Research Article

Utility of somatosensory and motor-evoked potentials in reflecting gross and fine motor functions after unilateral cervical spinal cord contusion injury

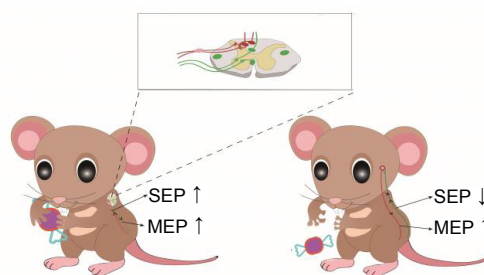Rong Li[1, 4], Zu-Cheng Huang[2], Hong-Yan Cui[1], Zhi-Ping Huang[2], Jun-Hao Liu[2], Qing-An Zhu[2, *], Yong Hu[1, 3, 4, *]

Graphical Abstract *Fine motor skills require coordinated control of sensory and motor pathways in rats following unilateral cervical spinal cord contusion injury*

Following left unilateral cervical spinal cord injury, significant recovery in SEP and MEP were accompanied by considerable recovery of grip skills in rats, while rats with significant MEP but SEP recovery had poor executive ability of grasping in ipsilateral paws

Abstract

Fine motor skills are thought to rely on the integrity of ascending sensory pathways in the spinal dorsal column as well as descending motor pathways that have a neocortical origin. However, the neurophysiological processes underlying communication between the somatosensory and motor pathways that regulate fine motor skills during spontaneous recovery after spinal cord contusion injury remain unclear. Here, we established a rat model of cervical hemicontusive injury using C5 laminectomy followed by contusional displacement of 1.2 mm (mild injury) or 2.0 mm (severe injury) to the C5 spinal cord. Electrophysiological recordings were performed on the brachial muscles up to 12 weeks after injury to investigate the mechanisms by which spinal cord pathways participate in motor function. After spinal cord contusion injury, the amplitudes of somatosensory and motor-evoked potentials were reduced, and the latencies were increased. The forelimb open field locomotion test, grooming test, rearing test and Montoya staircase test revealed improvement in functions. With increasing time after injury, the amplitudes of somatosensory and motor-evoked potentials in rats with mild spinal cord injury increased gradually, and the latencies gradually shortened. In comparison, the recovery times of somatosensory and motor-evoked potential amplitudes and latencies were longer, and the recovery of motor function was delayed in rats with severe spinal cord injury. Correlation analysis revealed that somatosensory-evoked potential and motor-evoked potential parameters were correlated with gross and fine motor function in rats with mild spinal cord contusion injury. In contrast, only somatosensory-evoked potential amplitude was correlated with fine motor skills in rats with severe spinal cord injury. Our results show that changes in both somatosensory and motor-evoked potentials can reflect the changes in gross and fine motor functions after mild spinal cord contusion injury, and that the change in somatosensory-evoked potential amplitude can also reflect the change in fine motor function after severe spinal cord contusion injury. This study was approved by the Animal Ethics Committee of Nanfang Hospital, Southern Medical University, China (approval No. NFYY-2017-67) on June 11, 2017.

Key Words: central nervous system; motor-evoked potential; motor function; regeneration; repair; somatosensory-evoked potential; spinal cord; spinal cord injury

Chinese Library Classification No. R454; R744; R338.8

[1]Institute of Biomedical Engineering, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China; [2]Department of Spine Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong Province, China; [3]Department of Orthopedics and Traumatology, The University of Hong Kong, Hong Kong Special Administrative Region, China; [4]Department of Orthopedics and Traumatology, The Hong Kong University Shenzhen Hospital, Shenzhen, Guangdong Province, China
*Correspondence to: Yong Hu, PhD, yhud@hku.hk; Qing-An Zhu, PhD, qinganzhu@gmail.com.
https://orcid.org/0000-0003-0305-5616 (Yong Hu); https://orcid.org/0000-0002-0412-0515 (Qing-An Zhu)

Research Article

Introduction

Upper limb motor skills are necessary for individuals with cervical spinal cord injury (SCI), and rehabilitation of these skills following SCI facilitates a substantive improvement in independent living (Anderson et al., 2007; Ding et al., 2019). The loss of proprioception can lead to deficits in fine motor control, which results from impaired integration of cortical sensory and motor signals (Akitake et al., 2015). However, the neurophysiological processes underlying the coordinated regulation of fine motor skills via links between the somatosensory and motor pathways during spontaneous recovery after SCI remain unclear.

Corticospinal circuits that descend from the motor cortex play an important role in the regulation of motor neuron activity, as has been reported by many classical studies (Isomura et al., 2009; Peters et al., 2017). Skilled voluntary movements (such as reaching and grasping) are generally considered to primarily rely on neural circuits located in the motor cortex, while stereotyped motor functions (such as locomotion and posture) are regulated by spinal motor circuits (Alstermark and Isa, 2012; Wang et al., 2018). However, these traditional views were challenged by the identification of the mechanisms underlying voluntary fine motor recovery after SCI in studies investigating behavioral deficits after limited interruption of the dorsal column in rodents and monkeys (Qi et al., 2014; Inácio et al., 2016). Some studies demonstrated that a preserved sensory pathway after partial or complete dorsal column lesion can facilitate reactivation of the cortex and behavioral recovery (Varejão and Filipe, 2007; Qi et al., 2014). Even with a normal corticospinal tract, loss of motor control may occur when proprioception is severely impaired by dysfunction of the sensorimotor network (Kato and Izumiyama, 2015). Additionally, a recent study showed that the modulation of walking speed, which is dependent on muscle activity and strength, requires proprioceptive feedback from the muscle spindle; in the absence of this feedback, rats cannot walk as fast as normal (Mayer et al., 2018). Similarly, a mouse study showed that animals without muscle spindle feedback could execute basic locomotor behaviors, but exhibited poor performance in precise motor tasks (Takeoka et al., 2014). These studies highlight the key role of sensory feedback in voluntary fine motor control, and underscore its importance in the development of interventions that enhance motor recovery after SCI.

Regardless of whether the SCI is incomplete or complete at any level of the cord, motor and sensory functions can spontaneously recover to varying degrees in both humans and animals because of the plasticity of the nervous system (Li et al., 2017; Wei et al., 2018; Harnie et al., 2019). This can involve various changes in the neuraxis, including alterations in the properties of the preserved neuronal circuit, axon collateral sprouting, and synaptic rearrangement (Fouad and Tse, 2008; Buen et al., 2017; Li et al., 2017; García et al., 2019). However, it is difficult to evaluate the contribution of these plastic processes that occur in specific sensory and motor systems to functional recovery through behavioral assessments alone. Electrophysiological examinations provide an objective and quantitative method to separately evaluate these plasticity-induced electrophysiological changes in sensory and motor pathways, and have been applied as a routine tool for intraoperative spinal cord monitoring (Hu et al., 2011). Therefore, complementary assessments of sensory and motor pathways after SCI, such as electrophysiology, can expand our understanding of their common and distinct contributions to voluntary sensorimotor control.

