

Regeneration Research NEWSLETTER



The Thirteenth International Symposium on Neural Regeneration

The Thirteenth International Symposium on Neural Regeneration (ISNR) was held at the Asilomar Conference Center in Pacific Grove, California from December 9-13, 2009. The meeting was co-sponsored by the U.S. Department of Veterans Affairs (Biological Laboratory Research and Development and Rehabilitation Research and Development Service), the Paralyzed Veterans of America (Spinal Cord Research Foundation), the National Institutes of Health (National Institute of Neurological Disorders and Stroke, Office of Rare Diseases and Eunice Kennedy Shriver National Institute of Child Health and Human Development), and the United Spinal Association.

The Symposium was organized by Dr. Roger Madison (Symposium Director, VA Medical Center and Duke University, Durham, NC), and the program planning committee which included Drs. John Steeves (International Collaboration on Repair Discoveries/University of British Columbia), Michael Sofroniew (University of California, Los Angeles), David Gardiner (University of California, Irvine), Jane Lebkowski (Geron Corporation), Jacqueline Bresnahan

(University of California, San Francisco), Keith Crutcher (University of Cincinnati) and James Guest (University of Miami). Guest attendees representing co-sponsoring institutions at the planning meeting were Drs. Vivian Beyda (United Spinal Association), Naomi Kleitman (National Institutes of Health - NINDS) and Patricia Dorn (Department of Veterans Affairs, Rehabilitation R&D Service).

The keynote speaker for this year's symposium was Jeff Lichtman from Harvard University. Featured talks were given by Ravi Bellamkonda from Georgia Tech/Emory University, Cheng-Ming Chuong from University of Southern California, Charles Howe from Mayo Clinic College of Medicine and Peter Ellaway from Imperial College London.

ISNR STAFF

Director: Roger Madison, Ph.D.

Coordinator: Frances Stuart

The Director's Chair
From the Office of the International Symposium
on Neural Regeneration
Roger D. Madison, Ph.D. - Director



2009 Highlights

The International Symposium on Neural Regeneration celebrated another successful gathering at the 2009 conference which was attended by 211 participants, including 75 students, with a record number of 115 posters being presented. All of the symposium events were of great interest to the participants with the debate and trainee awards being highlights of the meeting.

The focus of the debate was "Demyelination is a Therapeutic Target for Spinal Cord Injury" and was chaired by David Shine (Baylor College of Medicine). Representing the affirmative team was Hans Keirstead (University of California, Irvine), Michael Fehlings (University of Toronto), Jeff Kocsis (Yale University/VA Connecticut Healthcare System) and Blair Calancie (SUNY Upstate Medical Center). The opposing team consisted of James Guest (University of Miami), Philip Horner (University of Washington), Wolfram Tetzlaff (International Collaboration on Repair Discoveries/University of British Columbia) and Robin Franklin (University of Cambridge). The debate session had the highest attendance of any session at the symposium; standing room only. The format included a group presentation of each side's argument followed by a rebuttal period. The debate sparked much conversation throughout the remainder of the symposium.

The Fredrick Seil Trainee Award, established to honor the first director and founder of ISNR, was awarded for 2009 to Mihai Moldovan (University of Copenhagen) and Brian Sauer (Mayo Clinic). Each received a \$250 cash award and their names have been engraved on the Fredrick Seil Trainee Award perpetual plaque. Additional poster awards were presented to Ryan Williams (University of Miami), Jill See (Drexel University), Joseph Bonner (Drexel University), Jessica Alexander (Ohio State University) and Akshata Almad (Ohio State University). The United Spinal Association generously provided a \$50 cash award and a certificate for each of these winners. All awards were chosen by planning board members who reviewed the poster entries.



Mihai Moldovan and Brian Sauer

In addition to these poster awards, two individuals were selected to give platform presentations based on the abstracts they submitted. Frank Bradke (Max Planck Institute) and Rachel Bergstrom (Mayo Clinic) were chosen to give these talks in the final "Emerging Topics" conference session. ISNR congratulates all of these winners.

