Ahmed et al., J Spine Neurosurg 2013, 2:3
http://dx.doi.org/10.4172/2325-9701.1000115

Journal of Spine & Neurosurgery

Research Article

A SCITECHNOL JOURNAL

Spinal Cord Injury-Induced Discharges Modify the Function of Glutamatergic System and Improve Ground and Skilled Locomotor Control

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Abstract

Background: Recent data demonstrate that enhanced activity, induced by spinal cord injury (SCI) may accelerate functional recovery. This study was design to verify this assumption.

Methods: The effects of spinal cord injury with or without pharmacological suppression of injury-induced activity were investigated. In vivo method tested weekly locomotor activity of the animals during 7-week post-injury period. In vitro techniques characterized neuronal activity and properties of glutamatergic system.

Results: The sciatic nerve activity during SCI was categorized into sequentially occurring phases. The first phase, represented by mechanically-induced compound action potential, was followed by the second phase of high frequency discharges lasting approximately 2 min. These two phases were accompanied by enhanced glutamate efflux. The third phase lasting about 15 min with no discharges was followed by a hyperactive fourth phase with spasms and increased spontaneous activity. The application of lidocaine over the spinal cord before injury attenuated phasic activity. The ability of glutamatergic system to release glutamate in vitro 2 h, 24 h, and three weeks after the injury (chronic stage) from animals treated, or not treated with lidocaine was reduced, or elevated, respectively. The efficiency of glutamate uptake system in the animals not treated with lidocaine was elevated 2 h after injury and diminished later. In animals treated with lidocaine, the decline in the efficiency of glutamatergic system 2 h after the injury was followed by an increase. The expression of glutamatergic AMPA receptors in lidocaine-treated animals continued to rise from 2 h after the injury through a chronic stage. The expression of AMPA receptors in animals not treated with lidocaine was attenuated 2 and 24 h after the injury but elevated in the chronic stage. Blocking injury-induced activity with lidocaine impaired locomotion recovery. Conclusion: Neural discharges following the onset of SCI have beneficial effects on functional recovery.

Keywords

Spinal cord; Injury-induced activity; Recovery; Lidocaine; Plasticity; Glutamate; Behaviour

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Received: February 22, 2013 **Accepted:** June 20, 2013 **Published:** June 24, 2013

Abbreviations

AMPA: α-Amino-3-ydroxy-5-methyl-4-isoxazolepropionic acid; BMS: Basso muscle scale; CSI: Spinal cord injury; CNS: Central nervous system; D-Asp: 2,3^{3}H-D-Aspartic acid; LTP: long-term potentiation; mCAP: Mechanically-induced compound action potential; NMDA: N-methyl –D-aspartic acid; VGCC: Voltage gated calcium channel

Introduction

The injury of the spinal cord evokes intense neuronal activity of the spinal neurons. It has been always assumed that this excessive activity, correlated with a massive release of excitatory neurotransmitter, glutamate would impair functional recovery [1]. This assumption constituted a base for developing many pharmacological treatments for acute spinal cord injury (SCI) aimed at reduction of this hyperactivity. However, the idea of detrimental effects of hyperactivity is challenged by the data demonstrating that neuronal activity following injury may be beneficial for tissue regeneration [2] and functional recovery [3,4] activating intrinsic neuronal mechanism.

It is well known that brief but intense activity in the nervous system may lead to long-lasting physiological and structural changes in neurons. Examples include long-lasting increase in synaptic efficiency expressed as Long-Term Potentiating (LTP) [1], rapid changes of intrinsic neuronal excitability [5-10], enhanced sensitivity of postsynaptic membranes at individual synapses [11], and finally an increase in the number of active synapses, silent prior to enhanced activity [8]. All these changes represent neuronal plasticity which has been recognized as the most important phenomenon involved not only in the mechanisms of memory [12], but also recently implicated as one of the processes contributing to recovery after damage in the central nervous system (CNS) [13].

The spinal cord injury-induced discharges propagate quickly along spinal nerves and were shown to be correlated with long-term alterations in the spinal neurons and modification of behaviour. Among others, an increase in the expression of Fos protein [14], the voltage gated calcium channel (VGCC) [15], and triggering of autotomy (self-injury) [1,16,17] have been reported. Although these discharges are routinely observed as spasms at the onset of SCI in animals, their quantification, characteristics, and their effects on behavioural recovery are rarely investigated.

Most of spinal cord injuries are incomplete, sparing some functional connections [18] which could constitute anatomical substrate for functional recovery. We hypothesized that SCI by triggering an intense neuronal activity [15] would activate mechanisms involved in just described mechanisms of neuronal plasticity. Subsequently, the efficacy of spared connections between the injured spinal neurons would be enhanced initiating long-term processes leading ultimately to a better recovery. In order to evaluate the role of neuronal activity in the recovery process we compared the effects of spinal cord injury in animals, or in the tissue from the animals expressing injury-induced activity with the preparations where post-injury activity was attenuated by application of lidocaine.

As the first step of our investigation we decided to quantify the spinal cord injury-induced discharges and to characterize patterns of spontaneous activity of spinal motoneurons during the first hour following the impact. The effects of blocking injury-induced activity during and after spinal cord injury on ground and skilled locomotor control were also tested.

The locus of the changes in neuronal activity is expected to be mainly at the synapses involving modulation of synaptic transmission. Since glutamate is one of the major neurotransmitter in the spinal cord, and several activity-dependent alterations in glutamatergic synapses have been described [19-21], determination of changes in this system was the goal of our research as well.

To achieve our goals we have evaluated neuronal activity recorded electrophysiologically, and the properties of glutamatergic system tested biochemically. In addition, to obtain a comprehensive representation of investigated mechanisms, the performance of the animals with injury-induced activity blocked pharmacologically was compared with non-injured, control animals.

Our results reveal a beneficial effect of intense neuronal activity that occurs at the onset and after SCI [22,23].

Methods

Spinal cord contusion injury

Animals and experimental design: Experiments were carried out in accordance with NIH guidelines for the care and use of laboratory animals. Protocols were approved by the College of Staten Island IACUC. Adult CD-1 mice of both sexes (n=192) were used for this study. Animals were housed under a 12-h light-dark cycle with free access to food and water.

The experiments have been conducted according to acute (n = 35 animals), or chronic (n= 157 animals) paradigms (Figure 1). In acute experiments (Figure 1, Group A) the animals were anesthetized and the electrophysiological recordings of muscle and sciatic nerve activities were recorded immediately after injury. The animals were treated with the overdose of anesthetics just after recordings were completed. In experiments conducted according to chronic paradigm the animals were kept alive for 2 h (under anesthesia), 24 h and 7 weeks after spinal cord injury for electrophysiological recordings, behavioral tests and white matter evaluation (Figure 1, Group B). Biochemical tests were performed on the tissue obtained from animals surviving 2 h, 24 h and 3 weeks after the injury (Figure 1, Group C).

Surgery: Mice were deeply anesthetized with ketamine/xylazine (90/10 mg/kg i.p.). As needed, anesthesia was kept at this baseline level using supplemental dosages of anesthetics (~5% of the original dose). The vertebral column in anesthetized normal animals was clamped with a custom-made clamping system (Rutgers University), and spinal cord segments T10 –T13 were exposed by laminectomy and the animals were fixed with clamping system (Rutgers University, Figure 2) at the base of MASCIS/NYU impact or. Depending on type of experiment, either saline or lidocaine (5%) was applied for 7 min before surgery over the dorsal aspect of the exposed spinal cord. The whole animal was situated in NYU impact or base [MASCIS/NYU impact or [24] Rutgers University]. Next, a moderate injury was inflected at spinal cord level T13 using a 5.6 g rod dropped from 6.25 mm distance. The temperature of the animal was maintained at 37°C with the heating lamp.

