Longitudinal electrophysiological changes after cervical hemicontusion spinal cord injury in rats

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Highlight

1. Cervical hemicontusion SCI leads to ipsilateral forelimb impairment and spinal cord lesion.
2. SEP and MEP over-reaction after hemicontusion SCI suggests reliable electrophysiology beyond acute injury.
3. Longitudinal electrophysiology changes reflect the neurological impairment after hemicontusion SCI.

Abstract

Objective: To evaluate the longitudinal SEPs & MEPs characterization from acute to chronic injury following cervical hemicontusion spinal cord injury (SCI) in rats, and correlate the SEPs & MEPs to the behavioral outcomes.

Methods: Cervical hemicontusion spinal cord injury was performed on fifty adult male Sprague-Dawley rats. The electrophysiological assessment, including forelimb SEPs & MEPs was applied to 5 animals before injury and 3h, 1d, 3d, 1w, 2w, 4w and 8w after injury. Forelimb functional assessment of Montoya staircase task and cylinder rearing test were performed before injury and 2w, 4w and 8w after injury on other 10 animals, as well as histological analysis of the cord harvested at 8w after injury. A group correlation was performed between the SEPs & MEPs and behavioral outcomes.

Results: The hemicontusion injury was applied to all animals and resulted in tissue damage at the epicernter with loss of the ventral horns of gray matter and lateral funiculus of white matter. Both ipsilateral and contralateral forelimb MEPs showed latency prolongation and amplitude reduction at 3h after injury. The MEPs amplitude increased with time after injury, with the ipsilateral persistently lower compared to the contralateral. The ipsilateral MEPs latency increased over all the time after injury, with significant difference between sides. The SEPs amplitude dropped after injury and stayed at lower level until 8 weeks, with significant difference between sides. There was no difference in the SEPs latency among time points and between sides. The ipsilateral forelimb grasped and ate pellets 60% less after injury while the constralateral forelimb slightly less, with significant difference between sides. An obvious less usage of the ipsilateral paw in rearing test after injury while more usage of the contralateral paw, with significant difference between sides. The MEPs latency and amplitude correlated significantly to the forelimb motor function, while the SEPs did not to the forelimb motor function.

Conclusion: Cervical hemicontusion SCI led to persistent impairment of the ipsilateral forelimb in SEPs & MEPs and function, as well as tissue damage of the ipsilateral spinal cord. The "spinal shock" occuring at acute cervical hemicontusion spinal cord injury resulted in obvious SEPs & MEPs changes of both ipsialteral and contralateral forelimbs, suggesting reliable electrophysiology assessment beyond the acute injury. The behavioral assessment was correlated to the MEP, supporting the longitudinally electrophysiology assessment for neurological impairment after SCI.

Keywords: hemicontusion, spinal cord injury, Somatosensory evoked potentials, motor evoked potentials, behavioral assessments

1. Introduction

Cervical spinal cord contusion injuries are the most common type of traumatic spinal injuries causing severe deficits in arm and digit sensorimotor functions[1]. Recently, some rodent models replicate the pathophysiological and functional sequela of this injury, particularly using a hemicontusion SCI at lower cervical spine. The unilateral spinal cord injury models in rodent are ideal for evaluate particular ascending or descending pathways[2-5]. These studies developed and characterized cervical hemicontusion SCI models which were capable of producing consistent injuries of both proximal and distal forelimb functions while retaining substantial spared tissue capable of supporting new circuit formation for upper limb functional recovery[1]. This model would provide an useful tool for pre-clinical investigation of various new therapies of SCI. During the experiment studies, establishment of efficient, reliable and precise assessments of neurological function is an essential element. Behaviors observation and histology evaluation are commonly used methods for assessing injury severity and functional recovery. In addition, somatosensory evoked potentials (SEPs) and motor evoked potentials (MEPs) have been widely used to evaluate the neurological function in the spinal cord injury (SCI) models. However, many questions raised regarding the SEPs & MEPs evolution after SCI, such as effects of "spinal shock" on the SEPs & MEPs in acute spinal cord injury. In the cervical hemicontusion SCI rat model, longitudinal SEPs & MEPs changes in cervical spinal cord injury and its association with the behavior and histology, particularly during the acute and secondary chronic SCI, remain unknown.

To establish an experimental platform of cervical hemicontusion SCI, this study was designed to identify longitudinal change of SEPs & MEPs after SCI, and correlate the SEPs & MEPs with behavioral assays and histological outcome.

2. Materials and methods

2.1 Surgical procedure

All animal procedures were approval by the Committee on the Ethics of Animal Experiments of Southern Medical University and were conducted in accordance with the guidelines of caring for laboratory animals of the Ministry of Science and Technology of the People's Republic of China. This study was performed on adult male Sprague-Dawley rats (300~350g; provided by Laboratory Animals center of Southern Medical University). A total of 15 rats received a surgical procedure which was described previously[10]. A unilateral laminectomy was performed to the C5 vertebra, a designed clamp mount onto lateral transverse processes of C4 to C6, after the whole fixed device was settled, stereotaxic frame with the rat fixed in a frame tilted at a 22.5°angle under an ElectroPuls E1000 Mechanical Machine System (Instron, Canton, MA). The impactor tip was pointed to 1.4 mm to the left of midline, then lowered to make direct contact with the dural surface until preload force reach 0.01N. The impactor was then triggered to deliver a set displacement of 2.2 mm at 600mm/s. All impact biomechanical variables recorded were available immediately after the impact. After injury, 5 rats were randomly chosen to receive electrophysiological assessment, and other 10 rats receive behavioral assessment.

2.2 Electrophysiological assessment

In each recording session, the animals were anesthetized by inhalation with low dose of isoflurane. Core temperature was maintained at 37.5 °C with a homeothermic blanket. The scalp was shaved and cleansed with 70% ethyl alcohol. Somatosensory and motor evoked potential signals were collected from the rats in pre-surgery, 3 hours, 1 day, 3 days, 1 week, 2 weeks 4 weeks and 8 weeks post-SCI using Nicolet intraoperative neurophysiological monitoring (Nicolet, Madison, WI, USA). All the signal processing programmes were developed in the Nicolet Endeavor CR.

2.2.1 MEPs

Recording needle electrodes were inserted intramuscularly into the extensor muscle of the forelimbs. Two monopolar needle electrodes were inserted subdermally over the skull, the tips of the electrode respectively acting as the cathode anode were 1cm in front of C3 and C4. Single trial MEP were obtained with a current intensity of up to 16 mA and a pulse width of 50 μm at a frequency of 350 Hz for 1 minute duration. Peak-to-peak amplitudes of MEPs from 3 stimulations were recorded to assess intra-animal variability and confirm reproducibility

2.2.2 SEPs

A constant current stimulator was used to generate a 5.1-Hz square wave of 0.1 milliseconds to stimulate the median nerves in the forelimbs. The stimulation intensity was selected to cause a mild twitch of the forelimb. The SEP was recorded from the skull at sites corresponded to the right and left sensorinotor cortical hemisphere for forelimb. The signal was amplified 100,000 times with two amplifiers. To obtain a good signal-to-noise ratio for the SEP signals, a total of 300 SEP responses were averaged for each trial.