Following the format of preceding neural regeneration symposia, the program was divided into six sessions, which included: 1) Peripheral Nerve Regeneration, chaired by Rajiv Midha; 2) DEBATE: Demyelination is a Therapeutic Target for SCI, chaired by David Shine; 3) Retinal Regeneration, chaired by William Hauswirth; 4) PANEL DISCUSSION: Updating the Community - Progress and Suggestions Regarding SCI-Replication Studies (NIH-NINDS, ForeSCI), chaired and moderated by John Steeves and Naomi Kleitman; 5) Reorganization and Plasticity, chaired by Larry Jordan; and 6) Emerging Topics, chaired by Jane Lebkowski.

Brief summaries of the keynote and featured speakers presentations are given below followed by a listing of session chairmen and speakers.

KEYNOTE SPEAKER

Connectomics In the Developing Nervous System (Jeff Lichtman, Harvard University)



Dr. Lichtman began his keynote address by stating connectional maps of the brain may have value in developing models of both how the brain works and how it fails when subsets of neurons or synapses are missing or misconnected. Such maps might also provide more detailed information about how brain circuits develop and age. He noted an eagerness to obtain such maps from the developing nervous system because of a longstanding interest in the neuromuscular circuit changes during mammalian early postnatal life. In the neuromuscular system most axonal input to muscle fibers is pruned in early postnatal life. This so called 'synapse elimination' may be part of the process whereby the nervous system molds itself to a particular epigenetic landscape. The loss is driven by competition between multiple axons that temporarily share the same junction. The amount of resources available to each axon at a particular synapse may influence the competitive outcome. Because each axon has many branches all competing roughly at the same time, the resources available at one site are likely affected by the outcome of synaptic competitions at other neuromuscular junctions that are innervated by the same axons. Techniques have been developed to observe all these synaptic interactions at different sites simultaneously by computer assisted axonal tracing and the generation of transgenic mice in which different axons are labeled different colors. Dr. Lichtman referenced Brainbow mice (Livet et al., 2007), which provided an opportunity to see the entire connectional maps (or 'connectomes') for muscles and other neuronal circuits. He noted

that thin sectioning was required to disambiguate the many overlapping axons. Dr. Lichtman concluded by noting his colleagues have developed a new kind of microtome (and an electron microscope imaging strategy) that allows automated high resolution imaging of thousands of ultra thin (<30 nm) sections that are very large (~4 mm²). This approach aims at making serial microscopic analysis of large volumes of tissue routine.

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FEATURED SPEAKER

Micro and Nano-Scale Biomaterials That Facilitate Peripheral and Central Nerve Repair

(Ravi Bellamkonda, Georgia Institute of Technology/Emory University)



Dr. Bellamkonda reviewed evidence that injuries to peripheral and central nerves trigger a specific set

of cellular and molecular responses that in turn determines their regenerative capacity. The goal of using exogenously applied biomaterials is to allow for creating spatially and temporally controlled biological environments in vivo. Such materials may take the form of fibers, gels, or nanocarriers that may or may not be degradable or that are capable of incorporating biological elements such as nucleic acids, proteins and cells within them. Dr. Bellamkonda's presentation discussed the role of biomaterials in enhancing peripheral nerve repair and managing the complex post-injury CNS environment to create pro-regenerative environments. Oriented nanofiber based polymeric thin films create enhanced nerve guides that facilitate bridging of critically sized nerve gaps by facilitating Schwann cell migration and axonal regeneration. He also presented recent efforts using various biomaterials to enhance the limited regenerative potential following CNS injuries, focusing on hydrogels that carry polymeric nanoparticles or lipid-based microtubes, to decrease acute inflammation and digest astroglial scar tissue. He also presented a method of thermally stabilizing the enzyme chondroitinase ABC for polymeric sustained delivery systems of this enzyme.

