Published in final edited form as:

Exp Neurol. 2007 October ; 207(2): 238–247.

Loss and Spontaneous Recovery of Forelimb Evoked Potentials in both the Adult Rat Cuneate Nucleus and Somatosensory Cortex following Contusive Cervical Spinal Cord Injury

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Abstract

Varying degrees of neurologic function spontaneously recovers in humans and animals during the days and months after spinal cord injury (SCI). For example, abolished upper limb somatosensory potentials (SSEPS) and cutaneous sensations can recover in persons post-contusive cervical SCI. To maximize recovery and the development/evaluation of repair strategies, a better understanding of the anatomical locations and physiological processes underlying spontaneous recovery after SCI is needed. As an initial step, the present study examined whether recovery of upper limb SSEPs after contusive cervical SCI was due to the integrity of some spared dorsal column primary afferents that terminate within the cuneate nucleus and not one of several alternate routes. C5-C6 contusions were performed on male adult rats. Electrophysiological techniques were used in the same rat to determine forelimb evoked neuronal responses in both cortex (SSEPS) and the cuneate nucleus (terminal extracellular recordings). SSEPs were not evoked 2 days post-SCI but were found at 7 days and beyond, with an observed change in latencies between 7 and 14 days (suggestive of spared axon remyelination). Forelimb evoked activity in the cuneate nucleus at 15 but not 3 days post-injury occurred despite dorsal column damage throughout the cervical injury (as seen histologically). Neuroanatomical tracing (using 1% unconjugated cholera toxin B subunit) confirmed that upper limb primary afferent terminals remained within the cuneate nuclei. Taken together, these results indicate

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that neural transmission between dorsal column primary afferents and cuneate nuclei neurons is likely involved in the recovery of upper limb SSEPs after contusive cervical SCI.

Keywords

Dorsal Columns; Dorsal Column Nucleus; Medulla; Plasticity; Sensory; Somatosensory Evoked Potentials

INTRODUCTION

Persons with traumatic spinal cord injury (SCI) that are admitted to the hospital as sensory and motor complete (ASIA A) or as sensory incomplete and motor complete (ASIA B) have improvements of their neurologic function (Spinal Cord Injury Information Network, 2006; for a review, see Fawcett et al., 2007). Insights about the anatomical locations and physiological processes underlying spontaneous recovery after SCI could maximize the development of repair strategies (Kleitman, 2004).

Spinal cord injuries in the United States frequently occur at the cervical segments (Spinal Cord Injury Information Network, 2006). Damage to the adult human cervical spinal cord dorsal columns leads to sensory dysfunctions of the upper limbs as evidenced by transient exaggeration or abolishment of the cutaneous sensations of fine touch, vibration, and proprioception (for a review, see York, 1985; Nathan et al., 1986). Similarly (Metz et al., 2000; Norenberg et al., 2004), transient and persistent upper limb cutaneous sensation dysfunctions occur in adult rats with damage to their cervical spinal cord dorsal columns (Schrimsher and Reier, 1993; McKenna and Whishaw, 1999; Ballerman et al., 2001a,b; Onifer et al., 2005; Kanagal and Muir, 2007). Abolished upper limb somatosensory evoked potentials (SSEPs) also can recover spontaneously in persons with incomplete cervical SCI (Curt and Dietz, 1996). This has not been reported in rats with cervical SCI but has been observed after thoracic SCI (Nashmi et al., 1997).

The present study was an initial step towards elucidating processes underlying spontaneous sensory functional recovery after incomplete cervical SCI. In both humans (for review, see Kaas, 2004) and rats (for review, see Tracey, 2004), myelinated primary afferents in the cervical spinal cord dorsal columns transmit upper limb cutaneous sensory information to target neurons in the cuneate nuclei of the medulla. These dorsal column nuclei neurons then transmit this information to thalamic neurons where it is relayed to somatosensory cortical neurons. We previously reported that adult rat upper limb SSEPs can be persistently abolished by complete cervical spinal cord dorsal columns injury (Onifer et al., 2005). Based on these findings, we hypothesized that recovery of upper limb SSEPs after incomplete cervical SCI involves neural transmission between spared cervical spinal cord dorsal column primary afferents and cuneate nuclei neurons. To test this hypothesis, we used electrophysiological techniques in the same rat to determine the responses of both cuneate nuclei neurons (terminal extracellular recordings) and somatosensory cortical neurons (SSEPs) to forelimb evoked potentials after contusive cervical SCI. A neuroanatomical tracing study, using 1% unconjugated cholera toxin B subunit (CTB), was then done to confirm the electrophysiological findings. Taken together, the results indicate that neural transmission between spared dorsal column primary afferents and cuneate nuclei neurons is likely involved in recovery of upper limb SSEPs after contusive cervical SCI.

