Research Article • DOI: 10.2478/v10134-010-0044-5 • Translational Neuroscience • 1(4) • 2010 •268-278

VERSITA

Translational Neuroscience

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

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 covereslipped 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.

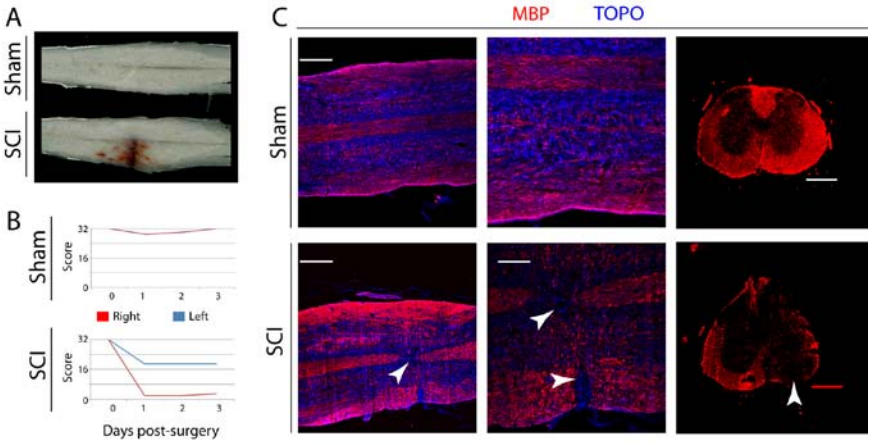

Figure 1. Lateral hemisection model of lumbar SCI. (A) Lateral hemisection SCI was induced in right side of the spinal cord (SCI), while spinal cord of sham operated control (Sham) was intact. (B) Behavioral scoring (Murine Locomotor Components Scale) showed decreased function of right leg (red) in SCI 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 bioinormatics analysis using