2.3 Behavioral assessment

The animals were tested on week 2, 4 and 8 post-operatively. Forelimb usage during rearing was done as described previously[11, 12]. Briefly, the rats were placed in a clear plexi-glass cylinder for 15 min. A mirror was placed at an angle behind the cylinder so that the forelimbs could be viewed at all times. The testing session was videotaped, and forelimb usage was scored blindly at a later date. Frame-by-frame analysis of the forelimb usage during 20 independent rears was performed. The animal would receive a score of one “contralateral” and one “ipsilateral” for that sequence.

The staircase reaching task was used to assess skilled forelimb reaching. Rats were trained for the staircase reaching task using the different colors pellet as described previously[13, 14]. The food pellets (45 mg, catalog F0299; Bioserve, Flemington, New Jersey, USA) were colored with a gel-based food paste from AmeriColor (12-pack kit and bright white). Each of the 6 steps of both stairs was filled with 4 color coded food pellets. Rats were food deprived the night before behavioral sessions. Animals were placed in the Montoya staircase apparatus for a period of 15 minutes, and the number of pellets eaten, misplaced, and displaced from each step was counted.

2.4 Histology

Animals survived for 8 weeks after injury. Rats were deeply anesthetized with sodium pentobarbital (Euthatal, 80 mg/kg, i.p.) and transcardially perfused with 0.1M of phosphate-buffered saline followed by ice-cold 4% paraformaldehyde. Immediately after perfusion, lesion site tissue was dissected, and post-fixed overnight and cryoprotected in graded concentrations of sucrose (12, 18, and 24%). 10 mm segment of cervical cord including the injury epicenter was sectioned using a cryostat (Leica) at 20 μm thickness in the transverse horizontal planes. Then the sections were stained with hematoxylin-eosin(H&E) and Eriochrome Cyanine (EC). Images were obtained using a Zeiss Axioplan 2 microscope. A customized script for the Northern Eclipse software (Northern Eclipse 6.0, Empix Imaging Inc., Mississauga, ON, Canada) captured images.

2.5 Statisticial Analysis

Statistical analysis was performed using the Statistica V7.1 software (StatSoft Inc., OK, USA). All data were expressed as the means ± standard error of the mean (SEM). SEPs & MEPs data and behavioral outcome measures were compared using two way ANOVAs. The factors were side (ipsilateral and contralateral side) and time (varied depending on test) with repeated measure. All post-hoc analyses were made using the Tukey's honestly significant test (Tukey's HSD), and $p < 0.05$ was considered to be significant for all tests. Because of variability in latency and amplitude and one case missed, SEPs & MEPs data at 3 hours and 1 day was excluded from the statistical analysis. Behavioral and electrophysiological correlation coefficients were calculated with a Pearson product moment correlation, and significance was set at $p < 0.05$.

3. Results

3.1 Mechanical parameters and histological feature

The average contusive displacement and speed were 2.196±0.003 mm and 598.5±1.4 mm/s, respectively, and very closed to the pre-set parameters with little variation. The average contusive compressive force was 1.462±0.117N following the inertial compensation with a blank hit.

Tissue damage was observed on the spinal cord transverse sections from 2 mm caudal to 2 mm cephalad to the epicenter. The most severe damage was at the epicenter, with loss of the ventral horns of gray matter and lateral funiculus of white matter (Fig. 1). However, the ipsilateral dorsal funiculus and horn were intact, as well as the entire contralateral cord.

3.2 Motor and somatosensory evoked potentials

Left and right forelimb MEPs were recorded from a single stimulation of the respective contralateral motor cortex via the subdermal cortical electrodes, while the SEPs from multiple stimulation to the forelimb musculus flexor. At baseline, similar latencies and amplitudes between sides were observed in the forelimb SEPs and MEPs of all animals as expected at this time point.

Both ipsilateral and contralateral forelimb MEPs showed latency prolongation and amplitude reduction at 3h after injury (Figure 2). The MEPs amplitude increased with time after injury, with the ipsilateral persistently lower compared to the contralateral (Figure 2A). At 8w after injury, the ipsilateral MEPs amplitude was s half of the baseline, while the contralateral MEPs amplitude restored to the baseline. The ipsilateral MEPs latency stayed greater than the baseline over all the time after injury, while the contralateral MEPs latency became shorter than the baseline beyond 1d after injury (Figure 2B). The MEPs latency was significantly different between sides, specifically at 3d and 2w after injury.

The SEPs amplitude after injury changed in a similar way as the MEPs amplitude (Figure 3). The SEPs amplitude dropped after injury and stayed at lower level until 8 weeks, particularly for the ipsilateral SEPs amplitude (Figure 3B). The SEPs amplitude was significantly different between sides, specifically at 1w and 8w after injury. It was difficult to see a trend of restoration of the contralateral forelimb SEPs amplitude as there was no difference in the SEP amplitude between sides at 2w or 4w after injury. In addition, there was no difference in the SEPs latency among time points and between sides (Figure 3A).

3.3 Forelimb motor function

We used Montoya staircase task and cylinder rearing test to evaluate the motor function after injury as they were designed to test the forelimb function in literatures (refs) (Figure 4). In the preoperative baseline staircase testing, all animal were able to reach, grasp and eat approximately 80% pellets from the first to the sixth step without difference between sides. After the cervical hemicontusion spinal cord injury, the ipsilateral forelimb grasped and ate 80% pellets compared to the baseline, while the constralateral forelimb slightly less pellets, with significant difference in number of pellets eaten between sides at 2w, 4w and 8w after injury, respectively (Figure 4A).

The cylinder rearing test was used to evaluate spontaneous forelimb usage for rodents. Before injury, all rats used both paws 80% simultaneously for the majority of weight support in the cylinder. An obvious less usage of the ipsilateral paw after injury, while slightly more usage of the contralateral paw as compensation to the injury (Figure 4B). There was significant difference in the paw usage between sides at 2w, 4w and 8w, respectively.

3.4 Correlation between SEPs & MEPs and forelimb motor function

The Pearson’s correlations between forelimb motor function (number of pellets eaten and paw usage) with latency and amplitude of SEPs and MEPs are summarized in Table 1 and Figure 5. A group average of MEPs latency and amplitude is correlated to an group average of number of pellets eaten and paw usage before injury and at 2w, 4w and 8w after injury, respectively (Figure 5). There is a significant correlation between the MEP (latency and amplitude) and forelimb motor function (number of pellets eaten and paw usage), while non-significant correlation between the SEPs and forelimb motor function (Table 1).

4. Discussion

In this study, we produce sustained functional deficits in the ipsilateral forelimb with unilateral spinal cord disruption. Multiple outcome measures are provided such as SEPs & MEPs, cylinder rearing, Montoya staircase and histology. We perform the longitudinal evaluation of SEPs & MEPs changes at a wider range from acute to chronic phase of spinal cord injury in cervical hemicontusion model. Meanwhile the results illustrate significant correlation of the MEP to the forelimb motor function after injury, whereas no significant correlation of SEPs to the forelimb motor function.