MATERIALS AND METHODS

Experimental Design

The methods described below were approved by the Institutional Animal Care and Use Committee at the University of Louisville. Twenty-four adult male (315-443g) Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were used for the experiments (Table 1). They were housed individually in standard cages with ALPHA-dri™ bedding on a 12-hour light-dark cycle. A clinically relevant model of contusion evolving to cavity formation cervical SCI (Bunge et al., 1993,1997) was used to test the hypothesis that recovery of upper limb SSEPs involves neural transmission between spared cervical spinal cord dorsal column primary afferents and cuneate nuclei neurons. SSEPs were assessed in all rats before cervical SCI and then at days 2, 7, 14, and 21 post-SCI. Terminal extracellular recordings in the medulla were performed on normal (sham) rats and at days 3 or 15 post-SCI. Neuroanatomical tracing was done in normal rats and at day 21 post-SCI.

SSEPs

As previously described (Onifer et al., 2005), 0.25mm diameter silver wires, each with a 1cm long and 0.31mm in diameter gold-plated pin soldered to one end, were wrapped around the top of sterile stainless steel self-tapping screws (#00 × 1/8). Each rat was anesthetized with a mixture of ketamine hydrochloride (50mg/kg), xylazine (5mg/kg), and acepromazine maleate (2.4mg/kg) injected intramuscularly. Gentozen™ antibiotic (10mg/kg IM) was injected. Lacri-Lube® ophthalmic ointment was placed onto the eyes. Core temperature was maintained at 37.5°C with a homeothermic blanket. The scalp was shaved and cleansed with both BETADINE® surgical scrub and 70% ethyl alcohol. The head was secured in a stereotaxic apparatus. Small incisions were made in the scalp to expose the cranium overlying the left and right forepaw somatosensory cortices. Two 0.8mm diameter holes were drilled through the cranium 3mm lateral at bregma, one each over the left and right hemispheres. Two of the screw electrodes were positioned in the holes such that their tips touched the dura. An additional hole was drilled into the cranium 1mm posterior to lambda and a screw without an electrode was positioned in it to secure the implant. Dental cement was applied to the cranium over the screws.

Two days post-surgery, each rat was anesthetized with a mixture of halothane (1.5%) and oxygen (30%) then restrained by a cloth stockinette wrapped around it. This was done so that we could perform the SSEP procedure in awake rats. The limbs, tail, and head extended from holes in the stockinette. It was safely suspended in the air so that the tail and limbs did not touch a surface. Two disposable stainless steel subdermal needle stimulating electrodes (0.32mm diameter × 28gauge × 12mm long, Cadwell Laboratories, Inc., Kennewick, WA) were inserted into the glabrous footpad of each forepaw and secured with adhesive tape. One subdermal needle electrode (0.36mm diameter × 27gauge × 12mm long, Cadwell Laboratories, Inc.) was inserted subcutaneously between the hips as a ground and one was inserted into the nose as a reference. The electrodes were connected to a Cadwell Sierra II (Cadwell Laboratories, Inc.) data acquisition system. Anesthesia was discontinued. The duration of anesthesia and the time between the end of anesthesia to the start of each SSEP procedure were the same for each rat. SSEPs were elicited 10min later by constant current stimuli delivered to each forepaw with a rate of 1.4Hz. The pulsewidth was 200μsec. The stimulus intensities were 1-, 2-, and 3-times threshold (normal threshold = 0.4 to 0.5mA). One hundred stimuli were averaged per waveform at each of the 3 stimulus intensities. The peak latencies and amplitudes of the P1 and N1 parameters of the SSEP waveform, the primary evoked cortical field potential (Jellema et al., 2004), after 1.5mA stimulus onset were measured (Fig. 1). This procedure was repeated for the injured rats at 2, 7, 14, and 21 days post-SCI.

Cervical SCI

Each rat was anesthetized with sodium pentobarbital (40-50mg/kg, IP). The antibiotic was injected and ophthalmic ointment was placed onto the eyes as described above. Core temperature was maintained with a homeothermic blanket. The nape was shaved and the skin was cleansed. An incision was made in the skin and the underlying muscles were dissected to expose the dorsal halves of the C2-7 vertebrae. Transverse vertebral process supports (Onifer et al., 1997; 2005) were placed bilaterally at the C4-5 vertebrae. Laminectomies were performed to expose the dura overlying the dorsal surfaces of the C5-6 segments. Contusion injuries of 176-201 actual kilodynes were produced dorsal to ventral between the C5 and C6 dorsal root entry zones with an Infinite Horizon SCI device (Scheff et al., 2003). The dura was intact during the SCI. The body was immobilized with a horizontal brace gently secured over the middle of the back to prevent it from moving upward during the SCI. Supplemental 100% oxygen, which did not improve neurologic outcome after contusion thoracic SCI (Salzman et al., 1990), was delivered to each rat while closing its incisions to compensate for potential respiratory dysfunction (El-Bohy et al., 1998).