FEATURED SPEAKER

Regulation of Stem Cell Regeneration In Skin Ectodermal Organs (Cheng-Ming Chuong, University of Southern California)



Dr. Chuong's presentation covered both neural tissues and skin derived from ectoderm; highlighting that neural tissues have limited regenerative power and skin organs have robust regener-

ative power. Skin regeneration comes in the form of continuous renewal (e.g., epidermis) or episodic organ regeneration (i.e., hair and feather follicles). Dr. Chuong discussed his work using the skin-organ-paradigm to decipher the fundamental principles of morphogenesis and regeneration. Skin appendage follicles are useful models because they go through regenerative cycling continuously in the adult as a physiological process. His research has developed this concept further to include: 1) organ shaping and topobiological arrangement of stem cells, 2) physiological and macro-environmental regulation of regenerative activities, and 3) re-establishing hair follicles after wound healing and periodic pattern formation. He noted how the simple difference in topological arrangements of stem cells can determine the radial or bilateral symmetry of the organ, and demonstrated this concept with the feather model (Yue et al., 2005, 2006). Dr. Chuong also reviewed data regarding how stem cell activity can be regulated by the macro-environment (e.g. subcutaneous adipose tissue, body physiological condition, and external environment) by using examples of regenerative hair waves (Plikus et al., 2008). Finally, he discussed how a "sustainable regenerative unit" has evolved from reptile scales to hairs and feathers. Dr. Chuong concluded by stating that in order to engineer stem cells into useful tissues/organs, one can learn by studying how development and regeneration occur in nature and from the regeneration of skin and ectodermal organs.

FEATURED SPEAKER

CD8+ Cytotoxic T Cells Acutely And Irreversibly Injure Demyelinated Axons In a Mouse Model Of Multiple Sclerosis (Charles Howe, Mayo Clinic College of Medicine)



Dr. Howe stated that while axon injury is a key factor in the loss of neurologic function associated with multiple sclerosis (MS), it is unclear whether damage to axons is an obligatory conse-

quence of demyelination or whether it is an independent process that occurs in the permissive environment of the demyelinated lesion. To explicitly test the role of CD8+ T cells in axon injury, his research has established a perforin-deficient mouse model on an H-2^d MHC background. Using the Theiler's murine encephalomyelitis picornavirus model of MS, he found that chronically infected perforin-deficient H-2^d mice exhibit robust preservation of spinal axons and motor function despite the presence of severe demyelination in the spinal cord that is indistinguishable from perforin-competent animals. In contrast to previous studies into the role of CD8+ T cells and perforin that used mouse strains normally resistant to TMEV infection, his observations in a susceptible MHC background directly test the hypothesis that demyelination is necessary but insufficient for axon injury by removing confounding factors related to viral biology. Using this model, Dr. Howe found that adoptive transfer of perforin-competent spinal cord infiltrating CD8+ T cells into demyelinated but functionally preserved perforin-deficient mice led to rapid loss of motor function, disruption of spinal motor conduction, and loss of medium- and large-caliber spinal axons. These acute effector CD8+ T cells were not antiviral but did exhibit a unique clonal expansion of the V β 5.1/5.2 T cell receptor locus not observed in splenocytes or peripheral blood T cells. This model directly compares demyelinated lesions between perforin-competent and perforin-deficient mice, revealing that the absence of perforin protects axons without influencing demyelination.

The findings suggest that perforin is a key mediator of axon injury, lending additional support to the hypothesis that CD8+ T cells are primarily responsible for the axon damage observed in MS.

FEATURED SPEAKER

Development of Reliable and Sensitive Physiological Assessments of Outcome After Treatments for Spinal Cord Injury (Peter Ellaway, Imperial College London and The London Spinal Cord Injuries Centre)



Experimentally induced regeneration of spinal cord axons in mammals has achieved growth over a few cm at most. Assuming that similar

