in control NPCs suppressed astrocytic differentiation and impaired neuronal synapse density. We also identified an interaction between MECP2 and the LIN28 promoter. These results indicate that a specific gene expression change in early development may lead to compromised glial and neuronal development in RTT.

This work was supported by grants from the California Institute for Regenerative Medicine (CIRM) TR2-01814 and TR4-06747 (to A.R.M), and RB3-05229 (to A.G. and J.J.K.). The Muotri lab is supported by National Institutes of Health (NIH) through the NIH Director's New Innovator Award Program (1-DP2-OD006495-01), an R01 MH100175-01 from NIMH and from the International Rett Syndrome Foundation (IRSF grant # 2915). J.N.S. is supported by an F. Hoffman-La Roche Postdoctoral Fellowship Award (SFP2063) and a 1K99DC013805-01. M.L.-A. is supported by a postdoctoral fellowship from the Fonds de recherche du Québec–Nature et technologies (FRQNT). The Yates' lab is supported by UOW/R41GM103533, MH–2 R01 MH067880-11 and 1 R01 MH100175-01.

Predicting Therapeutic Efficacy of Mesenchymal Stem Cells Based on Their Anti-Inflammatory Potential

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Over the past two decades, there hasn't been a clear decrease in mortality or improvement of outcome following traumatic brain injury (TBI), the leading cause of death and disability in children and adults from ages 1 to 44. Mesenchymal stem cells (MSC) arise as a promising TBI treatment. Paradoxically, the ease to isolate and culture MSC led to a wide range in efficacy from reports using MSC clinically and in pre-clinical models, likely due to differences in cell preparations and a significant amount of donor variability. Studies indicate MSC can treat TBI by counteracting neuroinflammatory responses following TBI, known to significantly broaden neurodegeneration.

In here, we hypothesized that the immunomodulatory potency of MSC would predict the therapeutic efficacy of MSC in TBI. To test our hypothesis, we first evaluated the anti-inflammatory effects of MSC both in vitro and in our TBI model. We then correlated these effects with the expression of known immunomodulatory factors and lastly, used a loss of function approach to determine the impact of such factors in our TBI model.

Our results show that MSC from human bone marrow (hBM), but not amniotic fluid (hAF), exert anti-inflammatory effects in vitro. Likewise, hBM-, but not hAF-MSC improve TBI outcome by decreasing BBB permeability and microglia activation and accumulation in the brain, markers of neuroinflammation. Among prominent anti-inflammatory factors investigated, there was a positive correlation between PGE2 and IDO expression and the anti-inflammatory potential of MSC seen *in vitro*. Current experiments suppressing expression of COX-2 and IDO using RNAi will reveal the true extent of the contribution of PGE2 and IDO in the therapeutic effects of MSC treatment following TBI.

Hence, the results here indicate that the anti-inflammatory potential of MSC, measured by the expression of PGE2 or IDO, could be critical for the prediction of therapeutic efficacy of MSC in TBI.

Funding from the TIRR Foundation and Mission Connect Award #13-110 and Glassell Family Pediatric Stem Cell Research Fund.

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Apcdd1 Stimulates Oligodendrocyte Differentiation after White Matter Injury

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Wnt signaling plays an essential role in developmental and regenerative myelination of the CNS, therefore it is critical to understand how the factors associated with the various regulatory layers of this complex pathway contribute to these processes. Recently, Apcdd1 was identified as a negative regulator of proximal Wnt signaling, however its role in oligodendrocyte (OL) differentiation and remyelination in the CNS remain undefined. Analysis of Apcdd1 expression revealed dynamic expression during OL development, where its expression is upregulated during differentiation. Functional studies using ex vivo and in vitro OL systems revealed that Apcdd1 promotes OL differentiation, suppresses Wnt signaling, and associates with β-catenin. Application of these findings to white matter injury (WMI) models revealed that Apcdd1 similarly promotes OL differentiation after gliotoxic injury in vivo and acute hypoxia ex vivo. Examination of Apcdd1 expression in white matter lesions from neonatal WMI and adult multiple sclerosis revealed its expression in subsets of oligodendrocyte (OL) precursors. These studies describe, for the first time, the role of Apcdd1 in OLs after WMI and reveal that negative regulators of the proximal Wnt pathway can influence regenerative myelination, suggesting a new therapeutic strategy for modulating Wnt signaling and stimulating repair after WMI.