Terminal Extracellular Recordings

Each rat was anesthetized with urethane (1.2 g/kg IP) the day after its last SSEP assessment. The jugular vein and trachea were intubated for supplemental urethane delivery and expired pCO₂ plus respiratory rate monitoring, respectively. Ophthalmic ointment was placed onto the eyes. Core temperature was maintained with a water circulating heating pad. The nape was shaved and the skin was cleansed. The head was secured in a stereotaxic apparatus. A midline incision was made in the skin and the underlying tissues were dissected to expose the dorsal surface of the brainstem between the caudal cerebellum and rostral C1 vertebra. A tungsten microelectrode (impedance 6-8MΩ, Frederick Haer and Co., Bowdoin, ME) attached to a hydraulic probe was used as previously described (Hubscher and Berkley, 1994; Hubscher and Johnson, 1996; Hubscher, 2006). Activity evoked by briefly touching the left or right neck, shoulder, forearm, forepaw, and hindlimb with a nylon brush was recorded between 100-1000μm below the dorsal surface in a grid of 15 electrode tracks (1.5, 1.3, then 1.1mm lateral to midline; 0.8, 0.6, 0.4, 0.2, then 0mm anterior to obex). The interstimulus interval was at least 1 second. Obex was 14.3mm posterior to bregma (Paxinos and Watson, 1998) and was 1mm (Beck, 1981; Maslany et al., 1992) and 0.96mm (Bermejo et al., 2003) posterior to the obex used in these previous studies. The electrode track placements were based on anatomical maps (Basbaum and Hand, 1973; Beck, 1981; Imamura et al., 1986; Webster and Kemplay, 1987; Pfaller and Arvidsson, 1988; Ygge, 1989; Arvidsson and Pfaller, 1990; Maslany et al., 1990; 1991; 1992; LaMotte et al., 1991; Bermejo et al., 2003) and electrophysiological assessments (Nord, 1967; Maslany et al., 1992) of the adult rat cuneate nuclei. Responses (single- or multi-unit) were determined at 100μm intervals and noted if the evoked excitatory activity was at least twice the background levels that immediately preceded stimulus onset or at least half if the response was inhibitory (Hubscher and Johnson, 2000; 2003). For units with no spontaneous discharge, a minimum of 3 spikes was required to be defined as excitatory.

Neuroanatomical Tracing

This was performed to determine whether forelimb primary afferents innervated the cuneate nuclei contralateral to the forelimb somatosensory cortices from which recovered SSEPs were recorded following contusive cervical SCI. Four normal rats and 4 rats at 21 days post-SCI were anesthetized with a mixture of ketamine hydrochloride (50mg/kg), xylazine (5mg/kg), and acepromazine maleate (2.4mg/kg) injected intramuscularly. The antibiotic was injected and ophthalmic ointment was placed onto the eyes as described above. Core temperature was maintained with a homeothermic blanket. The fur over the appropriate forelimb between the axilla and the elbow was shaved and the skin was cleansed. An incision was made in the skin

and the median nerve of each rat was exposed. A hole was made in the epineurium, the needle of a Hamilton syringe was inserted through the hole, and 10 μ l of 1% unconjugated CTB (List Biological Laboratories, Inc., Campbell, CA) in 0.01M sodium phosphate buffer, pH 7.5, was slowly injected (Baker and Hagg, 2005; Onifer et al., 2005; Massey et al., 2006, 2007).

Veterinary Care

After each surgical procedure, muscle and skin incisions were closed with silk sutures and wound clips, respectively. The wound clips were removed 7-10 days later. Bacitracin Zinc Ointment USP was applied to the sutured skin. Five milliliters of 0.9% sodium chloride were injected subcutaneously. Each rat was returned to its cage with clean bedding. One half of the cage was placed on a water circulating heating pad until the rat began weight support with its forelimbs and hindlimbs. Water bottles with straight spouts were used and standard rat chow was placed on the bedding. The state of hydration and gastrointestinal function were monitored daily. Lactated Ringer's solution was injected subcutaneously. ENSURE[®] and cereal were provided when necessary. Bladders were emptied with gentle pressure when necessary. Breathing was monitored twice daily during the first week post-SCI. The antibiotic was injected at 2 day intervals for 6 days.

