Enhancement of Bilateral Cortical Somatosensory Evoked Potentials to Intact Forelimb Stimulation Following Thoracic Contusion Spinal Cord Injury in Rats

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***Abstract*—The adult central nervous system is capable of significant reorganization and adaptation following neurotrauma. After a thoracic contusive spinal cord injury (SCI) neuropathways that innervate the cord below the epicenter of injury are damaged, with minimal prospects for functional recovery. In contrast, pathways above the site of injury remain intact and may undergo adaptive changes in response to injury. We used cortical somatosensory evoked potentials (SSEPs) to evaluate changes in intact forelimb pathways. Rats received a midline contusion SCI, unilateral contusion SCI, or laminectomy with no contusion at the T8 level and were monitored for 28 days post-injury. In the midline injury group, SSEPs recorded from the contralateral forelimb region of the primary somatosensory cortex were 59.7% (CI 34.7%, 84.8%; $c^2 = 21.9; \mathrm{dof} = 1; p = 2.9 \times 10^{-6}$) greater than the laminectomy group; SSEPs from the ipsilateral somatosensory cortex were 47.6% (CI 18.3%, 77%; $c^2 = 10.1; \mathrm{dof} = 1; p = 0.001$) greater. Activation of the ipsilateral somatosensory cortex was further supported by BOLD-fMRI, which showed increased oxygenation at the ipsilateral hemisphere at day seven post-injury. In the unilateral injury group, ipsilesional side was compared to the contralesional side. SSEPs on day 14 (148%; CI 111%, 185%) and day 21 (137%; CI 110%, 163%) for ipsilesional forelimb stimulation were significantly increased over baseline (100%). SSEPs recorded from the hindlimb sensory cortex upon ipsilesional stimulation were 33.9% (CI 14.3%, 53.4%; $c^2 = 11.6; \mathrm{dof} = 1; p = 0.0007$) greater than contralesional stimulation. Therefore, these results demonstrate the ability of SSEPs to detect significant enhancements in the activation of forelimb sensory pathways following both midline and unilateral contusive SCI at T8. Reorganization of forelimb pathways may occur after thoracic SCI, which SSEPs can monitor to aid the development of future therapies.**

***Index Terms*—Contusion spinal cord injury, cortical plasticity, electrophysiology, somatosensory evoked potentials, unilateral spinal cord injury.**

Manuscript received September 29, 2013; revised February 13, 2014, April 03, 2014; accepted April 06, 2014. Date of publication April 29, 2014; date of current version September 04, 2014. This work was supported in part by Maryland Stem Cell Research Fundunder Grant 2009-MSCRFII-0091-00 and Grant 2013-MSCRFII-0109-00 (PI A. H. All); in part by the National University of Singaporeunder Grant R-175-000-121-133, Grant R-175-000-121-733, Grant R-175-000-122-112, and Grant R-711-201-026-133 (PI A. H. All); and in part by the National Institutes of Healthunder Grant 5R01HL071568 (PI N. V. Thakor).

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Digital Object Identifier 10.1109/TNSRE.2014.2319313

I. Introduction

PATHWAYS of the spinal cord carry ascending sensory information and descending motor information between the peripheral nerves and the brain. Damage due to spinal cord injury (SCI) leads to a partial or complete loss of function below the site of injury. The majority of human SCIs are incomplete injuries, which leave a number of pathways anatomically intact but unable to conduct neural signals. Although the central nervous system (CNS) has a limited capacity for regeneration, it is able to adapt and reorganize following injury [1]–[3]. It has been shown that short-distance intra-spinal regeneration and plasticity can contribute to functional recovery [4]. For example, most patients who suffer from incomplete SCI show an improvement in daily activities over time despite the fact that there is no related improvement in spinal conductivity, measured by evoked potentials. These improvements in locomotion and motor performance have been attributed to a possible reorganization of spinal circuits [5]. Thus, locating the source of reorganization or plasticity and then identifying clinically relevant methods for monitoring are of great importance for developing therapeutic strategies that improve recovery.

In animal models of SCI, plasticity can also arise from spared or undamaged components of the CNS, which can contribute to functional recovery [6]. For example, damage to the thoracic spinal cord in rats has been shown to cause changes in the cortical response of forelimb sensory afferents, which innervate the spinal cord above the site of injury [7], [8]. Endo *et al.* also described changes in the forelimb cortical somatotopy in rats that occurred within days of a spinal transection [9]. In this study, fMRI showed that the sensory-deprived hindlimb region of the cortex due to SCI was invaded and partially taken over by the adjacent forelimb region. It has been suggested that such cortical rewiring could negatively impact repair of the spinal cord and possible rehabilitation of the lower body because the deprived hindlimb region may be permanently reallocated to forelimb use. On the other hand, reorganization or compensation could be the key to the daily functional improvements that patients with SCI experience, given the very low success so far of neuronal repair at the injury site. A better understanding of these endogenous changes will lead to a greater efficacy of therapies, such as rehabilitation and functional electrical stimulation (FES), in the future.

Somatosensory evoked potentials (SSEPs) quantitatively assess the integrity of ascending sensory pathways. After contusive SCI, the SSEP response through injured pathways exhibits reduced amplitude and increased latency, which grades with the severity of injury [10]. Therefore, SSEPs are widely used in both clinical [11] and research [12], [13] settings to monitor the injury and the progression of recovery. Whereas most studies of SCI in rodents use SSEPs to quantify the damage to sensory pathways by stimulating the hindlimbs [14], [15], we evaluated SSEPs that correspond to spared forelimb pathways. The afferents of forelimb peripheral nerves innervate the spinal cord in the cervical region, far above the site of the more prevalent injuries that occur in the thoracic areas such as at T8. For this purpose, we investigated time-dependent changes in SSEP amplitude upon electrical stimulation of the median nerves (forelimbs) in rats that received a midline spinal cord contusion, a unilateral spinal cord contusion, or a laminectomy with no contusion at the T8 level. Comparisons between midline and unilateral injury provided a powerful system for delineating whether cortical changes can occur unilaterally, specific in relation to the side of injury, or whether they always occur bilaterally.

This study demonstrates for the first time large enhancements in the SSEPs evoked from forelimb stimulation, not only in the contralateral hemisphere, but also in the ipsilateral hemisphere after either unilateral or midline injury. These results show that SSEPs can be used to evaluate cortical changes associated with intact, undamaged pathways that may be indicative of plasticity after injury. This work paves the way for future studies that seek to better define plasticity and to determine to what extent it may contribute to recovery from injury.

II. Materials and Methods

A. Animals

A total of 29 adult female Lewis rats weighing 200–225 g (Charles River Laboratories, Inc., Wilmington, MA, USA) were used. Rats were divided into groups receiving a midline contusion SCI ($N = 15$), unilateral contusion SCI ($N = 9$), or laminectomy with no contusion ($N = 5$). Animals were housed individually in ventilated rodent cages and allowed free access to food and water. One enrichment item (a nesting material block) was placed in each cage. The environment was kept on a light-dark schedule from 7 am to 7 pm (light cycle) and 7 pm to 7 am (dark cycle). The environment was also kept at a temperature of 22 °C and relative humidity between 50%–60%. Prior to any procedure, animals were allowed to acclimatize to the environment for at least two days after arrival. All procedures were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University.