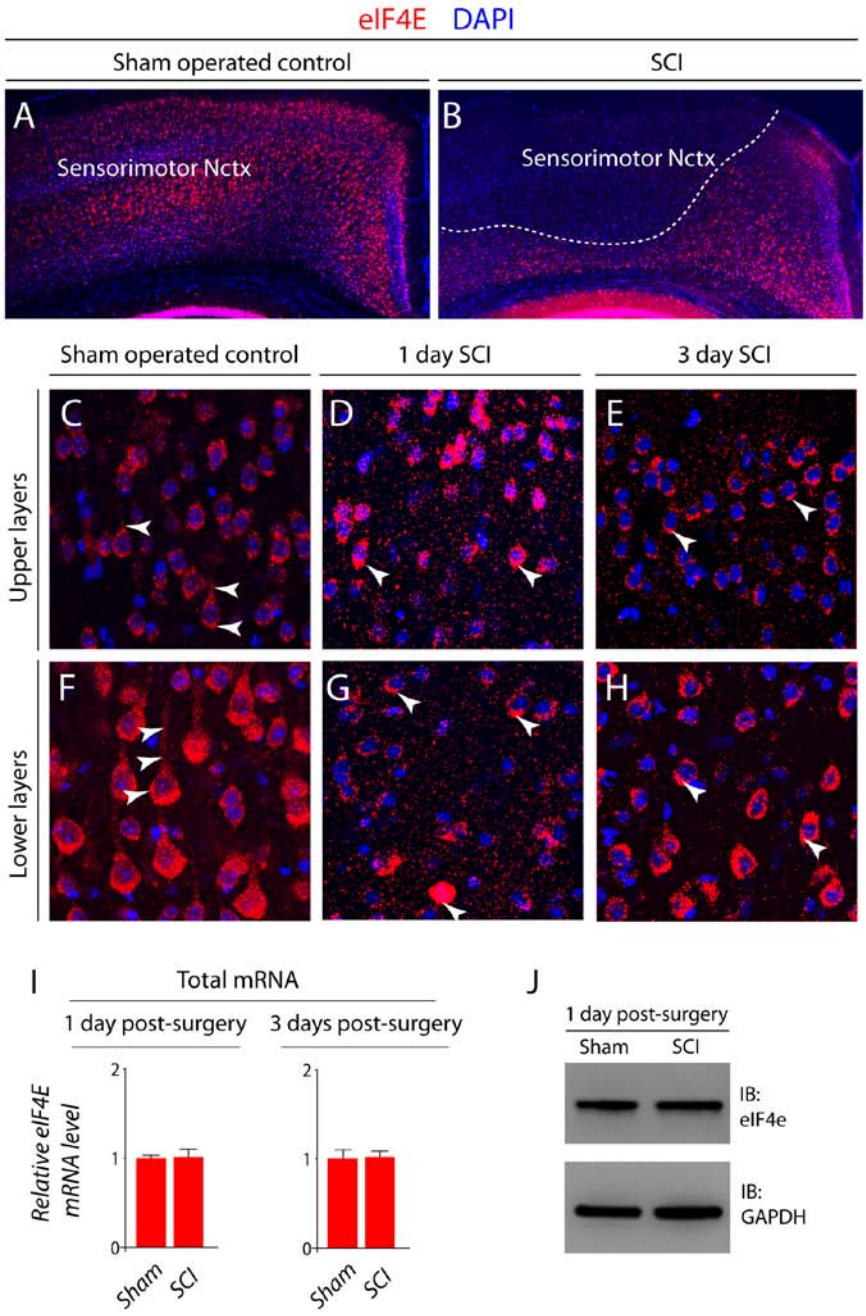

Figure 2. eIF4E is re-distributed in all projection neurons of sensorimotor neocortices contralateral to the SCI lesion site, while levels of eIF4E mRNA and eIF4E protein stay unchanged. (A,B) Representative low magnification images of eIF4E immunostaining showing a localized effect after SCI on eIF4E 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.

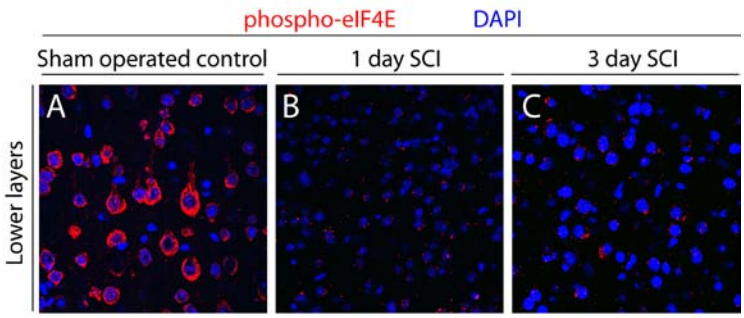

Figure 3. Expression of phospho-eIF4E is downregulated after SCI in all projection neurons of contra-lesional sensorimotor neocortices. (A-C) Representative confocal images of phospho-eIF4E immunostaining of sensorimotor neocortices showing downregulation of phospho-eIF4E expression 1 and 3 days after 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.

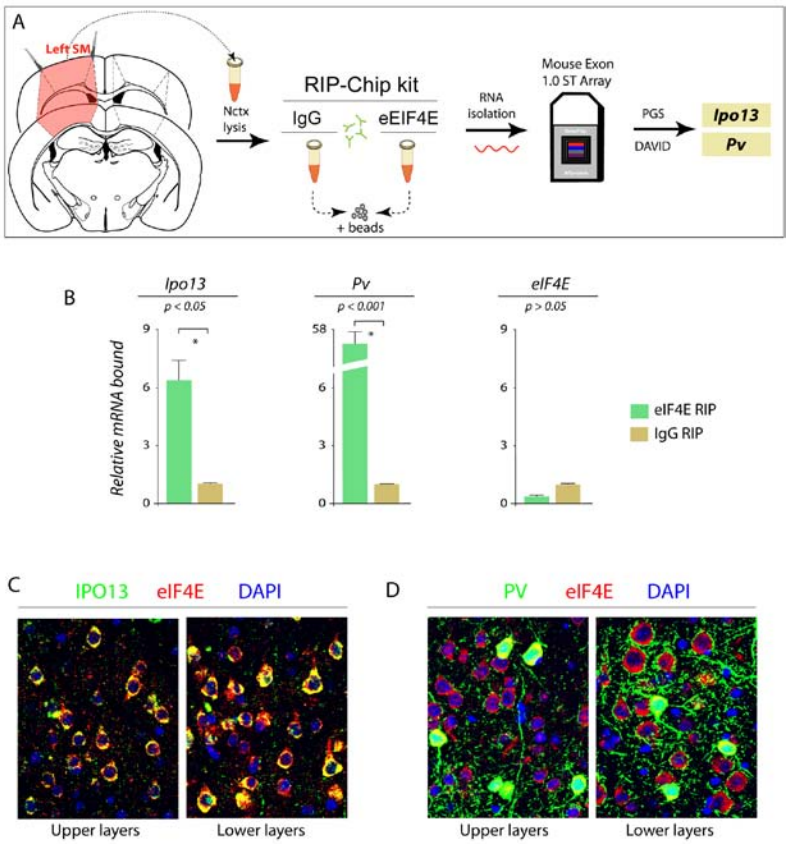

Figure 4. eIF4E binds *Ipo13* and *Pv* mRNAs. (A) Schematic representation of determination of mRNAs bound to eIF4E. eIF4E and bound mRNAs were precipitated from dissected developing neocortices using HuR RIP-Chip certified antibody and kit. Corresponding IgG was used as negative control. Isolated mRNAs 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.