In present study, we hypothesized that fine motor skills are associated with somatosensory and motor pathway recovery after SCI. To investigate whether sensory feedback regulation is involved in the control of fine motor function, we applied multiple behavioral analyses of gross to fine motor skills combined with serial recordings of motor-evoked potentials (MEPs) and somatosensory-evoked potentials (SEPs) following dorsal SCI in rats.

Materials and Methods

Animals

A total of 32 adult male Sprague-Dawley rats (weighing ~320 g, aged 3–4 months) were purchased from Guangdong Medical Laboratory Animal Center, Foshan, China (license No. SYXK (Yue) 2018-0002), and were randomly assigned to one of the following three groups: 1.2-mm SCI (n = 12, defined as mild), 2-mm SCI (n = 12, defined as severe), or sham (laminectomy, n = 8). The rats were acclimated in an environmentally controlled breeding room at 22°C and indoor humidity (30–50%) on a reverse light cycle (12/12 hours) with standard chow and filtered water for 7 days and fasted for 12 hours before the experiment. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978), and were approved by the Animal Ethics Committee of Nanfang Hospital, Southern Medical University, China (approval No. NFYY-2017-67) on June 11, 2017.

Cervical hemi-contusive SCI

All rats underwent a surgical procedure as previously described (Huang et al., 2018). Briefly, a C5 laminectomy was conducted to expose the cervical spinal cord, followed by immobilization from C4–6 with a customized vertebral clamp to a stereotaxic frame (68902; RWD Biomed Inc., Shenzhen, China). A 1.5-mm diameter impactor was placed over the dura at 0.25 mm right of the midline, and lowered vertically until the initial contact force reached 0.01 N. A target contusion displacement of 1.2 mm (mild injury) or 2.0 mm (severe injury) at 500 mm/s was applied to the C5 spinal cord by a servo-electromagnetic material testing machine (ElectroPuls E1000; Instron, Canton, MA, USA). Contusion displacement, speed and peak force during the time of impact were recorded and analyzed with Instron software (V1.5.6; Instron WaveMatrix, Instron).

Electrophysiological evaluation

SEP and MEP recordings were performed prior to SCI, followed by recordings collected at 10 minutes, 3 days, 1 , 2, 4, 8 and 12 weeks after injury. The MEP and SEP latencies were defined as the time from the stimulus to the first positive or negative deflection from baseline, and the amplitude was defined as the maximum amplitude between the maximum positive and negative peaks (**Figure 1**). A electrophysiological monitoring protocol was used for forelimb acquisition of SEPs and MEPs using a 16-channel monitoring system (YRKJ-G2008; YiRui Technology Co., Ltd., Zhuhai, China).

SEPs were recorded from two 1.2 mm needle electrodes (NE-S-1500/13/0.4; Friendship Medical Electronics Co., Ltd., Xi'an, China) inserted subdermally in the C3/4 region of the skull according to international 10–20 system electrode placement. The reference electrode was inserted subcutaneously over the skull at the frontal midline. Constant-current stimulation (5 mA, 0.1 ms) was applied to the median nerves via a pair of stimulating needle electrodes at a frequency of 5.3 Hz. SEP signals were bandpass-filtered between 30 and 3000 Hz, and 300 responses were averaged for each recording session. MEPs were evoked by constant electrical stimulation via two 1.2 mm needle electrodes (NE-S-1500/13/0.4; Friendship

Medical Electronics Co., Ltd.) inserted subcutaneously over the approximate location of the motor cortex corresponding to C3–4. Single-trial MEPs were obtained with a current intensity of 30–50 mA, a pulse duration of 1 ms, and a frequency of 300 Hz. MEPs were recorded using subdermal needle electrode pairs from the brachioradialis muscle, and signals were filtered at a bandpass frequency between 30 and 3000 Hz.

Behavioral testing
All behavioral tests were performed prior to SCI, followed by recordings collected at multiple time points up to 12 weeks after injury. Gross motor function was detected using forelimb open field test, grooming and rearing test, while fine motor skills were evaluated by Montoya staircase test.

Forelimb open field test
The forelimb locomotor rating scale scored from 1 to 17 was developed according to behavioral changes observed after unilateral injury (Singh et al., 2014). Briefly, rats were placed in an enclosure, and were allowed to move freely in the open field. A quantitative scale was used to measure movements of the shoulder, elbow and wrist joints, as well as forepaw position and digit placement in a minimum of three locomotor tests within 5 minutes. The higher the score, the better the motor function.

Grooming test
The grooming test was used to assess forelimb function according to the maximal area each forelimb independently touched during grooming (Plunet et al., 2008). Briefly, points were assigned with a 6-point scoring system on a scale ranging from the inability to touch the head or face (score of 0) to the ability to touch the head area behind the ears (score of 5).

Rearing test
The rearing test was employed to evaluate the coordination of the forelimbs. Briefly, video recordings were made of forelimb movement during exploratory activity in a transparent cylinder. A mirror placed behind the cylinder at an angle enabled to record movements of the forelimbs in all directions. The preference for use of one or both limbs was calculated as the percentage of total limb movement during wall climbing and landing. All animals were estimated for not less than 20 exploratory movements along the wall and at least 10 landing movements in each session.

Montoya staircase test
Forelimb reaching and grasping performance was assessed using the Montoya staircase test (Plunet et al., 2008). During this test, animals are freely allowed to reach down for colored pellets placed within six wells numbered 1 to 6 from near to far distance in a descending staircase on each side. Color-coded pellets were placed on each level of the well on the staircase to keep track of which pellets were obtained during scoring. During baseline and post-injury assessment, the pellets remaining in each well after 15 minutes were counted. Success rates were measured as the number of pellets eaten and the largest numbered well reached. The pellets on larger numbered well the rats eat, the greater distance they reach.

Histological evaluation
Histological quantifications were performed to demonstrate the effect of tissue preservation on pathway conductivity and motor function. Twelve weeks following injury, animals were deeply anesthetized by intraperitoneal injection of sodium pentobarbital (80 mg/kg). Transcardial perfusion was performed with 200 mL of 0.01 M phosphate-buffered saline,

sequentially followed by 200 mL 4% paraformaldehyde. Spinal cord tissue at the lesion site was removed and post-fixed in 4% paraformaldehyde overnight. Samples were cryoprotected at 4°C in ascending concentrations of sucrose solution (12%, 18% and 24% sucrose, in phosphate-buffered saline solution, 24 hours each). A 10-mm spinal cord sample was sliced into 20-μm sections using a cryostat (CM1950; Leica, Wetzlar, Germany). Sections were stained with Eriochrome Cyanine to identify pathological changes in gray and white matter areas. A microscope (Axioplan 2; Zeiss, Oberkochen, Germany) was used to obtain histological images. The damaged area of the spinal cord was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis
All statistical analyses were performed using SPSS 20.0 (IBM, Armonk, NY, USA). Between-group differences in SEP and MEP parameters were assessed using a multiple factor repeated-measures analysis of variance followed by Tukey's honestly significant test. Following the one-way analysis of variance, Bonferroni *post hoc* tests between groups were used to analyze the tissue lesion area and spared white and gray matter areas. An independent linear correlation analysis (Pearson's correlation coefficient) was performed between the individual behavioral outcomes and electrophysiological parameters. A value of $P < 0.05$ was considered statistically significant. All errors are given as the standard error (SEM).

