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Plasticity Associated Changes in Cortical Somatosensory Evoked Potentials Following Spinal Cord Injury in Rats

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Abstract— **Spinal cord injury (SCI) causes a number of physiological and neurological changes resulting in loss of sensorimotor function. Recent work has shown that the central nervous system is capable of plastic behaviors post-injury, including axonal regrowth and cortical remapping. Functional integrity of afferent sensory pathways can be quantified using cortical somatosensory evoked potentials (SSEPs) recorded upon peripheral limb stimulation. We implanted 15 rats with transcranial screw electrodes and recorded SSEPs from cortical regions corresponding to each limb before and after a mild or moderate contusion injury. We report a post-injury increase in the mean amplitude of cortical SSEPs upon forelimb stimulation. SSEP amplitudes for mild and moderate SCI groups increased by 183%±95% and 107%±38% over baseline, respectively, while hindlimb SSEPs decreased by 58%±14% and 79%±4%. In addition, we report increased SSEP amplitude measured from the anatomically adjacent hindlimb region upon forelimb stimulation (increase of 90%±19%). Our results show that previously allocated hindlimb cortical regions are now activated by forelimb stimulation, suggesting an expansion in the area of cortical forelimb representation into hindlimb regions after an injury. This result is indicative of adaptive plasticity in undamaged areas of the CNS following SCI.**

I. Introduction

Spinal cord injury (SCI) causes a number of anatomical, morphological, and physiological changes. These include cavity formation, disruption of neuropathways, inflammation, cell death, damage to myelin producing cells, and demyelination. The result is a loss of electrical signal conduction in the central nervous system (CNS). Once thought to be hard-wired, the brain and spinal cord are now considered capable of adaptation following neuronal injury [1]. Neural plasticity has been widely described as the capacity to adjust to injury through retained alterations of neural circuitry [2]. In animal models, training-induced functional recovery has been shown to parallel plastic changes in the brain and spinal cord following damage to spinal cord somatosensory pathways and the primary somatosensory cortex (S1) [3]. In humans, cortical reorganization has been linked with chronic neuropathic pain and possible structural abnormalities in motor regions [4, 5]. Somatosensory evoked potentials (SSEPs) have revealed short-term cortical modulation in patients who underwent percutaneous cervical cordotomies [6], and functional magnetic resonance imaging (fMRI) in SCI patients has revealed a correlation between temporal cortical plasticity and long-term recovery of movement [7].

CNS plasticity has also been described in recent rat studies that have shown a significant amount of spontaneous regrowth and axonal sprouting following injury to the spinal cord or brain [8-10]. In one study, anterograde tracers showed contralateral CST axons sprouting into denervated regions of the grey matter following traumatic brain injury [9]. Additionally, significant cortical reorganization has been identified following SCI. Blood oxygenation level-dependent (BOLD) fMRI is one popular modality that has helped reveal both short-term [8, 11] and long-term [10, 12, 13] cortical reorganization in macroscopic regions of the brain following SCI. These discoveries of brain plasticity and endogenous reorganization following SCI suggest that the sensorimotor system is more capable of reorganization, compensation, and rehabilitation than once believed. Therapies aimed to target or enhance this spontaneous recovery may prove promising. A better understanding of post-SCI plastic changes, particularly in the cortex, is an important step in facilitating spinal cord repair.

SSEPs are a quantitative way to assess the functional integrity of somatosensory pathways. SSEP monitoring is commonly used in clinical evaluation and in the operating room [14]. Damage to the spinal cord causes neuronal cell death and demyelination [15], resulting in loss of electrical functional integrity of somatosensory pathways. Therefore, SSEPs have been used to quantify both the amount of injury and spared function of injured pathways after SCI [16-18]. In addition, SSEPs can be used to study concurrent reorganization or compensation among spared pathways in response to SCI. Here we report changes in cortical SSEPs in rats after graded thoracic contusion injuries. These changes reflect a redistribution of forelimb and hindlimb cortical areas after thoracic SCI.