Histology and Microscopy

Each rat was anesthetized with sodium pentobarbital (120mg/kg IP). The heart was exposed and the rat was perfused transcardially with oxygenated, calcium-free Tyrodes solution then 0.1M phosphate buffer pH 7.4 (PB) containing 4% paraformaldehyde. The brainstem and C1-T2 spinal cord segments, with dorsal root ganglia attached, of each rat were removed and cryoprotected at 4°C for 3-7 days in PB containing 30% sucrose. Afterwards, all tissue was cut at 20 μ m in the transverse plane with a cryostat. The sections were mounted onto Fisherbrand[®] Superfrost[®] Plus microscope slides. These were stored at -80°C for staining.

Frozen brainstem and C1-T2 spinal cord sections were warmed to room temperature. The brainstem sections were stained with 0.03% cytochrome C (Sigma, St. Louis, MO) and 0.05% 3,3'-diamidinobenzidine tetrahydrochloride (Sigma) as described (Wong-Riley, 1979; Crockett et al., 1993) to see the electrode tracks in the dorsal column nuclei. The spinal cord sections were stained with 0.5% cresyl violet (Sigma) and 25% eosin y (Sigma) solutions as described (Onifer et al., 2005) to view the morphology of the injury site. Brainstem sections and C8 spinal cord sections also were immunostained for CTB as described (Massey et al., 2006, 2007) to view primary afferent terminals within the cuneate nuclei and the dorsal horn, so as to determine the success of the neuroanatomical tracing procedure. The sections were circled with a PAP pen after thawing. Following rinses in Tris-buffer with 0.9% saline, pH 7.4 (TBS), the sections were incubated for 24 hours at 4°C in TBS that contained 0.25% Triton X-100 (TBST), 10% normal donkey serum and an antibody directed against CTB (1:1000, List Biological Laboratories, Inc.). They were next washed at room temperature and incubated in TBST containing FITC-donkey anti-goat IgG (1:200, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 1 hour. Following washing, they were coverslipped with Mowiol mountant and stored at 4°C. The stained sections were examined with a Nikon Eclipse E400 microscope. Selected images were captured with a Nikon SPOT Insight 3.2 color camera connected to a Gateway Computer. The dorsal columns were outlined using Adobe Photoshop software (v.7.0.1.) in a bright-field image of a cresyl violet and eosin-stained section at the SCI epicenter of the 4 injured rats that underwent neuroanatomical tracing at 21 days and at the C5-6 spinal cord segment of the 9 normal rats. The Histogram tool was used to count the total number of pixels in each rat's outlined dorsal columns. The total number of pixels in each dorsal column was converted to area (mm²).

RESULTS

SSEP Recordings

SSEPs were recorded from all forelimb somatosensory cortices of all 24 normal (sham) rats (Table 1 and Fig. 1). The peak latencies (mean $\pm$ standard deviation) of the P1 and N1 components of the SSEP waveforms were 9.8 ± 0.7 ms and 14.7 ± 1.2 ms respectively. The amplitudes (mean $\pm$ standard deviation) of the P1 and N1 components were 24.5 ± 9.5 μ V and 45 ± 15.3 μ V respectively. There were no SSEPs recorded 2 days post-contusive C5-6 SCI from 27 of the 30 forelimb somatosensory cortices of the 15 injured rats (Table 1, Fig. 1). In 9 injured rats, SSEPs were observed at 7 and 14 days post-SCI from 12 of their 16 forelimb somatosensory cortices where SSEPs had not been found at 2 days post-SCI (Table 1, Fig. 1). No SSEPs were recorded from 4 (7 days post-SCI) and then 3 (14 days post-SCI) forelimb somatosensory cortices of these rats. At 21 days post-SCI, SSEPs were recorded in 4 injured rats from 5 of their forelimb somatosensory cortices where SSEPs were observed at 7 and 14, but not 2, days post-SCI (Table 1, Fig. 1).

The P1 and N1 peak latencies of the recovered SSEPs at day 7 post-SCI ($n = 12$ cortices from 9 rats and 5 cortices from 4 of these 9 rats that were assessed till 21 days) were significantly longer than at baseline (Table 2). The P1 peak latencies, expressed as percent of baseline, were significantly longer at day 7 versus 14 and 21 days post-SCI (Fig. 2A). The N1 peak latencies, expressed as percent of baseline, were significantly longer at day 7 than at day 14 post-SCI (Fig. 2C). The P1 and N1 amplitudes of the recovered SSEPs at day 7 post-SCI ($n = 12$ cortices from 9 rats and 5 cortices from 4 of these 9 rats that were assessed till 21 days) were significantly smaller than at baseline (Table 2). The P1 and N1 amplitudes, expressed as percent of baseline, were not significantly different at days 7 and 14 or at days 7, 14, and 21 post-SCI (Figs. 2B, D).