Funding: This work was supported by grants from the National Multiple Sclerosis Society (RG 4623A1/2 to BD), the Sontag Foundation (BD), and the National Institutes of Health (R01 NS071153 to BD, R01NS078294 to BA). This work was also supported in part by the Baylor College of Medicine IDDRC (HD024064).

Chronic Deep Brain Stimulation System

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In patients with Parkinson's disease, Deep Brain Stimulation is applied continuously over long periods. Typical experiments in rodent models rely on a tether to deliver stimulation current to the implanted electrode making studies involving long-term stimulation and complex behaviors difficult or impossible. While tether-free rodent-scale pulse generators have been developed to address this need, they have lacked the flexibility required to test novel patterns of stimulation. We have developed a flexibly programmable stimulator and a customizable 3-D printed housing that allow for chronic delivery of normal or novel patterns of DBS in rats. We will present three example scenarios that reflect our need for portable pulse generation that can last for extended periods without constant supervision. First, it is enabling new studies on the therapeutic value of more complex patterns of stimulation. Second, we are planning to use our device to investigate the therapeutic value of DBS in new genetic models that mimic the slow onset and progression of human symptoms. Finally, we are planning to use our device to test the biocompatibility of the new electrode materials under stimulation in order to mimic proper in vivo conditions. Thus, we anticipate that our low-cost, open source platform will provide tremendous value to our experiments and to future studies involving chronic stimulation in rodent disease models.

This work was funded by an NSF CAREER award (CBET-1351692) and a HFSP Young Investigators award (RGY0088).

Generation of Neural Lineage Knockin Reporters in Human Pluripotent Stem Cells by CRISPR/Cas9

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Objectives: Pluripotent stem cell technologies are powerful tools for modeling development and disease, and for drug screening and regenerative medicine. Faithful gene targeting in pluripotent stem cells greatly facilitates these applications. We have developed a fast and precise CRISPR(Clustered Regularly Interspaced Short Palindromic Repeats) /Cas9 (CRISPR-associated) technology-based method and obtained a collection of reporter constructs of neural lineage specific markers, including Neurogenin2 and Olig2, and generated neural lineage reporters. This platform allows us to directly purify transcription factor (TF)-defined specific progenitor populations by fluorescence activated cell sorting (FACS) for subsequent molecular characterization and high-throughput drug screening.

Methods: Knockin vectors were constructed by recombineering and multisite gateway. Single guide RNAs (sgRNAs) were designed with ZiFiT software and subcloned into sgRNA-expression vector using Golden-Gate assembly. Multiple sgRNAs were constructed and Surveyor assays were performed to identify suitable sgRNAs for subsequent transfection. Next, human iPSC line ND2.0 was electroporated with sgRNA, Cas9 and the TF targeting vector. Correctly targeted clones were verified by Southern blot analysis. Off-target prediction and analysis were carried out using CasOT, a Perl-based software, and PCR. Positive TF reporter cells were induced by dual SMAD inhibitor dorsomorphin and SB431542, followed by retinoid acid and purmorphamine (Shh agonist) in N2B27 medium. mCherry-expressing (Neurog2+) or GFP-expressing (Olig2+) cells were FACS-purified. A detailed differentiation time course of a specific TF-defined population was recorded and subsequent differentiation toward neurons and glia was performed. Furthermore, global gene expression profile of FACS-purified reporter cells was compared by bead based cDNA microarray.

