Cell Proliferation and Neurogenesis

Final Narrative: **NJCSCR grant # 04-3035-SCR-E-0**

1. Program Directors Name, Address, Telephone Number
David P. Crockett, MA, PhD
Department of Neuroscience and Cell Biology
675 Hoes Lane West
Piscataway, New Jersey 08854
Telephone: 1-732-235-5153
Crockett@umdnj.edu
Fax: 1-732-235-4029

Original Program Director: Richard S. Nowakowski, PhD. (retired from UMDNJ)

2. Name of Organization: UMDNJ-Robert Wood Johnson Medical School

3. Grant Title: Cell Proliferation and Neurogenesis

4. Grant Number: NJCSCR grant # 04-3035-SCR-E-0 **Banner Index: 502631**

5. Grant Period: 7/1/2004 – 1/31/11

6. Date of Submission of Report:

N.B. *The original PI on this project was Richard S. Nowakowski, PhD. Dr. Nowakowski retired during the summer of 2010 and has left the institution at which time I took of the lead. We were granted a 3 months no cost extension. However, this was not really enough time and therefore this report is late.*

Original Aims of Project:
There are 3 circumstances during which there is cell proliferation in the central nervous system (CNS) of mammals. Firs, there is a period of normal development of the CNS during which the neurons and glia of the brain are produced by stem/progenitor cells. Second, in the adult brain there are two generally accepted locations (the dentate gyrus and the subventricular zone) where there is a small amount of neurogenesis that persists throughout the lifetime of the animal (including humans). Third, following an injury to the brain there is a period during which there is a considerable amount of cell proliferation near the site of the injury and a variable amount of cell proliferation at sites distant from the lesion. Injury-induced proliferation both near the injury site and at a distance has received considerably less attention. This last factor was the main focus of the grant, which had three specific aims:

1) to establish when and where after a spinal cord injury cell proliferation occurs, what cell types are involved, and what cell types are produced, 2) to determine if there are genetic variants in cell proliferation following spinal cord injury and to identify the genetic loci involved, and 3) to use the specific results obtained to develop possible therapeutic approaches by either interfering with cell proliferation or by determining if we can target proliferating cells as a specific delivery mechanism for therapeutic agents.

After an injury to the spinal cord (or any area of the CNS) there are a number of changes that occur. Immediately after the injury there is damage to the cells and axons that pass through the area of injury. As time passes, some of the injured cells may die and the injured axons degenerate. In addition, other cells not directly damaged by the injury may die from sequellae related to the injury (e.g., exicitoxicity) and if there is swelling in the area of the injury other axons may be damaged secondarily. As additional time passes there are also changes in areas distant from the injury that occur as a result of the damage to the axons. Indeed several lines of research, including what will be presented here, suggest that there might be an injury signal retrogradely transported along the axons. The recovery from the lesion needs to include the production of new cells to replace those that die (including neurons and oligodendrocytes) and the rewiring of the connections that were lost from both the direct and indirect damage. Cell proliferation and related events (e.g., DNA synthesis, translation etc) play a role in both the injury and, in principle, in the events needed to provide recovery.

We have shown that proliferation after spinal cord injury occurs in sites quite distant from the site of injury at surprisingly short times after the injury, presumably signaled by damage to the axons that project to these distant areas. However, other areas such as the hippocampus, which do not connect directly to the spinal cord also show rapid alterations in ongoing cell proliferation rates following SCI. This suggests that there systemic signals or factors that can powerfully alter distant CNS functions as a result of the injury.

In addition to changes in proliferation, we have shown structural and functional changes at distant sites, again at surprisingly short periods of time. Namely, there is rapid rearrangement of local circuitry within the sensorimotor cortex, within days following SCI.

Project Successes:

Cell Proliferation Following Injury

An injury to the brain or spinal cord sets off a cascade of events that includes axonal degeneration, cell death, and inflammation. Associated with these events is the production of new cells, i.e., cell proliferation. Although there have been recent studies considering cell proliferation after SCI, much remains unknown regarding the diversity of cell proliferation after injury and if it exists in regions of the CNS distant from the lesion site. The initial aim of this research was to elucidate whether SCI elicits a proliferative response in the brainstem and forebrain, with the hypothesis that there is such a response within these regions distant from the site of injury. The rationale for this hypothesis is twofold: 1) any damage to the CNS, including degenerative changes, should stimulate the proliferation of glial cells and/or microglia involved in damage control, cleanup of debris,

and perhaps reorganization of motor/sensory pathways, and 2) the axons of many neurons damaged after SCI are projections from the brainstem.

To characterize the changes in cell proliferation within the brainstem, spinal cord, and dentate gyrus (DG) of adult, male C57BL/6J mice 96 hrs after a SCI (i.e., thoracic hemisection), a single injection of bromodeoxyuridine (BrdU) 30 minutes before sacrifice was employed to label cells within S-phase. Within the brainstem, there is nearly a 2-fold increase (173%) in the number of BrdU labeled cells in the caudal medulla; the region of the brainstem with the most BrdU labeled cells. The bulk of this increase is in the ventral region of the caudal medulla, which accounts for approximately 90% of the total number of BrdU labeled cells. Double labeling of BrdU (S-phase marker) with markers for glial cell types, i.e., GFAP+ (astrocyte marker) and NG-2+ (glial progenitor/oligodendrocyte precursor marker), confirms that this response is glial, and predominantly comprised of NG-2 expressing cells. An analysis of the asymmetry of the spinal cord lesion and the proliferative response in the caudal medulla shows that the asymmetry of the total area of white matter spared at the lesion epicenter correlates positively with the asymmetry of the total proliferative response in the caudal medulla ($R = 0.799$, $p < 0.02$), and specifically in the spinocerebellar tract (SCT; $R = 0.711$, $p < 0.05$). In other words, these results show that the more asymmetric the spinal cord lesion, the more asymmetric the proliferative response is in the caudal medulla, and particularly in the SCT. Out of the various pathways and structures in the ventral part of the caudal medulla that exhibit a significant increase in cell proliferation after SCI, only BrdU labeling in the dorsal SCT (dSCT) exhibits a correlation with measures of white matter spared within the spinal cord. The number of BrdU labeled cells in the dSCT correlates inversely with the volume of white matter within the dorsal column of the spinal cord ($R = -0.863$, $p < 0.007$). Overall, the direction of this correlation is simple to interpret – the more damage there is to the dorsal horn the more cell proliferation there is in the dSCT. Consistent with this interpretation, in all of the injured mice, damage to the dorsal column is accompanied by damage to the dorsal horn ipsilateral to the lesion, which houses the axons of the first order neurons that send sensory input to the second order neurons (Clarke's nuclei) whose axons form the dSCT. In order to assess whether the increase in cell proliferation following SCI is specific to the caudal medulla, a second region of the CNS distant from the site of the spinal cord lesion was examined. In the cervical spinal cord (C-1), there is a significant increase (162%) in the number of BrdU labeled cells following SCI as compared to sham-operated controls.

To further assess the specificity of the increase in cell proliferation following SCI, a third region of the CNS was examined. The dentate gyrus (DG) was chosen because there are no direct connections with the spinal cord or any part of the somatosensory and motor systems. Moreover, the hilus of the DG has a small population of stem/progenitor cells that persists through the lifetime of mammals, including humans and the mice employed in this study. On average, there is an ~17% decrease in the number of BrdU labeled cells in the stem/progenitor cell population of the DG of mice with moderate or severe SCI as compared to sham-operated controls. Importantly, the decrease in the number of S-phase cells in the DG is relatively symmetric, even though the spinal cord injury (i.e., left hemisection) is asymmetric. This symmetry indicates that the decrease in the number of

BrdU labeled stem/progenitor cells in the DG is due to a systemic signal elicited after SCI. In addition, the number of BrdU labeled cells in the DG of injured mice decreases in a severity-dependent manner following SCI. *It is important to note that the direction of the change in cell proliferation in the DG is the opposite of what was found in the brainstem and cervical spinal cord of the same animals*. This difference in direction and the symmetry both indicate that different mechanisms are involved in the regulation of cell proliferation after injury in these distinct populations.

In general, the results of this study indicate that within 96 hrs after SCI: 1) there are significant changes in the number of S-phase, i.e., BrdU labeled, cells in distinct regions of the CNS that are located quite distant from the spinal cord lesion; 2) these changes differ quantitatively and qualitatively in the areas studied; 3) different mechanisms are involved in the regulation of cell proliferation after injury in these distinct regions; and 4) areas of the CNS involved in learning and memory (i.e., the IO and cerebellum, as the olivo-cerebellar complex, and the DG) are highly responsive to changes in the CNS elicited after SCI. The significant decrease in cell proliferation within the DG of the hippocampus, point to a potential precursor to emotional/mood disruption, particularly depression as a result of SCI. This finding also indicates that SCI might lead to impairments in other hippocampal functions (i.e., learning and memory). Ultimately, the results of this study expand our understanding of the underlying biological changes in regions of the CNS beyond the spinal cord that have the potential to lead to anatomical and functional changes following SCI. Therefore, these findings need to be considered in the development of treatments for SCI, as well as when interpreting results from clinical trials.

Plastic Changes in Somatosensory Cortex After SCI

An SCI may destroy few to almost all descending corticospinal (CS) and ascending sensory axons traveling through the site of the injury. As a result, CS axons are irreversibly injured and cortical activity dependent on sensory afferents is altered.

These injuries and changes provoke adaptive reorganizational events in sensorimotor neocortices of all mammalian species that are distant to the injury site. Some of these reorganizational events contribute to functional recovery, but some may also provoke erroneous or maladaptive functions, resulting in chronic pain and/or spasticity.

Recently, improved regeneration after SCI was seen by manipulating a pathway in sensorimotor neocortices that is involved in phosphorylation of an RNA binding protein (RBP) required for mRNA translation--the Eukaryotic translation initiation factor 4E (eIF4E). Our data identifies rapid molecular alterations of eIF4E in the sensorimotor neocortices 1 and 3 days after a lateral hemisection SCI, used as a model for Brown-Séquard syndrome. The function of an RBP depends on both its distribution sites within the cell and its phosphorylation states. Indeed, we found both to be affected after SCI. There was a distinct subcellular redistribution of eIF4E and phosphorylated-eIF4E was reduced, indicating that the eIF4E's translation was disrupted. Upon identification and analysis of the mRNA cargo of eIF4E in uninjured sensorimotor neocortices, we found that eIF4E binds both *Importin-13* (*Ipo13*) and *Parvalbumin* (*Pv*) mRNAs, indicating a role in their translation. Remarkably, eIF4E's interaction with both *Ipo13* and *Pv* mRNAs

was disrupted 1 and 3 days after SCI, despite preservation of total *Ipo13* and *Pv* mRNA levels. Finally, we detected a selective loss of expression of both IPO13 and PV proteins in projection neurons of sensorimotor neocortices, as well as their disrupted dendritic polarity. Since IPO13 is predominantly expressed in neocortical projection neurons and PV in a subset of neocortical interneurons, these data suggest a strong acute effect of SCI on neocortical microcircuitry. Taken together, these data indicate that neocortical eIF4E and a subset of mRNAs may be rapidly recruited to translational machinery after SCI to promote adaptive regeneration response of sensorimotor neurons.

Implications for future research and clinical treatment:

The implications of these findings is that effective treatment for traumatic injury to the spinal cord must take into account that the consequences of the injury affect a number of areas within the central nervous system that are quite distant from the site of the injury.