B. Anesthesia

To induce general anesthesia during surgical procedures, 0.12 ml of a mixture of 30.4 mg/kg ketamine, 4.3 mg/kg xylazine, and 0.9 mg/kg acepromazine maleate was administered via intra-peritoneal injection. For SSEP recordings, rats were anesthetized with ∼80% room air, 20% oxygen, and 1.5% isoflurane. Rats were kept on a homeothermic blanket system (Harvard Apparatus Ltd., Kent, UK) to maintain their body temperature at 37 $\pm 0.5\,^{\circ}$C, as measured by a rectal probe. Lacrilube ophthalmic ointment (Allergan Pharmaceuticals, Irvine, CA, USA) was applied to the eyes to prevent drying.

C. Electrode Implantation

Five screw electrodes (E363/20, Plastics One, Inc., Roanoke, VA, USA) were implanted into the skull of each rat. Each rat was anesthetized, and its head region was shaved and aseptically prepared with chlorhexidine (Phoenix Pharmaceuticals, Inc., St. Joseph, MO, USA). A local anesthetic of 1% lidocaine HCl (Abbott Laboratories, North Chicago, IL, USA) was injected under the skin. After few minutes, an incision was made along the midline. The cranium was cleaned by removing the tissue under the skin. A dental drill (Fine Science Tools, North Vancouver, BC, Canada) was used to drill four burr holes into the exposed part of the cranium. Using a stereotaxic frame and the atlas by Paxinos and Watson, *The Rat Brain in Stereotaxic Coordinates*, the exact location of four holes were identified on the somatosensory cortex corresponding to the hindlimbs and forelimbs in both left and right hemispheres [16]. On each hemisphere, the forelimb sensory recording sites were located 0.2 mm posterior and 3.8 mm lateral from the bregma, and the hindlimb sensory recording sites were located 2.5 mm posterior and 2.8 mm lateral from the bregma. Transcranial screw electrodes were then screwed into the holes such that they made very light contact with the dura mater without causing compression of the brain tissue. This was verified by post-mortem inspection of the surface of the cortex to ensure no indentation or puncture of the dura mater or brain due to the screw electrodes. The distal end of each electrode was inserted into one of the slots of an electrode pedestal (MS363, Plastics One Inc., Roanoke, VA, USA). To secure the electrodes for long-term recording of cortical SSEPs, carboxylate dental cement (Durelon Carboxylate Cement, 3M ESPE, St. Paul, MN, USA) was used to hold the screw electrodes and the electrode pedestal in place. After hardening of

the cement, the skin incision was closed with a 4–0 suture. Following the electrode implantation surgery, 2% lidocaine gel was applied to the skin wounds. For pain relief, Tylenol (liquid) 0.5 cc per mouth was also given for up to 10 days.

D. Contusion Injury and Post-Injury Care

Following anesthesia, the back region of rat was shaved and aseptically prepared with chlorhexidine (Phoenix Pharmaceuticals, Inc., St. Joseph, MO, USA). After making a midline incision on the skin, two longitudinal incisions were made to the paravertebral muscles on the left and right sides of the spinous processes of the vertebrae from T6 to T10. The paravertebral muscles were then gently pulled away from the spine without damaging them. With retractors holding the muscle aside, a laminectomy was performed by cutting and removing the lamina at T8 to expose the dorsal surface of the spinal cord underneath, without opening the dura mater.

Spinal cord contusions were performed on deeply anesthetized rats at the mid-thoracic level (T8), as previously described [10], [17]. We used a standard MASCIS impactor for all contusion injuries. The rats were randomly divided into three groups: 1) 15 rats received a 12.5 mm midline moderate contusion injury following the laminectomy surgery, 2) nine rats received a unilateral moderate contusion injury randomly on the right or left of the midline, and 3) five rats received a laminectomy only as control.

For all contusion injuries, the spinous processes of the vertebrae at T6 and T10 were first secured with stabilization clamps to reduce the motion of the spinal column during the impact. The exposed dorsal surface of the spinal cord at T8 was then contused with the MASCIS weight-drop device by dropping a 10-g rod with a flat circular cross-section (tip diameter 2 mm) from a pre-calibrated height of 12.5 mm. For the subset of nine rats that received the unilateral moderate contusion injury, the tip of the rod was offset laterally by 0.8 mm to either right or the left, prior to the weight drop. This procedure created a precise contusion injury to only one-half of the spinal cord. The method of unilateral injury was described in detail and validated using histology in our previously published work [14]. To ensure consistence among all rats, biomechanical parameters including the impact velocity, height, time and the dynamic force applied to the cord were precisely recorded and monitored using the MASCIS Impactor software (Rutgers University). The variability in these injury parameters was less than 0.05%.

After the contusion injury, the paravertebral muscles were sutured in layers using absorbable sutures, and the skin was closed with a 4–0 suture. All rats were allowed to recover in their individual cages, warmed by a heat lamp, and food and water was easily accessible. Gentamicin antibiotic (5 mg/kg, intramuscular; Abbott Laboratories, Abbott Park, IL, USA) was administered immediately post-surgery and then daily for 4 days. The analgesic buprenex (0.01 mg/kg, intramuscular; Reckitt Benckiser Pharmaceuticals, Inc., Richmond, VA, USA) was delivered post-surgery daily for three days. After surgery, the rats' bladders were manually expressed at least two times per day until they regained control of urination.

E. Electrophysiology

For each rat, baseline SSEPs were recorded on two separate days, at least five days after electrode implantation. After two baselines were recorded, a contusion injury or laminectomy was performed within two days. Afterward, SSEPs were recorded on days 1, 4, 7, 14, 21, and 28 after injury. Prior to each recording session, intramuscular needle electrodes (Safelead F-E3-48, Grass Technologies, West Warwick, RI, USA) were manually inserted into the *flexor carpi radialis* and *flexor carpi ulnaris* muscles of the forelimb, and the *tibialis anterior* and gastrocnemius muscles of the hindlimb to electrically stimulate the median and tibial nerves, respectively. Care was taken to avoid direct contact of needle electrodes with the nerve bundle, by making sure that only the corresponding limb twitched lightly. An isolated constant current stimulator (Digitimer, Hertfordshire, U.K.) was used to deliver positive current pulses of 3.5 mA with 200 μs duration to one limb at a time. Pulses were delivered at a frequency of 1 Hz in a rotating fashion to each of the four limbs, such that each limb received one pulse every 4 s (0.25 Hz). This stimulation paradigm has been well established in our previous publications [12]–[15], [18]–[23]. For each pulse, the cortical SSEPs from the four areas of the sensory cortex representing each limb were simultaneously recorded via the four implanted cortical screw electrodes (Fig. 1). A fifth cortical screw electrode was used as reference. A subdermal needle electrode was placed at the back of the neck as ground. The signals were amplified by a gain of 20 000 (RA4PA Preamp, Tucker Davis Technologies, Aluchua, FL, USA), sampled at 4882 Hz, and recorded to a PC using OpenEx (Tucker-Davis Technologies, Alachua, FL, USA). Each SSEP sweep was recorded for 300 ms, where $t = 0$ corresponds to the instant the stimuli was delivered. For each recording session, at least 300 sweeps were recorded per electrode per stimulated limb.