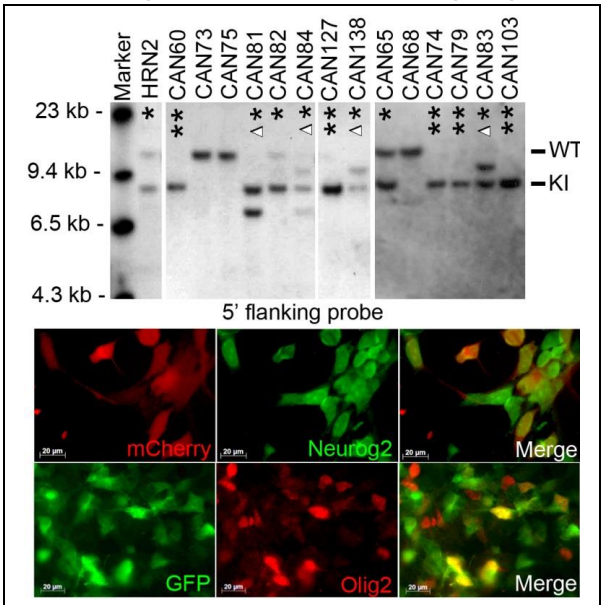

Fig 1. Generation of Neurog2-mCherry and Olig2-GFP knockin reporters in hiPSC ND2.0. (*Top*) Southern blot analysis for CRISPR-assisted Neurog2 targeting (designated as CAN clones) yielded a significantly improved targeting efficiency compared to conventional targeting (clone HRN2). * and **, targeting at one and two alleles respectively. Δ, deletion at one allele. (*Middle*) After differentiation, cells were purified by mCherry+ sort. Knockin mCherry expression recapitulated the endogenous Neurog2 expression (green). (*Bottom*) Similarly, Olig2-GFP cells were induced to express robust native GFP which recapitulated Olig2 (red) expression.

Results: Gene targeting efficiency was greatly improved in CRISPR/Cas9 mediated targeting (~36% correctly targeted clones) compared to conventional targeting protocol (~3%) at the same locus. Interestingly, ~33% of positive clones were targeted at both alleles (*Fig 1, top panel*). No off-target events were detected. Neurog2 and Olig2 reporter lines were induced and FACS-purified for mCherry (Neurog2+) or GFP (Olig2+) cells for further differentiation (*Fig 1, middle and bottom panels*) and gene expression analysis.

Conclusions: The characterization of purified Neurog2 and Olig2 cells provides novel insights on the complex and dynamic transcriptional regulation of human neural development, and provides a tool for future study on gene functions and neural circuit regulation. Overall, CRISPR editing coupled with FACS-sorting progenitor cells in a lineage reporter platform during stem cell differentiation should be broadly applicable in any stem cell derivatives and subpopulations of any lineages.

Funding sources: Staman Ogilvie Fund-Memorial Hermann Foundation, Mission Connect-TIRR Foundation, the Bentsen Stroke Center at the University of Texas Health Science center at Houston, and the National Institutes of Health (HHSN268201200154P).

Modification and Characterization of Novel Di-functional Hyaluronic Acid Using Michael's Addition and Click Chemistry for Neural Differentiation of Mouse Embryo Stem Cells

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Changes in the extracellular matrix (ECM) after injury impede natural cell migration into the lesion and inhibit survival and differentiation into mature neurons in the central nervous system. Hyaluronic acid (HA) is an important component of the brain extracellular matrix, and a regulator of cellular differentiation, migration, proliferation and angiogenesis. In this study, we modify hyaluronic acid to include 2 independent functional groups (thiol and azide) for Michael's addition and click chemistry. The addition of the thiol group was characterized by ^{1}H-NMR and colorimetric 2,4,6-trinitrobenzene sulfonic acid (TNBS) assay. The 2.8 ppm peak on the ^{1}H-NMR spectrum and positive response to the TNBS assay (Figure 1) were only present on HA samples functionalized with thiol, or thiol and azide groups. The presents of the azide group was characterized by ^{13}C-NMR, FT-IR, and isotopic dilution experiment with ^{68}Ga. The azide group peak was presented at 2096 cm^{-1} on the FT-IR spectrum, and at 50.1 ppm on the ^{13}C-NMR spectrum. The average number of chelating moieties capping the azide group in HA was 3.9 ± 0.09 on radio TLC on di-functional HA (diHA) samples. diHA and methacrylated HA (MHA) were used to fabricate hydrogels using photopolymerization with 0.1% Irgacure 2959 photoinitiator and 2.3 mJ cm^{-2} UVA light for 5 min. Hydrogels of varying composition ranging from 1:2 and 1:5, diHA: mHA were characterized for swelling ratio, water content, mechanical properties (Table 1). A higher ratio of mHA increased the Young's modulus and shear modulus. Neural differentiation of mouse embryo stem cells (mES) were examined in 3D HA hydrogels. Approximately 40% of mES on both formulations expressed β-tubulin III on day 3 of differentiation. These results indicated that thiol and azide functional groups were presented on the modified HA, and that mES could attached and differentiated on base matrix without additional signaling mechanism.

Figure 1. 2,4,6-trinitrobenzene sulfonic acid (TNBS) colorimetric assay for thiol detection on Hyaluronic acid (HA), mono-functional HA with thiol (HA-SH), di-functional HA with thiol and azide (HA-SH-AA).

