

# Electrophysiological evaluation of sensory and motor pathways after incomplete unilateral spinal cord contusion

## Laboratory investigation

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**Object.** Unilateral contusions represent an increasingly popular model for studying the pathways and recovery mechanisms of spinal cord injury (SCI). Current studies rely heavily on motor behavior scoring and histological evidence to make assessments. Electrophysiology represents one way to reliably quantify the functionality of motor pathways. The authors sought to quantify the functional integrity of the bilateral motor and sensory pathways following unilateral SCI by using measurements of motor and somatosensory evoked potentials (MEPs and SSEPs, respectively).

**Methods.** Eighteen rats were randomly divided into 3 groups receiving a mild unilateral contusion, a mild midline contusion, or a laminectomy only (control). Contusions were induced at T-8 using a MASCIS impactor. Electrophysiological analysis, motor behavior scoring, and histological quantifications were then performed to identify relationships among pathway conductivity, motor function, and tissue preservation.

**Results.** Hindlimb MEPs ipsilateral to the injury showed recovery by Day 28 after injury and corresponded to approximately 61% of spared corticospinal tract (CST) tissue. In contrast, MEPs of the midline-injured group did not recover, and correspondingly > 90% of the CST tissue was damaged. Somatosensory evoked potentials showed only a moderate reduction in amplitude, with no difference in latency for the pathways ipsilateral to injury. Furthermore, these SSEPs were significantly better than those of the midline-injured rats for the same amount of white matter damage.

**Conclusions.** Motor evoked potential recovery corresponded to the amount of spared CST in unilateral and midline injuries, but motor behavior consistently recovered independent of MEPs. These data support the idea that spared contralateral pathways aid in reducing the functional deficits of injured ipsilateral pathways and further support the idea of CNS plasticity.

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**KEY WORDS** • corticospinal tract • motor evoked potential • rat •  
spinal cord injury • somatosensory evoked potential • unilateral contusion injury

**C**ONTUSION models of SCI are used to study damage to a number of key motor and sensory pathways that provide regulation of motor movement. It is increasingly important to study and characterize these models because the majority of human SCIs are contusion injuries, whereas penetration injury and complete transection are quite rare.<sup>37,55</sup> Many recent studies have focused

on unilateral injury to the spinal cord.<sup>7,8,21,32,34,38,40,45,46,48</sup> These rodent models are ideal for evaluating specific motor pathways, such as the CST and RST, and for assessing the efficacy of rehabilitative therapies. It has been shown that disruption of the CST and RST in SCI results in behavioral motor deficits.<sup>38</sup> Despite this damage, however, motor behavior improves within several weeks postinjury.<sup>7</sup> Furthermore, it has been shown that forepaw gripping in rats with cervical SCI improves even when the majority of fibers in the CST are disrupted.<sup>8</sup> Rehabilitative training also improved recovery of forelimb and hindlimb function after unilateral contusion injuries.<sup>45</sup> Although these studies

*Abbreviations used in this paper:* BBB = Basso-Beattie-Bresnahan; CST = corticospinal tract; MEP = motor evoked potential; ROI = region of interest; RST = rubrospinal tract; SCI = spinal cord injury; SSEP = somatosensory evoked potential.

## Functional assessment of unilateral contusion injury

that point to recovery are promising, unilateral contusion studies rely heavily on motor behavior scoring and histological evidence to make their assessments.<sup>21,32,34,40,45,46,48</sup> Therefore, it is important to characterize the functional integrity of the motor pathways in addition to gross behavioral improvements.

Motor evoked potentials represent a reliable method of quantifying the functionality of motor pathways.<sup>17,18</sup> Specifically, MEPs have been extensively used to evaluate the integrity of descending motor tracts, such as the CST and RST, following SCI.<sup>1-6,17,18,26,31</sup> Motor evoked potentials are the electromyographic response of muscle in the periphery that are a result of stimulation of the motor cortex using electrical current or transcranial magnetic stimulation. Somatosensory evoked potentials represent an objective, quantitative measure of sensory pathway continuity<sup>2-5</sup> and are used by clinicians to assess spinal cord damage in humans.<sup>20</sup> Here, we present electrophysiological data in conjunction with histological quantification and behavioral scores that reflect the sparing and recovery of motor pathways after mild unilateral injury, an assessment that has not yet appeared in the literature. We also report that sensory pathways undergo less functional damage after a unilateral injury than a midline injury for the same amount of white matter tissue damage.

Both somatosensory and motor pathways have been shown to generate new lateral axonal sprouts following spinal cord lesions,<sup>19,39,56</sup> which supports CNS plasticity and functional repair. The CST in rats is located in the ventral part of the dorsal columns. Lesions to the dorsal CST in rats have been shown to lead to new collaterals that originate from myelinated axons,<sup>9,10</sup> and these new collaterals have been shown to form synapses with motor neurons.<sup>9</sup> Furthermore, regenerated axons through the lesion area have been reported to aid in open-field behavioral recovery.<sup>29</sup> In unilateral injuries, it has been shown that the outgrowth of CST axons terminates in the ipsilateral impaired side of the spinal cord.<sup>14</sup> The results presented here quantify the function of spinal tracts after a clinically relevant unilateral injury and support the idea of plasticity following injury.