II. Methods

A. Contusion Injury

Fifteen female Lewis rats (200 to 230 g) were randomly divided into three surgical groups: 6.25 mm (mild) contusion injury (n = 3), 12.5 mm (moderate) contusion injury (n = 7), and a laminectomy without injury (control, n = 5). Five transcranial screw electrodes were implanted on the cranium of each rat for SSEP recordings, as previously described [16-

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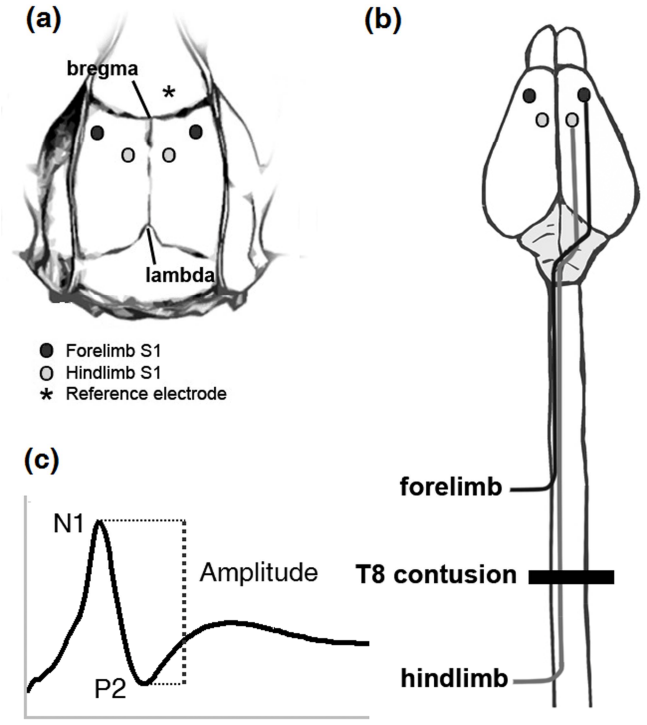

Fig. 1 (a) Dorsal view of the rat skull illustrating the locations of the electrodes placed for SSEP recording. (b) A schematic illustrating that somatosensory pathways cross above injury to the contralateral hemisphere and the respective SSEPs were measured from the four positioned electrodes. (c) Example of amplitude calculation.

20]. Briefly, an incision was made along the midline of the shaved head and a dental drill was used to drill five burr holes in the exposed part of the skull corresponding to the hindlimb and forelimb regions of the S1. Electrodes were inserted at five locations (Fig. 1a) and mounted on an electrode pedestal with dental cement to allow chronic longitudinal recordings.

Spinal cord contusion surgeries were performed on rats anesthetized with 1.5% isoflurane at the T8 level of the spinal cord and contusions were produced using the NYU-impactor weight drop device. Prior to injury, two baseline SSEP recordings were taken for each rat; recordings were then taken on days 4, 7, 14, and 28 following injury. After day 28, rats were perfused with 4% PFA and their spinal cords were harvested, cryoprocessed, and H&E stained for histological examination to verify the injury.

B. Electrophysiology

For SSEP recordings, rats were anesthetized with 1.5% isoflurane and intramuscular needle electrodes were used to electrically stimulate the median and tibial nerves of the left and right limbs, as previously described [16-20]. An isolated constant current stimulator (Digitimer, Welwyn Garden City, Hertfordshire, UK) was used to deliver positive current pulses of 3.5 mA with 200 μs duration at 0.5 Hz to one limb at a time while concurrently recording the evoked potential responses on the cortex. For a single session, SSEPs were recorded simultaneously from the four implanted cortical