approaches in man would not necessarily produce axonal regeneration over longer distances, protocols for assessing the outcome of such treatments would need to detect regeneration over one or two segments (Verma & Fawcett 2005, *Adv Biochem Eng Biotechnol* 94). This would be challenging using the American Spinal Injuries Association (ASIA) standard neurological classification of spinal injuries that is currently and widely employed for clinical evaluation. The sensory and motor assessments that constitute the ASIA standard neurological classification of spinal injuries have limited sensitivity and range. Despite many revisions to the ASIA standards, including refinements such as separate upper and lower extremity motor scores (Marino & Graves 2004, *Arch Phys Med Rehabil* 85), there remains a perceived need for sensitive, quantitative and objective outcome measures to supplement the ASIA standards clinical assessment. Initially, under a Clinical Initiative, physiological tools for improved assessment of sensory, motor and autonomic function were developed (Ellaway et al 2004, *Spinal Cord* 42). These tools have now been tested for sensitivity and reliability against treatments expected to produce functional improvements in those with incomplete spinal cord injury (iSCI). Repetitive transcranial magnetic stimulation in cervical iSCI was employed

with the aim of improving hand function and weight-assisted treadmill walking in iSCI with the aim of improving locomotion. The Electrical Perceptual Threshold test for cutaneous sensation, transcranial magnetic stimulation for evaluation of the corticospinal tract and the sympathetic skin response as an autonomic functional test were all evaluated and found to be potential tools to assess functional recovery from injury.

TOPIC SESSIONS

Peripheral Nerve Regeneration (Chaired by Rajiv Midha, University of Calgary)

Rajiv Midha (University of Calgary) presented "Elucidating Mechanisms Of Support Offered By Skin-Derived Stem Cells In The Repair Of Peripheral Nerve Injury". Christian Krarup (University of Copenhagen) presented "Changes In Axonal Properties During Maturation After Regeneration: A Study Using Threshold Tracking On Nerve In Vivo". Tessa Gordan (University of Alberta) presented "The Time Window Of Opportunity For Axon Regeneration In The Peripheral Nervous System". Roger Madison (Duke University/Durham VA Medical Center) presented "Why does a lower motor neuron regenerate an axon following a peripheral nerve lesion: and how can it be persuaded to grow back to its original terminal nerve branch to muscle?"

DEBATE - Proposition: Demyelination is a Therapeutic Target for SCI (Moderated by David Shine, Baylor College of Medicine)



l to r: H. Keirstead; J. Guest; W. Tetzlaff; R. Franklin; M. Fehlings; B. Calancie; P. Horner and J. Kocsis; kneeling - D. Shine

Hans Keirstead (University of California, Irvine), Michael Fehlings (University of Toronto), Jeff Kocsis (Yale University/VA Connecticut Healthcare System) and Blair Calancie (SUNY Upstate Medical University) presented an "Affirmative" position for the statement. While James Guest (University of Miami), Philip Horner (University of Washington), Wolfram Tetzlaff (ICORD/University of British Columbia) and Robin Franklin (University of Cambridge) presented a "Negative" position for the statement.

Retinal Regeneration (Chaired by William Hauswirth, University of Florida)

Daniel Goldman (University of Michigan) presented "Molecular mechanisms underlying Muller glia dedifferentiation and retina regeneration in Zebrafish". Thomas Reh (University of Washington) presented "Comparative Biology Of Retinal Regeneration". Raymond Lund (Oregon Health Sciences University) presented "Use of human forebrain-derived progenitor cells to stabilize photoreceptor degeneration and vision loss". William Hauswirth (University of Florida) presented "Leber congenital amaurosis gene therapy clinical trial".

PANEL DISCUSSION: Updating the Community - Progress and Suggestions Regarding SCI-Replication Studies (NIH-NINDS, Fore-SCI) (Chaired and Moderated by John Steeves and Naomi Kleitman)



l to r: D. Dietrich; N. Kleitman; P. Popovich; J. Steeves and O. Steward

Panel presenters were Oswald Steward (University of California, Irvine), Phillip Popovich (Ohio State University) and Dalton Dietrich (University of Miami).

An evening session was devoted to a discussion of the attempted replication of ten published studies, supported by a contract issued by the National Institute of Neurological Disorders and Stroke/NINDS. Drs. Naomi Kleitman (NINDS contract administrator) and John Steeves (ICORD, University of British Columbia) co-chaired the session, which included presentations by past and present contractors: Drs. Dalton Dietrich (University of Miami), Oswald Steward (University of California, Irvine) and Phillip Popovich (Ohio State University). The goals of the contract are to assess the reliability of promising preclinical strategies that appear to be translatable to human studies, if the results are found to be robust.