### Methods

All experimental procedures were in accordance with the guidelines provided in the Rodent Survival Surgery manual and were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University. We sought to evaluate the extent of damage to the CST as the result of a mild unilateral injury by using SSEPs and MEPs recorded from pathways both ipsilateral and contralateral to the SCI. We used histological data to supplement electrophysiological data to draw a link between the functional integrity of the spinal cord and anatomical preservation of the spinal tracts. Motor behavior of injured rats was rated using the BBB score. Seventeen adult female Lewis rats (Charles River Laboratories, Inc.), with an average body weight of 230 g, were used for this study. Prior to electrode implantation, each rat was randomly assigned to 1 of 4 injury groups: midline injury (3 rats), right unilateral injury (5 rats), left unilateral injury (4 rats), or laminectomy

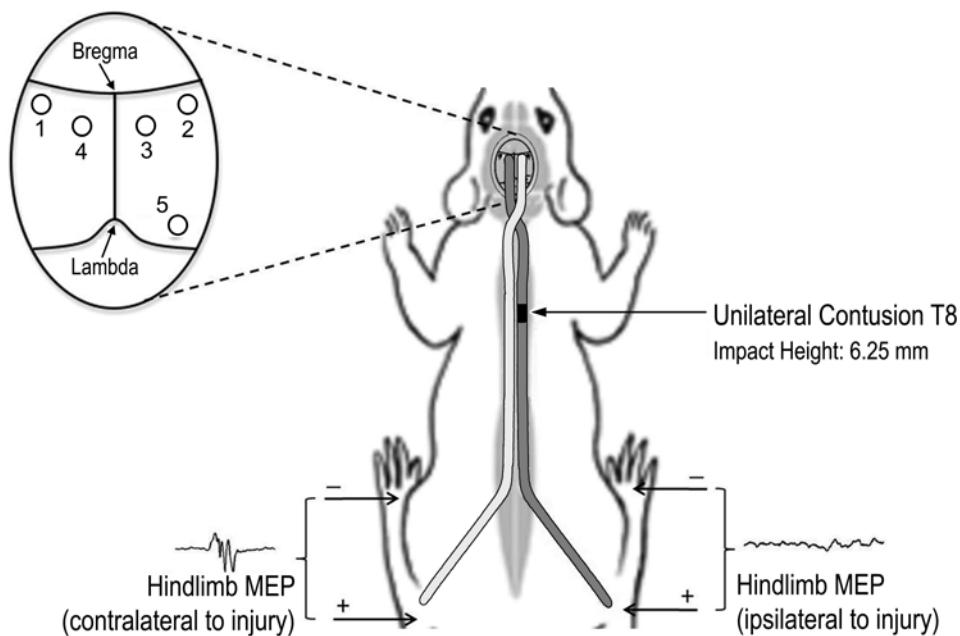
(5 rats). For data analysis, the right and left unilateral injury groups were combined (9 rats), and hindlimbs were considered as either ipsilateral or contralateral to the site of injury. The rats were housed in individual cages and given free access to food and water.

### Electrode Implantation

Rats were anesthetized and an incision was made along the midline of the skull. Five bur holes were drilled into the cleaned and exposed part of the cranium using a standard dental drill (Fine Science Tools, Inc.). As shown schematically in Fig. 1, 4 of these holes corresponded to the right and left hemisphere sensorimotor cortical areas for the hindlimbs and forelimbs. Forelimb sites are located 0.2 mm posterior to the bregma and 3.8 mm laterally from the bregma, and hindlimb sites are located 2.5 mm posterior to the bregma and 2.8 mm laterally from the bregma. The fifth hole was drilled 3.0 mm to the right of the lambda to serve as an intracranial reference. Transcranial screw electrodes (Plastics One, Inc.) were then screwed into the holes. Care was taken to ensure that the electrodes made very light contact with the dura mater without compressing the dura or cortex. Random histological examinations were performed to ensure that there was neither formation of hematoma nor damage to brain structure as a result of screw electrode implantation. The distal end of each electrode held an insulated 10-mm conductive wire with very low impedance and was inserted into the slot of an electrode pedestal (Plastics One, Inc.). Carboxylate dental cement (3M) was used to hold the screw electrodes and electrode pedestal permanently in place. The skin incision was closed with a 4-0 suture, and 2% lidocaine gel was applied.

### Spinal Cord Injury

A standard MASCIS impactor (W. M. Keck Center for Collaborative Neuroscience) was used to induce the contusive injury in rats. Laminectomy was performed to remove the laminae of T-8 and T-9 to expose the dorsal surface of the spinal cord. Laminae are the posterior pedicles of the vertebral arch. Stabilization clamps were used to immobilize the T-6 and T-12 vertebrae to support the spinal column during impact. The T-8 was placed directly under the vertical shaft of the mechanical impactor. In the case of a unilateral insult, we offset the position of the probe by 0.8 mm laterally (either left or right) to induce an insult weighted toward one side of the spinal cord. The impactor (2-mm gauge and 10 g) was adjusted to a height of 6.25 mm to induce a mild midline or unilateral injury. The probe was then released and allowed to fall freely onto the rat's spinal cord. The impact trajectory, height, velocity, and duration of impact were recorded by a computer and were compared with expected values to ensure consistency across all injuries. The rat was removed from the device, and its paravertebral muscles and skin were sutured. The entire procedure was performed while the rat was under gaseous anesthesia consisting of a mixed flow of 2% isoflurane, 80% oxygen, and room air at a flow rate of 2 L/minute. The rat was placed on an electric heating pad to maintain body temperature at 37°C ± 0.5°C throughout the experiment. An adequate level of anesthe-



**FIG. 1.** Schematic of the setup for unilateral injury at T-8 (right) and placement of cranial screw electrodes over the sensorimotor cortex (left). Numbered electrodes correspond to the right forelimb (1), left forelimb (2), left hindlimb (3), and right hindlimb (4) regions of the cortex and the reference (5). The example MEPs correspond to a right unilateral injury. “Contralateral to injury” or “ipsilateral to injury” refers to MEPs recorded from the limb respective to the side of unilateral insult. The *gray pathway* indicates the injured motor tracts corresponding to a right unilateral injury.

sia was determined by monitoring the corneal reflex and limb withdrawal to pinch stimuli.