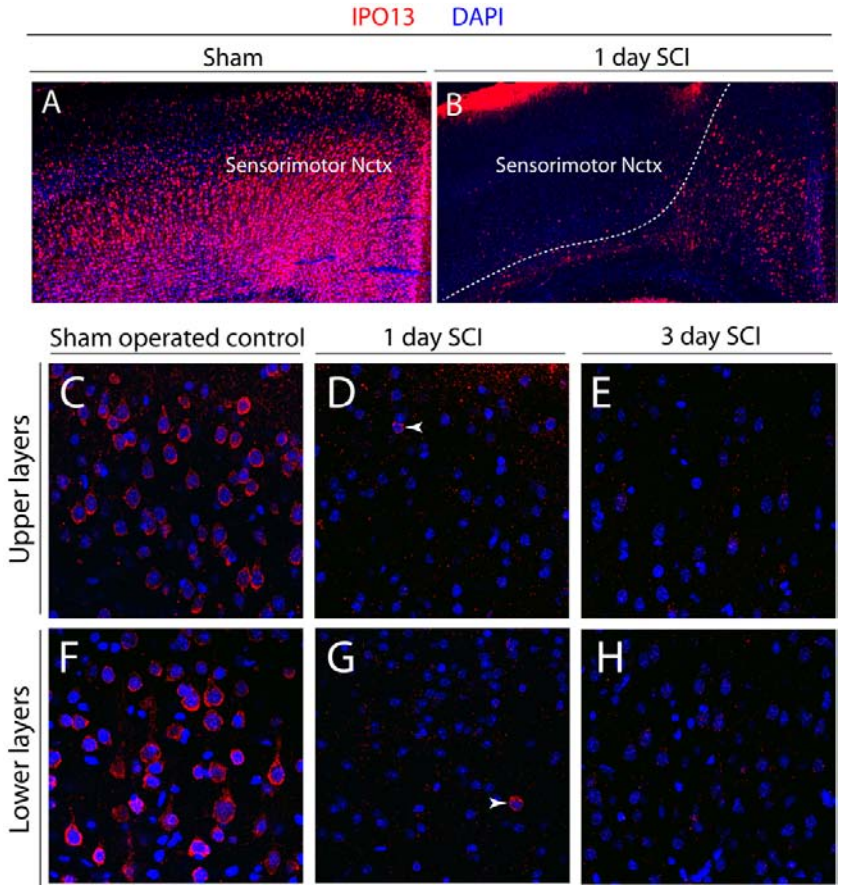

Figure 5. Expression of IPO13-protein is downregulated in all projection neurons of contra-lesional sensorimotor neocortices. (A,B) Representative low magnification images of *Ipo13* immunostaining demonstrate a localized effect on IPO13 expression after SCI selectively in contra-lesional sensorimotor 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).

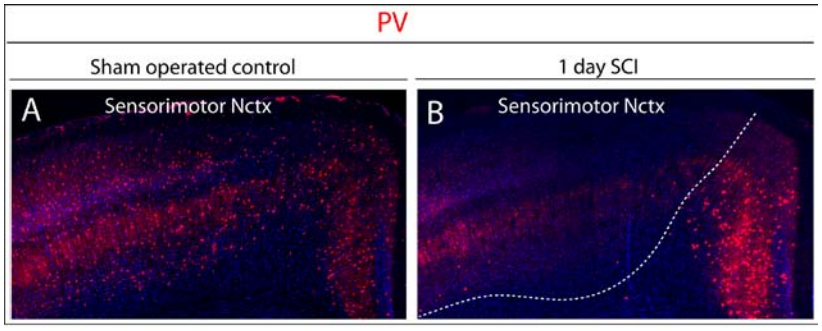

Figure 6. Expression of PV-protein is downregulated in interneurons after SCI in contra-lesional sensorimotor neocortices. (A,B) Representative low magnification images of PV immunostaining show a localized effect on PV expression after SCI selectively in contra-lesional sensorimotor neocortex (Nctx)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