Some of these distant changes may be adaptive and allow for re-establishment of function. However, many of these changes may be maladaptive. It is therefore critical that future research identify the maladaptive plastic response from the adaptive. Further, our most recent data suggest that not only must repair strategies focus on repairs at the injury site, but also attention must be paid to local circuits located at far distant sites. This may require complex multifactorial treatments that not only preserve local circuits in the sensorimotor cortex for example as well as treatments that allow for axonal growth accurse the injury site and the re-establishment of spinal circuitry.

Aside from the implications raised by changes in local circuitry distant from focal injury in the spinal cord, other less obvious consequences of spinal cord injury need to be explored. We have observed alterations in cell proliferation in areas of the CNS involved in learning and memory (i.e., the IO and cerebellum, part of the olivo-cerebellar complex, and the DG of hippocampus after SCI. The significant decrease in cell proliferation within the DG of the hippocampus may be a potential precursor to emotional/mood disruption, particularly depression following injury to the spinal cord. In addition, decreased cell proliferation within the SCI might also lead to impairments in other hippocampal functions (i.e., learning and memory). To our knowledge, this is the first report of a proliferative response in the brain following SCI.

A further implication of these finding is that following SCI, areas that are not directly connected to either the spinal cord or the sensorimotor cortex may be significantly affected. This implies that there potential exists a systemic signal that can affect other areas of the CNS not directly connected to the injury. This signal may be related to the inflammatory response characteristic of SCI. This undelineated systemic signal may have additional unknown affects on the CNS and should be taken into account in any effective treatment strategy or strategies that may be developed.

Plans for future research

We plan to pursue the analysis of SCI-induced plasticity within the sensorimotor cortex, correlating mRNA expression, protein expression and mechanism of translation over a wider range of times. We are also developing techniques to visualize the changes in the sensorimotor cortex following injury, in real time using two-photon confocal microcopy. This is being accomplished through new collaborations within our department.

We also plan to continue to study altered cell proliferation within the brain induced by SCI, particularly at longer time periods in order to determine whether these effects or permanent and to work out the factors responsible for injury induced reductions in hippocampal cell proliferation-inflammation, altered BDNF levels, etc.

Thesis Awarded, Presentations and Publications

Thesis

Carmichael, M. J. .2010. Distinct Changes in Cell Proliferation within the Dentate Gyrus, Medulla and Cervical Spinal Cord Follow a Thoracic Spinal Cord Injury (SCI). Dissertation presented to the faculty of *Biomedical Engineering, Joint Graduate Program of Rutgers the State University and UMDNJ – Graduate School of Biomedical Sciences.*

Publication:

Thompsn, K., DiBona, V.L., Dubey, A., Crockett, D.P., Rasin, M.R., 2010. Acute adaptive responses of central sensorimotor neurons after spinal cord injuryl. Translational Neuroscience, (4) 268-278. http://www.springerlink.com/content/2081-3856/1/4/

Presentations

Crockett, D.P., Carmichael, M.J., Gu, J., and Nowakowski, R.S. The Murine Locomotion Components Scale: a versatile behavioral tool to assess locomotion after spinal cord injury (SCI) and locomotor development in genetically modified mice. Program No. 600.26. 2007 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2007. Online

Carmichael, M.J., Crockett, D.P., Hayes, N.L., and Nowakowski, R.S. Development of a Novel Behavioral Scoring System to Assess Recovery of Locomotion Following Spinal Cord Injury (SCI) Abstract - NEA Science Day 2006

Carmichael, M.J., Crockett, D.P., Hayes, N.L., and Nowakowski, R.S. (2006) Strain Differences in Injury Phenotype, Cell Proliferation, and Behavioral Recovery after Spinal Cord Injury (SCI) in Mice. In press 36th annual meeting of the Soc for Neuroscience, Atlanta, GA.

Crockett, D.P., Son A., Carmichael, M.J., Jordan, M.E., Eang, R., Harris, S.L., Egger; M.D. (2006) A versatile behavioral scale for the analysis of locomotion following spinal cord injury (SCI): Functional recovery in the p27Kip1 knockout mouse. In press 36th annual meeting of the Soc for Neuroscience, Atlanta, GA

Dubey, A., Thompson, K., Diona, V.L., Rasin, M.R., Crockett, D.P. (2011) Adaptive cortical responses to spinal cord injury in the mouse in vivo. In Press submitted the Society for Neuroscience.

ABSTRACT OF THE DISSERTATION

Distinct Changes in Cell Proliferation within the Dentate Gyrus, Medulla and Cervical Spinal Cord Follow a Thoracic Spinal Cord Injury

by Marc J. Carmichael

Dissertation Advisor:

Richard S. Nowakowski, Ph.D.

An injury to the brain or spinal cord sets off a cascade of events that includes axon degeneration, glial scar formation, and inflammation. These events include increases in the proliferation of various cell types. Although there have been studies documenting cell proliferation in the spinal cord after spinal cord injury (SCI), much remains unknown regarding its broader distribution. The aim of this research was to investigate whether there is a proliferative response within regions of the CNS remote to the site of SCI, with the hypothesis that there is such a response in the brainstem.

To assess the proliferative response in the CNS of mice 96 hrs after SCI, a thymidine analogue, bromodeoxyuridine (BrdU), was employed to label proliferating cells within S-phase. The results of this study show that, following SCI, there is a significant increase in the number of S-phase cells within the brainstem and the first segment of the cervical spinal cord (C1). This increase is consistent with axon degeneration in the spinal cord. In the dentate gyrus (DG), there is a significant decrease in the number of S-phase cells after SCI. Importantly, this decrease in the DG is symmetric, in contrast to the asymmetry found in the SCI and proliferative responses in the brainstem and C1. This symmetry indicates that the decrease in the number of BrdU labeled stem/progenitor cells in the DG is due to a systemic signal elicited after SCI.

These differences in direction and symmetry of the response both indicate that different mechanisms are involved in the regulation of cell proliferation after injury in these distinct populations.

Taken together, the results of this study indicate that, within 96 hrs after SCI: 1) there are distinct changes in cell proliferation at multiple levels of the CNS distant from the site of SCI (i.e., within the forebrain, brainstem and cervical spinal cord), 2) these changes differ quantitatively and qualitatively, and 3) different mechanisms are involved in the regulation of the changes in cell proliferation within these distinct regions. To our knowledge, this is the first report of a proliferative response in the brain following SCI.

Translational Neuroscience

VERSITA

ACUTE ADAPTIVE RESPONSES OF CENTRAL SENSORIMOTOR NEURONS AFTER SPINAL CORD INJURY

Kevin Thompson[1],
Victoria Lynn DiBona[1],
Aditi Dubey,
David Paul Crockett[2],
Mladen-Roko Rasin[2,*]

Department of Neuroscience and Cell Biology
UMDNJ-Robert Wood Johnson Medical School
675 Hoes Ln West
Piscataway, NJ-08816, USA
[1] equally contributing co-authors
[2] corresponding co-authors

Abstract

Spinal cord injury (SCI) can be a lifelong, devastating condition for both the patient and the caregiver, with a daunting incidence rate. Still, there are only limited available therapies and the effectiveness of precise regeneration within the central nervous system is minimal throughout postnatal life. Recently, improved regeneration after SCI was seen by manipulating a pathway in sensorimotor neocortices that is involved in phosphorylation of an RNA binding protein (RBP) required for mRNA translation, the Eukaryotic translation initiation factor 4E (eIF4E). Our data identifies rapid molecular alterations of eIF4E in the sensorimotor neocortices 1 and 3 days after a lateral hemisection SCI, used as a model for Brown-Séquard syndrome. The function of an RBP depends on both its distribution sites within the cell and its phosphorylation states. Indeed, we found both to be affected after SCI. There was a distinct subcellular redistribution of eIF4E and phosphorylated-eIF4E was reduced, indicating that the eIF4E's translation was disrupted. Upon identification and analysis of the mRNA cargo of eIF4E in uninjured sensorimotor neocortices, we found that eIF4E binds both *Importin-13* (*Ipo13*) and *Parvalbumin* (*Pv*) mRNAs, indicating a role in their translation. Remarkably, eIF4E's interaction with both *Ipo13* and *Pv* mRNAs was disrupted 1 and 3 days after SCI, despite preservation of total *Ipo13* and *Pv* mRNA levels. Finally, we detected a selective loss of expression of both IPO13 and PV proteins in projection neurons of sensorimotor neocortices, as well as their disrupted dendritic polarity. Since IPO13 is predominantly expressed in neocortical projection neurons and PV in a subset of neocortical interneurons, these data suggest a strong acute effect of SCI on neocortical microcircuitry. Taken together, these data indicate that neocortical eIF4E and a subset of mRNAs may be rapidly recruited to translational machinery after SCI to promote adaptive regeneration response of sensorimotor neurons.

Keywords

mRNA translation • Injury • Corticospinal • Neocortex • Spinal cord • Parvalbumin
Importin • Interneuron • Pyramidal neuron • Dendrite

© Versita Sp. z o.o.

Received 16 December 2010
accepted 16 December 2010

1. Introduction

An SCI begins with a traumatic event in the spine that may cause widespread and sometimes irreversible changes in the central nervous system (CNS) [1,2]. The SCI trauma can be caused by a sudden external physical injury, or by internal hypoxia, inflammation or bleeding [1,2], making it difficult to design preventative treatments. An SCI may destroy few to almost all descending corticospinal (CS) and ascending sensory axons traveling through the site of the injury. As a result, CS axons are irreversibly injured and cortical activity dependent on sensory afferents is altered [1-3]. These injuries and changes provoke adaptive reorganizational events in sensorimotor neocortices of all mammalian species that are distant to the injury site [4-7]. Some of these reorganizational events contribute to functional recovery, but some may also provoke erroneous or maladaptive functions, resulting in chronic pain and/or spasticity [1,2,8]. Indeed, the impact of SCI can range from complete or partial paralysis of the body below the lesion site, to sometimes partial or complete recovery [1,2]. The incidence of SCI in the world ranges between 10.4 and 83 cases per million people per year, depending on the region analyzed [8-10]. However, in world regions affected by war, the incidence of SCI transiently multiplies [10]. Furthermore, care for people affected by SCI is predominantly given at home, the cost of which can run into millions of dollars and is a great strain on the patient and the caregiver. Moreover, due to limited capabilities of the CNS for repair, the effectiveness in precise regeneration after an SCI throughout postnatal life is minimal [1,2,8]. Therefore, the need to better understand SCI pathogenesis at a molecular level is even greater, and novel approaches are needed to enhance regeneration after SCI.

Recently, molecular manipulations of sensorimotor neocortices improved functional plasticity following an SCI [11,12]. These data indicate that increased understanding of molecular mechanisms underlying adaptive responses of sensorimotor neocortices after an SCI may propel ongoing regenerative efforts and identify early therapeutic targets. The sensorimotor neocortex is a six-layered structure, with each layer having a specified complexity of projection and interneuronal subtypes incorporated in highly organized microcircuitry. Neocortical neurons are critically involved in voluntary

* *E-mail: rasinml@umdnj.edu*

sensorimotor behavior, social abilities, learning and memory. Therefore, in the adult, the disruption of neocortical microcircuitry and CS axons is often extremely debilitating, resulting in lifelong disability or even death. An SCI induces adaptive plastic reorganization of the primary sensorimotor neocortex, which indicates microcircuitry reorganizational events [3-7]. Electrophysiological studies show that SCI produces an immediate functional reorganization of the neocortex, while an increase in fMRI signal occurs by the 3rd day after an SCI [3,7]. At the cellular level, dendritic spine morphology changes were found as early as 3 days post-SCI, and by 7 days post-SCI their number decreased [13]. Quantifiable changes in the reorganization of axonal and dendritic processes are expected to require a longer time interval after the SCI. For example, hindlimb CS neurons retract, sprout and incorporate into the sensorimotor circuits of the unaffected forelimb after several weeks [4]. Thus, detecting reorganization of neocortical microcircuitry in a restricted region less than 3 days after an SCI would be novel asset to the SCI field.