#### Electrophysiological Analysis

We used a neurophysiology monitoring setup for a multilimb acquisition of SSEPs and MEPs, as previously described.<sup>2–6,13,26,36</sup> Two baseline recordings were taken prior to injury or laminectomy, followed by recordings obtained at Days 7, 14, and 28 after injury.

For SSEPs, animals were anesthetized with 1.5% isoflurane, and evoked potentials were recorded simultaneously from each of the 4 implanted electrodes while sequentially stimulating each of the 4 limbs. Subdermal needle electrodes (Safelead F-E3–48, Grass Technologies) were used to stimulate the median and tibial nerves of the forelimbs and hindlimbs, respectively, and a reference electrode was placed subdermally at the neck. Current pulses of amplitude 3.5 mA and a duration of 200  $\mu$ sec were delivered at a frequency of 0.25 Hz so that each limb received a pulse once per second (1 Hz). For each recording session, 300 sweeps were recorded per electrode per limb.

For MEPs, animals were anesthetized via an intraperitoneal injection of a mixture of 43.5 mg/kg of ketamine, 4.3 mg/kg of xylazine, and 0.17 mg/kg of atropine sulfate. As isoflurane has been shown to severely depress MEP responses, this ketamine mixture was used for MEP recordings to acquire the most reliable signal possible.<sup>1</sup> Recording needle electrodes (Safelead F-E3–48) were inserted intramuscularly into the tibialis anterior muscle of the hindlimbs. Reference electrodes were inserted into the footpad of the corresponding limb, and a ground electrode was inserted subdermally at the base of the tail. Bipolar stimulation between 1 of the 4 cortical screw electrodes

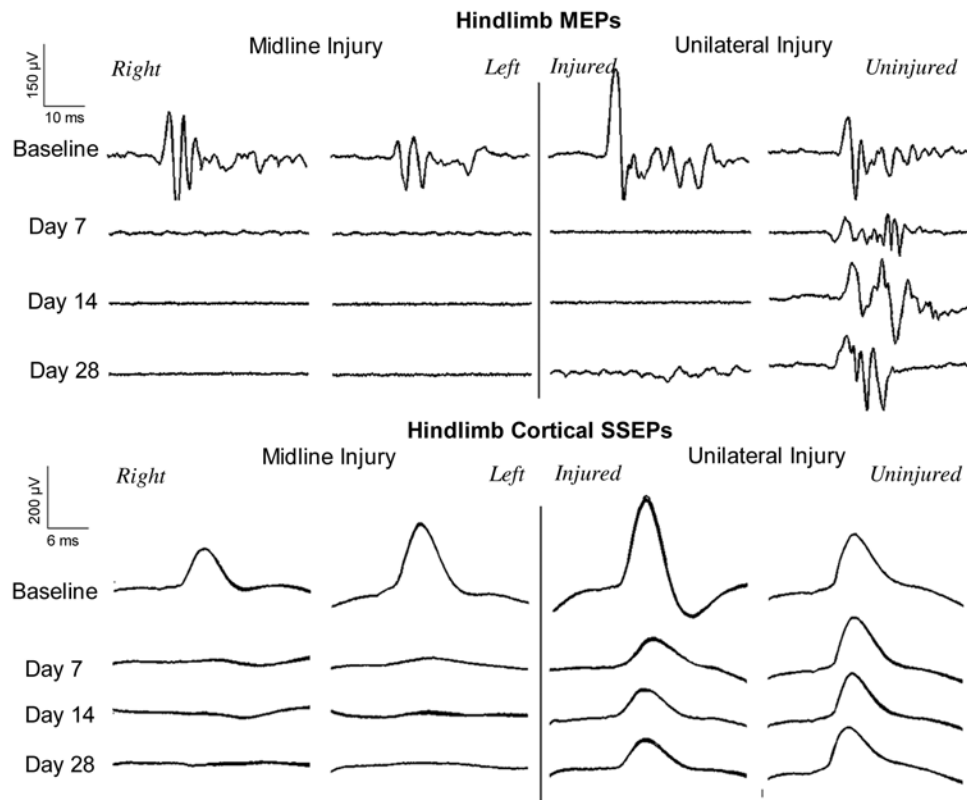
and the frontal screw was used to deliver a train of 5 pulses at 15.1 Hz, with each pulse 200  $\mu$ sec in duration. The simulation intensity varied between 3.5 and 12 mA, depending on the threshold stimulation at which muscle activity was found during baseline recordings; the threshold value was determined as the intensity eliciting an observable twitch in the limb but without excessive head shaking. Recordings were performed with an intensity 50% above threshold. Each cortical electrode individually applied a pulse train every 2 seconds to acquire a minimum of 30 sweeps from each limb per recording session.