Motor evoked potentials and somatosensory evoked potentials represent reliable methods of quantifying the functionality of motor and sensory pathways, and might have good potential as objective evaluation tool aiding in diagnosis prognostication of functional recovery [7, 9, 15]. There are some researches focus on longitudinal changes of SEPs or MEPs after spinal cord injury [7, 8, 16]. Walker et al described MEPs changes in 5 days and 4 weeks post-injury [7], while Redondo-Castro monitored SEPs & MEPs changes from 7 days to 8 weeks post-injury [16], but few studies evaluated SEPs & MEPs in acute spinal cord injury. The present study found notable prolongation of MEPs latency and reduction of MEPs amplitude in both sides of forelimb during acute injury phase, indicating the spinal cord shock symptom right after hemicontusion. The unstable MEPs changes may be contributed from white matter demyelination which begins within 24 hours and peaks at 2–3 weeks post-injury[17, 18]. In the present study, we observed large variation in SEPs &

MEPs latency and amplitude and suggested SEPs & MEPs monitoring from 3 days post-injury in order to response to severity of spinal cord injuries. We speculate that a plateau of the MEPs latency of ipsilateral side and a progressive decrease of the MEPs latency of contralateral side after 1 week post-injury may indicate a subacute phase of spinal cord injury. Hence, our study suggests optimal time to monitor SEPs & MEPs begin from 1 week post injury.

The unilateral contusive spinal cord injury deployed in the present study may contribute to quick recovery of the MEPs latency of contralateral side to the level of baseline. Walker et al conducted the cervical hemicontusion spinal cord injury in rats and found both isplateral and contralateral MEPs latency at 4 weeks post-injury was close to the pre-injury level [7], supporting the contralateral MEPs latency was unaffected in the hemicontusion injury. The present study further found that isplateral MEPs latency stayed longer as the anterior horn of gray matter was damaged more under an oblique hemicontusion model. On the other hand, the MEPs amplitude of right and left forelimbs was significantly lower following SCI than the baseline and increased with time, but the contralateral MEPs amplitude was comparable to the baseline at 8 weeks post-injury while the ipsilateral MEPs amplitude was significantly lower than the baseline. Previous study of cervical hemicontusion SCI also observed that only ipsilateral forelimb showed a significant reduction in the MEPs amplitude at 4 weeks following SCI, which was similar to the present results [7].

The present study deployed Montoya staircase task and cylinder rearing test to reflect the fine and gross components of the overall forelimb functions, and found that most of these tasks was abolished after injury, particulary for the the injured forelimb[19]. There was significant reduction in the number of pellets eaten in the staircase pellet test with the ipsilateral forepaw, and the deficits in ipsilateral forelimb usage were about 20%-30% for the Cylinder Rearing test after C5 hemicontusion. Despite our rats represented stable deficit forelimb function throughout entire experiment, a previous study with quite similar injury model to the present study showed more severe behavioral loss [3, 10]. The discrepency between two studies may related to the control mode used in the hemicontusion model where the force control was by Lee et al [3, 10]and the displacement control by us. The present study further found that these behavior assessement was significantly correlated to the MEP measurement. Although it is debatable whether the MEP reflects the motor function as the MEP represents the excitability of CST projections[20], we think that a link between the MEPs and the motor function exists in some fine spinal cord injury models.

Interestingly, SEPs & MEPs is related to the histopathology of hemicontusion spinal cord injury. Some studies reported effect of unilateral spinal cord injury severity on SEPs & MEPs waveforms[8, 16]. Severe damage to ipsilateral anterior gray matter and the lateral funiculus was observed in the present study using an oblique cervical hemicontusion spinal cord injury model. This hemicontusion model was first developed by Lee and his colleagues, who declare this model was aimed to injure the corticospinal and rubrospinal tracts of the ipsilateral side so that the deficit of motor function was confined to the ipsilateral forelimb. However, a branch of corticospinal tract is located in the dorsal column in rats which is different from in humans[21]. Accordingly, we consider that motor functional deficit of ipsilateral forelimb is caused by the completely destroying the anterior horn neurons. The dorsal funiculus that contain ascending sensory pathway is reserved in the present oblique hemicontusion model as th impact trajectory angled laterally 22.5°, which supports not obvious SEPs change both in latency and amplitude in our results. Loss of pathways of one side of the spinal cord in rats should be compensated by the exist pathway of the other side which cross at different levels of the descending systems in response to the injury[22, 23]. In our study, MEPs amplitude was feeble in the ipsilateral side from 3 hours to 3 days post-injury while recovered beyong 3 days in both side of forelimb. This result suggests that integrity of contralateral uninjury corticospinal and rubrospinal tract play a crucial part in the recovery, which is supported by the results of Krajacic's study[2].

In the present study, the ipsilateral side was compared the contralateral as the injury model is a hemicontusion to the cord. Therefore, a sham group was not included in the present study. For avoiding the influence of needle sting of electrophysiological assessment, we designed to do eletrophysiological and behavioral assessment separately using two groups. This design is of less power to detect a correlation between SEPs & MEPs and behavior and have more animals as a group-correlation with less number. In addition, other SCI model or difference severity should be considered in future.

In summary, cervical hemicontusion spinal cord injury led to persistent functional impairment of the ipsilateral forelimb, which was demonstrated by histological findings in tissue damage of the ipsilateral spinal cord. Montoya staircase task and cylinder rearing test, SEPs and MEPs can measure the functional changes after SCI. The "spinal shock" occurring at acute cervical hemicontusion spinal cord injury resulted in obvious SEPs & MEPs changes of both ipsialteral and contralateral forelimbs, suggesting reliable electrophysiology assessment beyond the acute injury. The behavioral assessment was correlated to the MEP, supporting the longitudinally electrophysiology assessment for neurological impairment after SCI.

Acknowledgment

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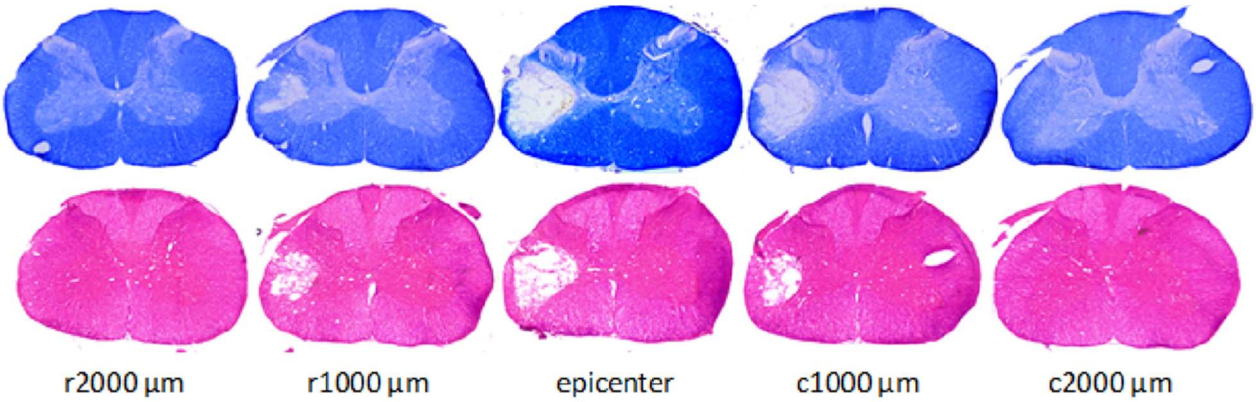