Temporal reorganizational events in sensorimotor neocortices depend on changes in the functional gene expression in many of their genes following an SCI. In particular, studies on altered gene expression in sensorimotor neocortices post-SCI revealed transcriptional decrease in mRNA expression of Nogo receptor and its co-receptor LINGO-I, while BDNF was up-regulated, as early as day 1 post SCI [7]. In addition, GAD67 mRNA stayed unchanged [7]. Axotomized CS neurons post-SCI have also shown decreased mRNA expression of cytoskeletal proteins such as tubulin [48]. However, functional gene expression is controlled not only at the DNA level, but also at the RNA level, particularly via RNA-binding proteins (RBPs). RBPs may guide groups of mRNAs through a sequence of post-transcriptional events; including splicing, nuclear export, stabilization and translation [14]. Consequently, sufficient levels of a functional protein can be rapidly produced for proper cellular events, such as axonal regeneration attempts after SCI. Thus, the functional disruption of a neocortical RBP in the sensorimotor neocortices after an SCI may affect the functions of many genes. However, presently very little is known about the role of RBPs in adaptive changes of sensorimotor neocortices after an SCI and its potential in regeneration of CS axons.

Function of an RBP depends on its phosphorylation states, which were shown to be strongly affected in lesioned peripheral neurons after an injury [15]. This data indicate that RBP-dependent mRNA translation may be central in acute responses of injured neurons after SCI. Genetic manipulation of PTEN/Akt/mTOR pathway in CS neurons improved the CS regeneration after SCI [12]. Importantly, it is known that both PTEN/Akt/mTOR signaling and neuronal activity, which is affected in sensorimotor neocortices after SCI, play a role in phosphorylation and, thus, in function of the Eukaryotic translation initiation factor 4E (eIF4E). However, the direct effect of SCI on eIF4E function in sensorimotor neocortices following SCI is unknown.

Therefore, in current study we examined acute adaptive changes in both eIF4E's distribution and its mRNA cargo after SCI in neurons of sensorimotor neocortices distant to injury site. We found that lateral hemisection SCI at T10 (used as a model for Brown-Séquard syndrome) leads to redistribution and de-phosphorylation of eIF4E, preventing its binding to target mRNAs, and thus resulting in aberrant mRNA translational control. Thus, after SCI, eIF4E and a subset of neocortical mRNAs in sensorimotor neocortices are accessible to be rapidly recruited to translational machinery to improve regeneration after SCI.

2. Experimental procedures

2.1 Animals and tissue

All procedures performed are in compliance with national regulations and UMDNJ-RWJMS policies and have been approved by the Institutional Animal Care and Use Committee and Institutional review board of UMDNJ-RWJMS. CD-1 and B6 black mice were used. Brains of 21-60 years old human specimens without neurological or psychiatric records were obtained from UMDNJ-RWJMS pathology.

2.2 Spinal Cord Injury: Mouse Model for Brown-Séquard Syndrom

All surgeries were performed under aseptic conditions. 12 adult mice of mixed genetic backgrounds were subjected to either a lateral hemisection of the spinal cord at T10 or a sham injury. Under deep anesthesia (Isoflurane) the mice were placed in a stereotaxic apparatus (Kopf Instruments, USA), designed for rodent spinal cord studies. The apparatus was equipped with non-traumatic ear bars and a custom-made mask for the delivery of Isoflurane. The animals were kept warm throughout the surgical procedures by a heating pad, filled with circulating water heated to 37oC. The skin over the thoracic spinal column was shaved and incised; the muscles were separated and cleared from vertebral column; and, the thoracic vertebrae were clamped using a pair of V-notched vertebral clamps. A small amount of bone at T10 was removed exposing the spinal cord. Using a No. 12 (hook-shaped) scalpel, a lateral hemisection was made. Immediately following the spinal cord incision, the wound was sutured and closed in layers. The animals were returned to fresh, warmed cages for recovery. The sham-operated mice received identical treatment to the injured mice except that they did not receive the spinal cord incision.

2.3 Behavioral Analysis and Neurological Assessment

The animals' locomotor abilities were assesed 1, 2 and 3 days post injury to determine the effectiveness of our injury paradigm. We observed our animals in a runway (100 X 6.4 X 10.5 cm). The runway had a "gated start box" at one end and a dark "goal" or "safety enclosure" at the other. The floor of the runway was made of clear Plexiglas. The run from start box to the goal was 75 cm. A mirror placed underneath the runway allowed the observer to detect foot positioning and aided in photographic documentation. Also, with the runway placed on a lab bench, toe clearance was easily observed through the clear viewing wall. We scored the animals using the conventional Basso Mouse Scale (BMS) [42-44]. Because the BMS scale was designed to assess a bilaterally

symmetrical contusion injury we in addition we used the Murine Locomotor Components Scale (MLCS), developed in our laboratory [45-47]. The MLCS, derived from the salient features of both the BMS and the earlier BBB scale [44], allowed user to assess each hindlimb independently as well as overall walking ability. As used in this investigation, the MLCS is a 32-point scale that considers murine locomotion as a hierarchical organization of components--hindlimb (HL) stepping, coordination, HL weight support, stability/posture, and balance. In addition, each of these components are based on subcomponents (e.g., toe clearance and foot position). Scores for each of these subcomponents may be summed to give an overall assessment of the animal's locomotor ability; or, they grouped to form a number of component subscores that may be used to monitor specific aspects of behavioral recovery and the assessment of asymmetry characterized by the hemisection injuries used in this study.

2.4 Immunocytochemistry

Adult CD-1 and B6 mice were perfused transcardially with PBS followed by 150 mL of fresh 4% paraformaldehyde (PFA; pH = 7.4-7.6). Brains were excised and postfixed in 4% PFA shaking at 4°C overnight and vibratome sectioned at 70 µm. Sections were then washed 3x in 1x PBS and immersed into blocking solution (0.3% Triton-X 100; 5% Normal Donkey Serum; 0.1% Glycine 0.1% L-Lysine in 1x PBS) for 1 hour shaking at room temperature followed by primary antibody overnight at 4°C shaking. Sections were washed next day 3x in 1x PBS and appropriate fluorescent secondary antibodies (Jackson Immunoresearch) were applied at room temperature 2 hours shaking. Sections were washed 3X PBS. DAPI was applied for 10 min before mounting and coverslipping in Vectashield (Vector Labs Inc.; Cat # H-1000). Sections were imaged on Olympus Fluoview FV10i confocal microscope at 10x and 60x. For human brain section immunostaining, brain was fixed in 10% formaldehyde. Medial primary motor cortex was isolated and vibratome cut at 70 µm. Sections were then washed 3x in 1x PBS and immersed into blocking solution (0.3% Triton-X 100; 5% Normal Donkey Serum; 0.1% Glycine 0.1% L-Lysine in 1x PBSTween) for 1 hour shaking at room temperature followed by primary antibody overnight at 4°C shaking. Sections were washed next day 3x in 1x PBS and biotinylated secondary antibody (Jackson Immunoresearch) was applied at room temperature, 2 hours shaking. Following secondary, sections were washed 3x 1x PBS and immersed into Vectastain elite ABC solution (Vector) for 2 hours. Following three washes in 1x PBS, reaction was visualized by 3,3'-diaminobenzidine (DAB) Peroxidase Substrate Kit. Sections were mounted, dehydrated for 5 min in increasing concentrations of ethanol, 50%, 70%, 90%, 100%, 100% and immersed for 1 hour in xylene. Finally, sections were coverslipped with permount (Fisher) and scanned with Aperio Scanscope. Primary antibodies used in this study: Chicken anti-myelin basic protein (Aves Labs; 1:100); Rabbitt anti-eIF4E (Cell Signaling; Cat # 2067S; 1:250 for immuno; 1:1000 for western blotting); Rabbitt anti-phospho-HuR (Cell SIgnaling; Cat # 9741S; 1:250); Goat anti-IPO13 (Santa Cruz Biotechnology; Cat # sc-68097; 1:250 for immuno); Mouse anti-parvalbumin (Swant; Cat # 235; 1:1000); Mouse anti-SMI32 (Sternberger monoclonals; Cat # SMI-32R; 1:1000).

2.5 RNA Isolation

Contra-lesional sensorimotor neocortices from Sham operated or SCI operated animals were excised and RNA was isolated from cortices using Ambion RNAqueous Midi (AM1911) or Ambion PARIS kit (AM1921).

2.6 RIP

Contra-lesional sensorimotor cortices were excised from animals. Tissue was subjected to lysis using MBL RiboCluster Profiler RIP-Chip kit lysis buffer by incubation on ice for 10 minutes, pipetting every 2 minutes to homogenize tissue. Then lysate was centrifuged at 500g for 1 minute to remove debris. Then pull-down from supernatant was performed using eIF4E RIP-Chip certified antibody (MBL; Cat # RN001P) and corresponding rabbit-IgG control following protocol from MBL RiboCluster Profiler RIP-Chip kit (RN1001), except step 34. The 3-hour incubation was extended to overnight due to time constraints. Isolated RNA was stored at -80°C until RT-PCR or microarray analysis.

2.7 Western blotting

Contra-lesional sensorimotor neocortices were dissected and Ambion PARIS kit (AM1921) was used to isolate total protein. Lysate was subjected to Invitrogen NuPAGE western blotting system using 12% Bis-Tris pre-cast gels. After protein transfer to nitrocellulose membranes, the membrane was immersed for 1 hour into 1x PBS with 0.4% Tween, 5% milk, 10% FBS. Primary antibody was incubated overnight 4°C. Next morning, after 3x washes in 1x PBS with 0.4% Tween, membrane was incubated with corresponding secondary antibody (HRP-conjugated Donkey anti-Rabbit;Jackson Immunoresearch; 1:5000) for 2 hours shaking at room temperature. Membrane was washed 3x in 1x PBS with 0.4% Tween and developed using ChemiGlow (Cell Biosciences) developing reagent. Membrane was imaged using G:BOX of Syngene.

2.8 Microarray and analysis

RNA precipitates were sent to UMDNJ-RWJMS transcriptional profiling facility to be analyzed on Affymetrix Mouse GeneChip 1.0 ST array. Obtained array data were analyzed using Partek's Genome Suite. Genes bound significantly higher ($p < 0.05$) by eIF4E than by IgG were considered further. Within these, cut-off was set at 1.5 fold change. Genes bound to eIF4E were further analyzed using functional annotation clustering analysis from DAVID (http://david.abcc.ncifcrf.gov/).

2.9 Real Time Quantitative RT-PCR

Applied Biosystems 1-Step master mix and Taqman probes were used according to protocol. Each assay was done in triplicate and quantitated using delta-delta CT method. Gapdh was used as a control.

2.10 Statistical analysis

For data analysis t-test was used. $p < 0.05$ was considered as statistically significant. In all figures error bars represent standard deviation.