Of the ten replication studies performed to date (including drugs, cells or antibodies/peptides), most have proven not to be reproducible in the hands of these experienced laboratories. In many cases no difference was observed between treated and control groups (a number of these replication studies have been published, and the others soon will be). In a few, initial indications of functional recovery disappeared when carefully randomized cohorts were run, or technical issues such as staining artifacts were identified; all of which might limit the original conclusions. Notably, positive findings were replicated for Schwann cell grafts (but not a combination of cells plus cAMP administration), and for glibenclamide treatment of sub-acute hemorrhage. The latter study also shed light on the difficulty of exact replication of the induced injury itself. Dr. Steeves noted that such replication difficulties are not unique to SCI research (as shown in recent ALS and stroke reviews), and emphasized the importance of instituting good laboratory practices and careful reporting of methodologies in all preclinical studies. The session provoked lively discussion between the panel and audience members about possible next steps and instances where replication studies have been or

could be attempted. An update on the state of replication studies is planned for the next ISNR meeting in December, 2011.

Reorganization and Plasticity (Chaired by Larry Jordan, University of Manitoba)

Michael Sofroniew (University of California, Los Angeles) presented "Recovery of function via pathway reorganization after spinal cord injury". Michael Beattie (University of California, San Francisco) presented "A link between synaptic plasticity and excitotoxicity in spinal cord injury". James W. Grau (Texas A&M University) presented "Learning within the spinal cord". Linda Sorkin (University of California, San Diego) presented "Peripheral inflammation induces acute changes in dorsal and ventral horn glutamate signaling".

Emerging Topics (Chaired by Jane Lebkowski, Geron Corporation)

Frank Bradke (Max Planck Institute) presented "Three-Dimensional Imaging Of The Entire Spinal Cord For Assessing Axon Regeneration And Glial Responses After Injury". Rachel Bergstrom (Mayo Clinic) presented "Analysis Of Immune-Mediated Axon Injury In An In-Vitro Microfluidic Chamber Model Of Multiple Sclerosis". Adam Ferguson (University of California, San Francisco) presented "Bioinformatics for Translational Spinal Cord Injury Research". Theo Hagg (University of Louisville) presented "PTPs and vasculature as neuroprotective targets".



ISNR Planning Board Members and sponsor representatives pictured with all of the 2009 ISNR poster award recipients. (L to R, back row - M. Sofroniew, D. Gardiner, R. Williams, J. Guest, J. Steeves, F. Bradke, D. Shine, J. Bonner and J. Bresnahan; center row - B. Sauer, M. Moldovan, R. Bergstrom, R. Madison, A. Kusiak, J. Lebkowski and N. Kleitman; front row - J. See, A. Almad, J. Alexander; lower center - M. Horner, the junior neuroscientist from the state of Washington.

For more information about the International Symposium on Neural Regeneration, please contact:

ISNR
Durham VAMC
508 Fulton Street - Building 16, Room 38
Durham, NC 27705
Phone: (919) 286-0411, ext. 7691/Fax: (919) 286-6811
Email: isnr@mc.duke.edu