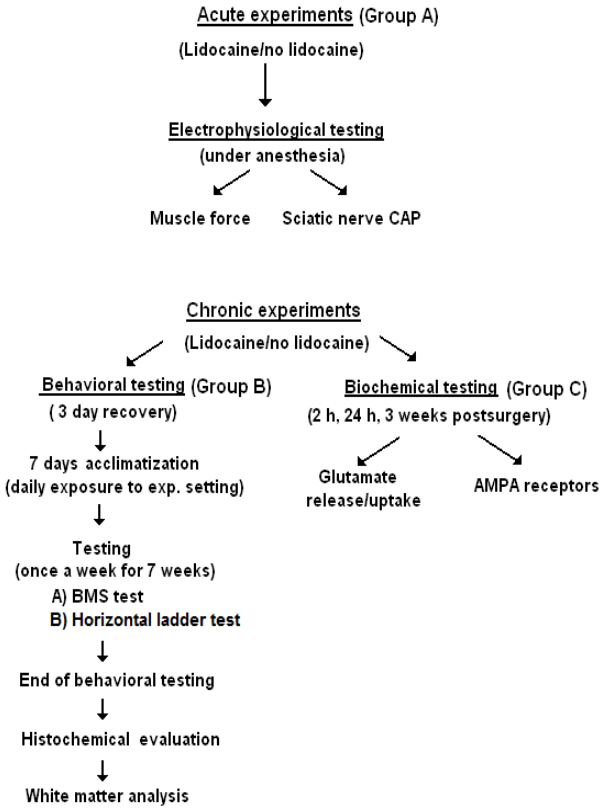

Figure 1: Experimental design. The experiments were conducted according to acute and chronic paradigms. In acute paradigm the neuronal activity and the force of muscle contraction were tested in anesthetized animals immediately after spinal cord injury (Group A). In experiments conducted according to chronic paradigm the locomotor activity of one group of the animals (Group B) was tested once week for seven weeks following spinal cord injury. The amount of white matter was evaluated in these animals after termination of testing. Another group of "chronic" animals (Group C) was used to evaluate properties of glutamatergic system (release/uptake and expression of AMPA receptors) 2 h, 24 h and 3 weeks post-injury. The experiments in all groups were conducted with or without application of lidocaine.

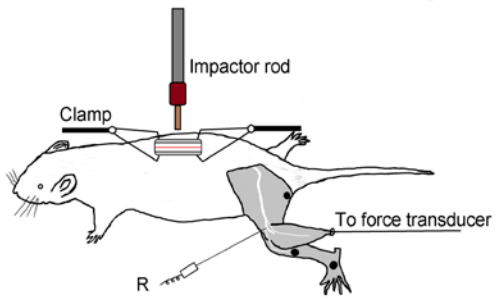

Figure 2: The experimental setup showing the animal prepared for spinal cord injury, for testing of the muscle contraction, and for recording of nerve activity. R, recording electrode.

The triceps surae (TS) muscle and sciatic nerve of hindlimb were separated from the surrounding tissue. The tendon of the muscle was attached to force transducer. Recording microelectrode was placed as shown in Figure 2.

Acute experiments: The recording of neuronal activity.

Extracellular recordings were made from the triceps surae muscle branch of the sciatic nerve with pure iridium microelectrodes (shaft diameter, 180 μm; tip, 1-2 μm; resistance, 5.0 MΩ; WPI, Sarasota, FL, USA). Tibial nerve potentials were recorded from the same location in all animals (approximately 3 mm from the TS muscle). The proper location was confirmed by penetration-elicited motor nerve spikes, which were correlated with muscle twitches [22,23].

Muscle force recording: The hindlimb and the proximal end of the tail bone were rigidly fixed to the base of the apparatus using dissecting pins. The knee was also fixed to the base to prevent any movements from being transmitted from the stimulated muscles to the body. The tendon of the triceps surae muscle (Figure 2) was attached to force displacement transducers (FT10, Grass Technologies), and the muscle length was adjusted to obtain the strongest twitch force (optimal length).

Data acquisition: The extracellularly-recorded potentials were passed through a standard head stage, amplified (Neuro Amp EX, ADInstruments, Inc., Colorado Springs, CO, USA), filtered (bandpass, 100 Hz to 5 KHz), digitized at 4 KHz, and stored in the computer for further processing. A PowerLab data acquisition system and LabChart 7 software (ADInstruments, Inc.) were used to acquire and analyze the data.

Chronic experiments: Mice were deeply anesthetized with ketamine/xylazine (90/10 mg/kg i.p.), and the spinal cord lesions were performed as described above for acute electrophysiological experiments. Before injury, saline or lidocaine (5%) was applied as described above. Following injury, the overlying muscle and skin were sutured, and the animals were allowed to recover at the temperature of 37°C maintained by heating pad. Mice were left to survive 2 h, 24 h (termed 2 h or 24 h animals), or more than three weeks (chronic animals). The animals were then anesthetized and the spinal cords were quickly dissected and used for biochemical experiments. Following recovery from anesthesia the 24 h and chronic animals were kept in separate cages with surgical padding for 3 days in Recovery Room at 23°C. These animals were given children Tylenol through a feeding tube to reduce pain, and hydrogel to facilitate feeding and keep them hydrated. Bladder was checked daily and manually emptied when required.

Behavioral testing

5.2.1. Testing Locomotor Activity (BMS): Experimenters who conducted behavioral testing were blinded to the status of the tested animals. Animals were acclimated to the testing environment by daily 30 min exposure to the testing procedure for a week before SCI. Animals were behaviorally tested on the first day after injury (Figure 9, Evaluation 1), and then once per week for seven weeks (Figure 9, Evaluations 2-7). Locomotor recovery of the hindlimbs was assessed by the motor rating according to BMS. The rating is as follows: 0, no ankle movement; 1-2, slight or extensive ankle movement; 3, planter placing or dorsal stepping; 4, occasional planter stepping; 5, frequent or consistent planter stepping; 6, inter-limb coordination; 7, parallel paw position; 8, trunk stability where the animal is able to keep the trunk region aligned and completely off the ground; 9, tail is up all the time. Each mouse was observed for 4 min in an open space, before a score was given.

Horizontal ladder walking test: While ground locomotion can be accomplished sufficiently by subcortical motor systems, skilled walking and inter-limbs coordination require voluntary control by the forebrain. Therefore, to evaluate the participation of the corticospinal system in the recovery we used the horizontal ladder walking test according to the modified procedure of Metz and Whishaw (2002). This test was shown to be sensitive to placing impairments of hindlimbs caused by lesions in the corticospinal system. We used a horizontal ladder (1 m long and 10 cm wide) with variable rungs spacing. Rung diameter was 3 mm, and the intervals between them were adjusted to 1-3 cm. A different template of irregular rung spacing was used in every testing session to prevent learning and to unmask impairments in limb use. To standardize the difficulty of the test, the same template was used across groups. Mice were required to cross the whole length of the ladder five times. The time of each run was recorded using a stopwatch. Runs were also video-recorded and analyzed in slow motion. The errors were defined as any miss or slip off the rung, and were counted for each hindlimb and calculated as a percent of the total number of steps. Mice were tested 3 times (once per day for three consecutive days) with the horizontal ladder one week before SCI. Results from these measurements were averaged and used as a baseline. All error data after SCI are presented as percent of total number of steps.

White matter measurement: The extent of the injury-induced lesion was verified by evaluation of the area occupied by spared white matter observable on the sections of the spinal cord. The animal was anesthetized and the segment of the spinal cord (1.5 cm) including the lesion epicenter was dissected out, and kept overnight in 4% paraformaldehyde containing 0.1 M PBS 4°C for 24 h. Next, the spinal column was freeze mounted, cut into 30 μm sections, and placed on poly-L-lysine-coated numbered glass slides to identify their locations in relation to the lesion epicenter. Four slides from each injured animal containing the lesion epicenter, and two slides containing tissue with no signs of damage obtained from the sites just above and below the lesion, were taken for Luxol fast blue (Sigma, St. Louis, MO) staining. The lesion epicenter was identified as the section containing the least amount of Luxol fast blue. Sections of the spinal cord dissected from the control animals at the T13 level were processed in an identical ways. The amount of spared white matter was measured using ImageJ software (www.imagej.nih.gov) and expressed as the percentage of total cross-sectional area of the spinal cord at the thoracic level in normal animals.

Analysis of Glutamatergic System

Release/uptake experiments

Changes in neuronal activity, recorded with electrophysiological methods, are probably interrelated with the modulation of synaptic transmission. Since glutamate is one of the major neurotransmitters in the spinal cord, it was reasonable to focus on changes in glutamatergic neurotransmission likely to occur during spinal cord injury. Investigators, who had performed biochemical experiments, were not informed about the origin of the tissue. We considered release and uptake of glutamate, correlated with the expression of ionotropic (AMPA) glutamatergic receptors, as critically important for the efficiency of glutamatergic transmission. Therefore we compared these three properties of glutamatergic neurotransmission in control, uninjured animals and in animals after SCI with and without application of lidocaine. This type of experiments would be very challenging to be performed *in vivo*, and we have selected two *in vitro* preparations, segments of the spinal cord and synaptosomes

to characterize glutamatergic system. To dissect the spinal cord the mice have been anesthetized and the segments of the spinal cord (approximately 2 cm long) were dissected and placed in custom-made perfusion chamber filled with constantly oxygenated Ringer's solution (in mM: NaCl 124, $NaHCO_3$ 25.9, glucose 10, KCl 3.1, KH_2PO_4 1.3, $MgSO_4$ 1.3, $CaCl_2$ 3.1). The perfusion chamber was connected to Brandel Suprafusion system and situated on the base of impactor used also to make lesions in anesthetized animals (see above). Following 3 hrs preincubation in oxygenated (95% O_2 /5% CO_2) Ringer's containing 3 μCi of radiolabeled glutamate analog, 2,3^{3}H-D-Aspartic acid (D-Asp, Radioactive Chemicals, USA), the perfusion of the spinal cord was initiated with simultaneous collection of 2 min fractions. Separate experiments were designed to test viability of the in vitro spinal cord preincubated in Brandel' chamber for 4 h. The stimulating and recording electrodes were placed on the ventral roots, and in the area containing cell bodies of alpha motor neurons, respectively. The electrical stimulation of the ventral roots evoked compound, antidromic potential generated by bodies of alpha motor neurons [25]. These results supported published reports [26-28], and confirmed viability of the spinal cord tissue used in our experimental setting. The time required for the molecules released from the spinal cord to reach collecting vials was approximately 3 min. At fraction number 8 the spinal cord was damaged with the rod released from the impactor according to the same procedure which was used for anesthetized animals. The amount of D-Asp was evaluated in all samples collected before and after injury in the scintillation counter. The increase in the release of D-Asp was expressed as the area under the peak of release divided by total area under the curve representing the release.