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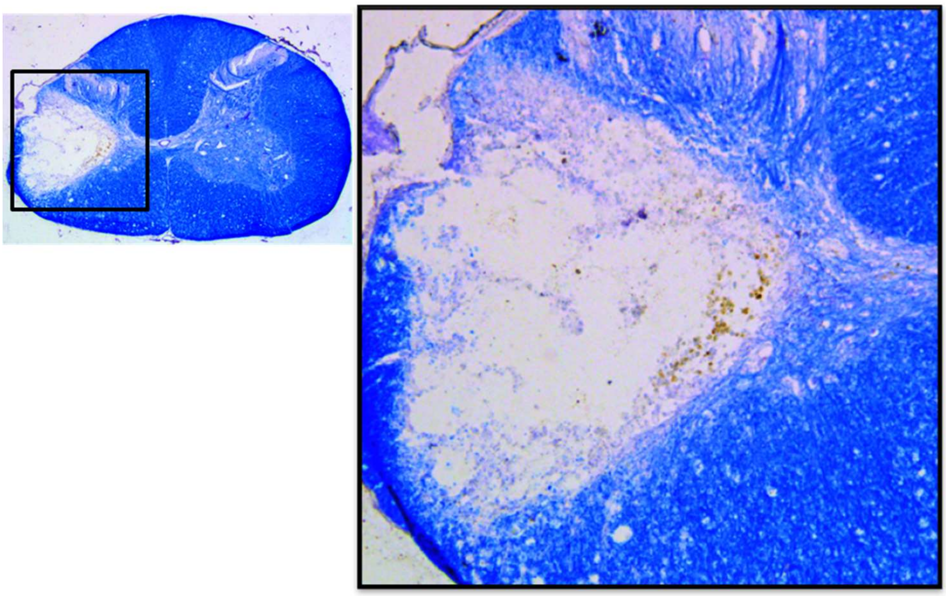

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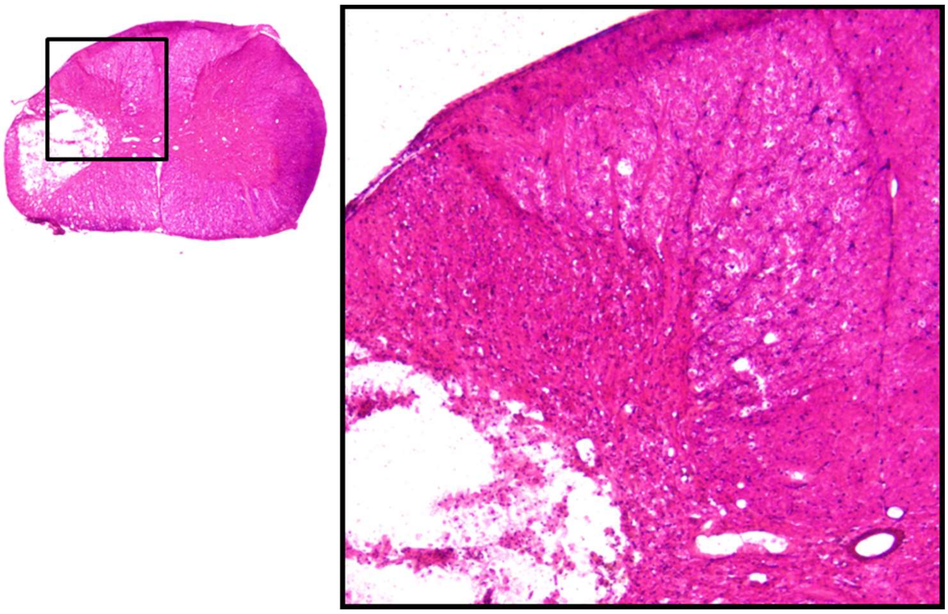
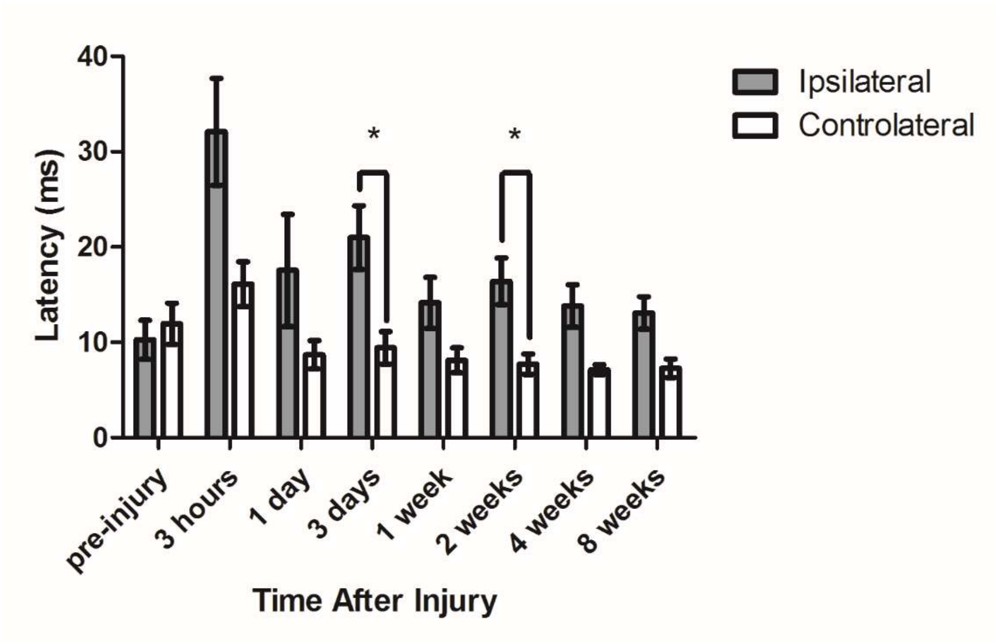


Fig. 1. The histology feature of hemicontusion SCI. Representative image of spinal cord cross-section from rostral 2000 μm to caudal 2000 μm by HE and EC stain (A). At 8 weeks

post-injury, HE and EC stained sections demonstrated the ipsilateral white matter and gray matter damage with extensive damage of the lateral funiculus (B) but complete sparing of the dorsal column, which contains the main contingent of ascending sensory axons and corticospinal tract(C).