Results
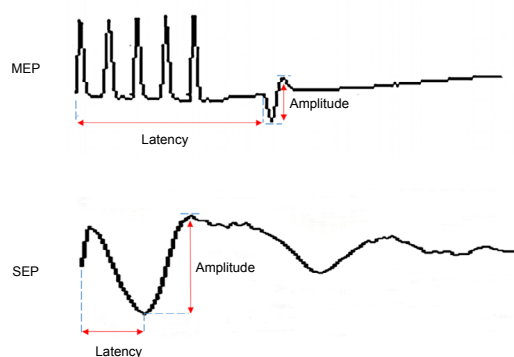
Contusion parameters in unilateral cervical SCI
No significant differences in impact velocity were found between the 1.2 and 2-mm SCI groups ($P > 0.05$). There was a significant increase in peak force during impact with increasing displacement of injury ($P < 0.05$). A summary of the contusion biomechanical parameters are presented in **Additional Table 1**.

Electrophysiological differences in rats with different severities of unilateral cervical SCI
SEP
Representative evoked potential waveform changes across time after cervical SCI in rats (**Additional Figure 1**). The SEPs in the 1.2 and 2-mm SCI groups showed extensive waveform changes compared with baseline. SEP amplitude and latency for the two sides in the 1.2 and 2-mm SCI groups differed significantly at all time points following injury ($P < 0.05$; **Figure 2**). The SEPs ipsilateral to the injury side were abolished in all rats with SCI at 10 minutes post-injury. At 3 days, SEP was partially restored in all 12 rats in the 1.2-mm SCI group, whereas 9 of the 12 rats in the 2-mm SCI group showed SEP activity by 1 week post-injury (**Figure 2A**). The amplitude and latency of SEPs recorded from the forelimbs on the opposite side of the injury exhibited a fast and substantial recovery to preoperative levels after a slight reduction post-injury (**Figure 2B** and **D**).

Moreover, both SEP latency and amplitude showed a significant but distinct trend toward recovery over time in the two injury groups (**Figure 2A** and **C**). Notably, SEP latencies in the two SCI groups were significantly different from baseline to 8 weeks post-injury ($P < 0.01$), but the significance was not maintained for the remaining time points. In contrast, the SEPs ipsilateral to the injury side in the 1.2-mm SCI group showed noticeably greater amplitude compared with the 2.0-mm SCI group for all recording days, except immediately post-injury ($P < 0.01$). Although the SEP amplitude recovered much better in the 1.2-mm SCI group compared with the 2-mm SCI group, it did not recover to preoperative levels.

Figure 1 | Illustrations for parameter measurements of SEP (upper) and MEP (lower) response waveforms in rats with unilateral cervical spinal cord contusion.
MEP: Motor-evoked potential; SEP: somatosensory-evoked potential.

MEP

As with the SEPs, MEPs in the contralateral forelimb showed a fast and substantial recovery to pre-injury levels after slight amplitude reduction and prolongation in latency (**Figure 2F and H**). MEP amplitude and latency were significantly different between the two sides in both SCI groups at all days following injury ($P < 0.01$; **Figure 2E–H**).

MEP amplitude and latency varied significantly with injury degree within each group ($P < 0.001$; **Figure 2E** and **G**). Twenty minutes after SCI, there was complete elimination of MEP activity in both injury groups. Rats subjected to the 1.2-mm SCI achieved a slight recovery in MEP activity by day 3, whereas rats with a 2-mm SCI exhibited recovery of MEP activity starting 1 week after injury (**Figure 2E**). MEP latencies were significantly longer in the two SCI groups compared with the sham group for all recording days ($P < 0.001$), and displayed substantial recovery over time, with better recovery in the 1.2-mm SCI group than the 2.0-mm SCI group. MEP amplitude also showed significant recovery in both SCI groups 4 weeks post-injury (1.2-mm SCI group: from no response to 434.60 ± 78.22 μV, $P < 0.001$; 2-mm SCI group: from no response to 413.54 ± 81.80 μV, $P < 0.001$). However, MEP amplitudes were not significantly different between the injury groups 4 weeks after injury (1.2-mm SCI group: 434.60 ± 78.22 μV; 2-mm SCI group: 413.54 ± 81.80 μV; $P > 0.05$). At 12 weeks, MEP amplitude reduction was significantly greater on the injured side, even though both the contralateral and ipsilateral spinal cords achieved a degree of recovery after hemi-contusive injury.

Behavioral difference in rats with different severities of unilateral cervical SCI
Forelimb open field locomotion test
All injured animals presented a clubbed ipsilateral forepaw during locomotion post-SCI. From 1 to 8 weeks post-injury, all injured animals exhibited a noticeable increasing trend in the forelimb locomotor rating scale score. At 8 weeks post-injury, all animals entered a stable stage and demonstrated relatively little improvements up to week 12. The forelimb locomotor rating scale scores varied with the degree of injury, and the scores in the 2-mm SCI group were greater than those in the 1.2-mm SCI group at all time points ($P < 0.01$; **Figure 3**).

Grooming test
At all time points after SCI, almost all animals demonstrated normal contralateral grooming behavior (data not shown), but exhibited obvious ipsilateral deficits. Significant functional improvements were displayed by the affected forelimbs in the 1.2- and 2-mm SCI groups from 2 to 8 weeks post-SCI ($P < 0.01$),

although they maintained only a limited ability to initiate grooming behavior from behind the ears. The grooming scores in the 2-mm SCI group were lower than those in the 1.2-mm SCI group at all time points following injury ($P < 0.01$; **Figure 4**).

Rearing test
All groups showed a near-complete loss in independent use of the affected paw (ipsilateral) during rearing post-injury (**Figure 5A**) and used the ipsilateral paw mostly in combination with the contralateral paw (**Figure 5B**). There was a significant increase in single limb usage to 37.76 ± 2.51% in the 1.2-mm SCI group from 2 to 8 weeks ($P < 0.001$). However, no increase in usage frequency of the ipsilateral paw was noted in the 2.0-mm SCI group over time, and the differences between the two SCI groups were still significant at 12 weeks post-injury (**Figure 5A**).