Table 1. Mechanical properties of varying composition ranging foam HA hydrogel.

di HA : mHA	Young's modulus	Shear modulus	Swelling ratio	Water Content
1 : 2	2.1 ± 0.4 kPa	100.4 ± 20.1 kPa	20.5 ± 2.5	94.9 ± 0.6 %
1 : 5	3.7 ± 0.07 kPa	162.9 ± 2.6 kPa	26.2 ± 3.7	95.9 ± 0.5 %

Acknowledgements: Funding for this study was provided by the Bentsen Stroke Center, University of Texas Health Science.

Mimicking Extracellular Matrix and Cell-cell Interactions with Bioactive Fragments of N-cadherin within Poly Ethylene Glycol Dimethacrylate Matrix for Accelerating Neural Differentiation of Mouse Embryo Stem Cell

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Bioactive signaling utilizing advanced tissue engineering approaches to optimize the artificial extracellular matrix is expected to lead to further improvements in the restoration of neurological function and cellular retention at the lesion site. In a previous study, Ile-Lys-Val-Ala-Val (IKVAV), bioactive peptide from laminin, was found to promote neuron survival, migration and axon extension *in vitro* in a concentration dependent manner. N-cadherin (NCAD) is a major adhesion molecule involved in the development and plasticity of the central nervous system, and participates in the organization of the adult neural tissue. NCAD may effect neural differentiation in a similar concentration dependent manner to IKVAV. Using polyethylene glycol dimethacrylate (PEGDM) hydrogels containing a continuous gradient of the NCAD derived peptide HAVDI, the optimal NCAD concentration to promote mouse embryoic stem cell (mES) survival, neurite extension and gene expression of neural markers was investigated. Gradient samples had a linear NCAD concentration (C_{NCAD}) gradient, and C_{NCAD} did not affect the Young's or shear moduli in the range tested. Pluripotency marker, alkaline phosphate, decreased at C_{NCAD} over 189 uM compared to mES. Gene expression of pluripotentency markers, oct3/4 and Nanog, significantly decreased on Day 3 and 6 of neural differentiation compared to mES (Figure 1). TUJ1 expression, an early neural marker, significantly increased on 292 and 467 uM C_{NCAD} compared to other tested C_{NCAD} (Figure 1). mES cultured on the 292 uM C_{NCAD} had significantly longer neurite lengths than mES cultured on other C_{NCAD} by Day 6. These results indicated that 292 to 467 uM C_{NCAD} is the optimal concentration range for enhancing neural differentiation of mES on PEGDM matrix.

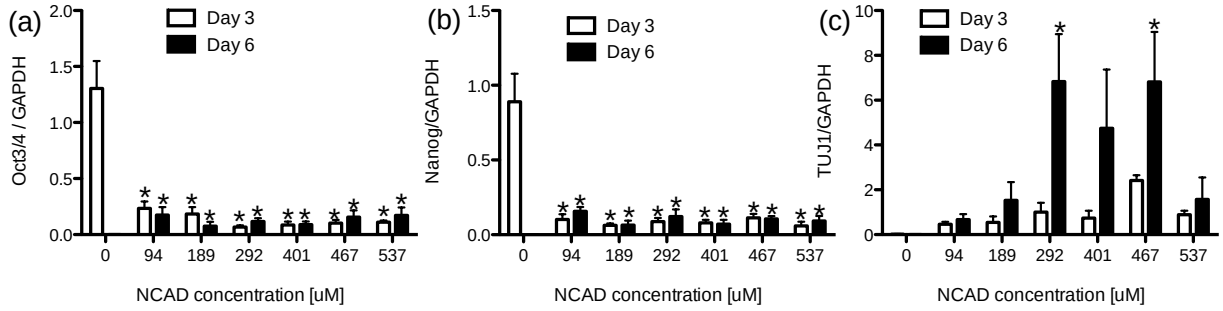

Figure 1. Gene expressions on differentiated mES on varying concentration of NCAD Day 3 and 6 after neural differentiation media treatment; (a) Oct3/4, (b) Nanog, which are mouse embryonic stem cell markers, and (c) TUJ1, which is early neural marker, expressions.

Acknowledgement: Mission Connect, a TIRR foundation program, the department of neurosurgery and William Stamps start up fund provided funding for this study.

The Social Networks of Neural Progenitor Cells

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Objective
Cell-cell communication among neural progenitor cells (NPCs) is essential for proper self-organization of the nervous system, a topic of great interest in regenerative medicine. Graph theory and network analysis have been used to study the functional connectivity of mature neuronal circuits but not developing networks, in which different modes of cell-cell communication exist. The objective of this study is to quantitatively describe the network structure of NPC communities at different stages of neural differentiation in order to uncover the role of cell-cell communication during neural network formation.