F. Signal Processing

All signal processing was performed in MATLAB 7.0 (MathWorks Inc., Natick, MA, USA). To improve signal-to-noise ratio, sweeps were bandpass filtered (20–1000 Hz), notch filtered to remove 60 Hz noise, and mean corrected. Outlier sweeps were identified by a simple condition: a sweep was omitted from analysis if the signal voltage $>3\times$ standard deviation of voltage of all sweeps within one session, taken at the preselected time of 240 ms post-stimuli, which is well beyond the duration of an expected SSEP response. The remaining sweeps were time-locked to the stimulus. A moving average with a window of 20 consecutive sweeps and an overlap of five sweeps was performed. Next, a custom peak detection algorithm was used identify the P1, N1, and P2 peaks of each averaged SSEP waveform. For each SSEP waveform, the N1 peak was used for latency calculations and the N1-P2 peaks were used for amplitude calculations. The values were averaged for all rats in each of the experimental groups.

Amplitude was normalized to the mean of each rat's respective baseline. Data was collected for each of the four limbs, and data for stimulation of the left and the right forelimbs of the

midline injury were averaged. For unilateral injuries, the forelimbs were grouped according to whether the stimulus was ipsilesional or contralesional. The recording channel electrodes are hereafter referred to as either ipsilateral or contralateral to the limb that was stimulated. Three channels relative to the stimulated forelimb were quantified: the contralateral response, ipsilateral response, and hindlimb-region response.

G. Statistical Analysis

To evaluate the effects of time, group, and time x group interaction in the amplitude of SSEP responses, we used the generalized estimating equations (GEE) to estimate the parameters of the linear regression because it accounts for the dependency of repeated-measures observations where both time and group were treated as factors. An interaction term in the model would indicate a difference in the temporal trend between the experimental and control groups. A group effect in a model with the interaction term would be assessed by taking the difference of the estimated marginal means (i.e., overall mean) between the experimental (injury) and control (laminectomy with no injury) groups. If the interaction term was found to be insignificant, the model including only time and group as main effects was considered. A group effect in a model without the interaction term would indicate the difference between the experimental and control groups having an independent effect on the outcome after factoring in the effect attributable to the day of recording. For all GEE analyses, an autoregressive correlation structure was assumed to account for decreasing correlation for farther time points. GEE models also robustly account for unrecorded data points, which is a key limitation of ANOVA methods.

To identify significance of a group effect, the Wald statistic was compared to a chi-squared distribution and the confidence interval, p-value, and degrees of freedom are reported. Next, to identify whether there was a difference when comparing any of the days within a group with respect to its baseline (i.e., the mean at each time point is significantly different from 100%), the confidence intervals of the estimated mean for each time point were calculated with Bonferroni correction to adjust for multiple comparisons. Therefore, a confidence interval for any day that does not include 100% indicates a post-SCI measurement that is significantly different from baseline.

H. Functional MRI

A randomly selected rat with moderate 12.5 mm contusive SCI underwent fMRI acquisition seven days after injury. A single-shot, gradient echo, echo planar imaging sequence was used to assess cortical responses to forepaw stimulations with the following parameters: effective echo time $= 21$ ms, repetition time $= 1\ 000$ ms, bandwidth $= 250$ kHz, field of view $= 1.92 \times 1.92$ cm, and matrix size $= 128 \times 128$. The paradigm consisted of 10 dummy MRI scans to reach a steady state followed by two epochs of 20 baseline and 20 scans during forepaw electrical stimulation. FMRIB Software Library (FSL, Oxford, U.K.) was used for analysis. Activation maps were obtained using the general linear model. Z statistic results were cluster-size thresholded for effective significance of $p < 0.05$. The activation threshold was set at 2.3. The number of activated pixels ($p < 0.05$) was calculated across three regions of interest representing the S1 according to the coordinates from Paxinos and Watson [16].

I. Histological Assessment

Rats were deeply anesthetized using isoflurane. Transcardial perfusion was performed with DPBS (14190, GIBCO, Grand Island, NY, USA) and paraformaldehyde (PFA) solution (4%; 15713-S, Electron Microscopy Sciences, Hatfield, PA, USA). The spinal cord was then carefully extracted from the vertebrae column and post-fixed in 4% PFA, followed by 30% sucrose solution for 24 h, and last embedded in paraffin. Spinal cords were sliced and fixed in glass slides. The slides were stained with hematoxylin and eosin (H&E) to assess the morphology of the injury.

III. Results

The experimental setup allowed us to simultaneously record from multiple regions of the cortex using permanently implanted screw electrodes while individually stimulating each limb (Fig. 1). This allowed us to evaluate changes in SSEPs recorded over 28 days for the three injury groups. These results are divided into two cases. In the first case, the midline injury group was compared with the laminectomy group. In the second case, the unilateral group was sub-divided such that forelimb stimulation of the injured side (ipsilesional) was compared with forelimb stimulation of the uninjured side (contralesional).

A. Midline Injury Group Versus Laminectomy Group

First, we studied the effects of forelimb response to stimulus following a midline contusion injury. Fig. 2 shows example SSEP waveforms recorded from the contralateral forelimb region, the ipsilateral forelimb region, and the hindlimb region of the somatosensory cortex during forelimb stimulation at specific time points after injury.

A GEE repeated measures model was constructed to compare the midline injury group with the laminectomy group, and then confidence intervals with Bonferroni correction were calculated to identify if any day of recording was significantly greater than 100% (baseline). The results of the model with estimates for SSEP amplitude at each day are shown in Fig. 3(a)–(c), presented according to the three regions of the cortex that were measured. The stimulation and recording scenarios are shown in Fig. 3(d) and (e), illustrating the contusion or laminectomy surgery and recording locations at the cortex upon forelimb stimulation (for simplicity, stimulation of only the left forelimb is shown). A significant group effect was observed for contralateral recordings, where the midline injury group was increased by 59.7% (CI 34.7–84.8%) over laminectomy ($\chi^2 = 21.86, p = 2.9 \times 10^{-6}, \mathrm{dof} = 1$). In the contralateral cortex of the midline injury group, a significant increase in SSEP amplitude of 143.3% (CI 101.6–185.1%) was first observed on day 4 and was sustained through day 28 (141.7%, CI 112.8–170.6). In the laminectomy group, only day 7 was significantly lower than baseline (70.4%, CI 40.9–99.9%), although the upper boundary of the confidence interval is borderline. There was also a significant group effect in ipsilateral recordings where midline injury group was increased by 47.6% (CI 18.3–77%) over laminectomy groups