A



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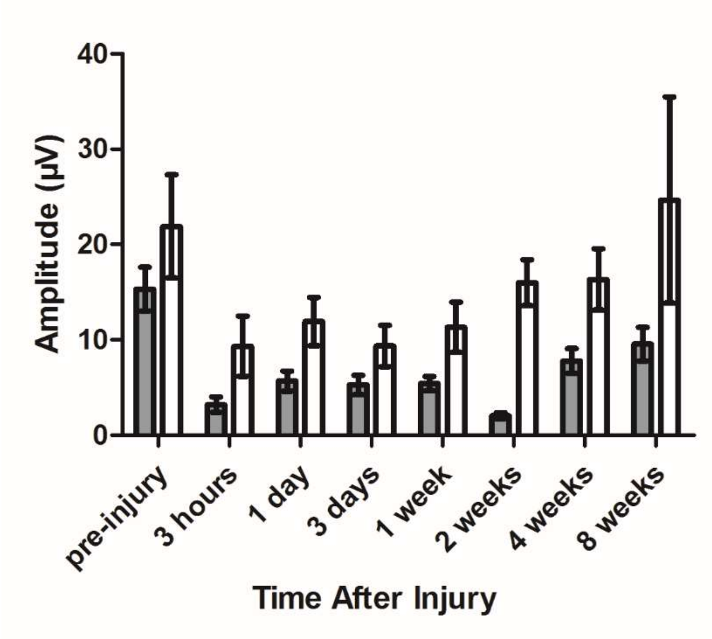

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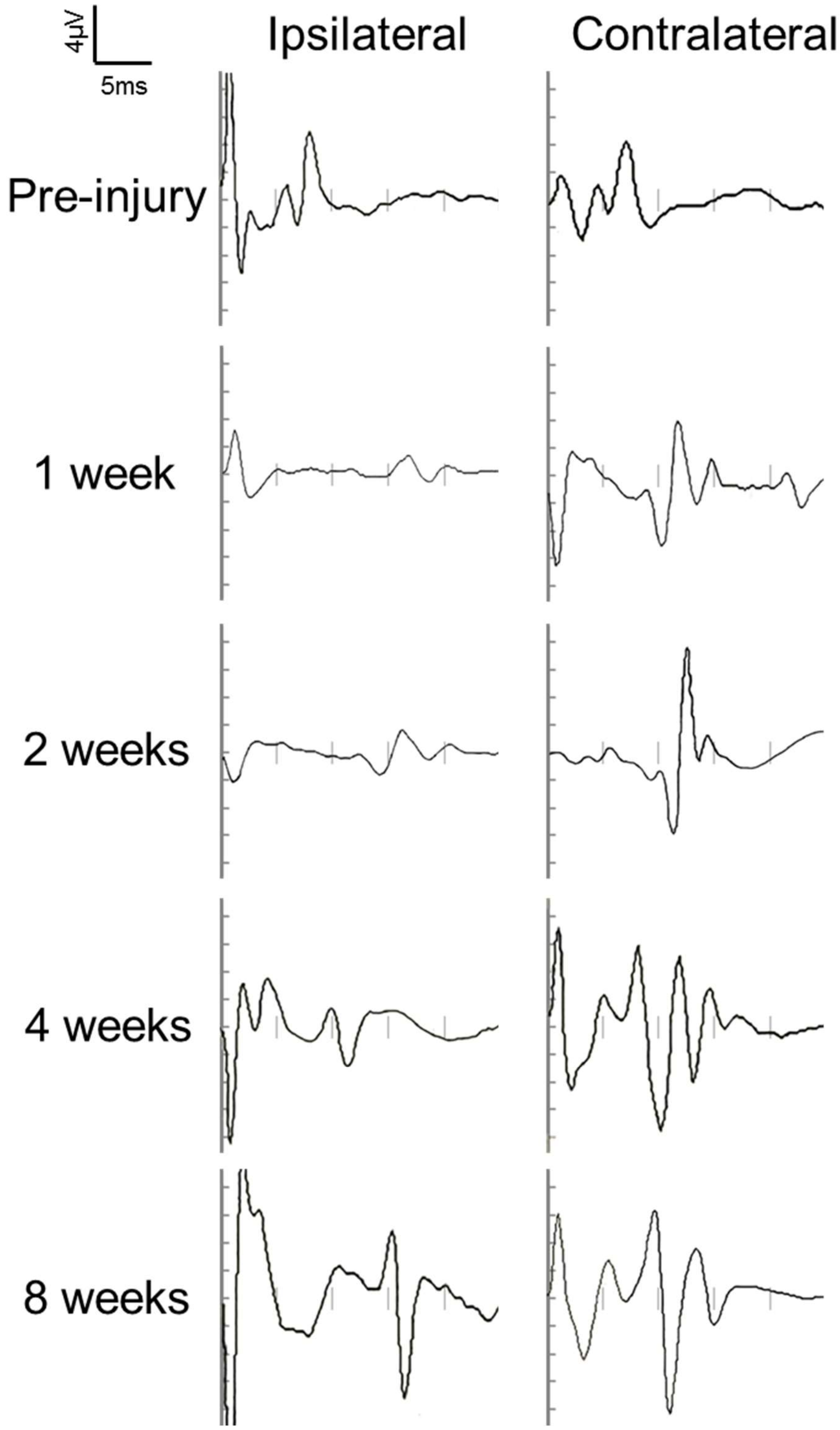
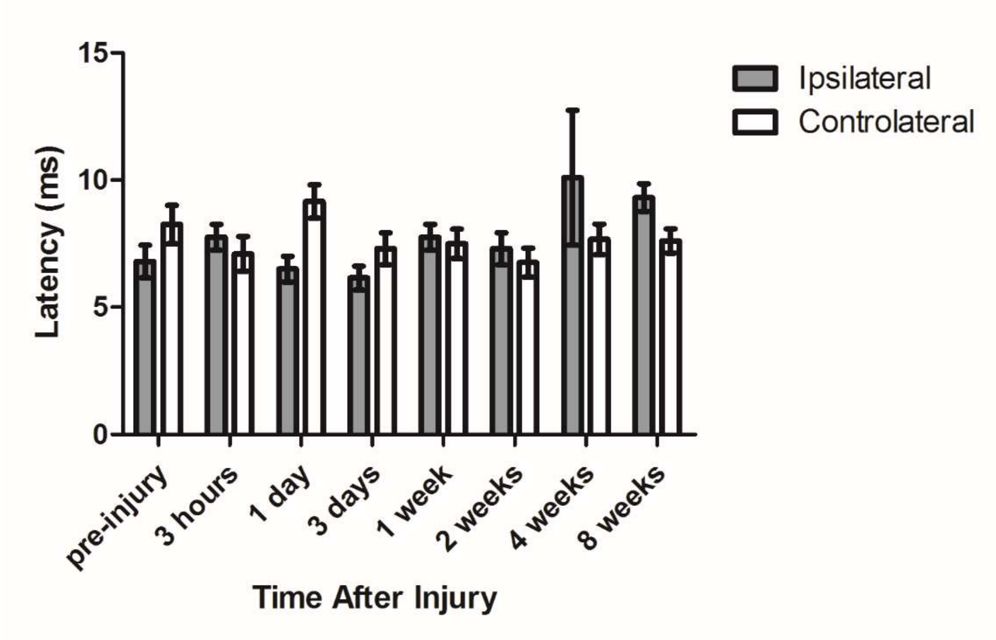
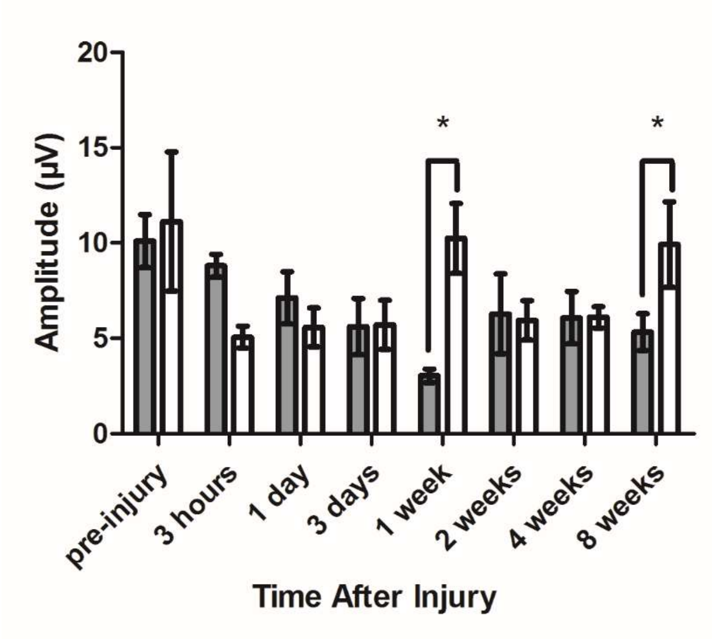