Somatosensory evoked potentials were recorded using an RA4LI headstage, and MEPs were recorded using an RA16LI-D low-impedance headstage (both from Tucker-Davis Technologies). All signals were sampled at 4882 Hz using an RA16 Medusa Base Station and digitally recorded using OpenEx recording software (Tucker-Davis Technologies) to a personal computer. Somatosensory evoked potential signals were additionally amplified with a gain of 20,000 using an RA4PA Medusa PreAmp (Tucker-Davis Technologies).

#### Signal Processing

To improve the signal-to-noise ratio, 3 mean sweeps were computed per limb per recording by averaging 100 consecutive stimulus-locked sweeps or 10 consecutive stimulus-locked sweeps for SSEP and MEP, respectively (Fig. 2). A custom peak detection algorithm was used for computing the peak-to-peak amplitude and latency for each mean sweep, within the window of 5 and 30 msec after the time of stimulus. The average amplitude and latency were computed for each experimental group. All data reported represent the means  $\pm$  standard error. Statistical analysis

## Functional assessment of unilateral contusion injury



**Fig. 2.** Representative MEP (upper) and SSEP (lower) response waveforms up to Day 28 after midline and unilateral SCI from 2 sample rats. Following midline injury, MEPs were undetectable, whereas SSEPs improved only marginally. Following unilateral injury, MEPs recorded from the hindlimb ipsilateral to injury (unilateral injured) exhibited a measurable recovery by Day 28, whereas those recorded from the hindlimb contralateral to injury (unilateral uninjured) showed a near full recovery to baseline levels. Somatosensory evoked potentials for stimulation of the ipsilateral injured hindlimb showed a slight decrease that remained constant over the 28 days, whereas those for stimulation of the contralateral uninjured hindlimb showed no decrease from baseline.

was performed using a 2-tailed unpaired t-test when comparing 2 injury groups and using a 2-tailed paired t-test when determining statistical significance over baseline values within 1 experimental group. Statistical significance was considered as  $p < 0.05$ .

### Motor Behavior Scoring

The 21-score BBB Locomotor Rating Scale<sup>1-6,11,12</sup> was used to validate the motor behavior of each animal before and after injury. Two well-trained examiners, blinded to the injury groups, evaluated and scored the motor behavior of each animal for the left and right sides individually. Scoring was performed before injury and on Days 1, 7, 14, 21, and 28 after injury.

### Histological Assessment

Rats were killed via transcardial perfusion with Dulbecco phosphate-buffered saline (GIBCO) and 4% paraformaldehyde solution (Electron Microscopy Sciences). The spinal cord was carefully extracted from the vertebral column, postfixed in 4% paraformaldehyde followed by a 30% sucrose solution for 24 hours, and embedded in paraffin. Spinal cords were sliced and fixed on glass slides. The slides were stained with H & E to assess the morphology of the injury and to quantify the percent damage of the

gray matter, white matter, and CST. Percent damage was calculated using the NIS-Elements Advanced Research software package (Nikon Instruments, Inc.). In brief, binary masks were created based on predetermined control threshold values for pixel composition, defining damage versus undamaged areas of the spinal cord slice image. For each histology image, individual ROIs were created by hand, outlining the gray matter, white matter, and CST. The binary masks were then restricted to each ROI to report the percent damage within the respective ROI, output in  $\mu\text{m}^2/\mu\text{m}^2$ . Three histological slices through the epicenter of injury were analyzed for each rat, and the mean percent  $\pm$  standard error was reported.

## Results

### Descending Motor Pathways

Following contusive SCI to one-half of the spinal cord, MEPs were recorded from each hindlimb either contralateral or ipsilateral to the injury during stimulation of the respective contralateral motor cortex via the implanted cortical electrodes (Fig. 1). In a similar fashion, SSEPs were recorded from the same cortical electrodes during stimulation of the tibial nerve either contralateral or ipsilateral to

injury. The MEPs and mean SSEPs are shown in Fig. 2 for representative rats from each injury group. Baseline evoked potentials recorded prior to injury are shown as a reference for each rat. Hindlimb MEPs for the midline-injured rat were completely abolished after the contusion injury and did not recover even by Day 28. Similarly, MEPs measured from the hindlimb ipsilateral to injury (unilateral injured) were also abolished after injury but showed a slight recovery by Day 28. Motor evoked potentials recorded from the hindlimb contralateral to injury (unilateral uninjured) showed substantial recovery of function.

Group results were quantified using mean amplitude (Fig. 3a). Motor evoked potential amplitudes for the 2 sides of the unilateral injured group were significantly different for all days following injury. Furthermore, although MEP amplitude disappeared in the midline-injured group and showed no observable recovery, the unilateral injured group displayed slight recovery by Day 28 ( $15 \pm 8 \mu\text{V}$ ,  $p = 0.007$ , compared with midline), suggesting partial recovery of motor tracts in a unilateral injury but not a midline injury. For all injury groups, the mean MEP amplitudes were significantly different from the corresponding baseline amplitude at all days postinjury. Although both left and right sides of the spinal cord, specifically the motor neuro pathways, sustained some degree of injury during unilateral impact, the injury was significantly greater on the injured side.