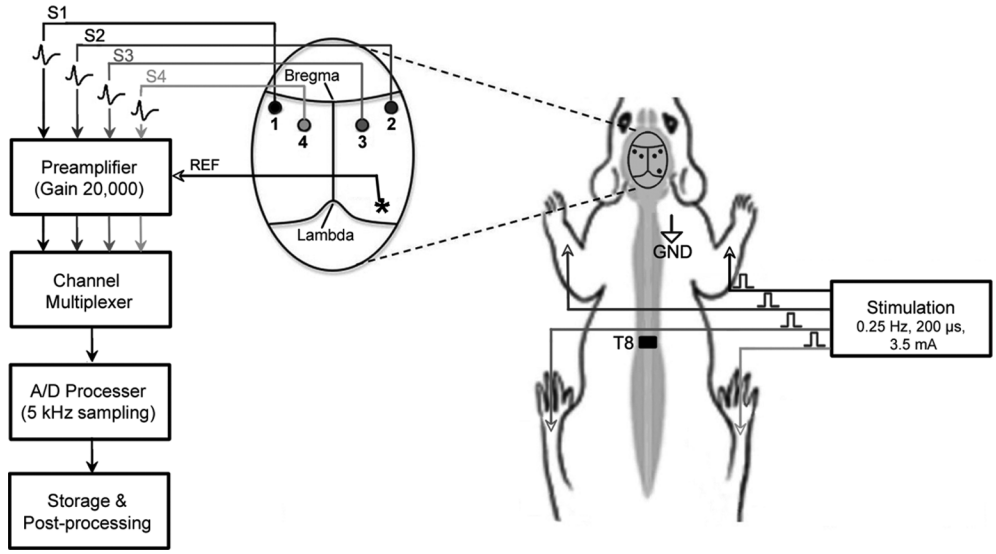

Fig. 1. Experimental setup for multichannel SSEP recording. A stimulus was delivered to each limb sequentially while multichannel cortical SSEPs were simultaneously recorded. The four cortical electrodes corresponded to the locations on the somatosensory cortex for each of the four limbs. S1–4 indicate the four simultaneously recorded SSEP signals.

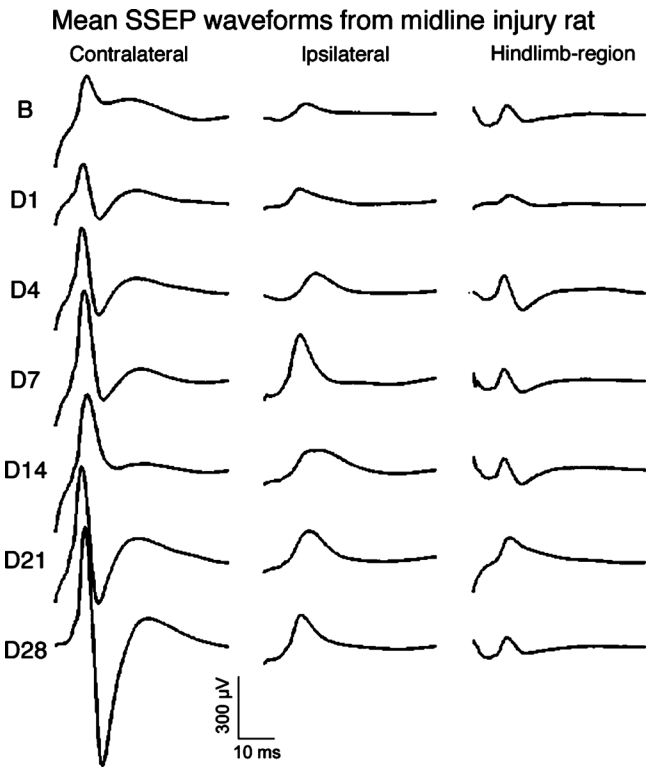

Fig. 2. Mean SSEP waveforms recorded from three regions of the cortex during forelimb stimulation of midline injury rat. Baseline SSEP waveforms recorded prior to contusion injury are shown in the first row. First column shows SSEP waveforms recorded from the contralateral somatosensory cortex during forelimb stimulation. Second column shows SSEP waveforms recorded from the somatosensory cortex ipsilateral to the stimulus. SSEP waveforms shown in the third column were recorded from the contralateral hindlimb cortex during stimulus. SSEPs from the three regions were recorded simultaneously upon administration of the same forelimb stimulus. B: Baseline. D: Day.

$(\chi^2 = 10.13, p = 0.0015, \mathrm{dof} = 1)$. Within the midline injury group, a significant SSEP amplitude increase was observed on day 7 (159%, CI 108.5–209%) and sustained through day 28 (150%, CI 102.1–198%). In the hindlimb cortex, the group effect was insignificant $(\chi^2 = 1.45, p = 0.23, \mathrm{dof} = 1)$. In addition, no time point for either group was significantly increased over baseline. These results show SSEP enhancements in both the contralateral and ipsilateral cortex due to forelimb stimulation after SCI, but no enhancement in the hindlimb region.

Given the significant changes in SSEP amplitude that occurred within the midline injury group over baseline, the N1 latency was assessed to determine if there were changes in the conduction speed of forelimb sensory signals after the midline T8 contusion injury. The N1 latency is defined as the time from stimulation to occurrence of the N1 peak (the first positive peak) of the SSEP waveform. GEE model revealed differences in latency for the contralateral, ipsilateral and hindlimb regions were highly significant $(\chi^2 = 445, p < 0.001, \mathrm{dof} = 2)$. The latency of SSEPs from the ipsilateral and hindlimb regions was compared to the latency of the contralateral region (Fig. 4). The latency of the hindlimb region was 0.7 ms longer than the contralateral region (CI 0.5–0.8 ms, $\chi^2 = 67, p < 0.001$) while the latency of the ipsilateral region was 3.90 ms longer (CI 3.5–4.4 ms, $\chi^2 = 344, p < 0.001$). The mean values of N1-latency recorded at each region of the cortex were 11.6 ms (CI 11.5–11.8 ms) for the contralateral cortex, 12.3 ms (CI 12.2–12.4 ms) for the hindlimb cortex, and 15.6 ms (CI 15.2–16.0 ms) for the ipsilateral cortex. The differing latency values show that the various regions of the cortex are activated at different times. The GEE model also revealed a borderline significant reduction in latency on day 1 (-0.5 ms; CI -1.0–0.0 ms, $p = 0.051$) and day 7 (-0.6 ms; CI -1.2–0.0 ms, $p = 0.053$) over baseline. However, after accounting for multiple comparisons, these days were insignificant.