Montoya staircase test
After C5 hemi-contusion injury, rats showed severe impairments not only in retrieval success with the ipsilateral paw, but also in maximum distance reached (**Figure 6**). From 2 to 8 weeks after 1.2-mm SCI, rats demonstrated a marked increase in reaching scores, which remained stable until 12 weeks post-injury ($P < 0.01$; **Figure 6A**). However, no recovery of reaching deficit was observed in the 2-mm SCI group. Increased maximum distance of reach was observed in rats with 1.2-mm SCI over time, while animals in the 2-mm SCI group could only retrieve pellets from well number 2, and occasionally, well number 3 (**Figure 6B**).

Histological features in rats with different severities of unilateral cervical SCI
After SCI, all rats developed typical cystic lesions and ipsilateral tissue atrophy (**Figure 7A–C**). Rats that received an extended 2-mm SCI showed greater ipsilateral tissue damage at the epicenter compared with the 1.2-mm SCI group ($P < 0.001$), and the average lesion areas in the 1.2 and 2-mm SCI groups were 34.2 ± 3.8% and 57.9 ± 6.8% of the hemicord ipsilateral to the injury, respectively (**Figure 7D**). At the epicenter in the 1.2-mm SCI group, damaged tissue included the total dorsal funiculus and parts of the dorsolateral funiculus (**Figure 7A**), whereas in the 2-mm SCI group, areas of spared tissue included a thin margin in the ventral funiculus. The gray matter in the dorsal and ventral horns was completely damaged (**Figure 7B**). The spared area of white matter at the injury epicenter (i.e., ipsilateral side to injury) in the 1.2-mm SCI group was significantly larger than that in the 2-mm SCI group ($3.39 ± 0.29$ mm^2 *vs.* $1.37 ± 0.19$ mm^2, $P < 0.01$).

Correlation between MEP/SEP and forelimb behavioral scores in rats with different severities of unilateral cervical SCI
The correlations between MEP/SEP and behavior scores were evaluated separately for the 1.2 and 2-mm SCI groups (**Figure 8**). When evaluating impairment in gross motor function, the latency and amplitude of SEPs and latency of MEPs in the 1.2-mm SCI group were significantly correlated with behavioral outcomes ($r = -0.80$, -0.72, -0.78 ($P < 0.05$), respectively, for correlation between forelimb locomotor rating scale and SEP latency, SEP amplitude and MEP latency; $r = 0.77$, 0.78, 0.81 ($P < 0.05$), respectively, for correlation between rearing and SEP latency, SEP amplitude and MEP latency; $r = -0.73$, -0.74, -0.61 ($P < 0.05$), respectively, for correlation between staircase and SEP latency, SEP amplitude and MEP latency). In contrast, these functions were not well correlated in the 2-mm SCI group ($P > 0.05$; **Figure 8**). Similarly, in the evaluation of fine motor skills with the staircase test, when the degree of SCI was low, a significant correlation was identified between
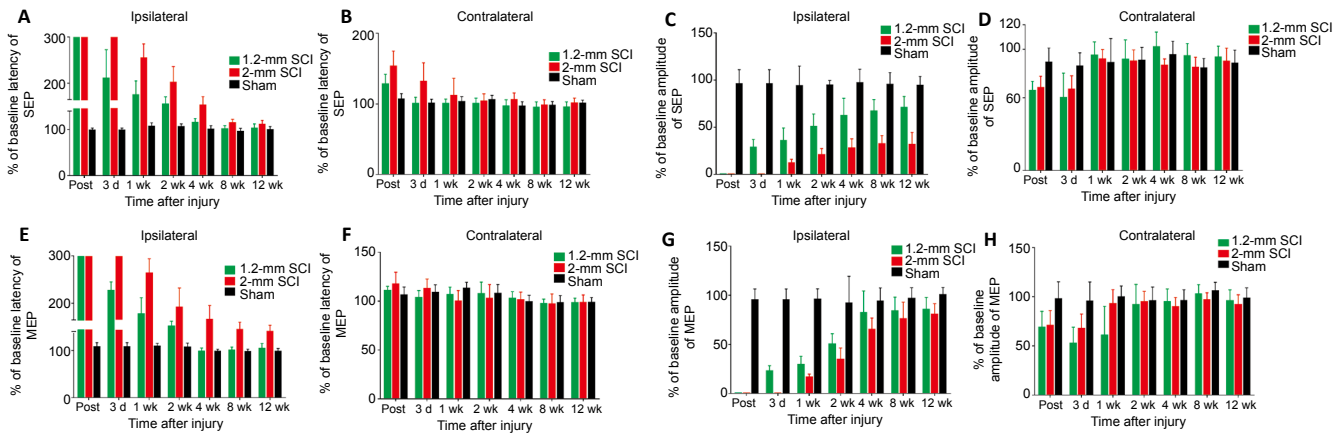
Figure 2 | Forelimb electrophysiological data of rats with unilateral cervical SCI.
(A) SEP latency in the two SCI groups showed a significant trend toward recovery over time. (C) The 2-mm SCI group showed very little recovery of electrophysiological response (SEP amplitude) after SCI. In contrast, the 1.2-mm SCI group showed a significant improvement over time. (E) MEP latency showed improvement and differed in all injury groups over time post-injury. (G) MEP amplitude in the two SCI groups showed significant recovery. (B, D, F, H) Both the amplitude and latency of SEPs and MEPs recorded from the forelimb contralateral to the injury showed a fast and substantial recovery to preoperative levels after a slight reduction post-injury. The data of the ipsilateral forelimb in the sham group was used as the baseline. Data are expressed as the mean ± SEM (1.2 and 2-mm SCI groups: n = 12; sham group: n = 8), and analyzed by repeated-measures analysis of variance followed by Tukey's honestly significant test. MEP: Motor-evoked potential; SCI: spinal cord injury; SEP: somatosensory-evoked potential.
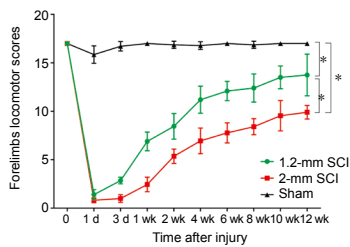

Figure 3 | Ipsilateral forelimb locomotor functional recovery is affected by the degree of displacement of the contusion in rats with SCI (forelimb open field test).
All SCI rats (1.2 mm and 2 mm) showed significant recovery before 6 weeks, and continued to slowly recover towards the end points. Data are expressed as the mean ± SEM (1.2- and 2-mm SCI groups: n = 12; sham group: n = 8). *P < 0.05 (repeated-measures analysis of variance followed by Tukey's honestly significant test). SCI: Spinal cord injury.
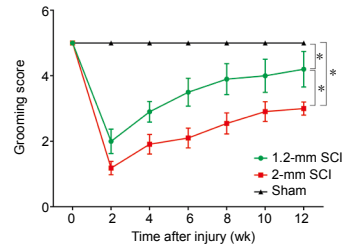