Terminal Electrophysiological Recordings

A total of 225 tracks in the cuneate nuclei of 15 rats (15 tracks per rat) were examined for single and/or multi-unit responses to mechanical stimulation (brush/touch only) of the upper body or hindlimbs. All of the responsive neurons were found to be excitatory, most had no background activity, and all had response latencies on the order of several milliseconds. Cuneate nuclei neurons, ipsilateral to the forepaws that were electrically stimulated for the SSEP procedure, in all 5 of the normal rats responded to hair movement and light skin contact of the dorsal and palmar surfaces of the ipsilateral forepaw, the ipsilateral forearm between the wrist and axilla, and the ipsilateral shoulder plus neck to the base of the ear (Figs. 3-5). No cuneate nuclei neurons in the group of 5 rats tested at 3 days post-SCI responded to hair movement or light skin contact of the dorsal and palmar surfaces of the ipsilateral forepaw or ipsilateral forearm between the wrist and axilla (Figs. 3-5). However, units were evoked by hair movement and light skin contact of the ipsilateral shoulder plus neck to the base of the ear (Figs. 3 and 4), that is, areas whose primary afferents enter the spinal cord rostral to the level of the injury. Cuneate nuclei neurons in all 5 of the rats examined at 15 days post-SCI responded to hair movement and light skin contact of the dorsal and palmar surfaces of the ipsilateral forepaw, the ipsilateral forearm between the wrist and axilla, and of the ipsilateral shoulder plus neck to the base of the ear (Figs. 3-5). The somatotopy of the cuneate nuclei of these injured rats appeared to be similar to that of the normal rats (Fig. 4). No cuneate nuclei neurons in all normal and injured rats responded to the cutaneous stimuli applied to the contralateral upper body and the hindlimbs.

The total percentage of recording sites from which units were recorded after hair movement and light skin contact of the ipsilateral forepaw at each cuneate nuclei recording site (0, 0.2, 0.4, or 0.6 mm anterior to obex) were not significantly different among and between the normal

and 15 days post-SCI rats (Fig. 5). There was no significant recording sites by groups interaction. The mean percentage of recording sites in the entire cuneate nuclei of the normal and day 15 post-SCI rats from which units were recorded ($15.58 \pm 6.70\%$ and $9.00 \pm 4.20\%$, respectively) were not significantly different. Furthermore, no significant relationships were found when lines of best fit were drawn for the normal and day 15 post-SCI rats' mean percentage of recording sites in the entire cuneate nuclei from which units were recorded and the P1 ($r^2 = .17$) or N1 ($r^2 = .22$) amplitudes of the last recorded SSEP waveform. The mean percentage of recording sites in the entire cuneate nuclei of the normal rats and the injured rats at day 3 and at day 15 post-SCI from which units were recorded after hair movement and light skin contact of the ipsilateral shoulder plus neck to the base of the ear ($15.50 \pm 5.70\%$, $22.28 \pm 12.4\%$, and $13.66 \pm 6.60\%$, respectively) also were not significantly different.

Histology

White and gray matter damage was seen in the cervical spinal cords of the 4 rats that survived for 1 month after contusive C5-6 injuries (Fig. 6A). This was most extensive at the epicenter. Here, white matter damage occurred in the dorsal funiculi, dorsolateral funiculi, lateral, and ventral funiculi. Damage was seen in the dorsal funiculi, particularly the dorsal columns, throughout the rostral and caudal extents of the injuries. An independent t-test revealed that the area (mean $\pm$ standard deviation) of the dorsal columns in the dorsal funiculi at the SCI epicenters ($1.02 \pm 0.29\text{mm}^2$) was significantly less ($P < .05$) than the area of the dorsal columns at the C5-6 segments of the 9 normal rats ($1.39 \pm 0.21\text{mm}^2$).

Importantly, CTB-immunopositive terminals were observed within the cuneate nuclei ipsilateral to the injected median nerves of these 4 rats (Fig. 6B). These cuneate nuclei were contralateral to the forelimb somatosensory cortices from which no SSEPs were recorded 2 days post-SCI then from which SSEPs were recorded 7, 14, and 21 days post-SCI. CTB-immunopositive dorsal column axon terminals were also seen within the cuneate nuclei ipsilateral to the injected median nerves of the 4 normal rats (Fig. 6C).