The concentration of extracellular glutamate, which is a result of the equilibrium between release and uptake processes [29] is critical for glutamate-evoked excitation. Therefore, we evaluated the efflux of D-Asp from perfused spinal cord segments in vitro as an indicator of changes in extracellular glutamate concentration, which we have termed "release". While the release experiments were carried out on the segments (T9-S4) of the spinal cord or synaptosomes, the characterization of the uptake system was performed on synaptosomes only. In a separate set of experiments the release and uptake studies were performed using synaptosomes obtained from spinal cord segments caudal to the injury (T12 to S4), excluding injured area.

Synaptosomes [30] were obtained according to Sawynok et al. [31] with the procedure modified and used by us before [32]. The tissue has been homogenized in 0.23 M sucrose (1:10, tissue/sucrose ratio) and centrifuged for 10 minutes at 5000 xg. The pellet has been discarded and the supernatant was centrifuged for 20 minutes at 19000 xg. The supernatant was discarded and the pellet (crude synaptosomal fraction) was suspended in Ringer's solution. It has been determined [32] that synaptosomes prepared in this way demonstrate specific Na^+-dependent accumulation, and partially Ca^{2+}-dependent release of D-Asp. For release experiments the synaptosomal fraction (200 μl corresponding to 50 mg of wet tissue) was transferred to the perfusion chamber of Brandel Suprafusion System. The suspension was preincubated for 1 hr with 3 μCi of D-Asp. Next, the perfusion of the synaptosomal suspension was initiated (1 ml/min) without fraction collection for the first 45 min followed by the collection of the samples every 2 min. The synaptosomes were stimulated electrically (10 mA, 10 Hz, 20 sec, duration of a single pulse = 200 μs) at sample # 10. The collection of the samples continued before, during and after electrical stimulation without any interruption. The magnitude of the release was evaluated comparing the background radioactivity before and after stimulation. The uptake experiments were performed on the synaptosomal fraction which was transferred to the filter placed in the manifold of the filtration system and incubated for 2 min with 3 μCi of D-Asp. The excess of D-Asp not accumulated by synaptosomes was removed by filtration under vacuum through GF/A filters. The filters were washed four times with three ml of cold Ringer's solution. The radioactivity, which remained on the filter represented mostly D-Asp taken up by synaptosomes, and was evaluated in the scintillation counter.

AMPA receptors analysis

AMPA receptors consist of four subunits (R1-R4) and all of them were recognized by polyclonal antibody used in our analysis. At various time points after SCI, the animals were anesthetized and the tissue of the spinal cord located 1 mm below injury site was isolated and taken for analysis. The tissue was lysed in 100 μl RIPA lysis buffer (PBS containing 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 0.5 mM Na_3VO_4 and freshly added protease inhibitor cocktail; Boehringer). Following sonication, the protein estimation was determined using the Bradford assay (Sigma, St. Louis, MI). The lysate (15 μg-25 μg protein) was resolved using a 10% SDS-polyacrylamide gel, protein bands transferred electrophoretically to a nitrocellulose membrane in a Tris-Glycine transfer buffer containing 10% methanol, then non-specific binding sites blocked in a blocking solution containing 5% nonfat dry milk in 0.1% Tween in 1X PBS (0.58M $Na_2HPO_4.4H_2O$, 0.17 M $NaH_2PO_4.H_2O$, 0.68M NaCl pH 7.4 for 10X PBS) (t-PBS). The membrane was probed with glutamate receptors rabbit polyclonal antibody (GluR (H-301), Santa Cruz USA) (1:1000), followed by the treatment with goat anti-rabbit IgG-HRP (1:5000). The immunoreactive bands were visualized using the Super signal Luminol kit (Pierce). Alpha InnotechFluroChem FC2 imaging system was used for image acquisition. The analysis and quantification of the bands were performed using Alpha View software. Blots were stripped and re-probed for β-actin. Data were normalized to β-actin and then normalized to values collected from control samples run on the same blot.

Statistical analysis

Statistical analysis was performed using One-way ANOVA, Repeated-measure ANOVA, Post hoc Holm-Sidak method, Pearson Product Moment Correlation. Paired t-test used to compare the data before and after lidocaine application. The specific statistical method is explained at each experiment.

Results

Quantification of neuronal activity following spinal cord injury

The recordings from the sciatic nerve before the injury showed the average spontaneous activity in the range of 8.5 ± 2.0 spikes/sec and the average spike amplitude of 25.7 ± .44 μV (Figure 3B and 4). Following injury this basic activity underwent dramatic changes which can be divided into several distinct phases indicated in Figure 3A1-4. The first phase, induced most likely by mechanical force, lasted less than a second, and was expressed as a few large spikes (1471.3 ± 318.6 μV) which appeared with the frequency of 12.8 ± 3.8 spikes/sec, average latency of 3.6 ± 0.4 ms, and duration of 55.0 ± 18.5 ms. Note the extended scale of the Figure 3A3 which depicts this phase in details.

The second phase was initiated by a rapidly progressing increase in the frequency of spontaneous activity to approximately 150 spikes/sec (Figure 3B) with simultaneous enhancement of the average amplitude to 435.5 ± 4.9 μV (Figure 3C) related to recruitment of additional units (Figure 3A2, lower panel). Both phases were accompanied by the muscle spasms. The variations in neuronal activity during these first 2 minutes (Figure 3B) were followed by a relatively longer (14.5 ± 4.6 min) silent phase when spontaneous activity (Figures 4A-4C) completely disappeared. In contrast the next phase was characterized by a dramatic enhancement of neuronal activity (33.8 ± 7.6 spikes/sec; $p<0.02$, Figure 4A and 4B, hyperactive period) and spike amplitude (505.4 ± 9.4 μV; $p<0.001$, Paired t-test, n=6, Figure 4C). Note that an increase in firing rate often occurred in bursts (Figure 4B), and was accompanied by muscle twitches and spasms. Figure 5A illustrates these events showing overlapping neuronal and muscle activities. Figure 5B depicts the shapes and amplitudes of the neuronal spikes recorded during a spasm that occurred during the hyperactive phase, as indicated by letters a-e in Figure 5A. Apparently, the injury modifies neuronal activity that changed from period of complete silence to hyperactivity.

Lidocaine and neuronal activity after spinal cord injury

The lidocaine applied topically on the surface of the spinal cord is able to penetrate meninges into the spinal cord [33], and to stop neuronal activity [34,35]. Figure 6 shows representative, injury-induced discharges recorded from injured animals (Injury) and from animals treated with lidocaine after injury (Injury+Lid). Lidocaine (5%) significantly reduced injury-induced neuronal activity (from 153.8 ± 3.9 to 27.9 ± 4.8 spikes/s; $p<0.05$, Paired t-test, n=6), eliminated impact-induced spasms, and markedly attenuated spontaneous activity for 45 minutes after the injury.