Figure 4 | Ipsilateral forelimb function after C5 hemicontusion differing in displacement extent (grooming test).
(A) Significant recovery was observed in grooming function in the 1.2-mm and 2-mm SCI groups. A more severe grooming deficit of the ipsilateral paw was observed when contusion injury was followed by 2-mm displacement injury. (B) For the contralateral paw, all groups showed similar grooming scores. Data are expressed as the mean ± SEM (1.2- and 2-mm SCI groups: n = 12; sham group: n = 8). *P < 0.05 (repeated-measures analysis of variance followed by Tukey's honestly significant test). SCI: Spinal cord injury.
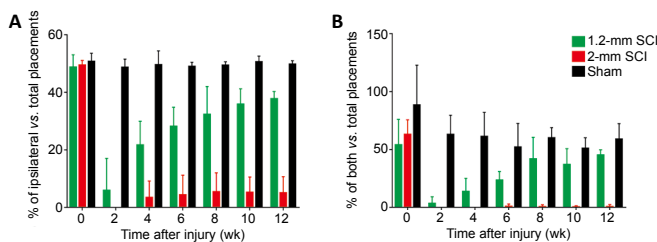
Figure 5 | Forelimb usage during rearing in C5 hemicontused SCI rats (rearing test).
The preference for use of one or both limbs in the rearing test was calculated as the percentage of total limb movement during wall climbing and landing. (A) Ipsilateral forelimb function recovery was evident in the 1.2-mm SCI group, while it was barely detectable in the 2-mm SCI group. (B) The ipsilateral paw was primarily used in the 2-mm SCI group when placed simultaneously with the contralateral paw (dependent use of both paws), and no recovery was observed over time; however, simultaneous usage of both paws as well as usage of the ipsilateral paw were significantly different over time in the 1.2-mm SCI group. Data are expressed as the mean ± SEM (1.2- and 2-mm SCI groups: n = 12; sham group: n = 8), and analyzed by repeated-measures analysis of variance followed by Tukey's honestly significant test. ipsi: Ipsilateral forelimb; SCI: spinal cord injury.
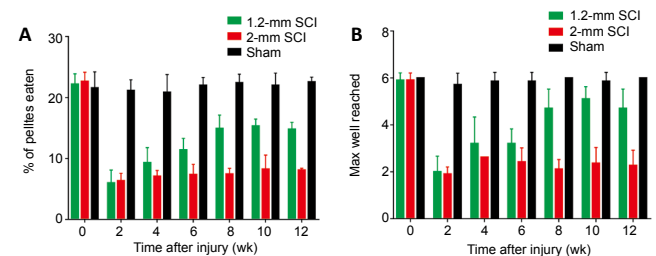
Figure 6 | Effect of C5 hemicontusion and displacement of the contusion on ipsilateral forelimb reaching and grasping performance (Montoya staircase test).
(A) Rats in the 1.2-mm SCI group demonstrated a marked increase in reaching scores; however, no differences in the time course of recovery or extent of reaching were observed in the 2-mm SCI group. (B) Increased maximum reach distance was observed in rats with 1.2-mm SCI over time, while animals in the 2-mm SCI group could only reliably retrieve pellets from well number 2 and sometimes well number 3. Success rates in the Montoya staircase test were measured as the number of pellets eaten and the largest numbered well reached. Data are expressed as the mean ± SEM (1.2- and 2-mm SCI groups: n = 12; sham group: n = 8). *P < 0.05 (repeated-measures analysis of variance followed by Tukey's honestly significant test). ipsi: Ipsilateral forelimb; SCI: spinal cord injury.

MEP/SEP and the number of pellets consumed, and when the degree of damage was severe, only the SEP amplitude was still correlated ($r = 0.67$, $P < 0.05$; **Figure 8**). In general, we noted that the amplitude of MEPs did not correlate well with behavioral outcomes, which may be a result of the large variation in MEP amplitude.

Discussion

Most rehabilitative interventions emphasize the improvement of motor function, but rarely address sensory functions and sensorimotor control. To maximize recovery and develop effective intervention strategies, the effect of the sensory system on the execution of fine motor control underlying spontaneous recovery after SCI needs to be clarified. In the current study, combined examination of SEP and MEP and changes in clinical neurological functions, ranging from gross to fine motor skills, were systematically analyzed in a rat model of SCI. In general, significant recovery of SEPs and MEPs were accompanied by considerable recovery of gross and fine motor skills in rats with mild SCI. In comparison, rats with severe SCI showed significant MEP recovery, but lower SEP recovery, and while these animals had the ability to execute basic motor tasks, their fine motor skills remained substantially impaired. These findings indicate that not only is the improvement of motor pathways crucial to the recovery of fine motor function, but that the sensory conduction system also plays a key role in the regulation of motor control. Moreover, the restoration of sensory signaling pathways in the spinal cord resulted in a better recovery of fine motor function than of gross motor function, which suggests that sensory regulation may be primarily involved in the feedback control of fine movements rather than gross motor function.

Like primates, the digital dexterity of rats in reaching for and grasping small pieces of food seems to be affected by the integrity of the dorsal spinal ascending sensory pathway and the descending motor pathway originating from the neocortex (Kanagal and Muir, 2007). In the present study, we found that the recovery of SEPs was associated with the recovery of fine motor function. Specifically, MEP and SEP responses recovered to more than 80% of baseline in rats with mild injury, which was followed by a marked increase in forelimb usage during wall placements, grooming, overground locomotion, and paw reaching and grasping. However, rats with severe SCI only showed recovery in locomotor ability and joint motion, and showed no recovery of skilled reaching and grasping functions; this was accompanied by significant improvements in MEPs, but a 70% reduction in SEP amplitude. This suggests that sensory pathway in the dorsal column are involved in providing and regulating unique sensory inputs for certain complex behavioral tasks or fine motor skills. Furthermore, the loss of sensory feedback is thought to be related to the reduction and elimination of the ability to distinguish tactile textures, touch frequencies, and the directions of moving tactile stimuli (Ballermann et al., 2001; Kanagal and Muir, 2007). Additionally, we found that MEP and SEP parameters were correlated with gross and fine motor functions in rats with mild injury. In contrast, only SEP amplitude was correlated with behavioral outcome in the severe SCI group, suggesting that SEP amplitude could be a potential indicator of fine motor function.