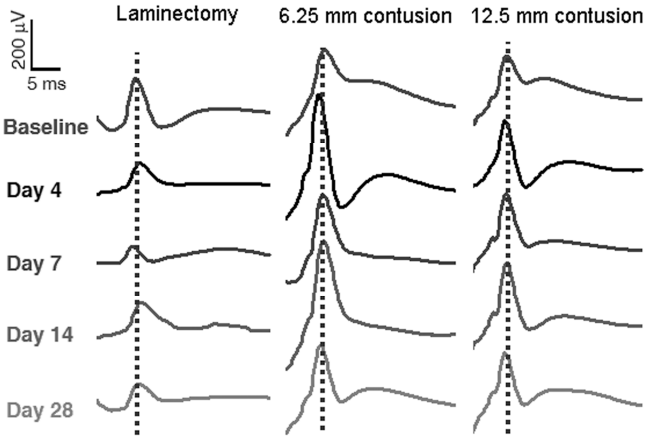

Fig. 2 Mean SSEPs in response to stimulation of forelimbs in three representative rats, one for each experimental group. Post-injury forelimb SSEP amplitude increased for both contusion levels; SSEP amplitude after laminectomy did not increase. SSEP latency did not change (dotted lines).

electrodes each time a limb was stimulated. Signals were amplified with a gain of 20,000 and sampled at 4882 Hz. SSEPs recorded from the two electrodes in the hemisphere contralateral to the stimulated limb were used for analysis. All signal processing was performed in MATLAB 7.0 (MathWorks Inc., Natick, MA). To improve signal-to-noise ratio, sweeps were high pass filtered (20 Hz cut off), notch filtered to remove 60 Hz noise, and mean corrected. Sweeps were time-locked to the stimulus and groups of 20 consecutive sweeps were averaged while shifting the averaging window by 5 sweeps at a time. A custom peak detection algorithm was used to calculate the N1P2 amplitude of each mean sweep (Fig. 1c), and the values were averaged for all rats in each experimental group. All reported values reflect group mean±SE. Statistical significance was calculated using an unpaired two-tailed t-test; $p < 0.01$ was considered significant.

III. Results

We used a commercial neurophysiology monitoring setup (Tucker Davis, Alachua, FL) to study the four pathways that correspond to the somatosensory function of four limbs' (Fig. 1b). Our setup is unique in that it allows simultaneous recording from all four cortical electrodes while stimulating a single limb. We were therefore able to evaluate changes in SSEP activity across multiple brain regions for a single stimulus. Following SCI in rats, we observed significant changes in SSEPs measured from the forelimb regions of the somatosensory cortex during stimulation of the corresponding forelimb whose afferents innervate the spinal cord above the location of injury at T8. Fig. 2 shows representative results for three rats, one from each of the three experimental groups. SSEP recordings were made from the cortical electrodes in forelimb regions of the S1 contralateral to the simulated forelimb. Rats from SCI groups exhibited SSEPs with increased amplitude, and changes were observed as early as day 4 after injury, lasting through day 28. In fact, the SCI groups showed a pronounced amplitude increase at day 4, suggesting rapid cortical remapping begins immediately following injury. In