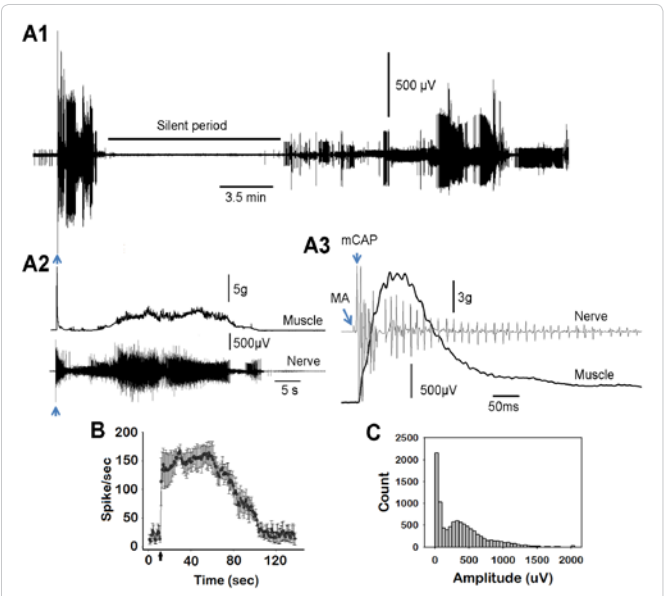

Figure 3: Injury-induced neuronal discharges and subsequent spasm of the muscle. A – examples of recordings observed in individual experiment; A1 - the activity of the sciatic nerve recorded during entire experiment; an arrow marks the time of injury. A2 - An example of injury-induced activity that is showing muscle spasms (upper panel) and nerve activity (lower panel). A3 - extended scale illustrating the mechanically-induced compound action potential (mCAP) and the corresponding muscle twitch. MA, mechanical artifact. Note the distinctly reduced activity following mCAP. B - Quantification of injury-induced neuronal activity (n=6). Arrow indicates the time of impact. C - a histogram of the amplitude of spikes recorded from the sciatic nerve during the injury-induced activity.

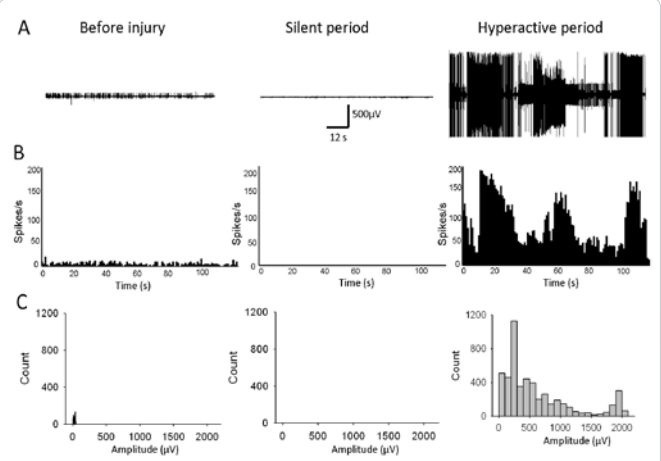

Figure 4: Spontaneous activity recorded from the sciatic nerve. The terms: Before injury, Silent period and hyperactive period indicate recordings collected during respective periods of nerve activity. A - Examples of spontaneous activities. B - a distribution of representative frequencies of spontaneous activity. C – A histogram depicting the amplitudes of spikes recorded during spontaneous nerve activity.

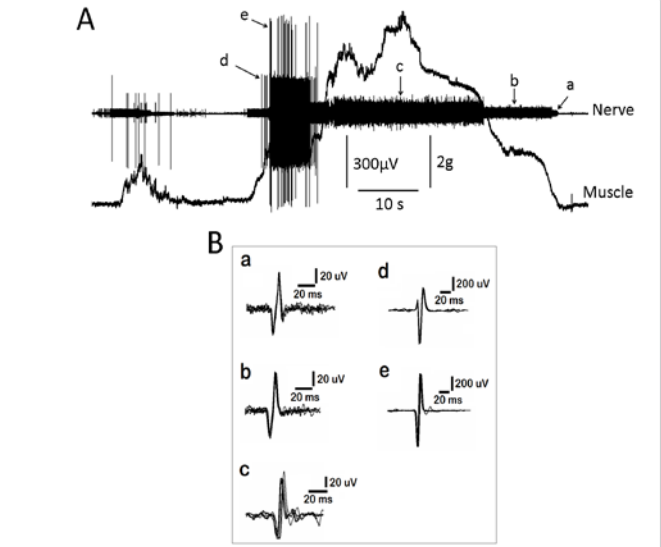

Figure 5: The analysis of individual motor units recruited during a spasm occurring in the hyperactive period. A – A representative recording of a spasm showing muscle contraction and nerve recordings. The letters indicate time of the recordings of individual motor units marked in B by letters corresponding to different units. B – The recording of motor units arranged according to the magnitude of the amplitudes from the lowest (a) to the highest (e). Note the difference in scale bars.

Impact-induced increase of extracellular glutamate analog, D-Asp

In an effort to correlate changes in neuronal activity with neurotransmitter turnover, we evaluated changes in the glutamatergic system following spinal cord injury. First, we determined D-Asp release from spinal cord during the impact. These experiments would be very challenging to be performed in vivo, and therefore they were conducted in vitro as described in the Methods. The

example of impact-induced release is shown in Figure 7. The average increase in the extracellular D-Asp concentration induced by the impact observed in four experiments was 16.7 ± 10.4%. The bell and asymmetrical shape of the release curve (with fast rising and slower falling phase) suggest that an impact-induced release was followed by activity-induced release. This may explain the progressive nature of injury-induced neuronal discharges.

It is well established [36-38] and supported by the above data that glutamate is intensively released after traumatic spinal cord injury. This intense release would most likely be accompanied by changes in the uptake of glutamate, and binding to its receptors.

D-Asp release was measured from synaptosomes that were prepared from the segments of the spinal cords located below injury from injury-only (n=18), and Injury + Lidocaine (n=18) groups of mice evaluated 2 h, 24 h, and three weeks after SCI. Synaptosomes were also prepared from laminectomy-only (Control/No Lid; Figure 8A-8C; n=6), and laminectomy + lidocaine (Control/Lid; Figure 8B and 8C; n=4) groups of animals evaluated at the same time intervals as injury-only group (2 h, 24 h, and Chronic). Injury-only group (No Lid) showed significantly higher ability to release D-Asp than control and injury + lidocaine groups (Lid, 2 h, 24 h, and Chronic; One-way ANOVA, F=10.4, p<0.001, Figure 8B). In injury-only group (No Lid), the ability of synaptosomes to release D-Asp was significantly higher only after 24h and in chronic stage (p<0.001, Holm-Sidak method), but not after 2 h (p>0.05, Holm-Sidak method) compared to control (Figure 8B). Synaptosomes from injury + lidocaine group (Lid) were significantly less able to release D-Asp than synaptosomes obtained from injury-only group (No Lid, p<0.001, Holm-Sidak method). There was no statistical difference between laminectomy-only and laminectomy + lidocaine groups (p=0.46, Holm-Sidak method). Note that these findings show the net concentration of extracellular D-Asp, which is the difference between its release and uptake.

We also tested the effect of SCI on D-Asp uptake at various time points after injury (Figure 8C). There was always a control tissue run alongside the samples. There were significant changes in uptake measured in synaptosomes from injury-only (No Lid, n=16) and injury + lidocaine (Lid, n=15) groups versus laminectomy-only groups (n=9, One-way ANOVA, F=34.3, p<0.001) (Figure 8C). The results of laminectomy + lidocaine (n=5) group were no different from laminectomy-only (p=0.8, Holm-Sidak method) group. The uptake was significantly higher in all groups of injured animals with no lidocaine (p<0.05, Holm-Sidak method) compared to control. In injury+ lidocaine group (Lid) the uptake was significantly less 2 h after injury (p<0.001), but higher in 24 h (p<0.001) and chronic groups (p<0.001) versus laminectomy-only group (Holm-Sidak method).

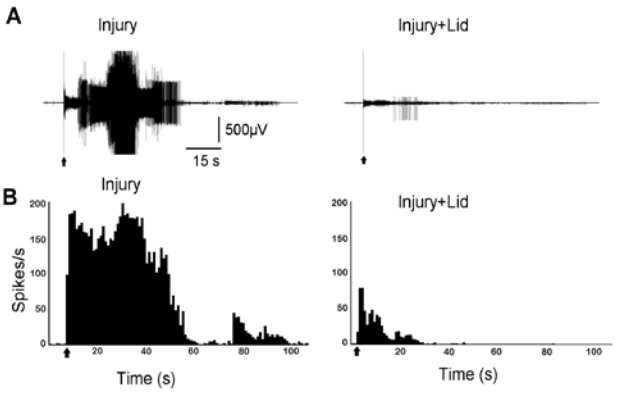

Figure 6: The reduction in injury-induced discharges by lidocaine. A – Sciatic nerve discharges following injury without (left, Injury only) and in the presence of lidocaine (Injury +Lid, right). B - Distribution of frequency of spikes recorded from sciatic nerve after spinal cord injury without (Injury only, left) and in the presence (Injury +Lid, right) of lidocaine. Arrows mark the time of the impact.

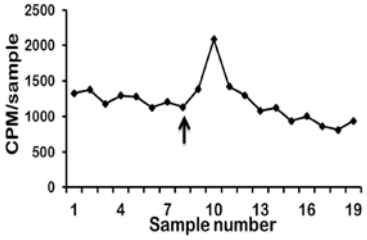

Figure 7: The representative experiment showing the impact-induced release of D-Asp from the segment of the spinal cord. The spinal cord was impacted at sample number 8.