Behaviorally, spinal dorsal column disruption in rats generates clear motor deficits. Similar to previous studies (Walker et al., 2016), our model exhibited sustained behavioral deficits in the ipsilateral forelimb, with parenchymal damage mainly confined to the ipsilateral side. Motor function deficits after injury were well predicted by the impact parameters, whereby a greater contusion displacement resulted in more severe neurological impairment. Moreover, impaired elbow aiming during limb movement resulted in some imprecise reaches, but less skilled actions, such as joint motion of the limbs, were not significantly affected during locomotion and grooming. However, rats with severe SCI also performed poorly in the rearing test, which may result from impaired feedback control of postural support during vertical exploration (Herter et al., 2015; Kang et al., 2018). In addition, the forepaws on the ipsilateral side of the lesion almost picked up food on the top two wells, without continuing to search along the stairs. In contrast, the forelimbs contralateral to the injury side palpated the well as if searching for the pellets. This indicates that rats with dorsal column lesions failed to realize their failure in detecting food targets. A similar deficit was reported in the probe test, where no food targets were presented, although the rats still reached out and checked for food in their paws (Anderson et al., 2007). These findings reveal that somatosensory feedback is crucial for limb targeting and adjusting movements related to grasping and retracting the forelimbs to bring food to the mouth.

Numerous examples of plasticity have been observed in a multitude of studies on motor recovery in human patients and animal models after SCI (Dunham et al., 2010; Streijger et al., 2013; Yang et al., 2020; Zheng et al., 2020). Dynamic electrophysiological evaluation of sensory and motor pathways has also been performed in various experimental rat models of SCI (Cao et al., 2005; Morris et al., 2017). In the current study, neither SEPs nor MEPs were detectable immediately after cervical SCI, but showed different levels of recovery in the two experimental groups over time, in concordance with previous findings in rats with unilateral graded contusion (Cao et al., 2005). The evoked potentials of rats with mild injury showed earlier signal activity and more obvious recovery compared with rats with severe injury. However, the electrophysiological improvement plateaued after 4 weeks and failed to return to the preoperative level. These results indicate that the rats had a limited ability to establish new connections in the dorsal column system. The reorganization that does occur is not sufficient to completely reactivate the deprived parts of the sensorimotor system (Schlag et al., 2001).

The mechanism underlying the compensation or recovery of interrupted pathways following SCI is not yet completely understood. However, several studies have demonstrated that the spinal cord has the capacity for plasticity and repair following SCI, especially via the lateral sprouting of axons. Indeed, axonal sprouting has been found in corticospinal tracts, reticulospinal tracts and somatosensory pathways following various types of SCI (Raineteau et al., 2002; Bareyre et al., 2005). Tissue staining in the present study revealed that extensive spinal cord tissue destruction was limited to one side, and resulted in white matter demyelination, a reduction in the number of neurons, and cavitation in the gray matter. Although only a small number of axons remained in the dorsal column, we observed considerable functional and electrophysiological recovery in both injury groups. While we did not provide data on plasticity, a previous study showed that the absence of a pathway on one side can be compensated for by the crossover of spontaneous sprouts of axons from the intact tracts on the other side at different levels (Filli et al., 2014).

A limitation of this study is that only general histological changes were observed; quantitative histological evaluation, such as immunofluorescence or immunohistochemical staining, will be carried out in future studies on the underlying mechanisms. In addition, collateral sprouting

in single pathways cannot be independently determined from histological findings, and further functional magnetic resonance imaging and neuroanatomical imaging studies are needed to more fully clarify the compensatory mechanisms.

In summary, we demonstrated correlations between electrophysiological findings and motor functional assessments after unilateral cervical spinal cord contusion in rats. A combination of SEP and MEP recordings can be used to evaluate gross and fine motor functional changes after mild contusion injury to the spinal cord. SEP is a potential tool for evaluating fine motor function in both mild and severe contusion injury. Notably, our findings imply that the ascending sensory pathway in motor regulation has a greater impact on the spontaneous recovery of deprived fine motor skills than gross motor function in the forelimbs.

**Additional files:**
*Additional Table 1:* The impact parameters of unilateral cervical spinal cord injury in rats.
Additional Figure 1: Representative SEP and MEP response waveforms up to 12 weeks after 1.2 mm and 2 mm cervical spinal cord injury from 2 sample rats.

References

Akitake B, Ren Q, Boiko N, Ni J, Sokabe T, Stockand JD, Eaton BA, Montell C (2015) Coordination and fine motor control depend on Drosophila TRPγ. Nat Commun 6:7288.

Alstermark B, Isa T (2012) Circuits for skilled reaching and grasping. Annu Rev Neurosci 35:559-578.

Anderson KD, Gunawan A, Steward O (2007) Spinal pathways involved in the control of forelimb motor function in rats. Exp Neurol 206:318-331.

Ballermann M, McKenna J, Whishaw IQ (2001) A grasp-related deficit in tactile discrimination following dorsal column lesion in the rat. Brain Res Bull 54:237-242.

Bareyre FM, Kerschensteiner M, Misgeld T, Sanes JR (2005) Transgenic labeling of the corticospinal tract for monitoring axonal responses to spinal cord injury. Nat Med 11:1355-1360.

Buen EP, Salgado-Ceballos H, González-Tapia D, Leal-Cortés C, Mondragón-Lozano R, Sánchez-Torres S, Álvarez-Mejía L, Fabela-Sánchez O, Martínez-Torres NI, González-Ramírez MM, Vázquez-Hernández N, González-Burgos I (2017) Spinogenesis and plastic changes in the dendritic spines of spinal cord motoneurons after traumatic injury in rats. Arch Med Res 48:609-615.

Cao Q, Zhang YP, Iannotti C, DeVries WH, Xu XM, Shields CB, Whittemore SR (2005) Functional and electrophysiological changes after graded traumatic spinal cord injury in adult rat. Exp Neurol 191 Suppl 1:S3-16.

Ding WB, Zhang SC, Wu DJ, Zhang YP, Ye HL (2019) Hand function reconstruction in patients with chronic incomplete lower cervical spinal cord injury by nerve segment insert grafting: a preliminary clinical report. J Neurorestoratol 7:129-135.

Dunham KA, Siriphorn A, Chompoopong S, Floyd CL (2010) Characterization of a graded cervical hemicontusion spinal cord injury model in adult male rats. J Neurotrauma 27:2091-2106.

Filli L, Engmann AK, Zörner B, Weinmann O, Moraitis T, Gullo M, Kasper H, Schneider R, Schwab ME (2014) Bridging the gap: a reticulo-propriospinal detour bypassing an incomplete spinal cord injury. J Neurosci 34:13399-13410.

Fouad K, Tse A (2008) Adaptive changes in the injured spinal cord and their role in promoting functional recovery. Neurol Res 30:17-27.

García E, Rodríguez-Barrera R, Buzoianu-Anguiano V, Flores-Romero A, Malagón-Axotla E, Guerrero-Godinez M, De la Cruz-Castillo E, Castillo-Carvajal L, Rivas-Gonzalez M, Santiago-Tovar P, Morales I, Borlongan C, Ibarra A (2019) Use of a combination strategy to improve neuroprotection and neuroregeneration in a rat model of acute spinal cord injury. Neural Regen Res 14:1060-1068.