Fig. 2. Motor evoked potentials (MEP) in the forelimb extensor muscles before and after spinal cord injury (SCI). Quantification of the latency and the amplitude of the MEP over time after injury (A and B). The ipsilateral forelimb showed a reduction in amplitude and an increase in latency follow SCI compared to the baseline. Representative MEP recording show changes or loss in MEP recording in the ipsilateral and contralateral forelimbs(C).

A


15
10
5
0
Latency (ms)
Ipsilateral
Controlateral
pre-injury
3 hours
1 day
3 days
1 week
2 weeks
4 weeks
8 weeks
Time After Injury

B


20
15
10
5
0
Amplitude (µV)
*
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pre-injury
3 hours
1 day
3 days
1 week
2 weeks
4 weeks
8 weeks
Time After Injury

C

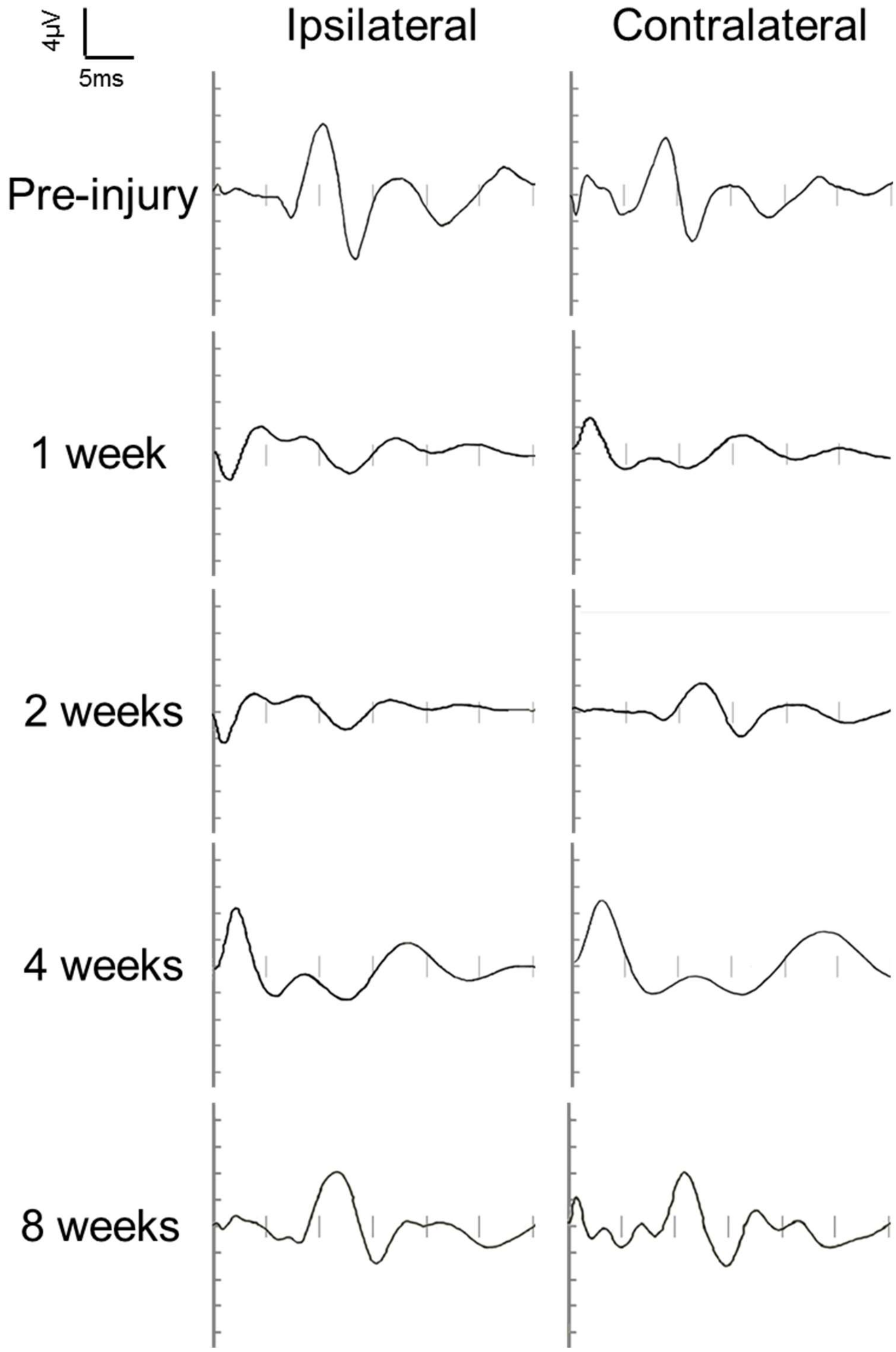
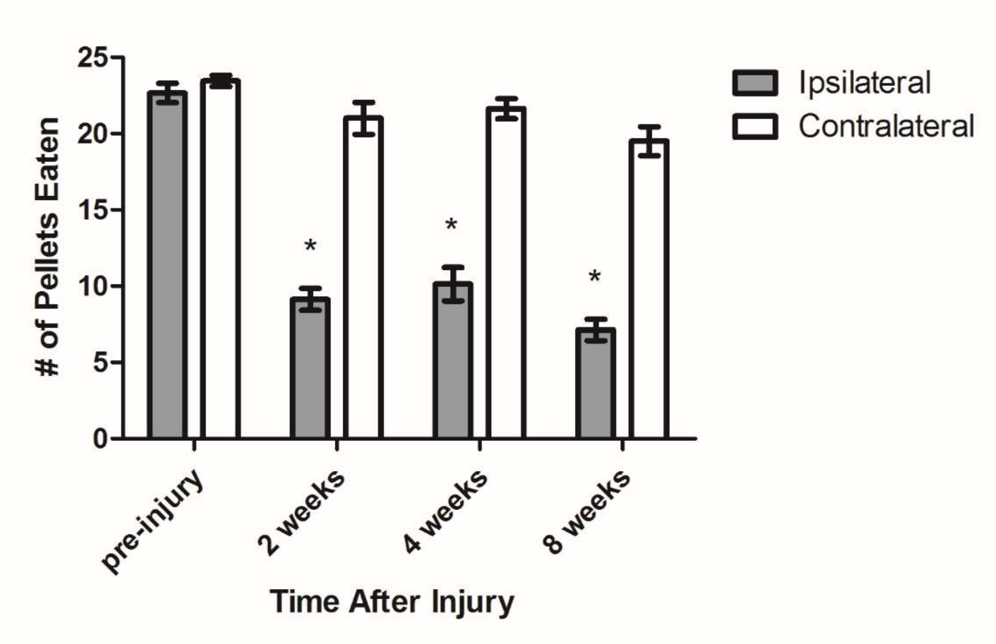