DISCUSSION

SSEPs can be abolished then spontaneously recover in humans with cervical SCI (Curt and Dietz, 1996). For the first time, the present study shows that when there is a loss then a return of evoked activity in the cuneate nucleus of adult rats with contusive C5-6 SCI there also is an abolishment then recovery of forepaw SSEPs. What are the potential anatomical locations and physiological processes of the loss and spontaneous recovery of function?

Activity in the cuneate nucleus evoked by cutaneous stimulation of shoulder and neck, that is, upper body areas with primary afferents entering the spinal cord above the C5-C6 injury, was not different from normal at 3 days post-SCI. This finding rules out the possibilities that spinal shock and transneuronal changes in the dorsal column nuclei after dorsal column injury (Bernstein and Ganchrow, 1981) played a role in the conduction deficit. Instead, the conduction deficit was likely due to the extensive cervical spinal cord dorsal columns damage produced by the primary contusive SCI, hemorrhage, and the secondary injury mechanisms, including edema, ischemia, oxygen free radical accumulation, lipid peroxidation, ionic imbalances, excitotoxicity, and inflammation (for reviews, see Tator and Fehlings, 1991; Anderson and Hall, 1992; McTigue et al., 2000; Nashmi and Fehlings, 2001; Park et al., 2004).

In the present study, forepaw SSEPs were recorded at day 7 post-SCI and beyond. Activity evoked by cutaneous forepaw stimulation was recorded in the cuneate nucleus at day 15 post-SCI. Moreover, CTB-immunopositive forelimb primary afferent terminals were seen in the cuneate nuclei when examined at 1 month post-SCI. We previously observed that complete adult rat cervical spinal cord dorsal column injury eliminated forepaw CTB-immunopositive

primary afferent terminals in the cuneate nuclei and abolished forepaw SSEPs up to 1 month post-SCI (Onifer et al., 2005). This finding rules out that alternate circuitry were transmitting cutaneous sensory information after SCI in the present study. Furthermore, it leads to the conclusion that the contusive injury did not completely damage the dorsal columns.

Previous experimental evidence indicate that sprouting of spared forelimb primary afferents within the cuneate nucleus likely was not a mechanism of the neural transmission recovery that occurred within the first week post-SCI. Data from other laboratories show, for example, that sprouting of spared primary afferents did not occur within the rat gracile nuclei between 5 days and 3 months after incomplete thoracic spinal cord dorsal column injury (Jain et al., 1995) nor within the non-human primate cuneate nuclei 5 days after incomplete cervical spinal cord dorsal column injury (Jain et al., 1997). Conditioning sciatic nerve injury to increase regenerative capacity (Richardson and Issa, 1984) failed to promote sprouting of spared primary afferents in the rat gracile nuclei during the first week after contusive thoracic SCI (Baker and Hagg, 2005). We have found that chondroitin sulfate proteoglycan (CSPG)-containing perineuronal nets are present at the adult rat dorsal column nuclei and that the CSPGs are altered during the first week after complete cervical spinal cord dorsal column injury (Massey et al., 2007). Moreover, we have seen that chondroitinase ABC digestion of the endogenous and injury-induced CSPGs in the rat cuneate nucleus after incomplete cervical spinal cord dorsal column injury was necessary for sprouting of spared primary afferents and for spreading of receptive fields to cutaneous forepaw stimulation (Massey et al., 2006).

The latencies of the P1 and N1 parameters of the recovered SSEPs were significantly faster at day 14 post-SCI than at day 7, however, they were still slower than normal. The change in latencies seen between days 7 and 14 post-SCI suggests that remyelination of spared primary afferents likely had occurred. This suggestion is supported by the report that a progressive oligodendrocyte and Schwann cell remyelination began in the adult rat spinal cord white matter during the first week following maximal demyelination at day 1 after contusive thoracic SCI (Totoiu and Keirstead, 2005). Additional support of this suggestion is the finding (Cao et al., 2005) that transcranial magnetic motor evoked potentials (Linden et al., 1999; Loy et al., 2002) that were abolished following contusive thoracic SCI recovered 1 week after transplantation of multineurotrophin-expressing glial-restricted precursor cells into the adult rat spinal cord ventrolateral funiculus (Cao et al., 2005). Their latencies significantly improved with time post-transplantation. Similar to the present findings, however, the latencies were slower than normal even at 3 weeks after the SCI (Cao et al., 2005).