3. Results

3.1 Subcellular distribution of eIF4E is disrupted after SCI, but eIF4E protein levels are unchanged

After an SCI, reorganization occurs in all layers of the sensorimotor neocortex [4-7]. To reveal changes in eIF4E in sensorimotor neocortices after an SCI, a sham-operated control and a model of unilateral spinal cord hemisection at T10 (Brown-Séquard syndrome) were used (Figure 1; n > 3 for each experimental condition). After confirmation of the SCI model efficiency (Figure 1), we examined the eIF4E-protein distribution and expression levels in sensorimotor neocortices of sham-operated controls and lateral hemisection SCI using immunohistochemistry. To analyze acute changes in mRNA and protein levels after SCI *in vivo*, neocortices were examined 1 day post-surgery. Three days post-surgery examination was additionally performed to see temporal changes in mRNA levels, and more importantly, to allow for protein turnover to assess if new protein synthesis is disrupted. We found that eIF4E-protein expression in the sensorimotor neocortices of sham-operated controls and in sensorimotor neocortices ipsilateral to SCI (ipsi-lesional) resembled the eIF4E-protein expression pattern from an uninjured animal (Figure 2 and data not shown). Remarkably, in sensorimotor neocortices contralateral to the SCI (contra-lesional), the eIF4E-protein was redistributed predominantly to the lower parts of cell body cytoplasm. The contra-lesional sensorimotor neocortices were then subjected to quantitative RT-PCR (qRT-PCR) analyses to determine *eIF4E* mRNA levels, and we further found *eIF4E* mRNA levels in this same region to be unchanged (Figure 2). Finally, to further determine that levels of eIF4E did not change in contra-lesional sensorimotor neocortices, we performed western blot analysis and no change was detected in levels of eIF4E protein (Figure 2). Thus, these results indicate that SCI causes redistribution of eIF4E protein in contra-lesional sensorimotor neocortices, and this redistribution may jeopardize the necessary localized translation selectively within sensorimotor neocortices.

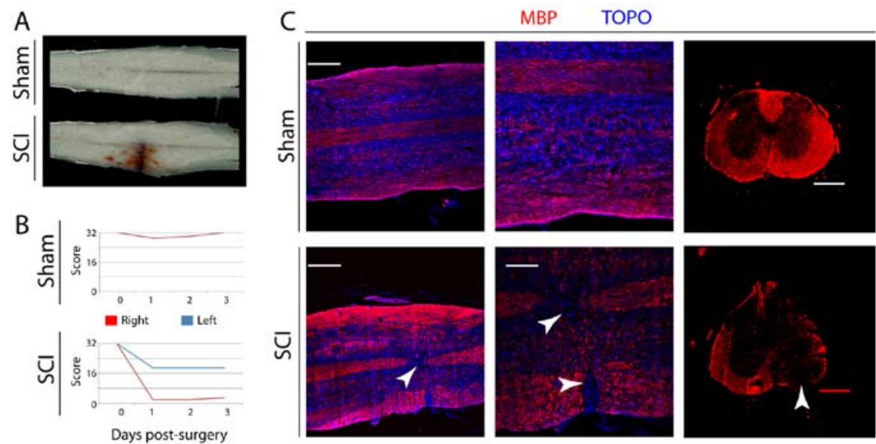


Figure 1. Lateral hemisection model of lumbar SCI. (A) Lateral hemisection SCI was induced in right side of the spinal cord (SCI), while spinal cord of sham operated control (Sham) was intact. (B) Behavioral scoring (Murine Locomotor Components Scale) showed decreased function of right leg (red) in SCI mice but not in Sham. The scoring was based on the salient features that constitute rodent walking behavior over a flat surface. This scoring separates walking into a number of elementary subcomponents that include foot position, toe clearance, plantar stepping, hind- and forelimb coordination. Scores for each of these subcomponents were summed to give an overall assessment of the animal's right and left leg's abilities. (C) Immunostaining for myelin basic protein (MBP; red) reveals disrupted right side of the SCI spinal cord (arrowheads), while spinal cord of sham operated control was intact. TO-PRO-3 iodide (blue) was used for nuclei.

To ensure that eIF4E redistribution was not an indirect consequence of neocortical neuronal cell death, as previously reported [16], we performed TUNEL assay (data not shown), but no significant increase in cell death in sensorimotor neocortices after SCI was seen. In addition, recent findings have suggested that retrograde cell death in the corticospinal neurons does not occur in SCI injury [17]. Collectively, these results suggest that lateral hemisection SCI does not cause significant cell death, but may affect the function of eIF4E leading to disrupted mRNA translation.

3.2 eIF4E phosphorylation is decreased after SCI

RBP distribution and function, such as selective mRNA translations, depend on RBP's phospho-states [14]. Neocortical re-distribution of eIF4E after SCI indicates that eIF4E's phospho-states may be altered. Therefore, to determine eIF4E's phospho-states after SCI, immunohistochemical analysis of sensorimotor neocortices contralateral to SCI site was performed using specific phospho-eIF4E antibody 1 and 3 days post-surgery (Figure 3). We observed that eIF4E phosphorylation is decreased in contra-lesional sensorimotor neocortices, but is preserved in ipsi-lesional sensorimotor neocortices (Figure 3). This selective decrease in eIF4E-phosphorylation suggests that its mRNA cargo is affected. Taken together, these data indicate a disruption in eIF4E-dependent local translation during the acute response of sensorimotor neocortices to SCI that is not recovered 3 days post-SCI.

3.3 eIF4E binds *Ipo13* and *Pv* mRNA in uninjured adult sensorimotor neocortices

eIF4E is known to bind a cargo of distinct mRNA transcripts and thus to regulate their translation rate [18]. This regulation may be lost after SCI, leading to disruption of translational events. Therefore, we first identified the mRNA cargo of eIF4E in sensorimotor neocortices using the RNA binding protein immunoprecipitation for microarray (RIP-Chip) kit (MBL), anti-eIF4E RIP-Chip certified antibody (MBL) and corresponding IgG as a negative control (Figure 4). Precipitates were then analyzed using Affymetrix Mouse GeneChip 1.0 ST array coupled to bioinformatics analysis using

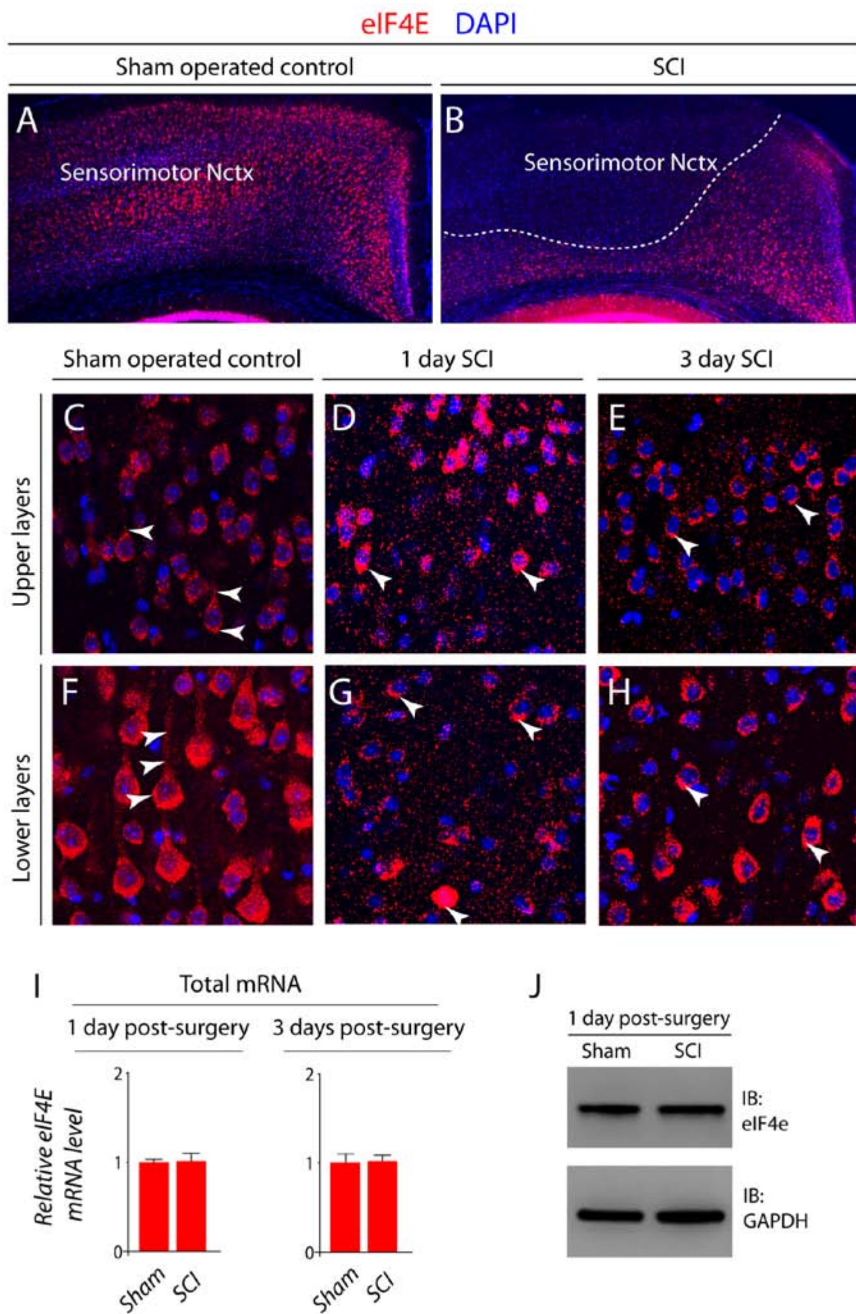


Figure 2. eIF4E is re-distributed in all projection neurons of sensorimotor neocortices contralateral to the SCI lesion site, while levels of eIF4E mRNA and eIF4E protein stay unchanged. (A,B) Representative low magnification images of eIF4E immunostaining showing a localized effect after SCI on eIF4E expression in the contra-lesional sensorimotor neocortex (Nctx), 1 day post-surgery (B). (C-H) High power magnification of eIF4E immunostaining of contra-lesional sensorimotor Nctx reveals subcellular re-distribution of eIF4E. In the sham operated control 1 and 3 days post-surgery, eIF4E was found equally distributed in cytoplasm and in initial parts of dendrites of upper and lower layer projection neurons (C,F; arrowheads). However, 1 and 3 days after SCI, no eIF4E was seen in dendrites, while remaining eIF4E was predominantly found to be lateralized in cell cytoplasm (D,E,G,H; arrowheads). (I) Quantitative RT-PCR analysis of contralateral to SCI lesion sensorimotor neocortices revealed no change in eIF4E mRNA levels 1 and 3 days after SCI. (J) Western blot analysis of contra-lesional sensorimotor Nctx reveals no change in eIF4E protein levels 1 day post-SCI.

Partek's Genome Suite and DAVID (http://david.abcc.ncifcrf.gov/). Since overexpression of neuronal calcium sensor-1 in sensorimotor neocortices promoted CS regeneration after SCI, we focused our analysis on a cluster of mRNAs bound by eIF4E that are characterized by calcium binding region domain (Figure 4) [11]. Surprisingly, within this cluster, we found *Parvalbumin* (*Pv*) mRNA, which is expressed in a subpopulation of interneurons [25-27]. Furthermore, given the dynamic changes in retrograde signaling after an injury [15,19], we were interested in cluster of mRNAs whose protein product plays a role in nuclear import function. Within this cluster, *Ipo13* was of particular interest since members of Importin family of nuclear receptors play a role in intracellular signaling after an injury and are involved in plasticity of neurons [20-22]. Importins also show stimulus-induced nuclear translocation and response to activity changes [21,23], allows for translocation of transcription factors into the nucleus [24], and thus may convey major intracellular signals to stabilize sensorimotor dendrites. Additional HuR and IgG RIPs coupled to qRT-PCR analysis of mRNA levels of *Ipo13* and *Pv* confirmed the microarray data. Finally, we determined if IPO13 and PV proteins co-localize in the same neurons as eIF4E using fluorescent co-immunohistochemistry. Indeed, we found that both proteins IPO13 and PV proteins co-localize with eIF4E in sensorimotor neocortices (Figure 4). These data suggest that functional eIF4E may be necessary for mRNA translation of both *Ipo13* and *Pv* in sensorimotor neocortices.