Fig. 3. Somatosensory evoked potentials (SEPs) in pre-injury and post-injury. No significant difference was observed in SEPs Latency at all time points (A). SEPs amplitude of the ipsilateral injury forelimb show a slight decrease(B). Typical SEP response waveforms up to 8 weeks post-injury (C).

A



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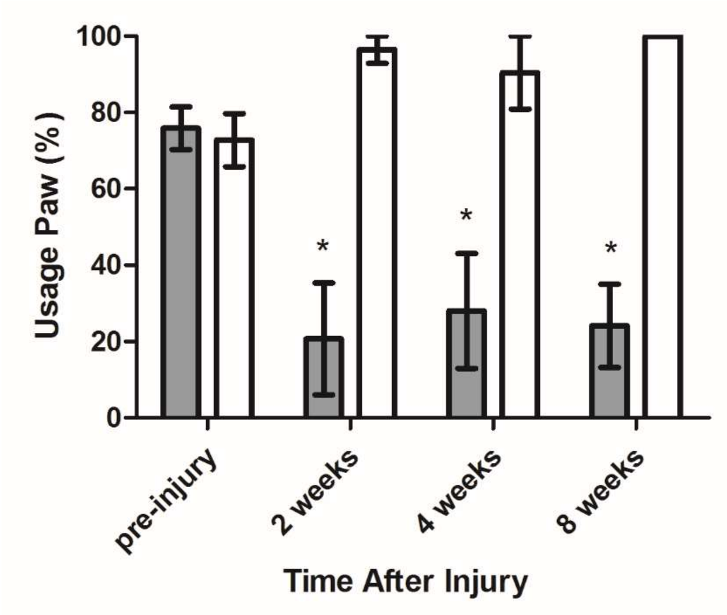


Fig. 4. Results for the staircase task and cylinder rearing test. Quantification of the number of pellets eaten using the ipsilateral and contralateral forelimb in the staircase tasks, respectively (A). Rats reveal notable impairments in reaching with the ipsilateral forelimb, as clarify by their decreased ability to retrieve pellets. Before injury, rats almost equally use both paws for spontaneous vertical exploration in cylinder rearing test (B). There is a significant decrease in independent usage of ipsilateral paw after injury.

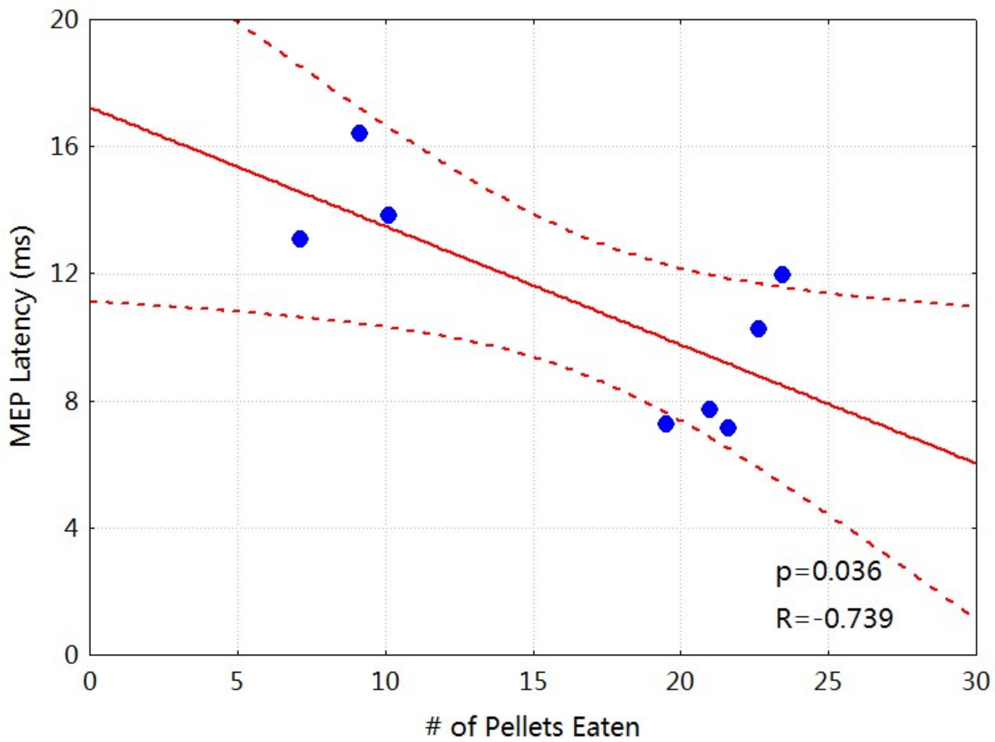
Tabe 1. Correlations Between Behavioral and Electrophysiological Assessment

	Staircase task	Cylinder rearing

	Pearson's r	p Value	Pearson's r	p Value
MEP Latency	0.803*	0.016	0.858**	0.006
MEP Amplitude	-0.739*	0.036	-0.935**	0.001
SEP Latency	-0.610	0.080	0.459	0.253
SEP Amplitude	0.650	0.108	-0.614	0.105

* $p < 0.05$ and ** $p < 0.01$ for linear relationship between variables.