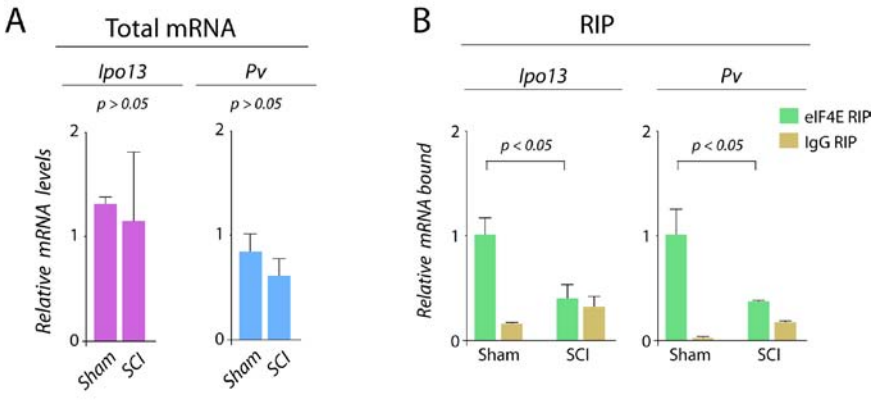

Figure 7. Levels of *Ipo13* and *Pv* mRNA are unchanged, but detached from eIF4E after SCI in contra-lesional sensorimotor neocortices. (A) Quantitative RT-PCR analysis of contra-lesional sensorimotor neocortices showed no change in *Ipo13* mRNA levels 1 and 3 days after SCI. (B) eIF4E RIP coupled to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

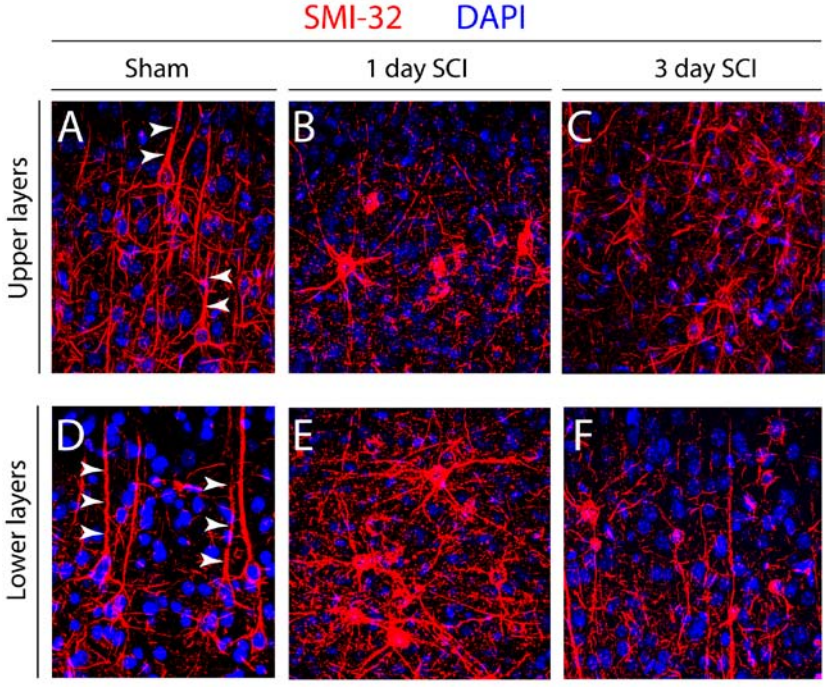

Figure 8. Dendritic polarity of projection neurons of sensorimotor neocortices is disrupted after SCI. (A-F) Representative confocal images of SMI-32 immunostaining of contra-lesional sensorimotor neocortices show disrupted polarity of dendrites of neocortical projection neurons 1 and 3 days after SCI.

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

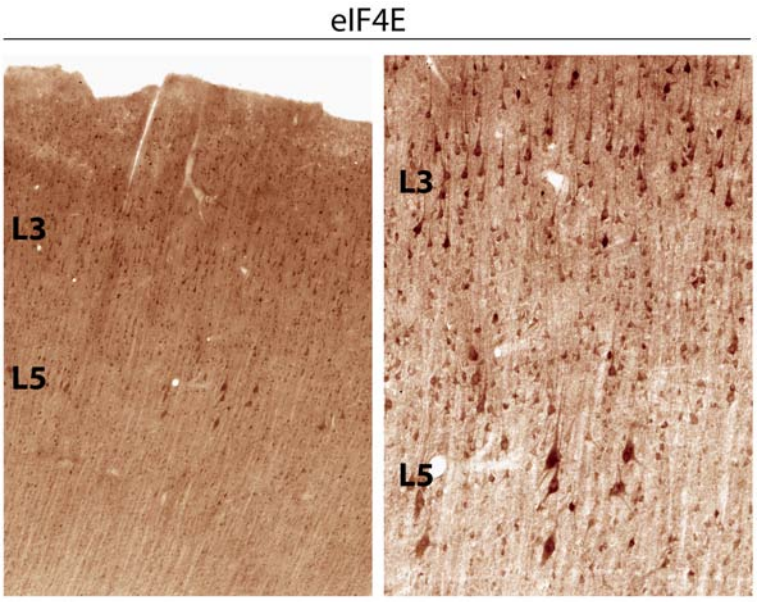

Figure 9. eIF4E is expressed in projection neurons of human adult primary motor cortex. Medial part of human primary motor neocortex, where legs are represented in human motor homunculus, was immunostained for eIF4E. eIF4E is expressed in projection neurons of all layers, mimicking the expression in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