We performed BOLD-fMRI acquisition on day 7 after injury in a randomly selected rat from the midline contusion injury group (Fig. 5). The purpose of BOLD-fMRI was to verify the response at the ipsilateral cortex was physiological, which can be

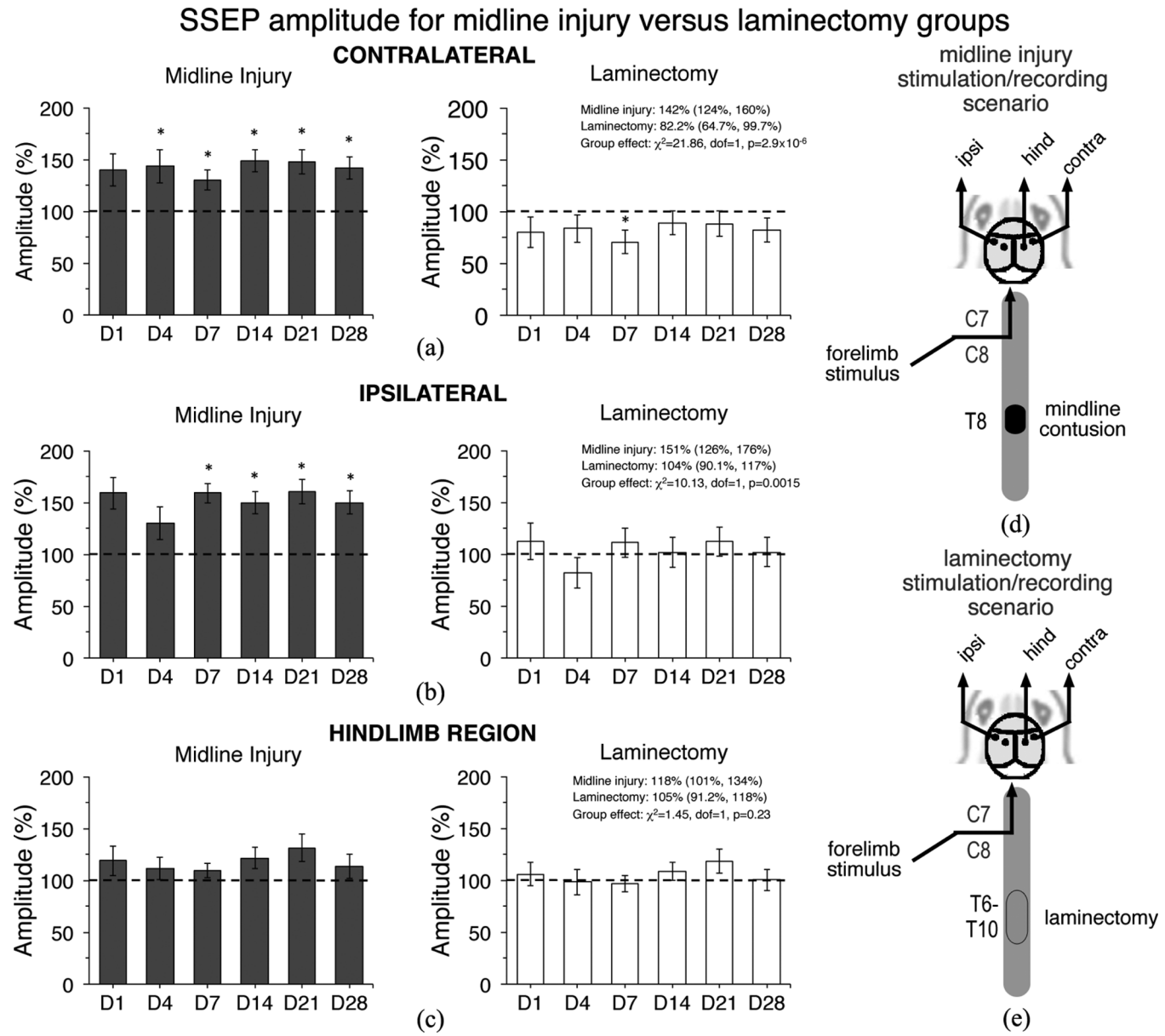

Fig. 3. Estimates derived from the GEE model of SSEP N1-P2 amplitudes for midline injury and the laminectomy groups. SSEPs were recorded from (a) the contralateral cortex, (b) ipsilateral cortex, and (c) hindlimb region for each group. A significant group effect was observed in contralateral ($p = 2.9 \times 10^{-6}, \mathrm{dof} = 1$) and ipsilateral recordings ($p = 0.0015, \mathrm{dof} = 1$). In addition, significant increases over 100% (baseline, dotted lines) were identified in both the contralateral and ipsilateral regions of the midline injury group. Hindlimb region was insignificant for both midline injury and laminectomy. Insets in upper right give the estimated group profile (95% confidence interval) and group effect. Schematics of the stimulus and recording paradigm for the midline injured (d) and laminectomy (e) groups are shown. $*p < 0.05$ within a group for days which were significantly different from 100% (baseline) with Bonferroni correction. Error bars represent standard error. $\mathrm{D} = \mathrm{Day}$; dotted line $= 100\%$ (baseline).

measured by changes in blood oxygenation during neural firing in the cortex. Fig. 5(a) shows the activation in four coronal slices through the forelimb somatosensory cortex upon stimulation of the left or right forelimb. A significant response was observed in the ipsilateral cortex upon stimulation of the right forelimb but not the left forelimb. Fig. 5(b) shows example SSEP waveforms recorded from a midline-injured rat. The responses at the ipsilateral cortex while either the left and right forelimb is stimulated are overlaid for comparison. In this rat, there is a marked increase at the ipsilateral cortex for the right forelimb but not the left forelimb on day 7. This result is one of several rats that exhibited an asymmetric response at the cortex when comparing the left and right forelimbs.

B. Unilateral Injury Group: Ipsilesional Versus Contralesional

The second set of experiments compared the ipsilesional limb (injured side) with the contralesional limb (uninjured side) of a group of rats that received a unilateral injury to one-half of the spinal cord. We hypothesized that increased contralateral and ipsilateral responses are due to adaptation or plasticity of injured pathways post-SCI. Therefore, increased contralateral and ipsilateral responses were expected to emerge for stimulation of the ipsilesional limb but not the contralesional limb. A GEE repeated measures model was constructed to compare the ipsilesional side with the contralesional side of the unilateral group. This stimulation/recording scenario is shown schematically in Fig. 6(d) and (e). Then, confidence intervals with

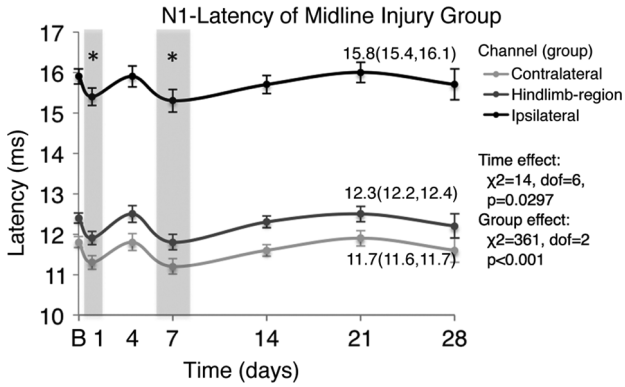

Fig. 4. N1 latency of SSEPs measured from the contralateral, ipsilateral, and hindlimb-regions of the cortex upon forelimb stimulation. Estimated group mean with confidence interval is denoted next to each curve. $*$*indicates significant time effect according to GEE but not significant after multiple comparisons*. Error bars represent standard error. B: Baseline.