#### Ascending Sensory Pathways

For the midline-injured rats, SSEPs were reduced in amplitude but still present, unlike the MEPs, which were completely abolished. The SSEPs corresponding to unilateral injured pathways as a result of stimulation of the injured hindlimb were reduced compared with baseline, although they maintained a consistent shape and amplitude for the duration of the study. Furthermore, SSEPs of the unilateral injured pathways exhibited noticeably greater amplitude than those of the midline-injured rats, even at

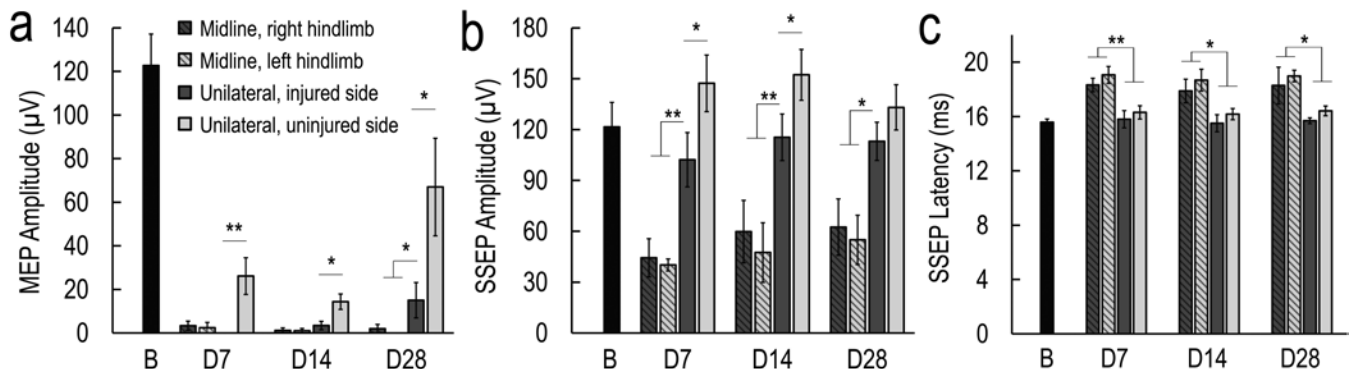
Day 7 after injury ( $42 \pm 8 \mu\text{V}$  and  $102 \pm 16 \mu\text{V}$  for unilateral injured and midline injury groups, respectively;  $p = 0.01$ ). Finally, we noted that SSEPs due to stimulation of the uninjured hindlimb in unilateral rats did not significantly differ from baseline ( $p = 0.80, 0.67, \text{ and } 0.77$  for Days 7, 14, and 28, respectively) and were consistent over the course of the experiment.

As with the MEPs, group results were quantified using mean amplitude; latency was used as a second parameter to evaluate the conduction rate (Fig. 3b–c). With respect to sensory pathways, SSEP amplitudes recorded from cortex were significantly different between the 2 sides of the spinal cord in unilateral injury at Days 7 and 14 after injury. However, the significance between these groups reduced over time and was not maintained at Day 28.

More notably, SSEP amplitudes of pathways ipsilateral to injury in the unilateral group were significantly greater than those in the midline-injured group for all recording days ( $p = 0.01, 0.008, \text{ and } 0.016$  for Days 7, 14, and 28, respectively). One can conclude that for the same severity of contusion, the unilateral injury undergoes less functional damage to somatosensory pathways. The latency of SSEPs, which conveys information about the speed at which the signal travels to the cortex, was not significantly different between the injured and uninjured sides of the unilateral group ( $p = 0.32$  at Day 7). Furthermore, neither side of the unilateral injury was significantly different from the baseline latency. However, the latency was on average 2.55 msec greater in the midline-injured group than in the unilateral group over the course of 28 days.

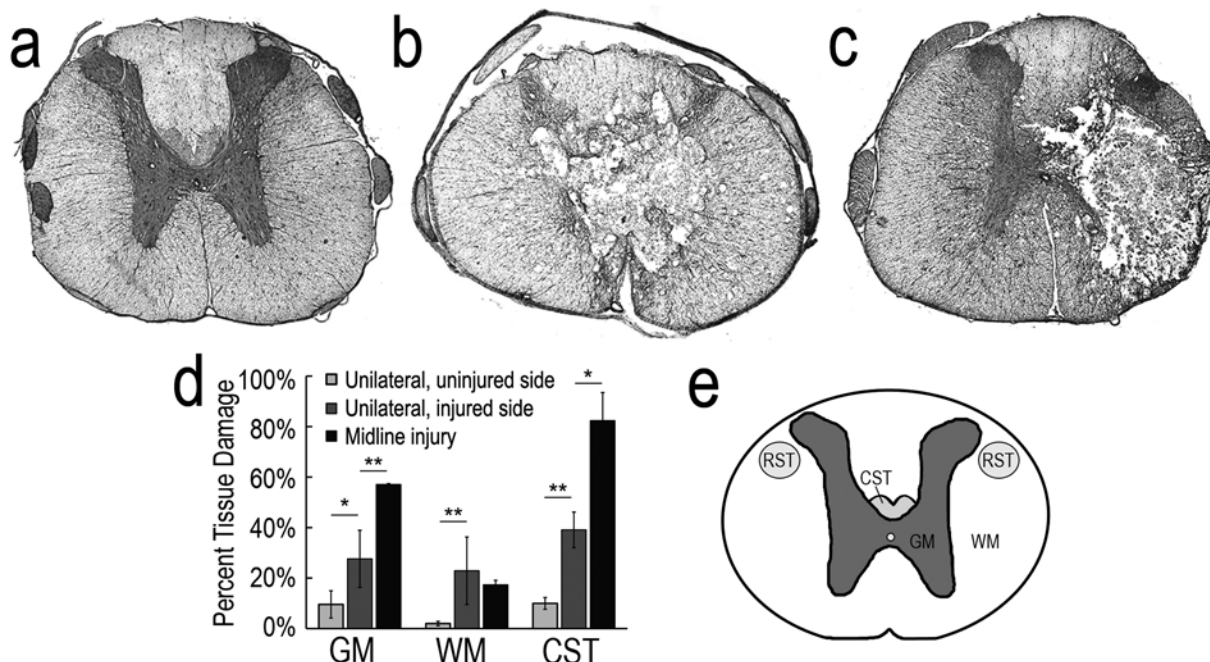
#### Histological Evaluation of Tissue Damage