Harnie J, Doelman A, de Vette E, Audet J, Desrochers E, Gaudreault N, Frigon A (2019) The recovery of standing and locomotion after spinal cord injury does not require task-specific training. Elife 8:e50134.

Herter TM, Takei T, Munoz DP, Scott SH (2015) Neurons in red nucleus and primary motor cortex exhibit similar responses to mechanical perturbations applied to the upper-limb during posture. Front Integr Neurosci 9:29.

Hu Y, Wen CY, Li TH, Cheung MM, Wu EX, Luk KD (2011) Somatosensory-evoked potentials as an indicator for the extent of ultrastructural damage of the spinal cord after chronic compressive injuries in a rat model. Clin Neurophysiol 122:1440-1447.

Huang Z, Li R, Liu J, Huang Z, Hu Y, Wu X, Zhu Q (2018) Longitudinal electrophysiological changes after cervical hemi-contusion spinal cord injury in rats. Neurosci Lett 664:116-122.

Inácio AR, Nasretdinov A, Lebedeva J, Khazipov R (2016) Sensory feedback synchronizes motor and sensory neuronal networks in the neonatal rat spinal cord. Nat Commun 7:13060.

Isomura Y, Harukuni R, Takekawa T, Aizawa H, Fukai T (2009) Microcircuitry coordination of cortical motor information in self-initiation of voluntary movements. Nat Neurosci 12:1586-1593.

Kanagal SG, Muir GD (2007) Bilateral dorsal funicular lesions alter sensorimotor behaviour in rats. Exp Neurol 205:513-524.

Kang Y, Ding H, Zhou HX, Wei ZJ, Liu L, Pan DY, Feng SQ (2018) Epidemiology of worldwide spinal cord injury: a literature review. J Neurorestoratol 6:1-9.

Kato H, Izumiyama M (2015) Impaired motor control due to proprioceptive sensory loss in a patient with cerebral infarction localized to the postcentral gyrus. J Rehabil Med 47:187-190.

Li X, Li G, Luk KDK, Hu Y (2017) Neurorestoratology evidence in an animal model with cervical spondylotic myelopathy. J Neurorestoratol 5:21-29.

Mayer WP, Murray AJ, Brenner-Morton S, Jessell TM, Tourtellotte WG, Akay T (2018) Role of muscle spindle feedback in regulating muscle activity strength during walking at different speed in mice. J Neurophysiol 120:2484-2497.

Morris SH, Howard JJ, El-Hawary R (2017) Comparison of motor-evoked potentials versus somatosensory-evoked potentials as early indicators of neural compromise in rat model of spinal cord compression. Spine (Phila Pa 1976) 42:E326-331.
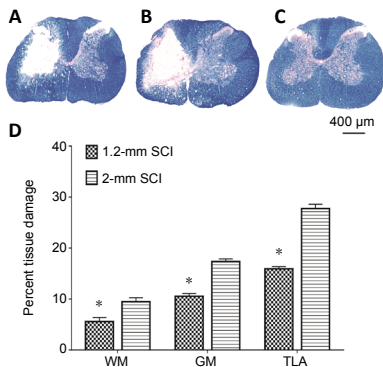
Figure 7 | **Histological changes in rats with different severities of unilateral cervical spinal cord injury.**
(A–C) Spinal cord slices stained with Eriochrome cyanine showing the injury in 3 representative rats: 1.2-mm SCI (A), 2-mm SCI (B), and sham (C) groups. Scale bar: 400 μm. (D) Quantification of the percent of damaged tissue in the 3 groups. Damage was quantified for the gray matter (GM), white matter (WM), and total lesion area (TLA) for each injury group. Laminectomy controls did not show any quantifiable damage (0%), and thus data for this group are not shown. The 1.2-mm and 2-mm injury groups were significantly different for all tissue types. Three slides per rat from the 1.2-mm ($n = 3$), 2-mm SCI ($n = 4$), and sham ($n = 1$) groups. Data are expressed as the mean ± SEM. *$P < 0.05$, *vs.* 2-mm SCI group (one-way analysis of variance followed by Bonferroni *post hoc* tests). SCI: Spinal cord injury.
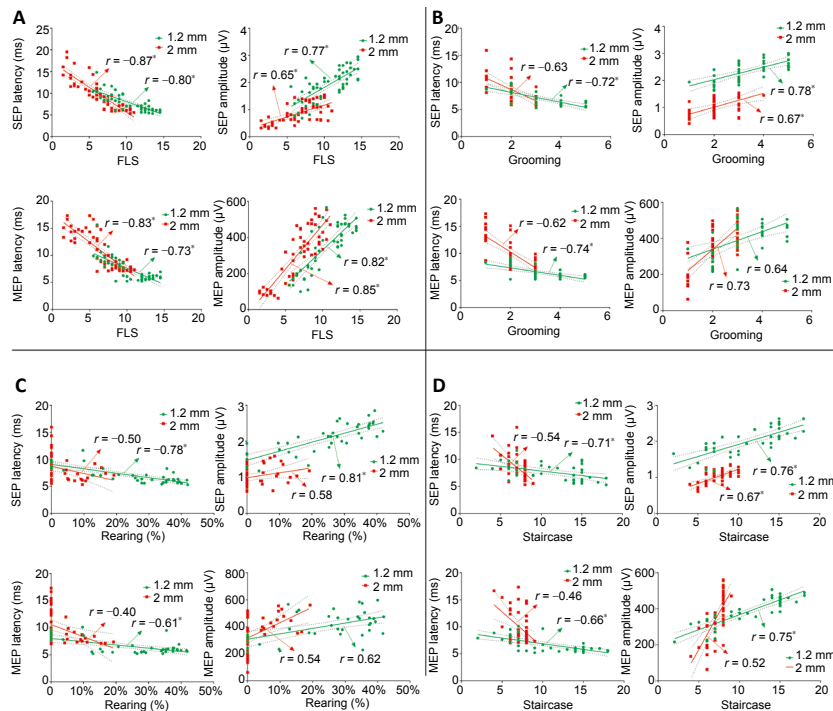
Figure 8 | **Intergroup correlations between electrophysiological assessment and behavioral outcome assessed with Pearson's correlation analysis.**
(A–D) There is a significant correlation between SEP and MEP and FLS, grooming, rearing and staircase in the 1.2-mm SCI group (1.2 mm), but not in the 2-mm SCI group (2 mm). Linear regression lines of best fit are plotted as solid lines, along with 95% confidence intervals as dashed lines. *$P < 0.05$. FLS: Forelimb open field test; MEP: motor-evoked potential; SCI: spinal cord injury; SEP: motor-evoked potential.

Peters AJ, Lee J, Hedrick NG, O'Neil K, Komiyama T (2017) Reorganization of corticospinal output during motor learning. Nat Neurosci 20:1133-1141.