3.4 Levels of IPO-13 and PV-proteins decreased, but levels of their mRNA stay unchanged

Since neocortical eIF4E-protein distribution and phosphorylation is affected in sensorimotor neocortices after SCI, we analyzed the expression of IPO13-and PV- proteins in this region in sham-operated control mice and SCI mice using immunohistochemistry.

IPO13-protein was expressed in all projection neurons of sensorimotor neocortices in sham-operated controls (Figure 5). Interestingly however, 1, 3 and 28 days after SCI, the

IPO13-protein expression was selectively decreased in similar neocortical regions where eIF4E-protein was redistributed and eIF4E's phosphorylation is decreased, i.e. in contra-lesional sensorimotor neocortices (Figures 2 and 5). PV-protein was expressed in a subset of interneurons of sensorimotor neocortices of sham-operated controls as described (Figure 5; 25-27]. Remarkably, the PV-protein expression was selectively decreased in contra-lesional sensorimotor neocortices where eIF4E- and IPO13-protein expression were affected after SCI (Figure 6).

Since eIF4E-protein is a translation initiation factor that binds *Ipo13* and *Pv* mRNA and is redistributed after SCI, we examined temporal changes in levels of *Ipo13* and *Pv* mRNAs. To do this, we isolated total RNA from sensorimotor neocortices of uninjured, sham-operated control mice and from sensorimotor neocortices contralateral to the SCI injury site 1 and 3 days post-surgeries. Unchanged levels of *Ipo13* and *Pv* mRNA were surprisingly detected in all sensorimotor neocortices using qRT-PCR analysis (Figure 7A). Collectively, these data indicate that eIF4E-dependent translation of *Ipo13* and *Pv* mRNAs is affected.

3.5 *Ipo13*- and *Pv*- mRNA detached from eIF4E in contra-lesional sensorimotor neocortices after SCI

The total levels of *Ipo13*- and *Pv* mRNAs did not change after SCI, but their protein levels were decreased. Therefore, we determined if *Ipo13*- and *Pv*- mRNA binding to eIF4E in contra-lesional sensorimotor neocortices is disrupted after SCI. RIP analysis was performed on contra-lesional sensorimotor neocortices with eIF4E RIP-Chip antibody and corresponding IgGs at 1 and 3 days post-surgery. Precipitates from sham-operated control and SCI were analyzed for levels of *Ipo13* and *Pv* mRNAs by qRT-PCR (Figure 7B). Remarkably, both *Ipo13* and *Pv* mRNAs were detached from eIF4E already at 1 day after SCI and did not re-attach by 3rd day post-SCI (Figure 7B). Collectively, these data suggest that mRNA cargo of eIF4E in sensorimotor neocortices may be disrupted after SCI, preventing selective mRNA translation.

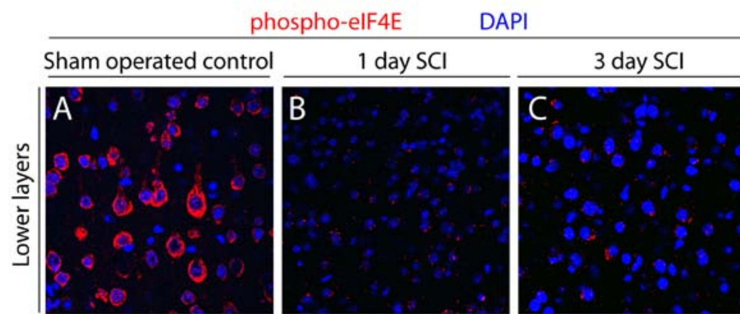
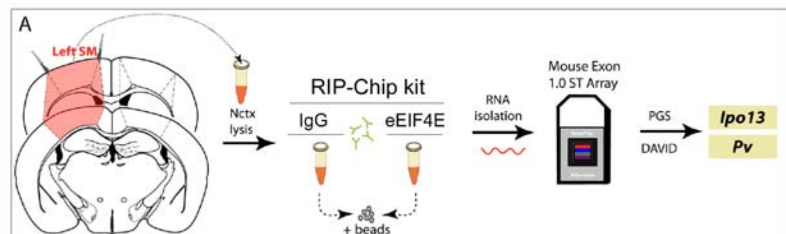
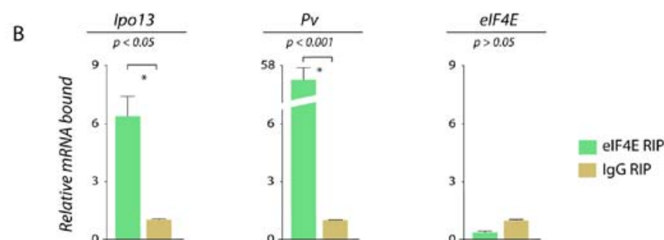


Figure 3. Expression of phospho-eIF4E is downregulated after SCI in all projection neurons of contra-lesional sensorimotor neocortices. (A-C) Representative confocal images of phospho-eIF4E immunostaining of sensorimotor neocortices showing downregulation of phospho-eIF4E expression 1 and 3 days after SCI. In sham operated control 1 and 3 days post-surgery, phospho-eIF4E was found equally distributed in cytoplasm and in initial parts of dendrites of neocortical projection neurons. However, 1 and 3 days after SCI phospho-eIF4E expression was downregulated in most of the contra-lesional sensorimotor neurons.





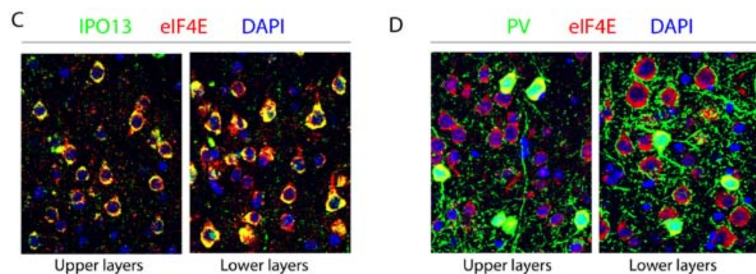


Figure 4. eIF4E binds *Ipo13* and *Pv* mRNAs. (A) Schematic representation of determination of mRNAs bound to eIF4E. eIF4E and bound mRNAs were precipitated from dissected developing neocortices using HuR RIP-Chip certified antibody and kit. Corresponding IgG was used as negative control. Isolated mRNAs from eIF4E and IgG precipitates were analyzed using Mouse Exon 1.0 ST arrays and PGS. mRNAs that are bound significantly higher by HuR than IgG were analyzed using functional annotation clustering of DAVID. We found that eIF4E binds *Ipo13* and *Pv*. (B) To confirm the RIP-Chip analysis, quantitative RT-PCR analysis on additional eIF4E RIPs confirmed high binding of both *Ipo13* and *Pv* mRNAs to eIF4E, while eIF4E did not bind eIF4E mRNA higher than control IgGs. The levels of mRNAs bound were normalized towards levels of mRNAs bound to control IgGs. (C,D) Immunostaining of adult sensorimotor neocortices revealed that IPO13 (C; green) and PV (D; green) are co-expressed with eIF4E in neocortical neurons of adult sensorimotor neocortices.

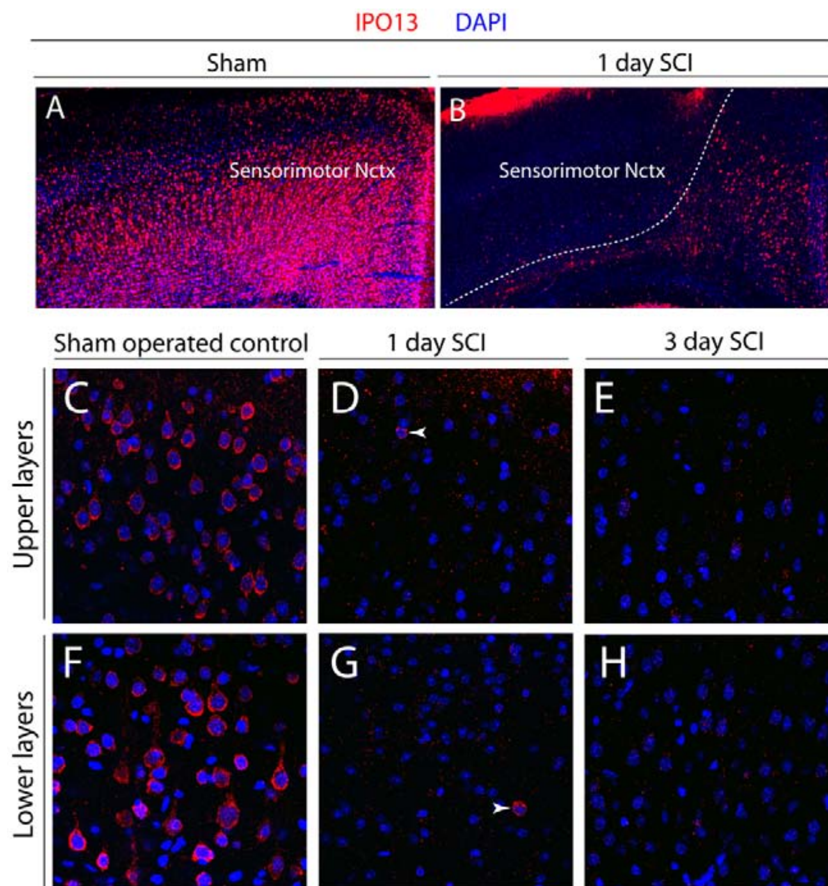


Figure 5. Expression of IPO13-protein is downregulated in all projection neurons of contra-lesional sensorimotor neocortices. (A,B) Representative low magnification images of *Ipo13* immunostaining demonstrate a localized effect on IPO13 expression after SCI selectively in contra-lesional sensorimotor neocortex (Nctx) 1 day after SCI (B), mimicking the effect site on eIF4E. (C-H) High power magnification of IPO13 immunostaining of sensorimotor neocortices reveal downregulation of IPO13-protein expression 1 and 3 days after SCI. In the sham-operated control 1 and 3 days post-surgery IPO13 was found equally distributed in cytoplasm and in initial parts of dendrites of upper and lower layer projection neurons. However, 1 and 3 days after SCI no IPO13 was seen in most of the sensorimotor neurons. Interestingly, rarely a cell can be found to express normal levels of IPO13 (G, arrowhead).

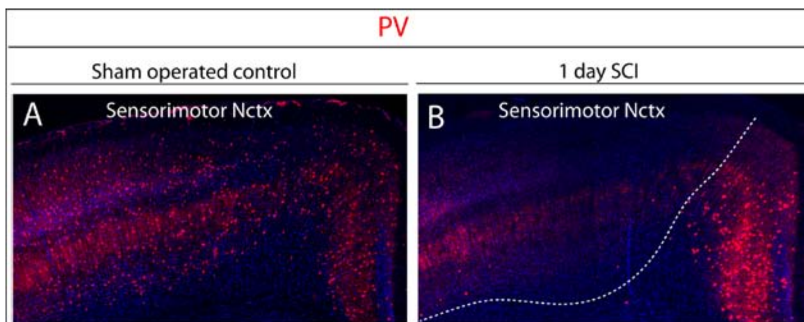


Figure 6. Expression of PV-protein is downregulated in interneurons after SCI in contra-lesional sensorimotor neocortices. (A,B) Representative low magnification images of PV immunostaining show a localized effect on PV expression after SCI selectively in contra-lesional sensorimotor neocortex (Nctx) 1 day after SCI (B), mimicking the effect site on eIF4E and IPO13.