Methods
hESC-derived neural progenitor cells were triggered to differentiate through withdrawal of bFGF from culture medium and imaged continuously for a period of 14 days. Image sequences were processed using custom-written algorithms, and spatial proximity of cell bodies was used to create graph representations of NPC spatial topology. Functional maturation of NPCs was analyzed using whole-cell patch clamp electrophysiology and immunochemistry.

Results
Our graph-based method enables quantification of known spatial arrangements of cells seen during neural differentiation such as neural rosettes, as well as morphological features that accompany differentiation like neurite extension. We show that the cell bodies of NPCs self-organize into smaller, more tightly connected cliques during differentiation, and uncover relationships between network efficiency and modularity in NPC networks. Further analysis of network metrics using dimensionality reduction shows that graph-derived metrics achieve good separation of functional differentiation stage, assayed independently through patch-clamp electrophysiology and immunochemistry.

Conclusions
Our results highlight the unique geometric arrangements of cells seen during neural differentiation and also provide new insights into the modularity and clustering of immature neural cells. The insights gained from this study can be used to further our understanding of the design principles involved in functional neural network formation.

New NSC-derived motor neuron model for ALS Generated using CRISPR/Cas9 Reveals Involvement of DNA Damage Response

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The recent breakthrough of creating patient-derived induced pluripotent stem cells (iPSC) has allowed researchers to study disease mechanism, therapeutic development and patient-specific drug screening in the context of identical genetic information. Particularly in the perspective of neuroscience, these iPSC lines are invaluable tools for scientists. At the same time, challenges exist with regard to transfections and generating disease-linked mutant cell lines. In case of a progressive motor neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS), several mutations in the RNA/DNA binding Tar DNA Binding protein 43 (TDP-43) have been implicated in disease development and progression. Till date no such patient-derived iPSC lines are available carrying etiologically linked mutant TDP-43 protein for studying the disease mechanism and therapeutic approach. Here, we utilized the recently developed gene editing technology (CRISPR/Cas9) to establish TDP-43 knock-out iPSC – derived neural progenitor stem cells (NSCs). We also generated Q331K mutant TDP-43 carrying progenitor neuronal cells. These two cell models provided tools for proof-of-principle in-depth mechanistic study of TDP-43 - associated ALS pathology mimicking identical genetic background. To reduce the off-target hitting of CRISPR/Cas9, we have employed cell penetrating peptide (CPP) tagged Cas9 protein and packaged the sgRNA with a highly cationic CPP, along with a short single-strand DNA as a template for gene editing. Moreover, we have created a cellular condition where homologous recombination would over-weigh non-homologous end joining after CRISPR/Cas9 mediated double-strand break generation enhancing the possibilities of "clean" gene editing in neural stem cells. Finally, these novel ALS cell models reveal that genome damage and DNA damage response (DDR) are not only involved in TDP-43 pathology-mediated cell death, but could also be potential therapeutic targets.

Supported by NIH/NINDS and MDA grants to MLH

Amelioration of Toxicity in a Neuron Model of Huntington's Disease by an Inhibitor of Sphingosine-1-phosphate-lyase

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Autophagy is an important homeostatic mechanism that eliminates long-lived proteins, protein aggregates and damaged organelles. Its dysregulation is involved in many neurodegenerative disorders. Autophagy is therefore a promising target for blunting neurodegeneration. We searched for novel autophagic pathways in primary neurons and identified the cytosolic sphingosine-1-phosphate (S1P) pathway as a regulator of neuronal autophagy. S1P, a bioactive lipid generated by sphingosine kinase 1 (SK1) in the cytoplasm, is implicated in cell survival. We found that SK1 enhances flux through autophagy and that S1P-metabolizing enzymes decrease this flux. In a neuron model of Huntington's disease, pharmacologically inhibiting S1P-lyase protected neurons from mutant-huntingtin-induced neurotoxicity. These results identify the S1P pathway as a novel regulator of neuronal autophagy and provide a new target for developing therapies for neurodegenerative disorders.

Funding: the Hereditary Disease Foundation

Functional Connectivity Signal Latency Predicts Laterality in Pediatric Medically-Refractory Temporal Lobe Epilepsy

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Introduction:

Temporal lobe epilepsy (TLE) affects resting state brain networks in adults. This study aims to correlate resting state functional connectivity MRI (rsfcMRI) signal latency in pediatric TLE patients with their laterality.