or visit our website: <http://www.rehab.research.va.gov/Neural/neural.htm>

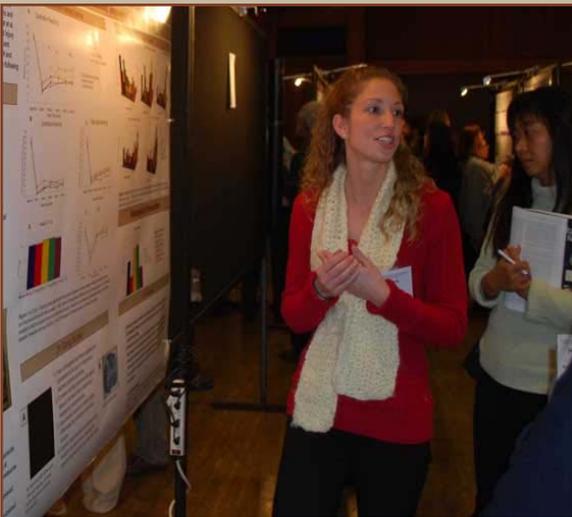
ISNR 2009



Chuck Howe and Roger Madison



Audrey Kusiak

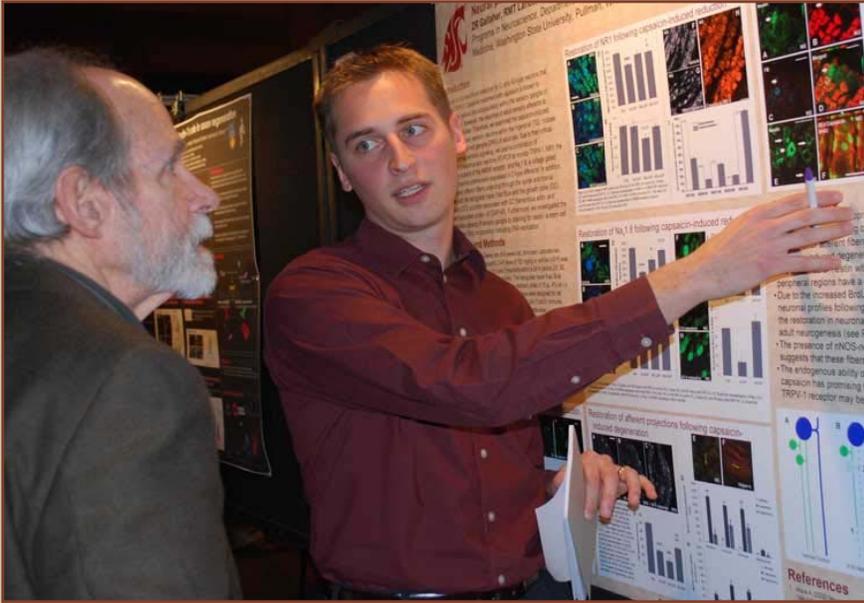


Laura Krisa

Rod and Melissa Williams



Jesse Owens and Stuart Hodgetts



ISNR 2009

Poster Sessions



Looking Ahead To ISNR 2011

Symposium Session Proposals

If your favorite topic has not recently been presented at one of the international symposia, we encourage you to submit a proposal for a symposium session. The program planning committee is meeting on September 10-12, 2010 to formulate the program for the Fourteenth International Symposium on Neural Regeneration (ISNR) scheduled for December 2011. Proposals may be received in the ISNR Office as late as August 15, 2010 and still be considered, but earlier submission is recommended for advance distribution to committee members. Proposals will not be considered, however, if they are not submitted in an appropriate format (see guidelines below).

Submission Guidelines

Symposium sessions are chosen for their timeliness, current interest and recent progress. One of the goals of these symposia is to cover the field of neural regeneration as broadly as possible. This cannot be done in a single year, and therefore an attempt is made to vary the programs in successive symposia so that eventually the spectrum of neural regeneration research is covered. A symposium session proposal should define a session topic and include a few words about why the topic should be presented. A chairman should be identified (often the symposium proposer), along with four to six first choice speakers. Information on the speakers should include institutional affiliation, email and mailing address, telephone number (if available) and a few words on what area this speaker would cover. At least one (preferably more) alternates should be listed for each speaker or chairman, along with institutional affiliation and other identifying data, as the first choice speaker is not always available.

In addition, you may submit suggestions for the Symposium Keynote speaker and possible Featured Speakers. Potential speakers should not be contacted in advance, as invitations are issued by the Director's office if the program planning committee accepts the session. Some thought should also be given to whether a potential speaker has previously been a recent symposium presenter, as an attempt is made to vary the individuals participating in the program. A list of speakers from past symposia are available from the ISNR office or on the ISNR Symposium website at www.rehab.research.va.gov/Neural/neural.htm.

Please submit session proposals via email to:

Roger Madison, Ph.D.
Director, International Symposium on Neural Regeneration
Email: madis001@mc.duke.edu