3.6 Dendritic polarity of projection neurons of sensorimotor neocortices is disrupted after SCI

Dendrites of projection neurons in sensorimotor neocortices show a highly polarized organization, with one apical dendrite and several basal dendrites [28,29] (Figure 8). The dendritic organization of mature neurons can be disrupted by an axonal injury, aberrant neuronal activity and/or disrupted mRNA translation events [28,30,33]. During SCI, both CS and sensory axons are injured and neocortical activity rapidly changes as a result (71-3). Furthermore, Importins are known to play a role in retrograde signaling, and PV interneurons target the initial segment of an axon of projection neurons. Both of these functions are also important for neuronal dendritic polarity [31,32]. Taken together with our results described above, the dendritic polarity of projection neurons is expected to be affected after SCI. Therefore, we first examined the acute effect of SCI on dendritic polarity of projection neurons in the contra-lesional sensorimotor neocortices. Immunostaining for SMI32, a marker for projection neurons, showed significant disruption of dendritic polarity of the sensorimotor neocortices contralateral to the SCI site, when compared to sham-operated controls 1 and 3 days post-surgery (Figure 8). These data suggest that SCI acutely disrupts the dendritic polarity selectively in contra-lesional sensorimotor neocortices, which does not recover after 3 days. Taken together, the alteration in dendritic morphology and the loss of PV-expression indicate an acute reorganization of microcircuitry of contra-lesional sensorimotor neocortices after SCI.

3.7 eIF4E is expressed in central neurons of human adult primary motor neocortices

Next, we determined the expression pattern for eIF4E in adult human sensorimotor neocortices. To examine this, we immunostained primary motor and primary sensory neocortices in a post-mortem sample of 46 year old individual without any neurological or psychiatric history (Figure 9). Indeed, the pattern of expression of eIF4E is analogous to our observations in mice and indicates that eIF4E may play a similar role in humans who have suffered an SCI.

4. Discussion

Our goal was to determine the effect of an SCI on eIF4E in neurons of sensorimotor neocortices that are distant from the injury site. In this study, we found that levels of eIF4E did not significantly change, but its subcellular distribution and phosphporylation were disrupted. These data suggest that function of eIF4E is disrupted. Indeed, we found that binding of distinct mRNAs, such as *Ipo13* and *Pv*, to eIF4E was acutely disrupted after SCI. Furthermore, total levels of mRNA of both *Ipo13* and *Pv* did not significantly change following SCI, while their protein was undetectable. Since we tested acute adaptive changes only after lateral hemisection SCI at T10, we can not exclude the possibility that after different types of SCI or after longer than 3 days post-SCI periods, IPO13- or PV-proteins will be re-expressed. Particularly because very often a spontaneous regeneration is seen after SCI [1,2].

eIF4E, an RBP member of the neocortical translational machinery, plays an essential role in the control of the initiation of mRNA translation and in the nucleocytoplasmic export of distinct mRNAs. In particular, eIF4E is crucial for cap-dependent mRNA translations, which are responsible for recruiting all nuclear transcribed mRNAs [34]. Both the mRNA and protein of eIF4E are found in dendrites as well as the cytoplasm [35,36]. Our results show a spatial re-distribution of this eIF4E expression after SCI in the contra-lesional sensorimotor neocortices. In addition, these sensorimotor neocortices have slower spontaneous activity due to deafferentation [3]. Thus, our finding of eIF4E's re-distribution after SCI is consistent with a recent report that neuronal activation promotes redistribution of eIF4E in neurons [35]. eIF4E is a major rate-limiting factor during protein synthesis; therefore, the SCI-triggered re-distribution of eIF4E may jeopardize the necessary local protein translation in neocortical dendrites. Local protein translation in dendrites is known to be essential for synaptic plasticity and is responsive to neuronal activity, which, in turn, affects the structural and functional plasticity of the dendrites [36].

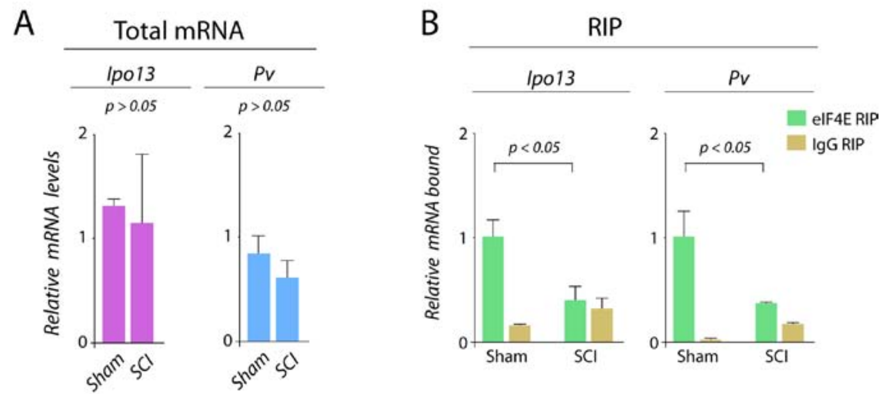


Figure 7. Levels of *Ipo13* and *Pv* mRNA are unchanged, but detached from eIF4E after SCI in contra-lesional sensorimotor neocortices. (A) Quantitative RT-PCR analysis of contra-lesional sensorimotor neocortices showed no change in *Ipo13* mRNA levels 1 and 3 days after SCI. (B) eIF4E RIP coupled to qRT-PCR showed decreased binding of both *Ipo13* and *Pv* mRNAs 1 and 3 days after SCI.

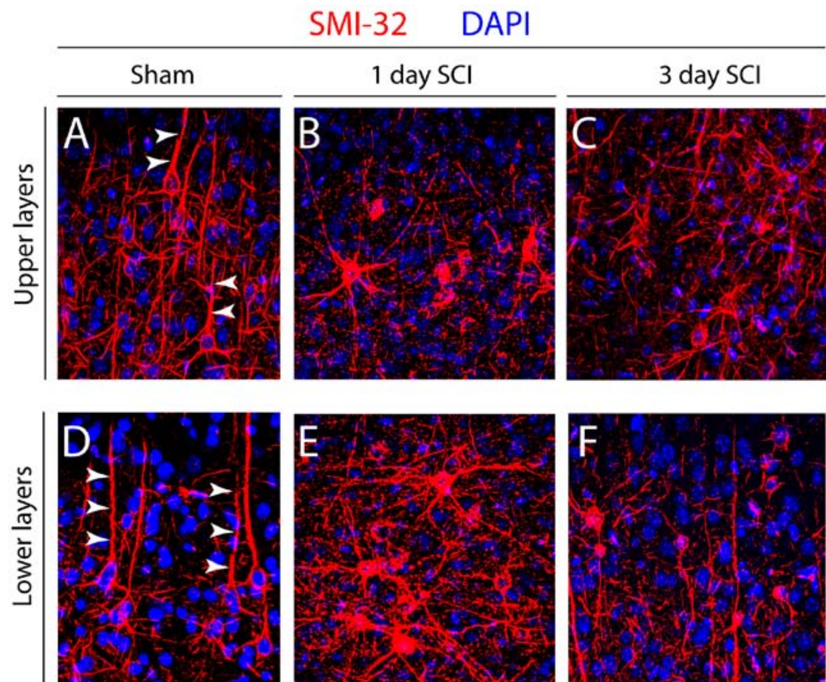


Figure 8. Dendritic polarity of projection neurons of sensorimotor neocortices is disrupted after SCI. (A-F) Representative confocal images of SMI-32 immunostaining of contra-lesional sensorimotor neocortices show disrupted polarity of dendrites of neocortical projection neurons 1 and 3 days after SCI.

Phosphorylation states of an RBP determine its function, such as mRNA translation. Importantly, phosphoproteins were shown to be strongly affected in lesioned peripheral neurons [6]. These data indicate that that RBP-dependent mRNA translation may be central in acute responses of injured neurons after SCI. In addition, it was recently shown that genetic manipulation of PTEN/Akt/mTOR pathway in CS neurons improved the CS regeneration after SCI [7]. Both PTEN/Akt/mTOR signaling and neuronal activity play a role in phosphorylation and, thus, in the function of Eukaryotic translation initiation factor 4E (eIF4E. Thus, a strong link may exist between CS axonal regeneration and proper eIF4E function. Recent reports show

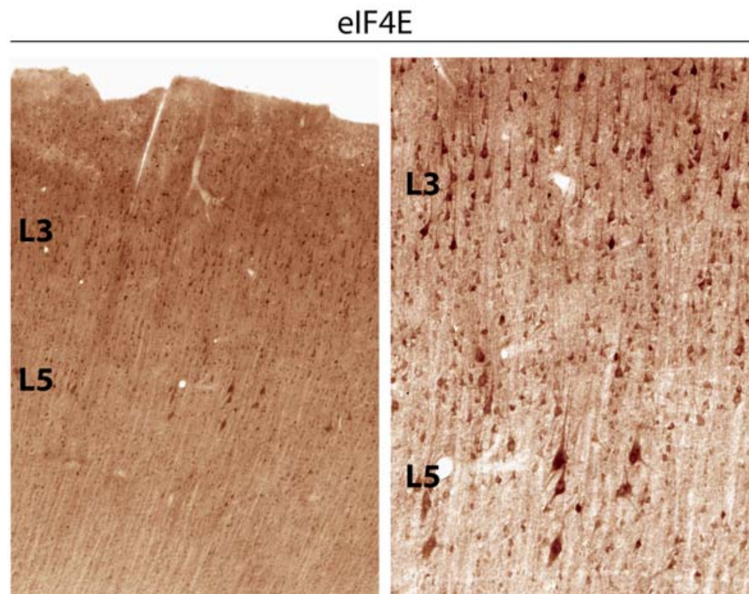


Figure 9. eIF4E is expressed in projection neurons of human adult primary motor cortex. Medial part of human primary motor neocortex, where legs are represented in human motor homunculus, was immunostained for eIF4E. eIF4E is expressed in projection neurons of all layers, mimicking the expression in mice sensorimotor neocortices.

that phosphorylation of eIF4E can be regulated by curcumin [37], a polyphenol derived from *Curcuma longa* that is usually used as a food spice. Beside its effects on eIF4E in cancer cells, curcumin was shown to provide neuroprotection in the spinal cord and to improve functional recovery after SCI [38,39]. Relevant to this study, it is interesting to note that in distinct type of cancer cells, curcumin increased levels of phospho-eIF4e [37], which we found to be downregulated in sensorimotor neocortices after SCI. Therefore, curcumin may promote some eIF4E-dependent events, which are lost after SCI, indicating its potential as a possible novel SCI drug that would be both widely available and affordable. Nevertheless, we found eIF4E phosphorylation to be decreased after SCI, further indicating that mRNA translation events are affected acutely post-SCI in contra-lesional sensorimotor neocortices.

Although neocortical projection neurons in different neocortical layers share the highly polarized morphology, each neocortical layer has a specific subset of projection neurons characterized by distinct functions, transcriptional programs, axonal connections and dendritic complexities. Critical to the function of each neocortical neuron is how it processes polarized synaptic inputs. The most polarized parts of neocortical projection neurons will receive input from distant brain regions, such as thalamus via thalamocortical axons [28]. Thus, one can reason that loss of polarity of apical dendrites of neocortical projection neurons in all layers of sensorimotor neocortices may also be affected after thalamic sensory input is disrupted due to SCI.