In addition to the electrophysiological analysis, we performed a histological assessment of spinal cord slices from the epicenter of injury after Day 28. Spinal cord slices for representative rats from the laminectomy, midline injury, and unilateral injury groups are shown in Fig. 4a–c. Laminectomy controls showed no visible damage to any



**Fig. 3.** Bar graphs showing group results comparing hindlimb electrophysiological data for midline (3 rats) and unilateral (9 rats) injuries. **a:** Motor evoked potential amplitude for the midline-injured group was significantly different from that for the uninjured limbs of the unilateral group for all days and was significantly different from the injured limbs of the unilateral group at Day 28, which suggests partial recovery of motor tracts. All injury groups were significantly different from baseline at all days. **b:** Somatosensory evoked potential amplitude was significant between the 2 sides of the unilateral injury group for all days, and those in the unilateral injured group and midline-injured group were significantly different for all days, and those in the midline injury group and baseline were significantly different for all days. **c:** Somatosensory evoked potential latencies between midline and both sides of the unilateral injury were significant for all days; differences between the 2 limbs in the unilateral group, as well as between the 2 limbs and baseline, were not significant for any day; and SSEP latencies for midline injury were significantly different from baseline for all days. Values are reported as the means  $\pm$  SE. \* $p < 0.05$ . \*\* $p < 0.01$ . B = baseline; D7, . . . 28 = Day 7, . . . Day 28.

## Functional assessment of unilateral contusion injury



**FIG. 4.** Stained spinal cord slices showing the injury of 3 representative rats: laminectomy control (a), midline injury (b), and unilateral injury (c). Bar graph (d) showing quantification of the percent of damaged tissue in the 3 groups. Schematic (e) of the rat mid-thoracic spinal cord. Damage was quantified for the gray matter (GM), white matter (WM), and CST for each injury group. Laminectomy controls did not show any quantifiable damage (0%), and thus data for this group are not shown. The 2 sides in the unilateral group were significantly different in all tissue types. The midline and unilateral uninjured groups were significantly different in all tissue types, whereas differences between the midline and the unilateral injured side were significantly different only in the gray matter and the CST. Three slides per rat from the midline contusion (2 rats), unilateral (4 rats), and laminectomy (1 rat) groups. \* $p < 0.05$ . \*\* $p < 0.01$ . H & E, original magnification  $\times 20$ .

area of the spinal cord. Midline-injured rats showed extensive damage localized to the midline area comprising the CST and gray matter, with considerable damage in the surrounding white matter tissue as well. Unilateral injury rats showed very similar damage on the injured side (extensive damage to the CST, gray matter, and white matter) with limited damage to some areas of the CST and gray matter on the side contralateral to contusion. These findings were quantified using imaging software that evaluated the pixel composition of each histological image, and the results are presented in Fig. 4d as the percent damage of each tissue. There was no quantifiable damage associated with the laminectomy group; therefore, this group was not included in the quantified results.

The percent damage found in the gray matter and the CST revealed similar trends among the remaining 3 groups (unilateral uninjured, unilateral injured, and midline groups). The uninjured unilateral side showed very little damage to either region, followed by the injured side, which showed  $28\% \pm 11\%$  and  $39\% \pm 7\%$  damage to the gray matter and CST, respectively. Finally, the midline contusion group showed the most damage with  $57\% \pm 1\%$  and  $82\% \pm 11\%$  damage to the gray matter and CST, respectively. Next, we compared the unilateral injured group with the midline injury group. The differences between these groups were significant in gray matter and CST ( $p < 0.001$  and  $p = 0.01$ , respectively); however, the difference in white matter damage was not significantly different between these groups ( $p = 0.38$ ).

### Motor Behavior Scores

We present gross motor behavior data, evaluated using the BBB scale, as supplemental data to the electrophysiological and histoanatomical characterizations. Two expert experimenters blinded to the type of injury independently scored the left and right hindlimbs of each rat. The scores for rats with a unilateral injury were divided respective to the side of injury, whereas the left and right hindlimb scores for the midline group were averaged. Figure 5 shows gradual motor recovery over the course of 28 days for all injury levels, with recovery beginning as early as Day 1 after injury. The BBB scores for the midline injury, unilateral injured, and unilateral uninjured group plateaued at  $16.0 \pm 0.5$ ,  $18.7 \pm 0.6$ , and  $19.7 \pm 0.3$  points, respectively. Accordingly, the trends for each injury group showed that BBB scores correspond to the severity of injury. On any given day, the unilateral injury groups had higher BBB scores as compared with the midline injury group. Moreover, uninjured limbs of rats in the unilateral group always exhibited higher BBB scores as compared with those in the unilateral injured group ( $p < 0.1$  at Days 4, 7, and 14).