The rats were largely immobile during the first days after SCI. They began to maintain forelimb and hindlimb weight support while in stance and locomotion during the first and second week post-SCI at the same time that forepaw SSEPs recovered. This recovery of forelimb and hindlimb weight support also was seen in rats with contusive C4-5 SCI (Schrimsher and Reier, 1993). Behavior was not assessed in the present study. However, it has been reported that rats with contusive SCI at their C5 segment improved to weight-supported locomotion in the open field between the first and second week post-SCI (Pearse et al., 2005). Rats with contusive SCI at their C4-5 segments also were unable to perform a pellet retrieval task until at least 3 weeks afterward due to a lack of limb elevation, shoulder extension, and elbow extension (Schrimsher and Reier, 1993). The lack of shoulder and elbow flexion impaired contact of an adhesive sticker on the forehead with the forepaw at week 1 post-SCI but this improved at week 2. In contrast, the injured rats could not grasp the sticker with their digits. The shoulder flexion deficit also limited vibrissae-induced placing at week 1 post-SCI but this also improved at week 2. Unilateral contusive C4-5 (Soblosky et al., 2001) and C5 (Gensel et al., 2006) SCI led to forepaw misplacements during locomotion on the horizontal ladder beam at week 1 post-injury but these decreased at week 2. The forelimb contralateral to the SCI was preferred more in an

asymmetry test at week 1 post-SCI and less at week 2. Grooming with the forelimb ipsilateral to the SCI was impaired at week 1 post-injury but less so at week 2.

A better understanding of the anatomical locations and physiological processes underlying spontaneous recovery after SCI could maximize both the recovery and the development/evaluation of repair strategies. The present study suggests that our future investigations of spontaneous sensory electrophysiology and behavior recovery after contusive SCI should focus at the injury site, especially on remyelination, because the remainder of the somatosensory neuraxis is intact.

ACKNOWLEDGEMENTS

This work was supported by NS40411 (SMO), RR15576 (SMO), and NS40919 (CHH) from the National Institutes of Health, KYNSF EPSCoR EPS-9874764 from the National Science Foundation (NGF Cooper for MJW and CMW), an Undergraduate Research Grant from the Office of the Vice President for Research at the University of Louisville (SMO for BNP), and the Friends for Michael, Inc. Spinal Cord Injury Organization (SMO for the Kentucky Spinal Cord Injury Research Center). The authors extend their appreciation to the Research Resources Center veterinarians and staff at the University of Louisville for their excellent assistance.

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Figure 1.

These SSEP waveforms were recorded from a representative rat's cortex after contralateral forepaw electrical stimulation prior to and following a contusive C5-6 SCI. The P1 and N1 components seen in the Normal SSEP waveform were absent at 2 days post-SCI. They were present with differing latencies (i.e., P1 L, N1 L) and amplitudes (i.e., P1 A, N1 A) at 7, 14, and 21 days. This rat's injury epicenter and cuneate nucleus contralateral to the somatosensory cortex from which these SSEPs were recorded can be seen in Figures 6A and 6B, respectively.

Figure 2.

These line graphs demonstrate that no SSEPs were recorded from 27 of 30 somatosensory cortices in 15 rats 2 days following a contusive C5-6 SCI. SSEPs were recorded 7 and 14 days post-SCI (from 12 of 16 cortices in 9 rats) as well as 21 days post-SCI (from 5 of 8 cortices in 4 rats). A repeated measures ANOVA (Time: $F = 16.4$, $df = 2,8$, $P = .001$) followed by Tukey HSD post hoc t-tests revealed that the mean percent (and standard deviation) of baseline (represented by a dashed line) P1 peak latencies of the recovered SSEPs (recorded from the 5 cortices in 4 rats) were significantly longer at day 7 than days 14 ($P < .01$) and 21 ($P < .05$) post-SCI (A). A paired t-test revealed that the mean percent of baseline N1 peak latencies of the recovered SSEPs (recorded from the 12 cortices in 9 rats) were significantly longer at day 7 than day 14 ($t = 2.4$, $df = 11$, $P < .05$) post-SCI (C). Significant differences were not found with repeated measures ANOVAs or paired t-tests between the mean percent of baseline P1 (B) or N1 (D) amplitudes of the recovered SSEPs recorded from the 12 cortices at days 7 and 14 post-SCI or from the 5 cortices at days 7, 14, and 21 post-SCI.

Figure 3.