Reorganizational changes depend on both the derived stimuli and the internal molecular programs that bring information from the membrane of the neuron to the nucleus. Importins are among the molecules that show stimulus-induced nuclear translocation and response to activity changes [21,23]. In particular, Importins may bind a nuclear localization signal (NLS) present in the cytoplasmic tail of synaptic molecules. This binding is crucial since it allows Importins to translocate transcription factors, such as CREB2, into the nucleus and induce transcription-dependent forms of neuronal plasticity [22-24]. In addition to this synapse-to-nucleus translocation during synaptic plasticity, Importins were shown to bring signals to the nucleus from injured axons [21]. Thus, Importins may convey major intracellular signals to stabilize sensorimotor dendrites, like IPO13 in our case. As shown by our experiments, an SCI induced loss of polarity of projection neurons in contra-lesional sensorimotor neocortices and IPO13-protein expression was decreased. Thus, alterations in IPO13, or other Importins, may ultimately lead to permanent plastic changes of neocortical dendrites and information processing after SCI.

Furthermore, within interneuronal subtypes, thalamocortical contacts predominantly target PV expressing interneurons [39]. These PV expressing interneurons target cell body and axon initial segments, forming so-called baskets around neocortical projection neurons, including CS. Surprisingly, these PV-positive basket cells excite rather than inhibit postsynaptic neurons [25]. Thus, beside both retrograde changes in CS neurons and changes induced via thalamocortical axons on projection neuron dendrites, an SCI will also likely induce significant changes in interneurons and provoke further the activity dependent dendrite changes of sensorimotor neocortices [30].

In summary, we have shown that a lateral hemisection SCI acutely disrupts eIF4E in contra-lesional sensorimotor neocortices, leading to disruption in eIF4E function, and preventing its binding to target mRNAs, such as *Ipo13* and *Pv*, thus resulting in aberrant mRNA translational control. Therefore, eIF4E and a subset of neocortical mRNAs after SCI may be rapidly recruited to translational machinery to induce regeneration. Thus, promoting the neocortical eIF4E-dependent mRNA translation in sensorimotor neocortices after SCI could improve the adaptive regeneration efforts of sensorimotor neurons.

Acknowledgments

We thank Suzan Harris, Althea Stillman and Erik DeBoer for technical support and helpful discussions. This work was supported by start-up funds from the UMDNJ-RWJMS.

References

[1] Raineteau O., Schwab ME. , Plasticity of motor systems after incomplete spinal cord injury, Nat. Rev. Neurosci., 2001, 2, 263-273

[2] Dobkin B.H., Havton L.A., Basic advances and new avenues in therapy of spinal cord injury., Annu. Rev. Med., 2004, 55, 255-282

[3] Aguilar J., et al., Spinal cord injury immediately changes the state of the brain., J. Neurosci., 2010, 7528-7537

[4] Ghosh A, et al., Rewiring of hindlimb corticospinal neurons after spinal cord injury. Nat. Neurosci., 2010, 13, 97-104

[5] Ghosh A., et al., Functional and anatomical reorganization of the sensory-motor cortex after incomplete spinal cord injury in adult rats. J. Neurosci., 2009, 29,12210-12219

[6] Jain N., Florence S.L., Kaas J.H., Reorganization of somatosensory cortex after nerve and spinal cord injury. News Physiol. Sci., 1998, 13, 143-149

[7] Endo T., Spenger C., Tominaga T., Brené S., Olson L., Cortical sensory map rearrangement after spinal cord injury: fMRI responses linked to Nogo signalling. Brain, 2007, 130, 2951-2961

[8] Yates C. C., Garrison K., Charlesworth A., Reese N. B., Garcia-Rill E., Therapeutic approaches for spinal cord injury induced spasticity. Transl. Neurosci., 2010, 1, 160-169

[9] Wyndaele M., Wyndaele J.J., Incidence, prevalence and epidemiology of spinal cord injury: what learns a worldwide literature survey? Spinal Cord., 2006., 44, 523-529

[10] Henigsberg N., Lagerkvist B., Matek Z., Kostovic I., War victims in need of physical rehabilitation in Croatia. Scand. J. Soc. Med., 1997, 25, 202-206

[11] Yip P.K., Wong L.F., Sears T.A., Yáñez-Muñoz R.J., McMahon S.B., Cortical overexpression of neuronal calcium sensor-1 induces functional plasticity in spinal cord following unilateral pyramidal tract injury in rat. PLoS Biol., 2010, 8, e1000399.

[12] Liu K., et al., PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat Neurosci., 2010, 13, 1075-1081

[13] Kim, B.G., Dai, H.N., McAtee, M., Vicini, S., Bregman, B.S., Remodeling of synaptic structures in the motor cortex following spinal cord injury. Exp. Neurol., 2006, 198, 401-415

[14] Keene, J.D., RNA regulons: coordination of post-transcriptional events. Nat Rev Genet., 2007, 8, 533-543

[15] Michaelevski I, et al., Signaling to transcription networks in the neuronal retrograde injury response. Sci. Signal., 2010, 3, ra53

[16] Hains B.C., Black J.A., Waxman S.G., Primary cortical motor neurons undergo apoptosis after axotomizing spinal cord injury. J. Comp. Neurol., 2003, 462, 328-341

[17] Nielson J.L., Sears-Kraxberger I., Strong M.K., Wong J.K., Willenberg R., Steward O., Unexpected survival of neurons of origin of the pyramidal tract after spinal cord injury. J. Neurosci., 2010, 30, 11516-11528

[18] McKendrick L., Pain V.M., Morley S.J., Translation initiation factor 4E. Int. J. Biochem. Cell. Biol., 1999, 31, 31-35

[19] Abe N., Cavalli V., Nerve injury signaling. Curr. Opin. Neurobiol., 2008, 18, 276-283

[20] Hanz S., Fainzilber M., Integration of retrograde axonal and nuclear transport mechanisms in neurons: implications for therapeutics. Neuroscientist., 2004, 10, 404-408

[21] Hanz S., et al., Axoplasmic importins enable retrograde injury signaling in lesioned nerve. Neuron., 2003, 40, 1095-1104

[22] Thompson K.R., et al., Synapse to nucleus signaling during long-term synaptic plasticity; a role for the classical active nuclear import pathway. Neuron., 2004, 44, 997-1009

[23] Jeffrey R.A., Ch'ng T.H., O'Dell T.J., Martin K.C., Activity-dependent anchoring of importin alpha at the synapse involves regulated binding to the cytoplasmic tail of the NR1-1a subunit of the NMDA receptor. J. Neurosci., 2009, 29, 15613-15620

[24] Lai K.O., Zhao Y., Ch'ng T.H., Martin K.C., Importin-mediated retrograde transport of CREB2 from distal processes to the nucleus in neurons. Proc. Natl. Acad. Sci. USA, 2008, 105, 17175-17180

[25] Szabadics J., Varga C., Molnár G., Oláh S., Barzó P., Tamás G., Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. Science., 2006, 311, 233-235

[26] Szabadics J., Varga C., Molnár G., Oláh S., Barzó P., Tamás G., Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. Science., 2006, 311, 233-235

[27] Petilla Interneuron Nomenclature Group, Ascoli GA et al., Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat. Rev. Neurosci., 2008, 9, 557-568

[28] Spruston, N., Pyramidal neurons: dendritic structure and synaptic integration. Nat Rev Neurosci., 2008, 9, 206-221

[29] Chen J.G., Rasin M.R., Kwan K.Y., Sestan N., Zfp312 is required for subcortical axonal projections and dendritic morphology of deep-layer pyramidal neurons of the cerebral cortex Proc. Natl. Acad. Sci. USA., 2005, 102, 17792-17797

[30] Groc L., Petanjek Z., Gustafsson B., Ben-Ari Y., Hanse E., Khazipov R., In vivo blockade of neural activity alters dendritic development of neonatal CA1 pyramidal cells. Eur. J. Neurosci., 2002, 16, 1931-1938

[31] Vrieseling E., Arber S., Target-induced transcriptional control of dendritic patterning and connectivity in motor neurons by the ETS gene Pea3. Cell., 2006, 127, 1439-1452

[32] Rasband M.N., The axon initial segment and the maintenance of neuronal polarity. Nat. Rev. Neurosci., 2010, 11, 552-562

[33] Morita T., Sobue K., Specification of neuronal polarity regulated by local translation of CRMP2 and Tau via the mTOR-p70S6K pathway. J. Biol. Chem., 2009, 284, 27734-27745

[34] Shatkin A.J., mRNA caps--old and newer hats. Bioessays., 1987, 7, 275-277

[35] Moon I.S., Cho S.J., Seog D.H., Walikonis R., Neuronal activation increases the density of eukaryotic translation initiation factor 4E mRNA clusters in dendrites of cultured hippocampal neurons. Exp. Mol. Med., 2009, 41, 601-610

[36] Bramham C.R., Wells D.G., Dendritic mRNA: transport, translation and function. Nat. Rev. Neurosci., 2007, 8, 776-789

[37] Chakravarti N., Kadara H., Yoon D.J., Shay J.W., Myers J.N., Lotan D., Sonenberg N., Lotan R., Differential inhibition of protein translation machinery by curcumin in normal, immortalized, and malignant oral epithelial cells. Cancer Prev. Res. (Phila)., 2010, 3, 331-338

[38] Cemil B., Topuz K., Demircan M.N., Kurt G., Tun K., Kutlay M., Ipcioglu O., Kucukodaci Z., Curcumin improves early functional results after experimental spinal cord injury. Acta Neurochir., 2010, 152, 1583-1590

[39] Lin M.S., Lee Y.H., Chiu W.T., Hung K.S., Curcumin Provides Neuroprotection After Spinal Cord Injury. J. Surg. Res., 2009, [Epub ahead of print]

[40] Sugiyama, S., Di Nardo, A.A., Aizawa, S., Matsuo, I., Volovitch, M., Prochiantz, A., Hensch T.K., Experience-dependent transfer of Otx2 homeoprotein into the visual cortex activates postnatal plasticity. Cell., 2008,134, 508-520

[41] Mikucki S. A., Oblinger. M.M., Corticospinal neurons exhibit a novel pattern of cytoskeletal gene expression after injury. J. Nsc Res., 1991, 30,213-225

[42] Basso D.M., Beattie M.S., Bresnahan, J.C. A sensitive and reliable locomotor rating scale foropen field testing in rats. J. Neurotrauma, 1995, 12, 1–21

[43] Basso D.M., Fisher L.C., Anderson A.J., Jakeman L.B., McTigue D.M., Popovich P.G., Basso Mouse Scale for locomotion detectds differences in recovery after spinal cord injury in five common mouse strains. J. Neurotrauma, 2006, 23, 635-659

[44] Engesser-Cesar E., Anderson A.J., Basso D.M., Edgerton V.R., Cotman C.W., Voluntary wheel running improves recovery from a moderate spinal cord injury. J. Neuotrauma., 2005, 22, 157-171

[45] Carmichael M.J., Crockett D.P., Hayes N.L., Nowakowski R.S., Strain differences in injury phenotype, cell proliferation, and behavioral recovery after spinal cord injury (SCI) in mice. Program No. 447.4 Neuroscience Meeting Planner, Atlanta, GA: Society for Neuroscience, 2006, Online

[46] Crockett D.P., Carmichael M.J., Gu J., Nowakowski R.S., The Murine Locomotion Components Scale: a versatile behavioral tool to assess locomotion after spinal cord injury (SCI) and locomotor development in genetically modified mice. Program No. 600.26/ BB22 2007 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2007, Online.