Plunet WT, Streijger F, Lam CK, Lee JH, Liu J, Tetzlaff W (2008) Dietary restriction started after spinal cord injury improves functional recovery. Exp Neurol 213:28-35.

Qi HX, Kaas JH, Reed JL (2014) The reactivation of somatosensory cortex and behavioral recovery after sensory loss in mature primates. Front Syst Neurosci 8:84.

Raineteau O, Fouad K, Bareyre FM, Schwab ME (2002) Reorganization of descending motor tracts in the rat spinal cord. Eur J Neurosci 16:1761-1771.

Schlag MG, Hopf R, Redl H (2001) Serial recording of sensory, corticomotor, and brainstem-derived motor evoked potentials in the rat. Somatosens Mot Res 18:106-116.

Singh A, Krisa L, Frederick KL, Sandrow-Feinberg H, Balasubramanian S, Stackhouse SK, Murray M, Shumsky JS (2014) Forelimb locomotor rating scale for behavioral assessment of recovery after unilateral cervical spinal cord injury in rats. J Neurosci Methods 226:124-131.

Streijger F, Beernink TM, Lee JH, Bhatnagar T, Park S, Kwon BK, Tetzlaff W (2013) Characterization of a cervical spinal cord hemicontusion injury in mice using the infinite horizon impactor. J Neurotrauma 30:869-883.

Takeoka A, Vollenweider I, Courtine G, Arber S (2014) Muscle spindle feedback directs locomotor recovery and circuit reorganization after spinal cord injury. Cell 159:1626-1639.

Varejão AS, Filipe VM (2007) Contribution of cutaneous inputs from the hindpaw to the control of locomotion in rats. Behav Brain Res 176:193-201.

Walker CL, Zhang YP, Liu Y, Li Y, Walker MJ, Liu NK, Shields CB, Xu XM (2016) Anatomical and functional effects of lateral cervical hemicontusion in adult rats. Restor Neurol Neurosci 34:389-400.

Wang WZ, Su YL, Li HF, Shen L, Chen JN, Pan XD, Jia TH (2018) miR-21a-5p knockdown promotes the locomotor function recovery of mouse models of spinal cord injury. Zhongguo Zuzhi Gongcheng Yanjiu 22:5180-5185.

Wei RH, Zhao C, Rao JS, Zhao W, Zhou X, Tian PY, Song W, Ji R, Zhang AF, Yang ZY, Li XG (2018) The kinematic recovery process of rhesus monkeys after spinal cord injury. Exp Anim 67:431-440.

Yang YW, Pan WX, Xie Q (2020) Combined effect of repetitive transcranial magnetic stimulation and physical exercise on cortical plasticity. Neural Regen Res 15:1986-1994.

Zheng Y, Mao YR, Yuan TF, Xu DS, Cheng LM (2020) Multimodal treatment for spinal cord injury: a sword of neuroregeneration upon neuromodulation. Neural Regen Res 15:1437-1450.

Additional Table 1 The impact parameters of unilateral cervical spinal cord injury in rats

Group	Speed (mm/s)	Displacement (mm)	Peak force (N)
1.2-mm SCI	503.25±1.74	1.20±0.01	0.99±0.14*
2-mm SCI	503.16±1.40	2.01±0.02	1.53±0.21

Data are expressed as the mean ± SEM (n = 12). *P < 0.05, vs. 2-mm SCI group (multiple factor repeated-measures analysis of variance followed by Tukey's honestly significant test).
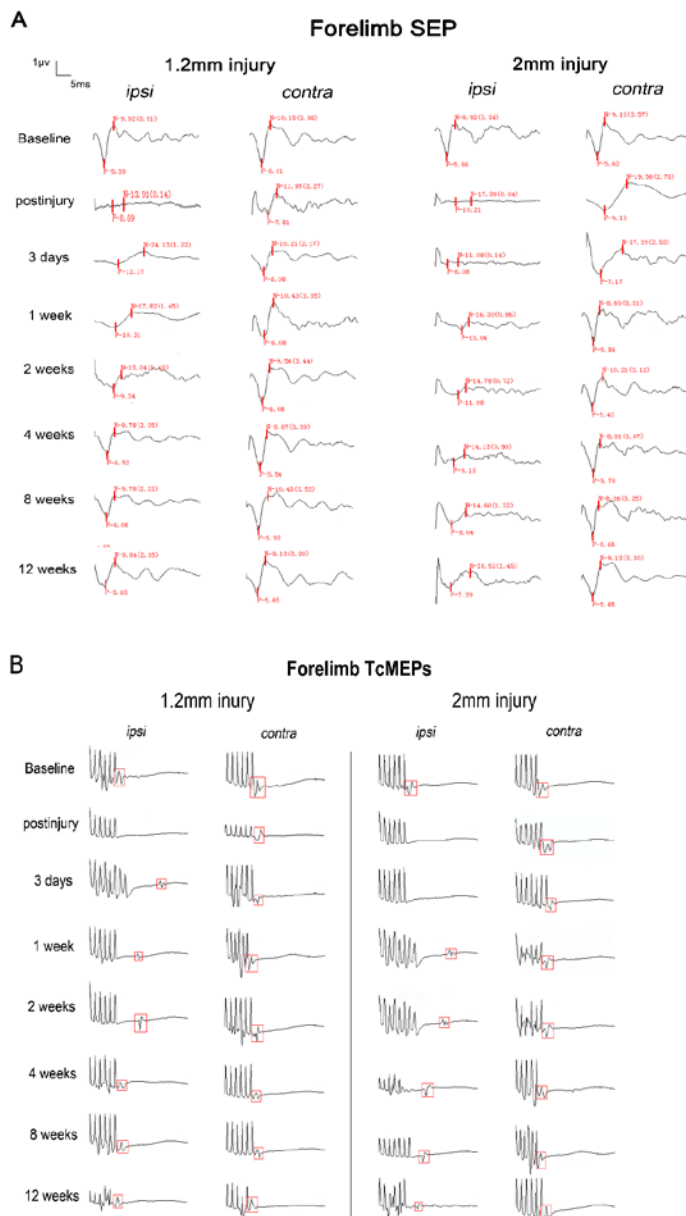
Additional Figure 1 Representative SEP and MEP response waveforms up to 12 weeks after 1.2 mm and 2 mm cervical spinal cord injury from two sample rats.

(A, B) Following 2 mm injury, SEP (A) and MEP (B) ipsilateral injured forelimb improved only marginally. (A) Following 1.2 mm injury, SEP for stimulation of the ipsilateral injured forelimb showed a significant trend toward recovery over time and remained stable over 4 weeks. (B) MEP recorded from the forelimb ipsilateral to injury exhibited a measurable recovery by 4 weeks, whereas responses for MEP and SEP contralateral to injured forelimb for both two injury groups showed no decrease from baseline. contra: Contralateral forelimb; ipsi: ipsilateral forelimb; MEP: motor evoked potential; SEP: somatosensory evoked potential.