Methods:

From 2006 to 2011, 16 surgical TLE patients (7 left, 9 right) with a mean age of 9.9 years (0.9-18) were studied. Preoperative rsfcMRI was obtained in patients with concordant lateralizing structural MRI, EEG and PET studies. After standard preprocessing techniques, the latency in rsfcMRI signal between each 6 mm voxel sampled was examined and mapped in each subject, the left and right cohorts as well as at the group level. Qualitative analyses were performed on the latency maps.

Results:

All 16 patients had improved seizure frequency postoperatively with a mean follow-up of 2.3 years (0.4-4.5), with 12 patients seizure-free. When grouped for epileptogenic laterality, the latency map qualitatively demonstrated that the right TLE patients had a relative signal source in the right temporal lobe whereas the left TLE patients had a relative signal sink in the right temporal lobe. When examined individually, the majority of left (5/7) and right (6/9) TLE patients followed this qualitative pattern.

Conclusion:

There are functional connectivity MR signal latency changes in medical refractory pediatric TLE patients. Specifically the signal in the right temporal lobe precedes the mean signal in right TLE patients and is delayed compared to the mean signal in left TLE patients. Preoperative rsfcMRI signal latency analysis could offer an inexpensive, noninvasive method of confirming laterality in pediatric TLE.

A Peripheral Neural Interface Communicating with the Regenerative Peripheral Nervous System

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In this study, a microchannel neural interface has been introduced that can be used in technologies such as prosthetics and muscle stimulator. Robotic prostheses and muscle stimulator which aim to acquire and process bio-signals to restore functionality to injured limbs must use advanced devices with high density recording. This requires bidirectional interfacing with the nervous system that can be attained by utilizing neural interfaces with recording and stimulating aptitudes. Though recent advancements in designing interfaces has improved enough to restore a high amount of functionality, however, restoring full functionality remains a challenge. The present study features an animal model where a regenerative microdevice is used for communicating with peripheral nerves.

As the axons of peripheral nervous system are regenerative in nature, many different techniques are used to interface with this nervous system. Detecting the small amplitude of nerves among the surrounding muscles increases the difficulty of acquiring relevant signals. Furthermore, small extracellular potentials and concentration of current at the nodes of Ranvier increases the complexity of recording from axons. The microdevice employed in this study is designed to obtain weak neural signals which are difficult to acquire using other techniques. In addition, axonal regeneration is confined to the microchannels and electrodes are long enough to record from the nodes of Ranvier. Traditional materials of electrodes such as metal and silicon are ineffective in part because they are more rigid than the surrounding tissue they try to record and stimulate. For this reason, instead of silicon, materials such as polyimide and other polymers like PDMS (polydimethylsiloxane) have been used for their flexible nature. We have developed PDMS microchannel structures with microwires embedded within the microchannels. The Silastic® tube and Sylgard 184® used in the fabrication of PDMS scaffolds are composed of the same PDMS material. The commercially available microwires (Stablohm 800A, California Fine Wires, CA) (75 µm diameter) which are used as recording electrodes, are embedded within the microchannels. Microwires are favorable because they are easy to implant, permit smaller wounds and create minimal obstruction to the regenerative path. The microdevice was successfully implanted in the rat sciatic nerve at the UTPA animal facility. All electrodes were guided subcutaneously to an incision made at the top of the head of the rat and henceforth attached to a connector. There are several types of electrodes for peripheral nerve interfaces, however, they record unwanted signals from surrounding musculature and are subject to crosstalk due to parasitic capacitances. To overcome this, neural regeneration is directed through this neural interface in sealed microfluidic channels. This feature aims to improve the design by reducing crosstalk between adjacent microchannels and increasing the signal-to-noise ratio.

The result of the present study can be used to increase the effectiveness in advanced prosthetics. The eventual application of the device is for using in a noninvasive brain-machine interface for bidirectional prosthetic arms. The microdevice has been developed and successfully implanted in the rat sciatic nerves. The device is designed as a general electrophysiological system for a small animal model covering all of the peripheral nervous system. The scaffold parameters can be modified to more closely match conditions that encourage the greatest amount of regeneration of neural tissue as the developed fabrication technique is simple and adjustable.