A



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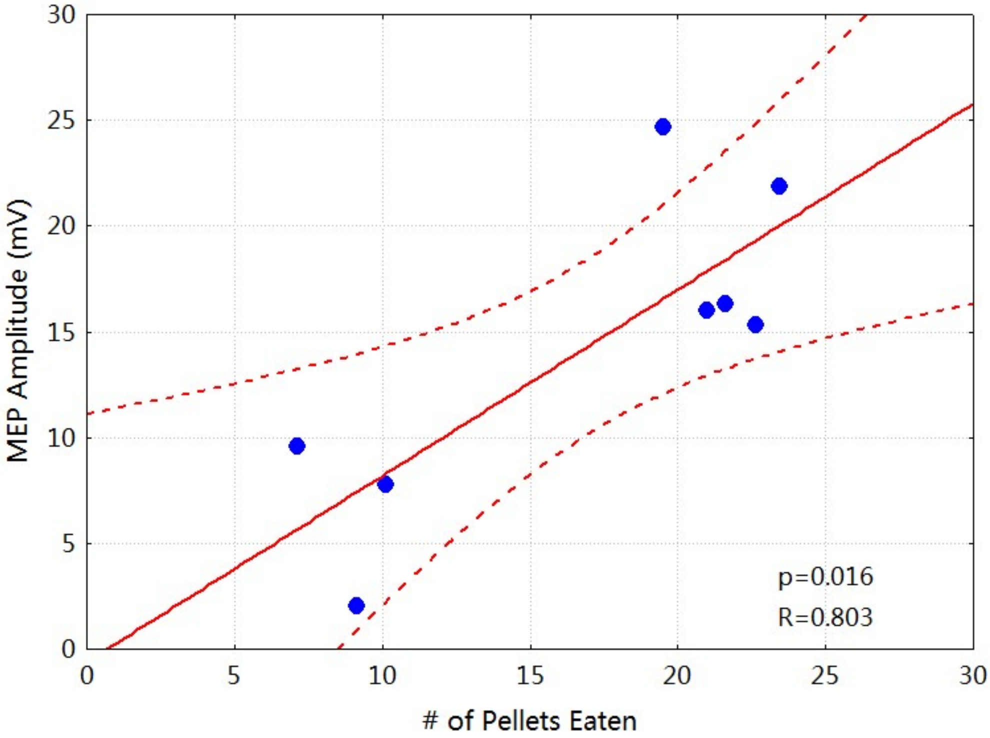
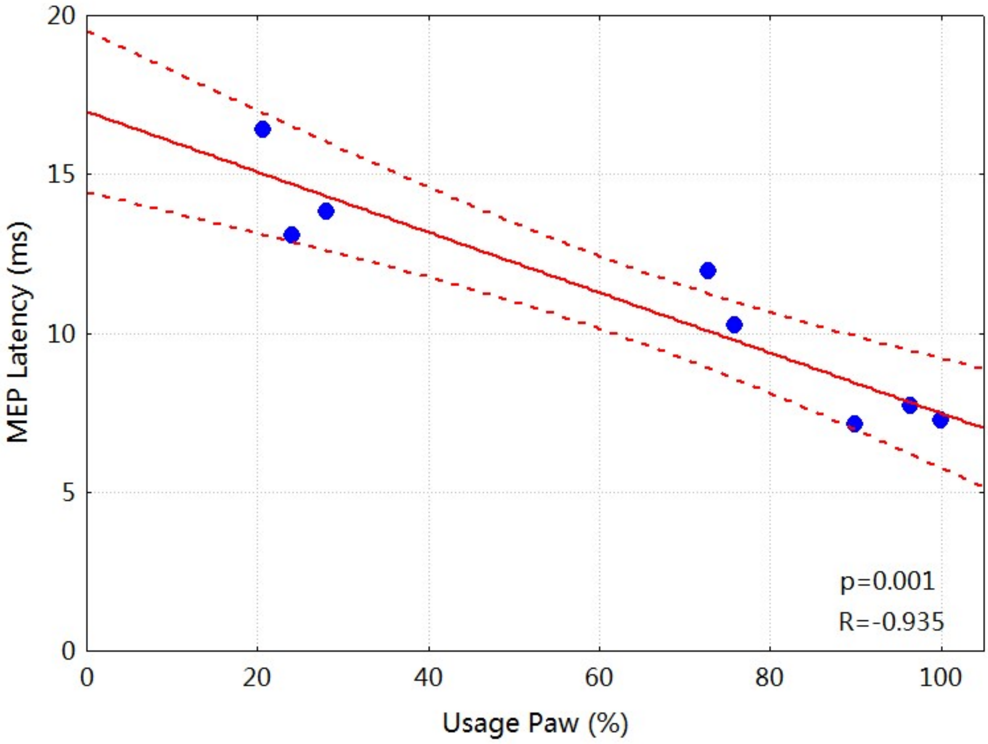


MEP Amplitude (mV)
# of Pellets Eaten
p=0.016
R=0.803

C



MEP Latency (ms)
Usage Paw (%)
p=0.001
R=-0.935

D

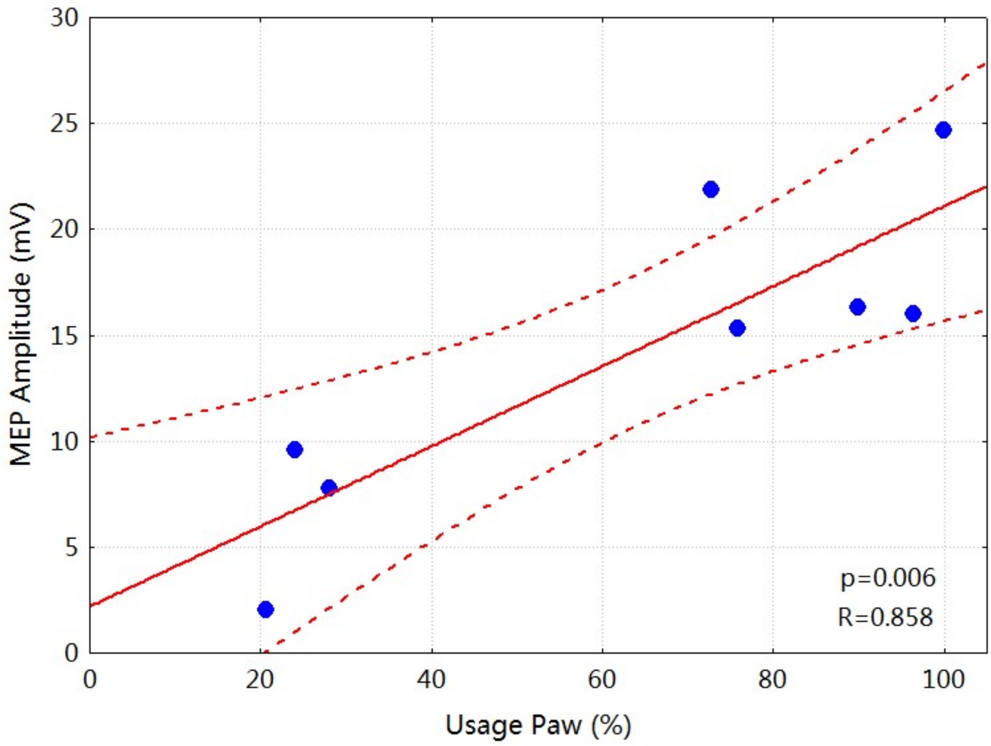


Fig. 5. Correlations between MEP and behavioral outcome. Linear regression was observed showing a significant positive correlation between (A and C) MEP latency and (B and D)MEP amplitude and behavioral assessment after SCI, as assessed with (A and B) staircase tasks and (C and D)cylinder rearing. Linear regression lines of best fit are plotted as solid lines, along with 95% confidence intervals as dashed lines

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