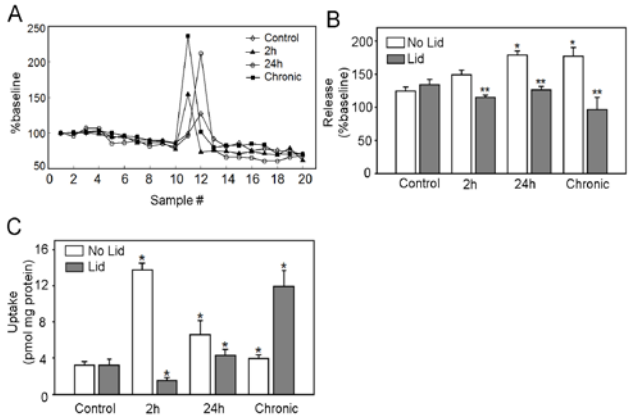

Figure 8: The influence of lidocaine on glutamate turnover in control and injured animals. A – stimulation-induced release of D-Asp from spinal cord synaptosomes prepared from control animals and injured animals evaluated at different times after injury without application of lidocaine. B – The averaged release of D-Asp from spinal cord synaptosomes prepared from control and injured animals treated, or not treated with lidocaine. C - The uptake of D-Asp by synaptosomes obtained from the spinal cord of the control and injured animals treated or not treated with lidocaine. *p<0.05, significantly different from injury-only; **p<0.05, significantly different from its respective, time-matched injury-only.

The Injury-induced modulation of AMPA receptors expression

The expression of AMPA receptors in spinal cord segments dissected from the area below the injury from animals with injury-only (2 h, 24 h, Chronic) and injury plus lidocaine groups (2 h+lid, 24 h+lid, Ch+lid) was tested using western blot. AMPA receptors expression in the injured groups was statistically different as compared with laminectomy-only group (control, Ct, n=6) (One-way ANOVA, F=21.7, p<0.001) (Figure 9). In injury-only groups (n=17), AMPA receptor expression was significantly less 2 h (n=5) and 24 h (n=5) after injury (p<0.05, Holm-Sidak method) compared to control. However, in mice with chronic injury (Ch, n=7), AMPA receptor expression was significantly higher versus laminectomy-only

group (control) (p<0.001, Holm-Sidak method). In mice treated with lidocaine (n=18), AMPA receptor expression was slightly higher 2 h (n=6) after injury but the difference was not statistically significant (p=0.3, Holm-Sidak method). AMPA receptor expression was significantly higher in 24 h (n = 5; p < 0.005) and in chronic injury + lidocaine groups (n=7; p<0.001, Holm-Sidak method) compared to control. Comparing injury only (2 h, 24 h, Ch) with injury + lidocaine (2 h+lid, 24 h+lid, Ch+lid) groups showed significantly higher AMPA receptors expression in injury + lidocaine versus injury only at 24 h after injury (p<0.001 Holm-Sidak method), and in chronic stage (p<0.001, Holm-Sidak method, Figure 9).

Lidocaine hinders ground and skilled locomotion

To determine whether there is a correlation between blocking of the injury-induced neuronal discharges following contusive spinal injury, and functional recovery, we tested motor performance in mice with injury+lidocaine (n=19), and mice with injury only (n=11) using BMS scale, and horizontal ladder test.

As shown in Figure 10A, mice treated with lidocaine before injury induction showed significantly worse recovery in ground locomotion skills than injury only group (repeated-measures ANOVA, F=19.6, p<0.001). Although the injury-only group showed better recovery one day following injury than lidocaine-treated group, the difference was not statistically significant (p=0.2, Holm-Sidak method). Injury-only group performed significantly better at evaluation 3 (one week following injury) (p=0.029, Holm-Sidak method) as well as in all subsequent evaluations (2-5 weeks after injury, evaluations 4-7 in Figure 10) than lidocaine-treated group (p<0.001, Holm-Sidak method). Injury-only group showed significant improvements in evaluations 3 to 7 (2-5 weeks after injury) compared to evaluation 2 (one week after the injury, repeated measure ANOVA, F=4.8, p<0.001). Lidocaine-treated group showed significant improvement in evaluations 4 to 7 (3-6 weeks after the injury) compared to evaluation 2 (first week after the injury; repeated measure ANOVA, F=6.5, p<0.001).

Skilled locomotion recovery was also significantly retarded in the lidocaine-treated group versus injury-only group (Figure 10B) (repeated measures ANOVA, F=12.6, p<0.001). The number of errors was significantly lower in the injury-only group versus lidocaine-treated group in all evaluations (the first - 7th week after the injury, evaluations 2 to 7, p<0.05, Holm-Sidak method). In injury-only group, evaluations 5, 6, and 7 (4-7 weeks after the injury) showed significant improvement in the rate of errors compared to rate of error in evaluation 2 (first week after the injury; p<0.001, Holm-Sidak method,). In the lidocaine-treated group, evaluations at 3 – 7 weeks after the injury (evaluations 4, 5, 6, and 7, Figure 10B) showed significant difference in the rate of errors compared to evaluation 2 (first week after the injury; p<0.001, Holm-Sidak method).

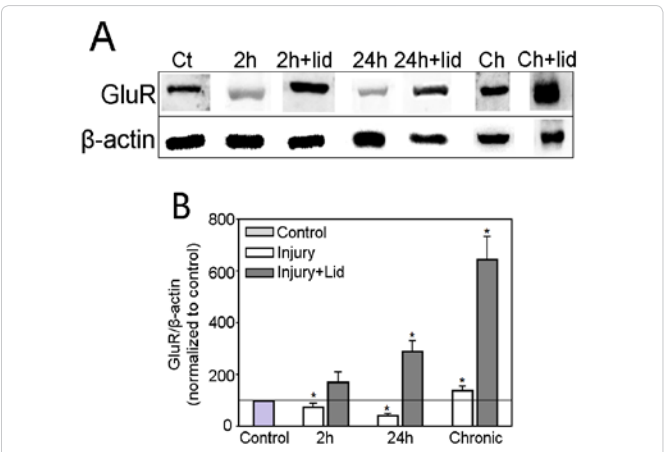

Figure 9: Glutamate receptor (GluR 1 to 4) expression was changed in injury-only and injury + lidocaine groups. A - Representative of western blot bands showing typical glutamate receptor changes with and without application of lidocaine evaluated at different times after injury. B - Averages of GluR expression evaluated with and without application of lidocaine determined at different times after the injury. All values are normalized to a control spinal tissue run alongside the samples. *p<0.05 significant from control.

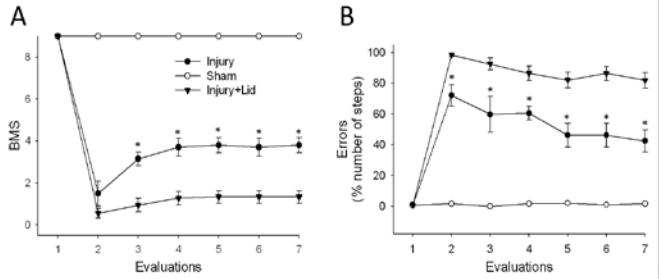

Figure 10: Blocking injury-induced discharges by lidocaine application retarded ground and skilled locomotor recovery after SCI. A – Ground locomotion evaluated with Basso moue scale (BMS). Injury-only mice (Injury) showed significant improvement in ground walking in the first week following injury compared to lidocaine-treated animals. B – Skilled locomotor activity assessed with horizontal ladder walking test. Injury-only animals show significant reduction in the numbers of errors compared to animals treated with lidocaine. Sham animals underwent laminectomy but no injury. *p<0.05 significant from injury plus lidocaine mice.

Sham-treated group (laminectomy plus lidocaine) showed no changes in neither ground nor skilled locomotion (p>0.05, Holm-Sidak method).

White matter analysis

Lesion verification revealed that the tissue obtained from both, the injury-only, and injury+lidocaine groups has significantly less white matter as compared to the tissue from control animals (One-way ANOVA, F=52.7, p<0.001). The amount of white matter in the tissue from control group (42.4 ± 2.2%) was significantly larger than in the tissue from either injury-only group (17.7 ± 1.4%), or injury+lidocaine group (17.1 ± 1.5%) (Holm-Sidak method, p<0.001). There was no difference in the amount of white matter observed in the injury-only compared to injury+lidocaine groups (p=0.9, Holm-Sidak method) (Figure 11). Further analysis shows that BMS scores are significantly and positively correlated with the volume of spared white matter in injury-only (Pearson Product Moment Correlation, r=0.8, p=0.007), but there was no significant correlation in injury+lidocaine group (Pearson Product Moment Correlation, r=0.36, p=0.3). This data excludes the possibility that the difference between injury-only and injury+lidocaine groups in motor recovery to be due to the difference in injury size.