Funding Sources: DoD Army Research Office DURIP

Neuroimaging Neural Substrates of Trust in Veterans with PTSD

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Objectives: This study was designed to evaluate the impact of Cognitive Processing Therapy (CPT) on behavioral improvements in interpersonal trust and Post Traumatic Stress Disorder (PTSD) symptoms, and the concomitant neural changes after treatment. Our research focuses on the neural impact of treatment for PTSD, and prospectively--the use of neuroimaging data to guide psychotherapy treatment.

Methods: We recruited sixty-one Veterans with PTSD (50 male OEF/OIF/OND Veterans and 11 OEF/OIF/OND and Persian Gulf female Veterans) to participate in the study. Eighteen Veterans completed group CPT, 20 completed a treatment as usual (TAU) control group and 23 dropped out of one of the two conditions. CPT is a manualized 12 session, cognitive behavioral therapy treatment for PTSD. Veterans also completed an economic exchange game, the Iterated Trust Game in an fMRI scanner before and after a 12 week treatment or TAU period.

Results: Preliminary Neuroimaging findings. In preliminary, partial data analysis from this study, Post-doctoral Fellow, Jason Aimone analyzed neural data from Veterans engaging in the Iterated Trust Game, before and after group-based CPT. Preliminary analyses (see **Figure 3**) on the first 13 subjects reveal significant increases in the striatal signal, suggesting that CPT treatment may be correcting deviations in a neural prediction error signal in the striatum which Co-Is King Casas and Chiu have found is related to interpersonal trust and the severity of PTSD symptoms. These preliminary results point to the value of combining the iterated Trust Game and fMRI data as measures that mark changes in social functioning in psychotherapy.

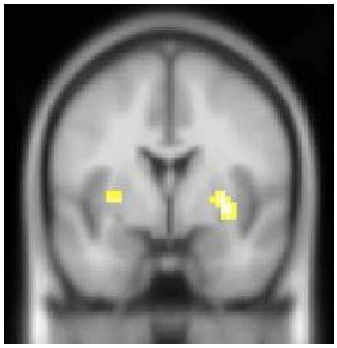

Figure 1. Neural responses to benevolent social gestures (+ prediction error) in the striatum increased following completion of group-based CPT by 8 OEF/OIF/OND Veterans with PTSD, relative to 3 treatment as usual control subjects and 2 treatment non- completers, all of whom met criteria for PTSD ($p < .001$ unc, (Full sample will be corrected for multiple comparisons, Family Wise Error-ratio .05))

Conclusions: We found that: (1) group CPT was effective in reducing the symptoms of PTSD and depression, (2) almost a third of treatment dropouts also may have been treatment successes, and (3) pre-treatment trust was linked to treatment success. Our preliminary neural data indicated that CPT treatment may be correcting deviations in a neural prediction error signal in the striatum which Co-Is King Casas and Chiu have found is related to interpersonal trust and the severity of PTSD symptoms.

Funding Sources: We are grateful for the following research support: Williams, Grant B7760-P, from VA Rehabilitation Research and Development; King-Casas, Grant D7030R, from VA Rehabilitation Research and Development; and Chiu, MH087692, from the National Institutes of Mental Health.

Transplantation of Human Inducible Pluripotent Stem Cell-derived Neuronal Progenitor Cells Promotes Locomotor Recovery after Spinal Cord Injury

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Objectives: Human inducible pluripotent stem cells (hiPSC), the remarkable pluripotent embryonic stem cell (ESC)-like cells reprogrammed from embryonic or adult somatic cells by over-expression of four developmental/pluripotency transcription factors, offer tremendous potential for individualized patient- and diseased-specific therapy. Transplantation of hiPSC-derived neural stem cells could be one of the most promised novel reparative strategies to promote functional recovery after spinal cord injury (SCI). However, one of the major challenges to fully realized the full therapeutic potential of hiPSC for SCI and other neurological diseases is to direct hiPSC to differentiate into desired neural stem or precursor cells in vitro and then purify these cells before transplantation. In this study, we developed a standard in vitro protocol to differentiate and purify neuronal precursor cells from hiPSCs and then tested its survival and differentiatation following traumatica SCI in mice.

Methods: Fibroblasts were obtained from the skin biopsies of spinal cord injury patients and then reprogrammed into iPSC by overexpressing four transcript factors, sox 2, c-myc, oct4 and klf4 in fibroblasts using retroviral method. SCI patient-specific iPSCs were induced to differentiate into neuronal precursor cells and purified by FACS. The survival and differentiation of purified neuronal presursor cells were tested on traumatically injured spinal cords of severe-combined immunodeficiency mice.