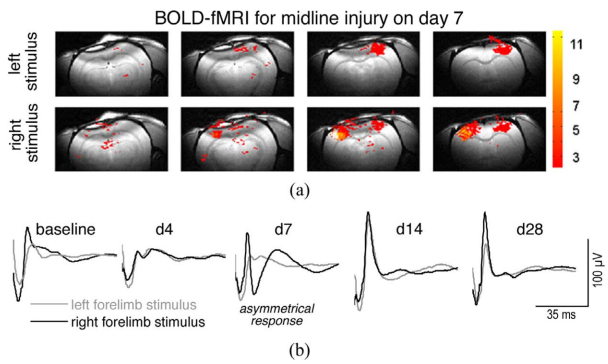

Fig. 5. One session of BOLD-fMRI on day 7 after a midline injury. The images show the activation in four coronal slices through the forelimb somatosensory cortex upon stimulation of the left or right forelimb. A BOLD response was observed in the ipsilateral cortex upon stimulation of the right forelimb, verifying that the ipsilateral SSEP responses are a function of hemodynamic changes from neural activity in the ipsilateral somatosensory cortex. No ipsilateral response was observed for stimulation of the left forelimb. (b) A sample rat from the midline injured group illustrating that asymmetric responses were also observed in SSEPs. Waveforms show the response at the ipsilateral cortex upon stimulation of the left and right limbs, overlaid for comparison. At day 7, there is a marked increase at the ipsilateral cortex for the right limb but not the left for this particular rat.

Bonferroni correction were calculated to identify if any day of recording was significantly greater than 100% (baseline). The results of the model with estimates for SSEP amplitude at each day are shown in Fig. 6(a)–(c). The model identified that the group effect comparing the ipsilesional and contralesional groups was insignificant for both the contralateral recording $(\chi^2 = 0.63, p = 0.43, \text{dof} = 1)$ and the ipsilateral recording $(\chi^2 = 0.63, p = 0.43, \text{dof} = 1)$. However, for contralateral recordings, there were significant increases over baseline in the ipsilesional group on day 14 (148%, CI 111.1–185%) and day 21 (137%, CI 109.6–163%), which were not present in the contralesional group. Interestingly, for ipsilateral recordings, there was a significant group x time interaction, which is represented by the opposing polarity of the linear trend for the ipsilesional (-1.65%/day) and contralesional (1.32%/day) groups (Difference -2.97%/day; CI $-5.38, -0.564\%/\text{day}$; $\chi^2 = 5.85; p = 0.08; \text{dof} = 1$). At day 4, the ipsilesional group is significantly higher than the contralesional ($+70.8\%$; CI 7.7, 134%; $p = 0.019$), but at day 28 the ipsilesional group is significantly lower (-110.0%; CI $-30.4, -189.6\%, p = 0.002$) than the contralesional. Lastly, there was a group effect in the hindlimb region where the ipsilesional group was increased by 33.9% (CI 14.3, 53.4) over the contralesional group $(\chi^2 = 11.6, p = 0.0007, \text{dof} = 1)$. For the ipsilesional group, SSEPs recorded from the hindlimb region were significantly greater than baseline on day 14 (128.2%; CI 100.8, 155.7%) and day 21 (126.1%; CI 104.0, 148.3%). The accuracy of the unilateral injuries was validated by histological evaluations of the spinal cords, performed after sacrificing the animal on day 28, to ensure only pathways on one-half of the spinal cord were damaged (Fig. 7).

IV. Discussion

Spinal cord injury at T8 is known to cause significant impairment to hindlimb motor and sensory function. Previous studies have provided evidence of two modalities of reorganization within the adult CNS following SCI, compensatory mechanisms and endogenous plasticity [24]–[28]. Plasticity can be defined as the formation of neuronal circuits in both lesioned and unlesioned fibres [1]. Compensation refers to improvements in function without any corresponding change in neuronal deficit [5]. Because the brain may be learning new ways to achieve the same task, compensation may also result in cortical changes. However, there are a number of hurdles in understanding how plasticity and compensation translate to outcomes for SCI patients. For example, not all such changes may be beneficial: reorganization of the brain to compensate for a loss of function could lead to overuse and overreliance on healthy structures and thus inhibit repair of injured structures [29]. Furthermore, the potential for axonal regeneration through the site of injury is limited due to the formation of a glial scar and cavity at the epicenter and the limited ability of remyelination by oligodendrocytes [30]–[32]. Thus, a complete neuronal repair after injury has proven extremely difficult [33]. Therefore, a significant focus of research efforts is to understand and enhance mechanisms that may aid recovery. In addition, the majority of past studies on plasticity after SCI make use of complete transection or hemisection models in rats, despite the fact that complete transection SCIs in humans are rare [34]. Although transection studies have provided information regarding response of the spinal cord to injury, these models are unable to recapitulate the plastic responses that may occur after contusion. We therefore studied a midline and unilateral contusion injuries, which mimic the majority of incomplete spinal cord traumas in humans.

First, we presented evidence for an enhanced activation of the forelimb sensory pathways that ascend from the cervical region after a thoracic SCI in rats. We showed that cortical SSEPs recorded from the contralateral cortex upon forelimb stimulation had sustained increases in amplitude from day 4 to day 28. The increase in the contralateral SSEPs could indicate CNS reorganization following injury to the hindlimbs. After an incomplete spinal cord injury, there exist a number of spared afferent hindlimb fibers that remain anatomically complete although functionally disconnected from their source of sensory