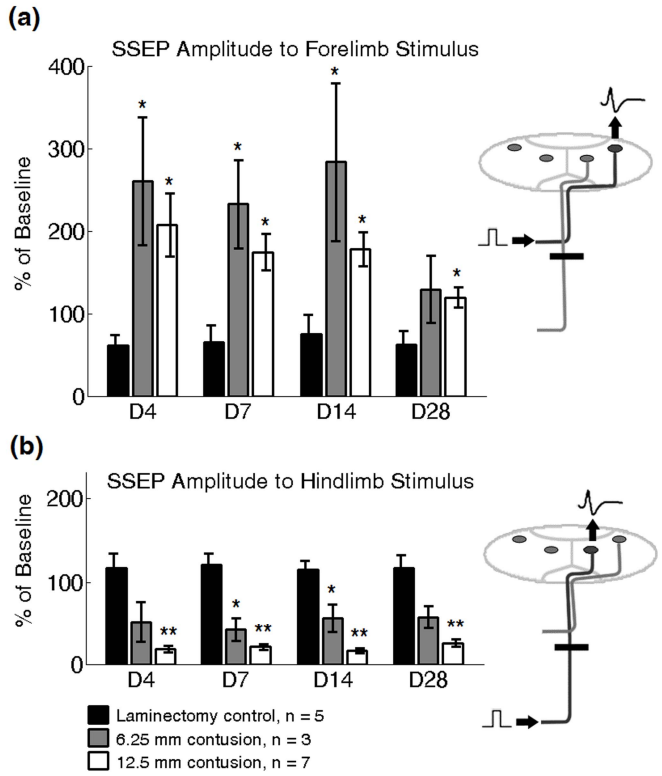

Fig. 3 Mean SSEP amplitude post-injury (normalized to baseline) during forelimb (a) and hindlimb (b) stimulus for the three experimental groups: laminectomy, 6.25 mm contusion, and 12.5 mm contusion; schematic of recording scenarios shown to the right. * $p < 0.01$; ** $p < 0.001$.

contrast, the laminectomy group (control) showed stable forelimb SSEPs throughout the duration of the study. N1 peak latency, defined as the time delay from the stimulus to the N1 peak, was not significantly affected by injury (Fig. 2).

Summarized results of SSEP amplitude for each of the three experimental groups are shown in Fig. 3. The mean amplitude of contralateral SSEPs measured from the forelimb region during forelimb stimulation significantly increased following injury for both contusion levels (Fig. 3a). At day 4 post-injury, the forelimb region SSEPs had increased by 160%±77% and 107%±38% for the 6.25 mm and 12.5 mm contusion groups, respectively. The greatest increase in SSEP amplitude for the 6.25 mm contusive injury was seen at day 14 with a 183%±95% increase and for the 12.5 mm contusive injury at day 4 with a 107%±38% increase. For both experimental groups the increase in amplitude was sustained through day 28. Amplitudes at days 4, 7, and 14 were all significantly greater than laminectomy control for both SCI groups ($p < 0.01$), and significantly greater at day 28 for the 12.5 mm contusion group.

We have previously reported that SSEPs recorded upon hindlimb stimulation after a thoracic SCI are significantly reduced in amplitude immediately following injury, then partially recover over several weeks post-injury [16-19]. As an internal control, we verified our current injury model by reporting SSEP amplitudes recorded during hindlimb stimulation (Fig. 3b). Amplitude decreased significantly following injury whereas the laminectomy control showed no decrease. The amplitude reductions were graded with the severity of injury. At day 7, SSEP amplitude was decreased by 58%±14% ($p < 0.01$) and 79%±4% ($p < 0.001$) for 6.25 mm and 12.5 mm contusion groups, respectively, whereas laminectomy controls did not exhibit a significant reduction in SSEP amplitude (121%±14% of baseline).

Our data suggest an increase in cortical forelimb representation following SCI. This increase was quantified by the increase in SSEP amplitude recorded from the forelimb region upon forelimb stimulation. However, the magnitude and direction of such an expansion remain unknown. One possibility is an expansion of the forelimb region into the adjacent hindlimb region [10], as these regions are anatomically adjacent to one another. To evaluate this possibility, we looked at the change in SSEP amplitude recorded from electrodes located at the hindlimb region during stimulation of the contralateral forelimb. An expansion of the forelimb representation into the hindlimb region after injury could be verified by recording SSEPs via electrodes implanted at the hindlimb region upon stimulation of the contralateral forelimb. Indeed, we observed an increase in SSEP amplitude measured from the hindlimb electrode (Fig. 4). Results are normalized to the amplitude of residual SSEPs measured pre-injury under the same scenario. The increase in SSEP amplitude, reported as the percent of baseline, was significantly greater than the laminectomy control for the 12.5 mm contusion group at days 4, 7, and 14 ($p < 0.01$) with increases of 90%±19%, 52%±11%, and 58%±12%, respectively. The 6.25 mm contusion group followed the same trend and we would expect significant results for an increased sample size. Day 28 was not significant, although still elevated with respect to laminectomy control ($p = 0.06$ and 0.03 for 6.25 mm and 12.5 mm groups).