These representative single units were recorded in the cuneate nucleus at obex and 1.3mm lateral to midline of a Normal rat and two rats after a contusive C5-6 SCI. Responses to hair movement and light skin contact (arrows) of various upper body parts are shown. Units were recorded in the cuneate nucleus of the Normal rat (e.g., at 800 μ m ventral to the medulla's surface) and the 15 Days Post-SCI rat (e.g., at 400 μ m ventral to the medulla's surface) after ipsilateral forepaw digits and palms plus forearm stimulation but not in the cuneate nucleus of the 3 Days Post-SCI rat (e.g., at 500 μ m ventral to medulla's surface). In contrast, units were recorded after shoulder and neck stimulation in the cuneate nuclei of all rats (Normal: e.g., at 400 μ m ventral to the medulla's surface, 3 Days Post-SCI: e.g., at 300 μ m ventral to the medulla's surface, 15 Days Post-SCI: e.g., at 400 μ m ventral to the medulla's surface). The cuneate nuclei of the injured rats were contralateral to the forelimb somatosensory cortices from which no (2 Days Post-SCI) or recovered (14 Days Post-SCI) SSEPs were recorded. The cytochrome-

oxidase-stained transverse section of the Normal rat's medulla at obex (−14.3mm posterior to bregma, Paxinos and Watson, 1998) shows an electrode track (black arrow) in the right cuneate nucleus (white arrow). The hypoglossal nucleus (12) and gracile nucleus (Gr) are also indicated.

Figure 4.

These bar graphs display the average total percentage of responsive recording sites in the Normal rats' ($n = 5$), Chronic SCI rats' (15 days post-SCI, $n = 5$), and Acute SCI rats' (3 days post-contusive C5-6 SCI, $n = 5$) cuneate nuclei at 0, 0.2, 0.4, and 0.6 mm anterior to plus 1.1, 1.3, and 1.5 mm lateral to obex where units were recorded after hair movement and light skin contact of the ipsilateral forepaw (Fp), forearm (Fa), as well as neck and shoulder (NS). The cuneate nuclei of the injured rats were contralateral to the forelimb somatosensory cortices from which no (Acute SCI) or recovered (Chronic SCI) SSEPs were recorded.

Figure 5.

This bar graph shows the mean total percentage and standard deviation of responsive recording sites to hair movement and light skin contact of the ipsilateral forepaw in the cuneate nuclei of the Normal rats ($n = 5$) and Chronic SCI rats (15 days post-contusive C5-6 SCI, $n = 5$) at 0, 0.2, 0.4, and 0.6 mm anterior to obex. The cuneate nuclei of the injured rats were contralateral to the forelimb somatosensory cortices from which recovered SSEPs were recorded. There were no significant differences found with repeated measures ANOVA for recorded single and multi-units between the Normal and injured rats at the various posterior to anterior levels.

Figure 6.

A representative cresyl violet-eosin-stained, transverse section at the epicenter shows that the contusive C5-6 SCI evolved over 1 month to extensive white and gray matter damage with cavity formation. In particular, damaged occurred in the dorsal columns (A). Representative CTB-immunostained coronal sections of cuneate nuclei at obex from an injured rat (B), whose injury epicenter is shown in (A) and SSEPs are shown in Figure 1, and a Normal rat (C) that had CTB injected into their ipsilateral median nerves. CTB-immunofluorescent axon terminals were seen in the injured rat's cuneate nucleus. The dorsal columns (DC) and dorsal horn gray matter (GM) are indicated. Scale bars: (A) = 1 mm and (B, C) = 100 μ m.

Table 1

Summary of Experimental Group Procedures

Groups	Days Post-SCI	Cortical SSEPs	Terminal Recordings in the Medulla	Neuroanatomical Tracing
C-SCI(N=15)	2	n=15 *		
	3		n=5	
	7	n=9		
	14	n=9		
	15		n=5	
	21	n=4		n=4
Sham(N=9)		n=9	n=5	n=4

* One rat was euthanized on day 2 post-SCI after the SSEP procedure due to morbidity.

Table 2
Baseline and week 1 post-SCI means and SD for SSEP waveform components of normal and injured rats (n=12 and 5 cortices).

Component	Baseline(n=12)	Week 1(n=12)	P value	Baseline(n=5)	Week 1(n=5)	P value
PI Peak Latency(ms)	9.7 ± 0.3	12.5 ± 1.6	$P<.001^1$	9.8 ± 0.3	12.9 ± .8	$P<.005^2$
PI Amplitude(µV)	27.4 ± 10.3	14.9 ± 6.9	$P<.005^4$	28.3 ± 3.9	20.6 ± 7.1	ns
NI Peak Latency(ms)	14.7 ± .8	16.3 ± 1.9	$P<.05^3$	14.8 ± 0.7	17.1 ± 2.0	ns
NI Amplitude(µV)	49.1 ± 13.6	22.2 ± 9.8	$P<.001^5$	50.1 ± 10.7	29.0 ± 9.3	$P<.01^6$

Independent t-tests:

ns=not significant

- ¹ $t_{(12)} = 5.9$
- ² $t_{(4)} = 6.8$
- ³ $t_{(15)} = 2.8$
- ⁴ $t_{(22)} = 3.5$
- ⁵ $t_{(22)} = 5.6$
- ⁶ $t_{(4)} = 4.5$