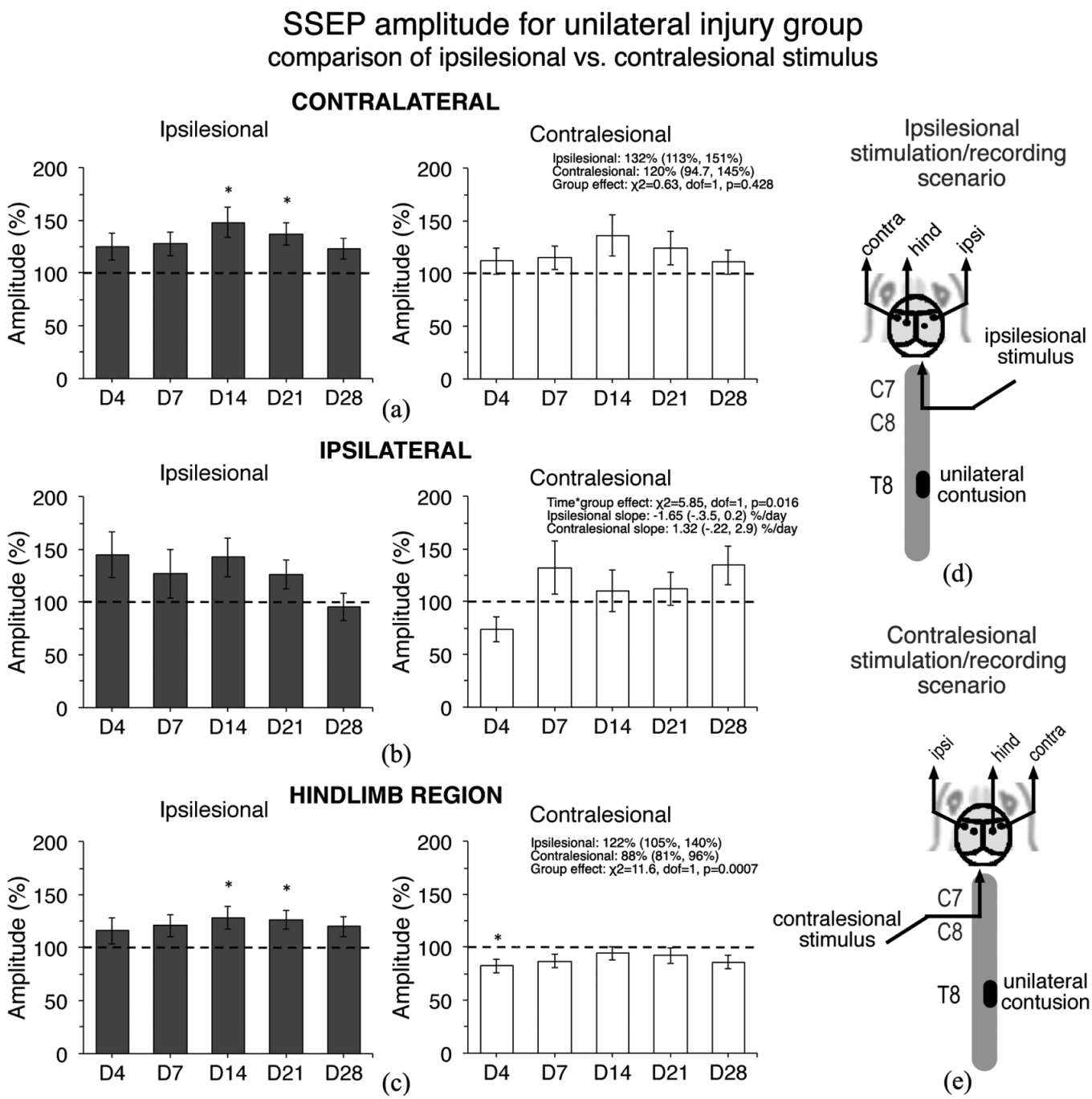

Fig. 6. Estimates derived from the GEE model of SSEP N1-P2 amplitudes for the unilateral injury group. Responses were grouped depending on whether the ipsilesional or contralesional limb was stimulated. SSEPs were recorded from (a) the contralateral cortex, (b) ipsilateral cortex, and (c) hindlimb region. In contralateral recordings, there was no group effect, but a significant effect over 100% was observed in selected days of the ipsilesional stimulus. In ipsilateral recordings, the response was dependent on both group and time; ipsilesional exhibited a negative slope while contralesional exhibited a positive slope. In the hindlimb-region recordings, there was 34% increase in ipsilesional compared with contralesional stimuli ($p = 0.0007, \mathrm{dof} = 1$). Schematics of the stimulus and recording paradigm for the ipsilesional (d) and contralesional (e) stimuli. $*p < 0.05$ within a group for days which were significantly different from 100% (baseline) with Bonferroni correction. Error bars represent standard error. D = Day; dotted line = 100% (baseline).

information in the periphery, for example due to de-myelination [6], [13]. These viable axons from the hindlimb pathways may reorganize and form new connections with the forelimb sensory afferents after injury, thus leading to increased inputs to the forelimb-region of the somatosensory cortex and a resulting increase in SSEP amplitude. These new connections may be recruited towards enhancing forelimb sensory function as a method of compensating for the loss of sense in the hindlimbs, wherein the existing axons and hindlimb cortex are reallocated for use by the forelimbs.

Cortical reorganization following SCI has been reported in similar studies that used voltage-sensitive dye imaging [35] and fMRI [36], [37]. The hindlimb somatosensory cortex is located anatomically adjacent (medial and posterior) to the forelimb region. Ghosh *et al.* reported that after a unilateral hemisection in rats, the forelimb region enlarged and partially expanded into

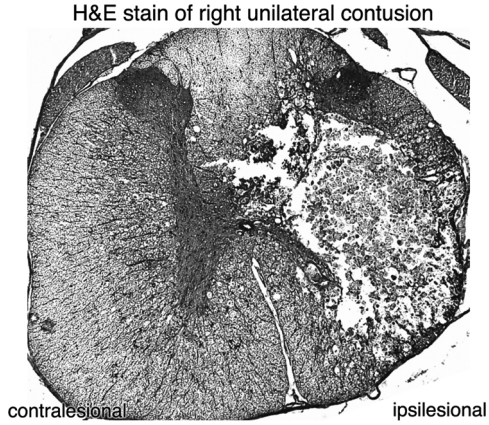

Fig. 7. Hematoxylin & Eosin stained spinal slice taken one month post-SCI through the epicenter of injury for a unilateral injured rat to verify localization of damage to one-half of the cord.

the former hindlimb region [35]. Other studies have shown a similar forelimb expansion using fMRI [36], [38]. In our present study, SSEPs measured from the adjacent hindlimb region in the midline injury group were not found to increase in amplitude over the course of our study. However, we did find a significant increase in SSEP amplitude at the hindlimb region on day 14 and 21 of the unilateral injury group to an ipsilesional stimulus. This finding corroborates Ghosh *et al.*, who also found a large expansion of forelimb activation via BOLD-fMRI on week 4 [35] after a unilateral hemisection. It seems reasonable to suggest that the increase in amplitude we recorded at the hindlimb region after unilateral injury could be due to this forelimb cortical expansion. However, our results suggest that the expansion into hindlimb regions may not be as prominent as hypothesized, as the effect is not significant by day 28. Interestingly, significantly increased SSEP amplitude at the hindlimb region was only found in the ipsilesional pathways of the unilateral injury group, but not the midline injury group. This suggests that the hindlimb region was being invaded by forelimb somatosensory cortex only in the hemisphere that was associated with injured pathways. These findings support the need for future studies incorporating electrophysiological methods with a unilateral hemisection to elucidate the differences seen here between midline and unilateral injuries.

In addition to the contralateral cortex, we observed enhancements in the ipsilateral SSEPs recorded upon forelimb stimulation. In order to demonstrate that the ipsilateral response was physiological rather than a possible artifact of SSEPs in the ipsilateral cortex, we performed a BOLD-fMRI acquisition on day 7. The positive BOLD-fMRI response that is seen in the ipsilateral cortex upon stimulation of the right forelimb provides strong evidence that the reported SSEP responses are indeed a function of emerging neural activity associated with hemodynamic changes in the ipsilateral cortex, rather than any possible artifact in recording. Rao *et al.* have also shown the bilateral activation of the cortex upon forelimb stimulation in non-human primates that underwent unilateral thoracic SCI [38]. In their study, cortical reorganization was linearly correlated with time after injury, where the greatest reorganization was found at 12 weeks post-SCI. Interestingly, the BOLD-fMRI response that we identified is asymmetrical: stimulation of the left forelimb did not elicit ipsilateral activation. We noted similar findings in the rats that underwent SSEP. The day of the maximum ipsilateral SSEP response may not necessarily occur at the same time for the right and left limbs. These asymmetrical changes could be a result of the nature of the incomplete contusion injury.