[47] Crockett D.P., Son A., Carmichael M.J., Jordan M.E., Eang R., Harris, S.L., Egger; M.D., A versatile behavioral scale for the analysis of locomotion following spinal cord injury (SCI): Functional recovery in the p27Kip1 knockout mouse. Program No. 586.5 Neuroscience Meeting Planner, Atlanta, GA: Society for Neuroscience, 2006, Online

[48] Mikucki S. A., Oblinger M.M., Corticospinal neurons exhibit a novel pattern of cytoskeletal gene expression after injury. J. Nsci Res, 1991, 30, 213-225

Program#/Poster#: 600.26/BB22

Title: The Murine Locomotion Components Scale: a versatile behavioral tool to assess locomotion after spinal cord injury (SCI) and locomotor development in genetically modified mice

Location: San Diego Convention Center: Halls B-H

Presentation Start/End Time: Tuesday, Nov 06, 2007, 9:00 AM -10:00 AM

Authors: ***D. P. CROCKETT**[1], M. J. CARMICHAEL[1,2], J. GU[1], R. S. NOWAKOWSKI[1,2];
[1]Dept Neurosci & Cell Biol, UMDNJ-RW Johnson Med. Sch., Piscataway, NJ; [2]Dept. of Biomedical. Engin., UMDNJ-Graduate Sch. of Biomed. Sci., Piscatway, NJ

We have developed a new scale, the *Murine Locomotion Component Scale* (*MLCS*), for the behavioral assessment of the recovery of function in mice after SCI. The MLCS incorporates a hierarchical design in considering locomotion as set of 5 components: (1) hindlimb (HL) stepping, (2) coordination, (3) HL weight support, (4) stability/posture and (5) balance. Each of these components consists of a set of subcomponents that can easily be observed, differentiated and independently scored. These subcomponent scores generate the 5 component scores, the summation of which provides an overall locomotion rating (OLR). The hierarchical structure of the MLCS and its systematic approach to locomotion enhances its versatility in applying the scale to a wide range of injury models that may produce varying deficits and recovery scenarios. We have demonstrated the utility of the MLCS in several animal models of SCI (dorsal hemisection, compression and chemical demyelination). Here, we have extended the use of the MLCS to study locomotion of mice lacking the common neurotrophin receptor, p75NTR. At postnatal day 19 (P19), the p75NTR knockout mice consistently exhibited significantly lower scores than their wild-type littermates in 3 out of 5 of the MLCS components (stepping, posture/stability and balance). By adulthood, the p75NTR knockout and WT mice exhibited significant differences only for the balance component (i.e., the assessment of locomotion along bars of varying diameters, 5.0 - 0.5 cm). Additionally, heterozygotes did not differ from WT littermates on any of the measures. Importantly, behavioral observations were performed blind to the animals' genotypes. In addition to being useful in the analysis of a wide range of injury models, our current application of the MLCS suggests that its breadth of application and versatility enhances the characterization of the phenotype of genetically modified mice.

Disclosures: **D.P. Crockett**, None; **M.J. Carmichael**, None; **J. Gu**, None; **R.S. Nowakowski**, None.

Support: To RSN from the NJ Comm on Spinal Cord Res

[Authors]. [Abstract Title]. Program No. XXX.XX. 2007 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2007. Online.

2007 Copyright by the Society for Neuroscience all rights reserved. Permission to republish any abstract or part of any abstract in any form must be obtained in writing by SfN office prior to publication.

OASIS - Online Abstract Submission and Invitation System™ ©1996-2011, Coe-Truman Technologies, Inc.

Program#/Poster#: 447.4/S22

Title: Strain differences in injury phenotype, cell proliferation, and behavioral recovery after spinal cord injury (SCI) in mice

Location: Georgia World Congress Center: Halls B3-B5

Presentation Start/End Time: Monday, Oct 16, 2006, 4:00 PM - 5:00 PM

Authors: ***M. J. CARMICHAEL**[1,2], D. P. CROCKETT[2], N. L. HAYES[2], R. S. NOWAKOWSKI[1,2];
[1]Biomedical Engineering, UMDNJ-Graduate School of Biomedical Sciences, Piscataway, NJ,
[2]Neuroscience & Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

Inbred strains of mice that are resistant to kainic acid (KA) induced excitotoxicity (e.g., C57BL/6) have been shown to develop significantly larger lesion volumes after SCI than strains that are KA-sensitive (e.g., FVB/N, Inman *et al.,* 2002). Proceeding from the hypothesis that lesion size is likely to correlate with potential for recovery, we are extending this analysis with fine spatial and temporal resolution to quantify the rate and extent of recovery of locomotion, cell proliferation and differentiation, and other morphological characteristics such as lesion and cyst volumes, white matter sparing, etc. after SCI. Our preliminary analysis used two groups of male mice: 21 week old FVB/NJ and 48 week old C57BL/6J. Our first step was to develop a new behavioral scoring system, the Murine Locomotion Component Scale (MLCS) which we have used in parallel with the standard Basso Mouse Scale (BMS) and BBB scale. The hierarchical design of the MLCS provides an informative scoring system for mice that considers locomotion to be the result of a set of 5 components that can be easily observed, differentiated and scored independently: hindlmb (HL) stepping, coordination, HL weight support, stability/posture, and balance. Our preliminary results suggest that there are strain- and/or age-dependent differences in the behavioral recovery of these mice within the initial 2 week period following SCI ($p < 0.01$, MLCS and BMS). In the same animals, we have begun to analyze cell proliferation using the S-phase marker bromodeoxyuridine (BrdU) in combination with markers for specific cell types. This information will be integrated to identify post-SCI phenotypes that will be used as quantifiable traits to analyze the effect of genetic background on the development of morphological sequelae and functional recovery from SCI. In principle, this will ultimately provide new insight into the biological mechanisms related to variability in functional recovery after SCI and will assist in the development of genetically personalized therapies.

Disclosures: **M.J. Carmichael**, None; **D.P. Crockett**, None; **N.L. Hayes**, None; **R.S. Nowakowski**, None.

Support: NJ Commission on Spinal Cord Research

[Authors]. [Abstract Title]. Program No. XXX.XX. 2006 Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience, 2006. Online.

2006 Copyright by the Society for Neuroscience all rights reserved. Permission to republish any abstract or part of any abstract in any form must be obtained in writing by SfN office prior to publication.

OASIS - Online Abstract Submission and Invitation System™ ©1996-2011, Coe-Truman Technologies, Inc.

Program#/Poster#: 586.5/OO66

Title: A versatile behavioral scale for the analysis of locomotion after spinal cord injury (SCI): functional recovery in the p27Kip1 knock-out mouse

Location: Georgia World Congress Center: Halls B3-B5

Presentation Start/End Time: Tuesday, Oct 17, 2006, 8:00 AM - 9:00 AM

Authors: ***D. P. CROCKETT**, A. SON, M. J. CARMICHAEL, M. E. JORDAN, R. EANG, S. L. HARRIS, M. D. EGGER;
Dept Neurosci & Cell Biol, Univ Med & Dentistry, Piscataway, NJ.

Stimulating endogenous oligodendrocyte progenitor cells (OPCs) to replace lost oligodendrocytes following SCI may aid recovery of function. To determine whether increasing the size of the progenitor pool proves beneficial, we have been studying mice lacking the cell-cycle inhibitor p27Kip1. Following SCI, increased numbers of proliferating NG2-labelled cells were detected in the p27Kip1-deficient mice when compared with WT controls. Because there are a number of difficulties in applying the BBB scale to mice, we have developed a new scale, the *Murine Locomotion Component Scale* (*MLCS*), for the clinical assessment of mice that have undergone SCI. This new scale considers murine locomotion as a hierarchical organization of components--hindlimb (HL) stepping, coordination, HL weight support, stability/posture, and balance. In turn, each of these components consists of a set of definable subcomponents. Unlike the BBB scale, which relies on complex descriptions of constellations of symptoms in order to derive a score, the MLCS rates each of the individual subcomponents of locomotion independently, providing component scores that sum to give an overall rating for walking. This allows for increased versatility in applying the scale to a wide range of injury models that may produce varying deficits and recovery scenarios. We have applied the new scale to analyze recovery of function following traumatic compression injuries and chemically induced demyelinating injuries. After compression injury, we found preliminary evidence of enhanced functional recovery in mice lacking p27Kip1 when compared with WT littermates, using both the BBB scale and our new MLCS. Although highly correlated with the BBB scale, the MLCS proved easier to use. In addition, the MLCS was useful in assessing subtle behavioral deficits that followed lysolecithin-induced demyelination of the ventral white matter, which did not match any of the scenarios described in the BBB scale.

Disclosures: **D.P. Crockett** , None; **A. Son**, None; **M.J. Carmichael**, None; **M.E. Jordan**, None; **R. Eang**, None; **S.L. Harris**, None; **M.D. Egger**, None.

[Authors]. [Abstract Title]. Program No. XXX.XX. 2006 Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience, 2006. Online.

2006 Copyright by the Society for Neuroscience all rights reserved. Permission to republish any abstract or part of any abstract in any form must be obtained in writing by SfN office prior to publication.

OASIS - Online Abstract Submission and Invitation System™ ©1996-2011, Coe-Truman Technologies, Inc.

Print this Page for Your Records Close Window

Control/Tracking Number: 2011-S-6682-SfN
Activity: Scientific Abstract
Current Date/Time: 5/12/2011 1:46:40 PM

Adaptive cortical responses to spinal cord injury in the mouse in vivo

AUTHOR BLOCK: *A. DUBEY, K. THOMPSON, V. L. DIBONA, M. R. RASIN, D. P. CROCKETT;
Dept. of Neurosci. and Cell Biol., Umdnj-Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: The rate of incidence of spinal cord injury (SCI) is about 10,000 new cases per year in the U.S. alone, but there are only limited available therapies throughout postnatal life. SCI results in trauma that affects the entire central nervous system, rather than just the spinal cord. Our laboratories have recently shown that after a lateral hemisection SCI, a model for Brown-Séquard syndrome, rapid changes occur in the contralesional sensorimotor neocortices. In particular, the mRNA cargo of Eukaryotic translation initiation factor 4E (eIF4E), an RNA binding protein that regulates the rate of mRNA translation, and the neocortical microcircuitry were already disrupted at 1 day post-SCI. To investigate mechanisms underlying these observations, we are currently performing in depth analysis of molecular changes involving mRNA translation in contralesional sensorimotor neocortices induced by SCI. Moreover, we are performing in vivo analysis of changes induced by lateral hemisection SCI in contralesional sensorimotor neocortices, using multiphoton microscopy on transgenic mice with fluorescently labeled projection neurons and interneurons. Our preliminary results indicate that eIF4E-dependent mRNA translation is affected after hemisection SCI in contralesional sensorimotor neocortices in both projection neurons and parvalbumin-expressing interneurons. The dendritic organization of projection neurons was disrupted and number of parvalbumin-expressing cell bodies was decreased. Therefore, these data implicate eIF4E to be involved in mechanisms underlying disrupted mRNA translation and microcircuitry changes of injured neocortical neurons after an SCI, which may contribute to their adaptive responses that are hitherto unknown.
:

Presentation Preference (Complete): Poster Only
Linking Group (Complete): None selected
Nanosymposium Information (Complete):

Suggest the order of presentations by entering the presenting author of each submission below separated by a comma. :
Aditi Dubey

Theme and Topic (Complete): C.10.d. Spinal cord: Cellular and molecular mechanisms ; C.10.d. Spinal cord: Cellular and molecular mechanisms
Keyword (Complete): dendrites ; CORTICAL PLASTICITY ; Post-transcriptional regulation
Support (Complete):
Support: Yes
Grant/Other Support: : UMDNJ Start-up Funds to MRR
Grant/Other Support: : New Jersey Commission for Spinal Cord Injury Research to DPC

Special Requests (Complete):
Is the first (presenting) author of this abstract a high school or undergraduate student?: None

Religious Conflict?: No Religious Conflict
Additional Conflict?: No

Status: Finalized

OASIS Helpdesk

Leave OASIS Feedback

Powered by OASIS, The Online Abstract Submission and Invitation System SM
© 1996 - 2011 Coe-Truman Technologies, Inc. All rights reserved.