Results: Human iPSC expressed all undifferentiated hESC markers suggesting that it is fully reprogrammed. We then induced these iPSC into neural differentiation using our established neural differentiation protocol. Human iPSC differentiated into neural stem cells expressing sox1 and pax6, the two early neural stem cell markers, at 15 days post-differentiation (PD) and into neuronal precursor cells (NPC) expressing β-tubulin III and nestin at 26 days PD. At this stage, many β-tubulin III+ cells also expressed a membrane antigen, A2B5. We purified A2B5+ NPC by fluorescence-assist-cell-sort (FACS) using A2B5 antibody. The purified cells were all expressed β-tubulin III without contamination of other types of cells including undifferentiated hiPSC. These cells can be proliferated in vitro in the presence of mitogens and differentiate into mature neurons after further neuronal differentiation for 10 days in vitro. NPCs were labeled by infection of retroviruses expressing GFP in vitro and then transplanted into the injured spinal cord of severe-combined immunodeficiency mice at 8 days after moderate contusion. The transplanted NPCs differentiated into mature neurons expressing NeuN and graft-derived neurons sent processes into gray matter and formed connections with host neurons. Importantly, recovery of hindlimb locomotor function is significantly enhanced in animals receiving grafted of hiPSC-derived NPCs. No teratoma formation is observed in any animals receiving hiPSC-derived NPCs.

Conclusions: we established an in vitro protocol to direct hiPSC to differentiate into NPCs and develop a FACS method to purify these NPCs. Purified NPCs can survive and differentiate into mature neurons after transplantation into the traumatically injured spinal cord and promote locomotor functional recovery. These results suggest that hiPSC-derived NPCs have great therapeutic potential for neuronal replacement after SCI and other neurological diseases.

Acknowledgments: This research was supported by The Staman Ogilvie Fund, NS061975 and the Senator Lloyd & B.A. Bentsen Center grant for Stroke Research.

Regulation of Neurocognition by Nuclear Receptor Corepressors (NCORs)

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Objectives: Rett syndrome, an autism spectrum disorder characterized by mental retardation and metabolic dysfunction, is caused by mutations in the gene methyl CpG binding protein 2 (MeCP2) that binds methylated DNA. MeCP2 interacts with nuclear receptor corepressors (NCORs) and missense mutations on MeCP2 affecting such interaction cause Rett syndrome in human and mouse, suggesting that NCORs could be required for proper function of MeCP2. However, neuronal functions of the NCOR complex have not been investigated. Histone deacetylase 3 (HDAC3) is the only HDAC that confers enzymatic activity to the NCOR complex. The activity of HDAC3 requires binding to the Deacetylase Activating Domain (DAD) of NCOR and its homolog SMRT. Here we use whole-body knock-in mice with mutated DAD for both NCOR and SMRT (nsDAD), brain cell-specific and region-specific knockout mice to study neuronal functions of the NCOR complex.
Methods: 3-4 months old mice were used in this research. To knockout NCOR and SMRT in hypothalamus, $NCOR^{flox/flox}$/$SMRT^{flox/flox}$ mice were intracranially injected with adeno- associated virus (AAV) expressing Cre under the Synapsin1 promoter. Elevated Plus Maze (EPM), Open Field Arena test (OFA), Light-Dark test (LD), Social Interaction test (SI), Rotarod test, Novel Object Recognition Test (NOR), Morris Water Maze (MWM), and Plenthsmography were used to test the function of NCOR and SMRT.
Results: nsDAD mice displayed hyperactivity (OFA), improved motor coordination (rotarod), decreased anxiety-like behavior (EPM, OFA, LD), reduced social interaction (SI), and impaired memory (NOR and MWM). Gene expression analysis identified a battery of genes downregulated in hippocampus and hypothalamus in the absence of active HDAC3 enzymes. Golgi staining revealed altered neuron population in multiple brain regions in nsDAD mice. Depletion of NCOR and SMRT specifically in hypothalamus impaired special memory (MWM) without obvious changes in other neurobehaviors, but caused rapid body weight gain.
Conclusion: The findings demonstrate pivotal roles of neuronal NCORs in neurocognition and metabolism, which is independent of MeCP2 and vice versa considering the overall distinct behavioral phenotype and gene expression alterations between NCORs-manipulated mice and MeCP2 null mice. Further studies are ongoing to decipher the underlying mechanism using epigenomic and metabolomic approaches.
Funding: NIH R00DK099443, pilot project DRC/BCM.