The rat midline contusion model represents an incomplete injury with a hostile microenvironment that mimics SCI in human. Thus, one may question whether the asymmetry of the midline contusion or axonal degradation post-injury contributed to the observed ipsilateral responses. To account for asymmetric injuries and to verify that the plasticity-associated responses are localized specifically to injured pathways, we performed a series of unilateral contusion injuries on rats, which randomly received a contusion injury to either the right or left side of the midline. We sought to ensure that no damage was sustained to the contralesional pathways, as the objective of this study was to verify that plastic responses are indeed an effect of injury. In the case of unilateral injury, stimulation of the ipsilesional limb resulted in significant SSEP amplitude increase in the contralateral cortex. In contrast, no increase in SSEP amplitude was found following injury upon stimulation of the contralesional limb. Interestingly, for ipsilateral SSEPs, a significant difference in the slopes for amplitude between ipsilesional and contralesional stimuli was found. For example, amplitude upon ipsilesional stimulus begins enhanced and decreases over time, while the amplitude upon contralesional stimulus increases over time. This result may be attributed to damaged pathways on the ipsilesional side that contribute to the heighted ipsilateral response, although this response fades over time.

A novel finding by Côté *et al.* identified enlarged evoked responses recorded from the forelimb cortex upon stimulation of hindlimb sciatic nerve after a cervical hemi-contusion [39]. Interestingly, Côté *et al.* reported an increase in evoked response regardless of whether the ipsilesional or contralesional sciatic nerve is stimulated, which they attributed to activation of long ascending propriospinal neurons. Aguilar *et al.* also identified bilateral changes in cortical evoked potentials upon stimulation of the ipsilesional hindpaw in a unilateral hemisection model [40]. Stimulation of uninjured pathways even below the site of injury contributed to an enhancement of evoked potentials. They suggested an immediate hypersensitivity of the primary somatosensory cortex in response to preserved (ipsilesional) spinothalamic inputs. These studies taken together with our findings suggest that some reorganization or plasticity may occur within the spinal columns, both above and below the site of injury. However, as our study only performed measurements at the cortex, further studies involving measurement of evoked potentials from the spinal cord and brainstem are required to determine whether spared pathways or plasticity within the spinal cord contributed to these changes.

For instance, new spinal circuits could be generated post-injury, leading to a rewiring of sensory pathways that activates ipsilateral cortical regions. The N1-latency of SSEPs recorded at each region of the cortex could shed light on the speed of transmission to the respective cortical regions upon forelimb stimulation. By comparing the latency of SSEPs measured in each cortical region, we can infer that SSEPs initially reach the

contralateral cortex, spread next to the hindlimb region, and later arrive in the ipsilateral cortex. Therefore, it is plausible that the changes in SSEPs are due primarily to cortical reorganization rather than development of new spinal circuits. However, the absolute path by which SSEPs reach the ipsilateral cortex cannot be elucidated here, as recordings from other brain structures would be required. Second, because the contusion occurred below the cervical level of the spinal cord at T8, any changes in latency must be due to physiological changes that effect conduction speed of forelimb sensory input rather than damage to axons of sensory neurons, as no intraparenchymal injury occurred between the location of forelimb innervation in the spinal cord and the somatosensory cortex. In fact, we observed a borderline significance of latency at the ipsilateral cortex on day 7, although not significant after accounting for multiple comparisons. This observation may predict the peak of period plastic changes following trauma, during which time the brain is undergoing the most adaptation and may be a promising time window for therapeutic strategies such as rehabilitation and functional electrical stimulation as well as hypothermia [21], [41], remyelination strategies [31], [42], or administration of anti-inflammatory drugs [43].

Finally, it remains unclear if our reported increases in SSEPs due to forelimb stimulation are beneficial for recovery or how they may possibly play a role in gait improvement. In rats, constraint-induced movement therapy applied by restricting use of the healthy limb in order to force the use of its injured limb after unilateral SCI aided in long-term functional recovery [44]. Constraint-induced movement therapy and forced-use therapy targeted at debilitated limbs are also beneficial for neurological treatment in humans [45]. In our study, compensation due to over-use of uninjured forelimbs may explain this forelimb enhancement and could inhibit the ability of hindlimbs to recover. In this regard, SSEPs can be used to monitor how plasticity is being modulated to gain the maximum beneficial outcome in patients. For example, SSEP monitoring can aid in rehabilitation therapies in the future to prevent overcompensation with uninjured limbs; therapies can then be continuously adapted to emphasize the use of injured limbs in order to promote rehabilitation. A recent study which developed a spinal electrochemical prosthetic for SCI rehabilitation showed that recovery of hindlimb stepping may be possible by training the cortex to actively use paralyzed hindlimbs and to develop new circuitries for controlling gait [46]. In some studies, positive outcomes following SCI have been associated with rehabilitation regimes that commence within the first 1–2 weeks after SCI [47]. Interestingly, the height of the ipsilateral response in both midline injury group occurred at day 7 and in the unilateral injury group at day 14. It could be argued that the observed responses mark the 'end' of the critical period, before which rehabilitation would be most beneficial.

Nevertheless, this study illustrates how SSEPs can be used to quantitatively assess plastic changes at the cortex following an SCI. These methods can be applied to both animal models and patients with SCI for structuring therapies, especially rehabilitative medicine or functional electrical stimulation treatments. In the future, this work can be extended to determine how rehabilitation or gait improvement are correlated with cortical plasticity in order to better understand how it contributes to, or inhibits, recovery.

V. Conclusion

We identified time-dependent changes in the activation of sensory regions of the brain that may be indicative of cortical plasticity. These changes were observed upon stimulation of the forelimbs, whose afferents innervate the spinal cord rostral to the site of injury and remain undamaged after a thoracic spinal cord contusion. The increase in the amplitude of the ipsilateral SSEPs may suggest increased connectivity between the left and right hemispheres or the formation of new intraspinal circuits as a result of forelimb compensation. Furthermore, we used a unilateral injury model to show that increased activity at the contralateral and hindlimb cortex is related to ipsilesional stimulation but not contralesional stimulation. Our findings suggest that despite the focal nature of the injury, post-SCI plasticity can involve system-wide changes that affect other CNS components, including uninjured spinal cord circuits and both brain hemispheres. Finally, our study illustrates the use of SSEPs, a modality that is already routinely used to assess patients with SCI, to monitor the natural time-course of injury, and they may be used to assess treatments that are aimed at modulating plasticity after SCI. In the future, this work can be also extended to understand how rehabilitation or gait improvements modulate plasticity and recovery.

Acknowledgment

The authors would like to thank Dr. G. Pelled and Y. Han of the Krieger Kennedy Institute, Johns Hopkins University for help with BOLD-fMRI acquisition, and C. Hu of the Johns Hopkins University for help with the animal experiments.

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Angelo H. All started his higher education in pharmacy school, graduated from medical school, continued his career as a surgery fellow and received the MBA degree from the Johns Hopkins University, Baltimore, MD, USA.

His translational research is focused on spinal cord injury pathology and processes involved after injury in axonal remyelination during recovery. His lab conducts *in vivo* longitudinal assessments of long-term functionality following therapeutic based approaches. These approaches include acute hypothermia, human embryonic and iPS stem cell transplantation therapy, specifically how oligodendrocyte cells regenerate myelin. This is in combination with somatosensory and motor evoked potential assessments and novel bio-signal processing methods.