### Discussion

Hindlimb motor function in mammals is primarily controlled by 2 descending motor tracts in the spinal cord: the CST and the RST.<sup>41</sup> In rats, the CST is located in the ventral part of the dorsal column of the spinal cord and

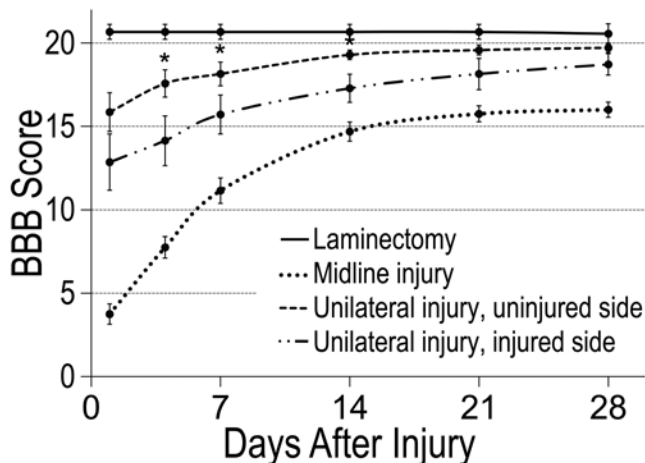


Fig. 5. Graph showing the BBB scores for hindlimb motor function at 4 weeks postinjury. Limbs were scored independently by observers unaware of the rats' injuries. For midline and laminectomy groups, the average of the 2 limbs was used; for the unilateral groups, limb scores were plotted separately, according to side. At Day 28, the mean BBB scores plateaued at  $20.6 \pm 0.6$  for the laminectomy (control) group,  $16.0 \pm 0.5$  for the midline injury group,  $19.7 \pm 0.3$  for the unilateral uninjured group, and  $18.7 \pm 0.6$  for the unilateral injured group. A statistically significant difference was found between midline and unilateral injuries. \* $p < 0.05$ .

can be easily damaged by any dorsal impact or contusive injury. Rubrospinal tracts are located in dorsolateral sides of the spinal cord in rats (Fig. 4e). Many studies have reported that even slight damage to the rat spinal cord results in the complete disappearance of MEP responses, whereas motor behavior recovers during the weeks following injury.<sup>31,47</sup> Several studies specifically modeling unilateral contusion injuries have focused on behavioral and histological data characterizing the extent of injury and recovery.<sup>21,32,34,40,45,46,48</sup> We sought to determine the extent of damage to these pathways in a mild unilateral contusion injury, an injury that is clinically relevant in a large number of patients with SCI. We assessed the functional integrity of the descending motor pathways as well as the ascending somatosensory pathways by using electrophysiology, and we quantified the percent of anatomical tissue damage in stained slices of each injured spinal cord that had been taken through the epicenter of the injury.

Our results showed that after a mild midline contusion injury, MEP responses are completely absent. However, after a unilateral injury, MEP responses measured from the hindlimb of the uninjured side during stimulation of the respective contralateral sensorimotor cortex recover significantly by Day 28 after injury. Even pathways through the injured side of the spinal cord show a partial recovery by Day 28 (Fig. 3a). Furthermore, the MEP recovery trends of these 3 groups closely corresponded with the amount of spared tissue quantified in stained spinal cord slices (Fig. 4); that is, a larger amount of tissue damage led to a greater reduction in MEP amplitude.

In the midline injury group, the contusion injury resulted in about 90% damage to the CST, and we observed no recovery of MEPs. This result was expected, as the large majority of CST fibers were compromised during injury. However, in the unilateral injury group, the side

contralateral to the contusion sustained only about 10% damage to the CST, and yet we still observed significantly reduced MEPs at Day 28. Therefore, 10% damage to the CST resulted in an approximately 50% reduction in MEP responses. Similarly, on the side ipsilateral to the unilateral contusion, although more than half of the CST tissue was spared ( $39\% \pm 7\%$  damage), MEPs only marginally recovered. These data suggest that although MEPs do correspond to the amount of spared CST fibers, small amounts of anatomical damage to the CST may cause more pronounced functional deficits in the pathways.

Although damage contralateral to injury was initially unintended, our subsequent study using MEPs revealed that the CST is sensitive to even mild tissue damage. We also showed that by using our well-calibrated weight drop—whose speed, impact moment, and cord compression were monitored—we were able to detect and quantify the small amount of damage that was incurred contralateral to the contusion. This is clinically important because it will allow the natural progress of human SCIs to be precisely monitored. These findings should be considered in future studies that seek to induce a unilateral injury, and care should be taken when assessing functional outcomes for both the ipsilateral and contralateral sides. It is worth noting that human CSTs are situated in the ventral and lateral columns of the spinal cord. This anatomical difference between rats and humans will play an important role in the interpretation of therapeutic outcomes aimed at the restoration of CST neurons, for which preliminary data are acquired using a rat model.