Discussion

A synopsis of the results

The properties and effects of spinal cord injury-induced discharges recorded from the sciatic nerve have been characterized. The compound action potential appeared as an immediate effect of

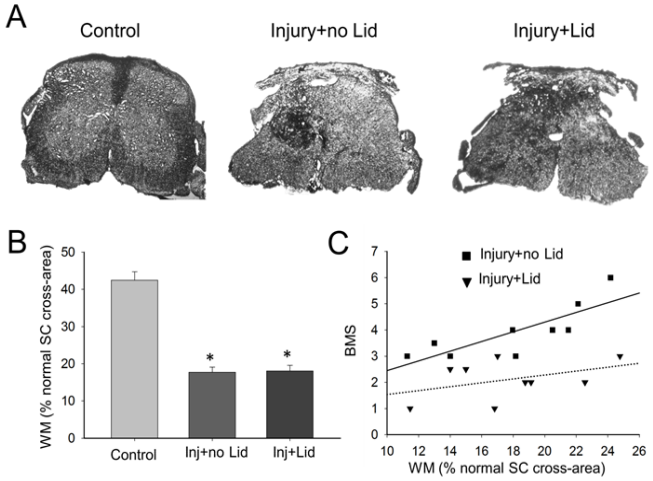

Figure 11: White matter analysis. A, Representatives of spinal cord transverse sections. B, Summary plot showing the volume of white matter as a percent of total cross-area of thoracic spinal cord in control. C, A scatter plot showing the relationship between the spared white matter volume and BMS score.

the impact and was followed by the period of short (approximately 2 min) phase of intense activity of motoneurons. In vitro experiments demonstrated a significant injury-induced increase in the efflux of glutamate analog from the segments of the spinal cord during these early, post-injury periods. The activity of motoneurons disappeared during the next silent stage lasting about 15 minutes. Finally, the hyperactive phase ensued for the remaining time (1 h) of the experiment time. Topical application of lidocaine on spinal cord dorsum reduced both injury-induced and spontaneous activity. This also attenuated ground and skilled locomotor recovery. Electrophysiological and behavioral assessments were supplemented by characterization of glutamatergic system in vitro using synaptosomes and segments of the spinal cord. The ability to release neurotransmitter was either not changed, or potentiated in synaptosomes from animals treated, or not treated with lidocaine, respectively. The efficiency of glutamate uptake system in synaptosomes obtained from lidocaine treated animals was reduced immediately after the injury, but it continued to recover exceeding considerably the control values in chronic stage. In contrast, the efficiency of the uptake system in animals not treated with lidocaine increased immediately after the injury, but declined later to slightly exceed control values in chronic stage. The expression of AMPA receptors in the tissue from the animals not treated with lidocaine was somewhat reduced at the first 24 h after injury and then slightly elevated in the chronic stage. However, the expression of AMPA receptors in animals treated with lidocaine started to increase immediately after injury and continue to rise reaching statistically significant higher level at 24 h and in the chronic animals.

The selection of anesthetic: The anesthetic used in our experiments, ketamine acts as NMDA receptor antagonist, and is also able to block voltage-gated Ca^{2+} channels [39,40]. Those actions of ketamine could influence our data. However, NMDA receptor does not play any role in the pathophysiology of traumatic spinal cord injury [41,42], and action of ketamine on calcium channels is exerted by much higher concentrations than those used in our experiments [43]. Ketamine also acts as agonist of opioid receptors [43-45] which worsen spinal cord injury [46,47]. Nevertheless, in the present study, lidocaine treated group was compared to injured control group, which was anesthetized in similar way. This excludes any influence of ketamine use on our results.

The role of mechanical impact during SCI

The spinal cord is suspended in cerebrospinal fluid and encased in dura, a tough fibrotic tissue. This anatomical arrangement would allow mechanically induced pressure wave generated by the impact (observed as mechanical artifact, Figure 3), to propagate quickly throughout the spinal cord and cerebrospinal fluid activating neurons situated in its path. The first observable effect of this wave was the large, mechanically-induced compound action potential (mCAP) recorded from sciatic nerve, followed by the muscle contraction (Figure 3). The sciatic nerve neurons are located 3-4 segments away from the impact site (T13-L1). The short latency of mCAP indicates that the pressure wave would activate these neurons by direct mechanical stimulation. Alternatively, short latency of mCAP could be also due to the activation of short or long-axon propriospinal projections that cross the impact site [48]. Currently, it is not known if travelling mechanical forces contribute to the initiation of other event like the rise of extracellular potassium concentration [49], and the massive release of glutamate observed during traumatic injury [37,50,51], which accompany SCI.

Possible mechanisms involved in SCI-induced changes of neuronal activity. here are several likely mechanisms modulating neuronal activity. It is well known that following axonal injury, current will flow into the cut area [52]. That will depolarize the membrane and generate ectopic action potential, which would subsequently trigger synaptic release of glutamate followed by increase in neuronal activity. There are only a few reports describing the physiological responses to mechanical compression of the spinal cord [53-60] and this important issue unquestionably deserves future investigation.

There are also additional probable mechanisms initiated by enhanced neuronal activity during phase two leading to subsequent cessation of neuronal activity observed in phase three. One of them is reduction in extracellular Na^+concentration which would compromise the ability of Na^+ inward current to depolarize the membrane. Consequently, the ability of neurons to generate action potentials would be distinctly reduced and expressed as silent period. One can also assume that repeated activity leads to reduction in intracellular ATP, activation of ATP-sensitive K^+ channels (KATP), increasing the K^+ leak current and subsequent compromising of the initiation and propagation of the action potential.

Injury-induced changes in glutamatergic system

The molecule used in the present investigation to characterize glutamatergic system was D-Asp. It is an endogenous molecule in mammalian central nervous system and is considered a putative neurotransmitter operating within glutamatergic synapses [53]. It has been documented in the past [54] and subsequently confirmed [55] that D-Asp is a reliable chemical, which can be used for characterization of synaptic glutamate turnover. Therefore, we will interpret all the results as functional indicators of the glutamatergic system.

Stimulation-induced glutamate release became elevated already 2h after the injury. That enhancement was persistent reaching statistically significant level at 24 h following injury as well as in chronic phase. While this increase of glutamate release is relatively modest, it

appears very quickly after the lesion and is practically permanent. The enhancement in glutamate release was accompanied by significant increase in the uptake. It can be considered as compensatory mechanisms initiated by neurons and/or glia to counteract elevated glutamate release. However, the fact that in our experiments we could observe enhanced release in spite of parallel elevation in the uptake indicates that this new high activity of glutamate transporters was unable to cope with new challenging concentration of glutamate. Our results contest the report by Olsen and collaborators [56] who observed a massive and persistent loss of glutamate transporter 1 after spinal cord injury. However, glutamate transporter 1 is limited to astrocytes, and therefore the modifications in its activity do not provide a complete picture of changes in the total capacity of neurons and glia to accumulate glutamate. The crude synaptosomal fraction, tested in our experiments was likely to contain gliosomas, vesicles made of fragments of glia cells containing glutamate transporters [29]. Therefore, the amount of glutamate detected by us was indeed a net result of equilibrium between the efficiency of release and all uptake mechanisms, reflecting a real, elevated concentration of extracellular glutamate. Moreover, in the opposite to our experimental paradigm, Olsen and collaborators [56] included the mechanically damaged, nonfunctional tissue from site of the lesion into analyzed samples. This procedure could result in underestimation of the expression of glutamate transporters [60-64].

The significance of alterations in expression of AMPA receptors. In the current study, both, neurotransmitter release and uptake were elevated immediately following SCI and this enhancement persisted into the chronic stage. The AMPA receptor expression was reduced in first 24 h and increased in the chronic stage. The reduction in AMPA receptor in the first 24 h after injury is probably a desensitization response due to the increase in extracellular glutamate [57]. We hypothesize that injury-induced discharge might initiate some of the long-term potentiation-like mechanisms [65-70] including enhancement of presynaptic neurotransmitter release [19,58-60], glutamate uptake [61,62], and increase in postsynaptic AMPA receptors [63-66].