IV. Discussion & Conclusion

It has been reported that spared hindlimb somatosensory pathways (anatomical complete but functionally incomplete) and forelimb somatosensory pathways innervating the spinal cord rostral to the site of injury reorganize in response to SCI [10-12]. For example, Ghosh et al. have reported an increase in cortical fMRI responses in the forelimb somatosensory cortex due to forepaw stimulation after a spinal cord hemisection [21]. They also report that the area of activation in the cortex expands into the previously mapped hindlimb area, suggesting hindlimb neurons are rewired to forepaw neurons following injury. Although fMRI makes it possible to distinguish changes in the boundaries of cortical activation, SSEPs presented here ensure spatial precision in that the electrodes are positioned at precise coordinates of the cortex describing the forelimb and hindlimb S1. Furthermore, very few human SCIs are characterized by complete transections; thus here we studied mild and moderate contusion injuries. Our data suggest an enlarged forelimb representation in the somatosensory cortex that partially expands into the pre-injury hindlimb region.

We observed significant increases in SSEPs measured in response to forelimb simulation after contusion SCI, but no increase for laminectomy group (control). The SSEP

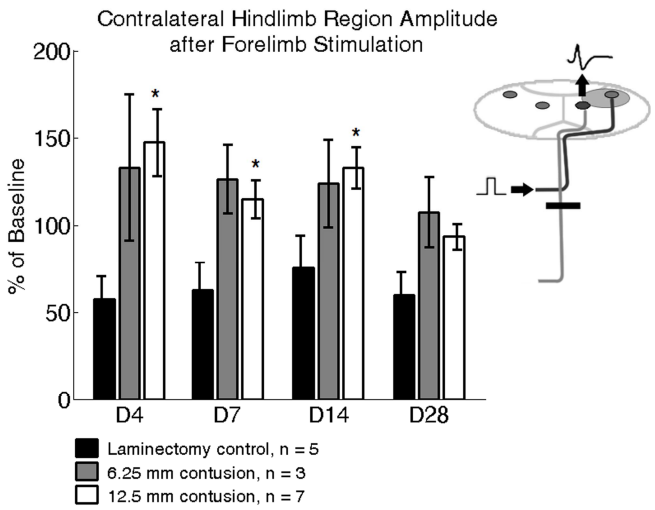

Fig. 4 Mean SSEP amplitude measured at the contralateral hindlimb region of the brain, anatomically adjacent to the forelimb region during respective forelimb stimulation; schematic of the recording scenario shown to the right. Shaded oval denotes an expanded forelimb representation into the hindlimb region. * $p < 0.01$

amplitude changes presented here suggest somatosensory changes in the acute period (within 4 days) post-injury that are sustained over the following four weeks. These data point to a cortical remapping of forelimb representation that corresponds to spared somatosensory pathways proximal to injury. To further study this possibility, we analyzed SSEPs recorded from the anatomically adjacent hindlimb region to contralateral forelimb stimulation, with the hypothesis that an expanded forelimb representation would yield SSEPs of increased amplitude at these hindlimb sites. Our results support this hypothesis, as hindlimb region SSEP amplitude was nearly 150% of baseline at day 4 after injury and sustained through day 28.

Although the underlying mechanisms are not completely understood, the increases in forelimb SSEPs presented here provide compelling electrophysiological evidence that the brain may employ compensatory mechanisms to make up for the loss of hindlimb function. An expansion of forelimb regions could also be a response mechanism to maintain the integrity of hindlimb regions of the brain whose inputs have been partially compromised due to injury; this could then allow later endogenous recovery. These findings support the idea that the CNS is capable of adaptation and reorganization early after injury, and that the brain and spinal pathways may exhibit plastic behaviors to compensate for lost function, retain cortical viability, and aid future recovery.

V. Acknowledgment

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