Using our controlled impactor, we were able to deliver injury to either the right or left side of the spinal cord, damaging essentially one-half of the dorsal ascending sensory pathways, as indicated by the histological tissue evaluation (Fig. 4d). To determine the functional effects of unilateral injury on the sensory pathways, we also evaluated SSEPs upon stimulation of each limb, measured from the respective contralateral sensorimotor cortex. The midline injury group displayed SSEPs with significantly reduced amplitude and increased latency with respect to baseline, in agreement with previous reports.<sup>2,4,5,24</sup> However, SSEPs recorded from the pathways corresponding to the injured side of the unilateral injury, which received the same contusion injury as the midline group, exhibited SSEPs with significantly greater amplitudes as compared with the midline-injured group. Strikingly, the latency of SSEPs for the uninjured unilateral side was not significantly different from baseline. Therefore, although the signal was slightly reduced in amplitude, it was not delayed with respect to baseline SSEPs. Combining these findings with histological data, the midline-injured spinal cords and the injured side of the unilateral injured spinal cords showed comparable levels of white matter damage (no significant difference,  $p = 0.38$ ). Yet, for the same amount of tissue damage, the unilateral group showed SSEPs with higher amplitude and lower latency, 2 indicators of preserved functional integrity of sensory pathways. In the uninjured side of the unilateral injury group, the percent of damaged white matter was negligible, and similarly SSEP amplitude and latency were not significantly different from baseline, as expected. These findings suggest that the uninjured, spared

## Functional assessment of unilateral contusion injury

pathways on the side contralateral to contusion injury in unilateral injured spinal cords may contribute to the functional recovery of contralateral limbs.

The complete mechanism used by the spinal cord to promote compensation or recovery of injured pathways has yet to be fully elucidated. However, a number of studies have demonstrated that the spinal cord is capable of plastic behavior and repair following SCI, particularly via the lateral sprouting of axons. Axonal sprouting has been observed in the CST,<sup>9,10,14,22,34,53,54</sup> RSTs,<sup>42–44,51</sup> and somatosensory pathways,<sup>27,52</sup> following a variety of SCIs. Training and exercise have also been shown to promote plasticity and axonal sprouting in the spinal cord after SCI.<sup>23,30,35,49</sup> Although we do not present explicit data that illustrate plasticity, our current findings are in line with our preliminary data<sup>13</sup> and data from other previous studies that support spinal plasticity after a mild injury. We observed reduced deficits of electrical conductivity for ipsilaterally contused pathways in comparison with midline-contused pathways. Despite the same magnitude of contusion injury and the same degree of white matter tissue damage in the midline and unilateral injuries, the unilateral group displayed significantly reduced deficits in neurotransmission: the amplitude of unilateral SSEPs for the injured side was more than 50  $\mu$ V greater than the midline SSEP amplitude ( $p < 0.05$ ), and the latencies of unilateral SSEPs were not increased from baseline. Latency measurements reflect the speed at which the neural signals reach the brain after peripheral nerve stimulation. The lack of increased latencies in the unilateral injured group may suggest that SSEPs are rerouted through uninjured pathways. The number of spared fibers after SCI is inversely proportional to the severity of the contusion. Hence, depending on the severity of contusion injury, a number of pathways that span the lesion are always spared postinjury and may remain functional. The latencies of both the injured and uninjured sides of the unilateral contusion did not increase over baseline. These SSEPs may be traveling through spared pathways contralateral to the injury or simply through spared ipsilesional pathways. Similar to other authors,<sup>25</sup> we have observed the white matter and confirmed the presence of spared fibers in both sides of the spinal cord. However, the individual pathways cannot be determined from the histological data alone. Further studies, such as neuroanatomical imaging and functional MR imaging, will be needed to completely elucidate this mechanism.

Finally, we used BBB scoring to assess the gross motor behavior of the rats in each injury group (Fig. 5). Saporita et al.<sup>47</sup> reported an improvement in BBB scores after complete contusion, despite the lack of recovery of MEPs. Several previous studies of unilateral SCIs have documented intrinsic improvement in BBB scores after injury.<sup>2,4,45,48</sup> It is therefore important to characterize BBB scores together with functional electrophysiology in unilateral injuries, given the clinical relevance of the model and the increased number of studies using unilateral injuries to study SCI. Our BBB results following a mild unilateral contusion injury are consistent with previous reports in that the scores increased and plateaued after injury. However, these data did not correspond to the MEP results, which showed only marginal improvement for unilateral injuries and no recovery

for the mild midline injury. Therefore, we emphasize the need for electrophysiology in addition to BBB scoring when assessing unilateral SCIs.<sup>4,5</sup>

### Conclusions

Although BBB motor behavior scoring has been widely reported in rat SCI experiments, our results are the first to characterize via electrophysiology the functional integrity of motor and somatosensory pathways in a mild unilateral contusion injury. In the present study, we showed that the sparing of pathways in the spinal cord following a unilateral contusion injury might be enough to promote the recovery of both motor and somatosensory transmission. We also showed that MEP recovery closely corresponded to the amount of anatomical damage sustained by the CST, which was quantified in stained spinal cord slices. However, after injury, rats are able to develop a step reflex that uses spinal circuits to coordinate walking independently of the brain.<sup>50</sup> Therefore, the BBB motor scores improved in all injury groups regardless of MEP recovery. Lastly, many recent studies utilizing novel therapies, such as stem cells,<sup>28,33</sup> to address SCI have reported behavioral and electrophysiological improvements.<sup>15,16</sup> In light of the results in the current study, which show endogenous behavioral and electrophysiological recovery for a mild injury, care should be taken when distinguishing among endogenous recovery, plasticity, and the effectiveness of cell replacement. Our results emphasize the importance of using precise electrophysiological techniques, together with behavioral assessments, to study recovery after SCI.

### Disclosure

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## Functional assessment of unilateral contusion injury

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