The consequence of lidocaine-mediated attenuation of neuronal activity. Activation of glutamatergic mechanisms could increase the spinal cord neurons excitability setting the fate of subsequent functional recovery at the onset of injury. Indeed, the animals treated with lidocaine, which blocked sodium channels attenuating injury discharge demonstrated impaired recovery. Although there are reports of beneficial effects of lidocaine infused after ischemic injury of the spinal cord [67,68], they are not supported by other data showing lack of facilitatory effects of lidocaine on the return of spinal cord function following contusive [69], or ischemic injury [70]. Attenuation of neuronal activity by phenytoin, another blocker of sodium channels was found to be effective in improving functional recovery after contusive spinal cord injury [71], although contradictory data have been also reported [72]. Although an increase in intracellular sodium concentration following injury was detrimental for recovery causing axonal degeneration [71,73], Borgens and collaborators [74-76] demonstrated beneficial effect of enhanced concentration of intracellular sodium after spinal cord injury. In support of this claim, early increase in intracellular sodium ions transported by voltage-gated sodium channels was required for initiating regeneration following Xenopus laevis tail amputation [2].

We would also challenge the view that the beneficial effect of these activity blockers is exerted by attenuation of neuro-excitotoxicity [77]. Instead, we propose that their action would deprive the tissue its activity, which leads afterwards to enhancement of neuronal excitability [78].

Conclusion

The current findings demonstrate that blocking activity during and within the first hour after contusive spinal cord injury, is detrimental to functional recovery. They also imply that external stimulation or even controlled application of exogenous glutamate in clinical setting could mimic or enhance the effects of injury-induced activity promoting better recovery. We postulate that injury-induced activity has beneficial effects on functional return through two main mechanisms: 1) strengthening spared connections, and/or activation of silent synapses; 2) preservation and enhancement of injury-induced sodium currents that are very important for axonal outgrowth. The postulated contribution of the injury-induced activity has obvious and very significant clinical implications in designing therapeutic treatment of acute spinal cord injury.

Acknowledgment

Support for this research was provided by grants from the New York State Department of Health Grant # CO23684 and PSC CUNY grants #$ 62610-0041 to Z. Ahmed and # 63644-0041 to A. Wieraszko.

References

1. Seltzer Z, Cohn S, Ginzburg R, Beilin B (1991) Modulation of neuropathic pain behavior in rats by spinal disinhibition and NMDA receptor blockade of injury discharge. Pain 45: 69-75.
2. Tseng AS, Beane WS, Lemire JM, Masi A, Levin M (2010) Induction of vertebrate regeneration by a transient sodium current. J Neurosci 30: 13192-13200.
3. Ahmed Z, Wagdy M, Benjamin M, Mohamed S, Mohamed H, et al. (2011) Therapeutic effects of acrobatic exercise and magnetic field exposure on functional recovery after spinal cord injury in mice. Bioelectromagnetics 32: 49-57.
4. Ahmed Z, Wieraszko A (2008) Combined effects of acrobatic exercise and magnetic stimulation on the functional recovery after spinal cord lesions. J Neurotrauma 25: 1257-1269.
5. Debanne D, Poo MM (2010) Spike-timing dependent plasticity beyond synapse - pre- and post-synaptic plasticity of intrinsic neuronal excitability. Front Synaptic Neurosci 2: 21.
6. Belmeguenai A, Hosy E, Bengtsson F, Pedroarena CM, Piochon C, et al. (2010) Intrinsic plasticity complements long-term potentiation in parallel fiber input gain control in cerebellar Purkinje cells. J Neurosci 30: 13630-13643.
7. Hanse E (2008) Associating synaptic and intrinsic plasticity. J Physiol 586: 691-692.
8. Triesch J (2007) Synergies between intrinsic and synaptic plasticity mechanisms. Neural Comput 19: 885-909.
9. Desai NS, Rutherford LC, Turrigiano GG (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. Nat Neurosci 2: 515-520.
10. Ahmed Z, Wieraszko A (2009) Activity-dependent axonal plasticity: The effects of electrical stimulation on compound action potentials recorded from the mouse nervous system in vitro. Open Neurosci J 3: 1-12.
11. Kennard JT, Guévremont D, Mason-Parker SE, Abraham WC, Williams JM (2009) Increased expression, but not postsynaptic localisation, of ionotropic glutamate receptors during the late-phase of long-term potentiation in the dentate gyrus in vivo. Neuropharmacology 56: 66-72.
12. Richter-Levin G, Canevari L, Bliss TV (1995) Long-term potentiation and glutamate release in the dentate gyrus: links to spatial learning. Behav Brain Res 66: 37-40.
13. Clarkson AN, Overman JJ, Zhong S, Mueller R, Lynch G, et al. (2011) AMPA

receptor-induced local brain-derived neurotrophic factor signaling mediates motor recovery after stroke. J Neurosci 31: 3766-3775.

14. Chi SI, Levine JD, Basbaum AI (1993) Effects of injury discharge on the persistent expression of spinal cord fos-like immunoreactivity produced by sciatic nerve transection in the rat. Brain Res 617: 220-224.

15. Boroujerdi A, Kim HK, Lyu YS, Kim DS, Figueroa KW, et al. (2008) Injury discharges regulate calcium channel alpha-2-delta-1 subunit upregulation in the dorsal horn that contributes to initiation of neuropathic pain. Pain 139: 358-366.

16. Cohn S, Seltzer Z (1991) Inherited propensity for neuropathic pain is mediated by sensitivity to injury discharge. Neuroreport 2: 647-650.

17. Seltzer Z, Beilin BZ, Ginzburg R, Paran Y, Shimko T (1991) The role of injury discharge in the induction of neuropathic pain behavior in rats. Pain 46: 327-336.

18. Field Fote EC (2009) Spinal cord injury: An overview, in Spinal cord injury rehabilitation. (1stedn) F. A. Davis Company, UK.

19. Ghijsen WE, Besselsen E, Geukers V, Kamphuis W, Lopes da Silva FH (1992) Enhancement of endogenous release of glutamate and gamma-aminobutyric acid from hippocampus CA1 slices after in vivo long-term potentiation. J Neurochem 59: 482-486.

20. Bliss TV, Errington ML, Laroche S, Lynch MA (1987) Increase in K+-stimulated, Ca2+-dependent release of [3H]glutamate from rat dentate gyrus three days after induction of long-term potentiation. Neurosci Lett 83: 107-112.

21. Dolphin AC, Errington ML, Bliss TV (1982) Long-term potentiation of the perforant path in vivo is associated with increased glutamate release. Nature 297: 496-498.

22. Ahmed Z, Freedland R, Wieraszko A (2010) Excitability changes in the sciatic nerve and triceps surae muscle after spinal cord injury in mice. J Brachial Plex Peripher Nerve Inj 5: 8.

23. Ahmed Z (2011) Trans-spinal direct current stimulation modulates motor cortex-induced muscle contraction in mice. J Appl Physiol 110: 1414-1424.

24. Gruner JA (1992) A monitored contusion model of spinal cord injury in the rat. J Neurotrauma 9: 123-126.

25. Ahmed Z, Wieraszko A (2012) Trans-spinal direct current enhances corticospinal output and stimulation-evoked release of glutamate analog, D-2,3-^{3}H-aspartic acid. J Appl Physiol 112: 1576-1592.

26. Jiang M, Schuster JE, Fu R, Siddique T, Heckman CJ (2009) Progressive changes in synaptic inputs to motoneurons in adult sacral spinal cord of a mouse model of amyotrophic lateral sclerosis. J Neurosci 29: 15031-15038.

27. Moghaddasi M, Velumian AA, Zhang L, Fehlings MG (2007) An ex vivo preparation of mature mice spinal cord to study synaptic transmission on motoneurons. J Neurosci Methods 159: 1-7.

28. Jiang MC, Heckman CJ (2006) In vitro sacral cord preparation and motoneuron recording from adult mice. J Neurosci Methods 156: 31-36.

29. Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65: 1-105.

30. Jones DG (1975) Synapses and Synaptosomes: Morphological aspects: Chapman and Hall, London.

31. Sawynok J, Downie JW, Reid AR, Cahill CM, White TD (1993) ATP release from dorsal spinal cord synaptosomes: characterization and neuronal origin. Brain Res 610: 32-38.

32. Wieraszko A, Armani J, Maqsood N, Raja H, Philip S (2005) Modification of the synaptic glutamate turnover in the hippocampal tissue exposed to low-frequency, pulsed magnetic fields. Brain Res 1052: 232-235.

33. BROMAGE PR, JOYAL AC, BINNEY JC (1963) Local anesthetic drugs: penetration from the spinal extradural space into the neuraxis. Science 140: 392-394.

34. Foffani G, Humanes-Valera D, Calderon-Muñoz F, Oliviero A, Aguilar J (2011) Spinal cord injury immediately decreases anesthetic requirements in rats. Spinal Cord 49: 822-826.

35. Kuno M, Matsuura S (1982) Sites and mechanisms of action of lidocaine upon the isolated spinal cord of the frog. Brain Res 249: 87-93.

36. McAdoo DJ, Hughes MG, Nie L, Shah B, Clifton C, et al. (2005) The effect of glutamate receptor blockers on glutamate release following spinal cord injury. Lack of evidence for an ongoing feedback cascade of damage --> glutamate release --> damage --> glutamate release --> etc. Brain Res 1038: 92-99.

37. Xu GY, Hughes MG, Ye Z, Hulsebosch CE, McAdoo DJ (2004) Concentrations of glutamate released following spinal cord injury kill oligodendrocytes in the spinal cord. Exp Neurol 187: 329-336.

38. Park E, Velumian AA, Fehlings MG (2004) The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma 21: 754-774.

39. Connelly TJ, Ahern C, Coronado R (1995) Ketamine, at clinical concentrations, does not alter the function of cardiac sarcoplasmic reticulum calcium release channels. Anesth Analg 81: 849-854.

40. Kaye AD, Banister RE, Anwar M, Feng CJ, Kadowitz PJ, et al. (1998) Pulmonary vasodilation by ketamine is mediated in part by L-type calcium channels. Anesth Analg 87: 956-962.

41. Agrawal SK, Theriault E, Fehlings MG (1998) Role of group I metabotropic glutamate receptors in traumatic spinal cord white matter injury. J Neurotrauma 15: 929-941.

42. Lips J, de Haan P, Bodewits P, Vanicky I, Dzoljic M, et al. (2000) Neuroprotective effects of riluzole and ketamine during transient spinal cord ischemia in the rabbit. Anesthesiology 93: 1303-1311.

43. Gupta A, Devi LA, Gomes I (2011) Potentiation of μ-opioid receptor-mediated signaling by ketamine. J Neurochem 119: 294-302.

44. Sarton E, Teppema LJ, Olievier C, Nieuwenhuijs D, Matthes HW, et al. (2001) The involvement of the mu-opioid receptor in ketamine-induced respiratory depression and antinociception. Anesth Analg 93: 1495-1500, table of contents.

45. Nemeth CL, Paine TA, Rittiner JE, Béguin C, Carroll FI, et al. (2010) Role of kappa-opioid receptors in the effects of salvinorin A and ketamine on attention in rats. Psychopharmacology (Berl) 210: 263-274.

46. Winkler T, Sharma HS, Stålberg E, Olsson Y, Nyberg F (1994) Opioid receptors influence spinal cord electrical activity and edema formation following spinal cord injury: experimental observations using naloxone in the rat. Neurosci Res 21: 91-101.

47. Bakshi R, Ni RX, Faden AI (1992) N-methyl-D-aspartate (NMDA) and opioid receptors mediate dynorphin-induced spinal cord injury: behavioral and histological studies. Brain Res 580: 255-264.

48. Conta AC, Stelzner DJ (2009) The Spinal cord. (1stedn), Acadmic Press, USA.

49. Chesler M, Sakatani K, Hassan AZ (1991) Elevation and clearance of extracellular K+ following contusion of the rat spinal cord. Brain Res 556: 71-77.

50. Katayama Y, Becker DP, Tamura T, Hovda DA (1990) Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. J Neurosurg 73: 889-900.

51. Katayama Y, Kawamata T, Tamura T, Hovda DA, Becker DP, et al. (1991) Calcium-dependent glutamate release concomitant with massive potassium flux during cerebral ischemia in vivo. Brain Res 558: 136-140.

52. Borgens RB (1988) Voltage gradients and ionic currents in injured and regenerating axons. Adv Neurol 47: 51-66.

53. Hamase K, Homma H, Takigawa Y, Fukushima T, Santa T, et al. (1997) Regional distribution and postnatal changes of D-amino acids in rat brain. Biochim Biophys Acta 1334: 214-222.

54. Savage DD, Galindo R, Queen SA, Paxton LL, Allan AM (2001) Characterization of electrically evoked [3H]-D-aspartate release from hippocampal slices. Neurochem Int 38: 255-267.

55. Fleck MW, Barrionuevo G, Palmer AM (2001) Synaptosomal and vesicular accumulation of L-glutamate, L-aspartate and D-aspartate. Neurochem Int 39: 217-225.

56. Olsen ML, Campbell SC, McFerrin MB, Floyd CL, Sontheimer H (2010) Spinal cord injury causes a wide-spread, persistent loss of Kir4.1 and glutamate transporter 1: benefit of 17 beta-oestradiol treatment. Brain 133: 1013-1025.

57. Papke D, Gonzalez-Gutierrez G, Grosman C (2011) Desensitization of

neurotransmitter-gated ion channels during high-frequency stimulation: a comparative study of Cys-loop, AMPA and purinergic receptors. J Physiol 589: 1571-1585.

58. Sola E, Prestori F, Rossi P, Taglietti V, D'Angelo E (2004) Increased neurotransmitter release during long-term potentiation at mossy fibre-granule cell synapses in rat cerebellum. J Physiol 557: 843-861.

59. Koyano K, Kuba K, Minota S (1985) Long-term potentiation of transmitter release induced by repetitive presynaptic activities in bull-frog sympathetic ganglia. J Physiol 359: 219-233.

60. Bliss TV, Douglas RM, Errington ML, Lynch MA (1986) Correlation between long-term potentiation and release of endogenous amino acids from dentate gyrus of anaesthetized rats. J Physiol 377: 391-408.

61. Tsvetkov E, Shin RM, Bolshakov VY (2004) Glutamate uptake determines pathway specificity of long-term potentiation in the neural circuitry of fear conditioning. Neuron 41: 139-151.

62. Levenson J, Weeber E, Selcher JC, Kategaya LS, Sweatt JD, et al. (2002) Long-term potentiation and contextual fear conditioning increase neuronal glutamate uptake. Nat Neurosci 5: 155-161.

63. Correia SS, Bassani S, Brown TC, Lisé MF, Backos DS, et al. (2008) Motor protein-dependent transport of AMPA receptors into spines during long-term potentiation. Nat Neurosci 11: 457-466.

64. Williams JM, Guévremont D, Mason-Parker SE, Luxmanan C, Tate WP, et al. (2007) Differential trafficking of AMPA and NMDA receptors during long-term potentiation in awake adult animals. J Neurosci 27: 14171-14178.

65. Malinow R (2003) AMPA receptor trafficking and long-term potentiation. Philos Trans R Soc Lond B Biol Sci 358: 707-714.

66. Yang Y, Wang XB, Frerking M, Zhou Q (2008) Delivery of AMPA receptors to perisynaptic sites precedes the full expression of long-term potentiation. Proc Natl Acad Sci U S A 105: 11388-11393.

67. Apaydin AZ, Büket S (2001) Regional lidocaine infusion reduces postischemic spinal cord injury in rabbits. Tex Heart Inst J 28: 172-176.

68. Kobrine AI, Evans DE, LeGrys DC, Yaffe LJ, Bradley ME (1984) Effect of intravenous lidocaine on experimental spinal cord injury. J Neurosurg 60: 595-601.

69. Haghighi SS, Chehrazi BB, Higgins RS, Remington WJ, Wagner FC (1987) Effect of lidocaine treatment on acute spinal cord injury. Neurosurgery 20: 536-541.

70. Robertson CS, Foltz R, Grossman RG, Goodman JC (1986) Protection against experimental ischemic spinal cord injury. J Neurosurg 64: 633-642.

71. Hains BC, Saab CY, Lo AC, Waxman SG (2004) Sodium channel blockade with phenytoin protects spinal cord axons, enhances axonal conduction, and improves functional motor recovery after contusion SCI. Exp Neurol 188: 365-377.

72. Schwartz G, Fehlings MG (2001) Evaluation of the neuroprotective effects of sodium channel blockers after spinal cord injury: improved behavioral and neuroanatomical recovery with riluzole. J Neurosurg 94: 245-256.

73. Waxman SG (2006) Axonal conduction and injury in multiple sclerosis: the role of sodium channels. Nat Rev Neurosci 7: 932-941.

74. Borgens RB, Blight AR, Murphy DJ, Stewart L (1986) Transected dorsal column axons within the guinea pig spinal cord regenerate in the presence of an applied electric field. J Comp Neurol 250: 168-180.

75. Borgens RB, Roederer E, Cohen MJ (1981) Enhanced spinal cord regeneration in lamprey by applied electric fields. Science 213: 611-617.

76. Borgens RB, Jaffe LF, Cohen MJ (1980) Large and persistent electrical currents enter the transected lamprey spinal cord. Proc Natl Acad Sci U S A 77: 1209-1213.

77. Obrenovitch TP, Urenjak J (1997) Is high extracellular glutamate the key to excitotoxicity in traumatic brain injury? J Neurotrauma 14: 677-698.

78. Trasande CA, Ramirez JM (2007) Activity deprivation leads to seizures in hippocampal slice cultures: is epilepsy the consequence of homeostatic plasticity? J Clin Neurophysiol 24: 154-164.

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