Alterations in Burst Firing of Thalamic VPL Neurons and Reversal by Nav1.3 Antisense After Spinal Cord Injury

Bryan C. Hains,1,2 Carl Y. Saab,3 and Stephen G. Waxman1,2
1Department of Neurology and Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven; 2Rehabilitation Research Center, Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut; and 3Department of Surgery, Rhode Island Hospital, Brown University School of Medicine, Providence, Rhode Island

Submitted 26 September 2005; accepted in final form 7 February 2006


INTRODUCTION

Experimental spinal cord injury (SCI) produces behaviors indicative of chronic neuropathic pain (Hains et al. 2001; Hulsebosch et al. 2000), concomitant with development of hyperresponsiveness in spinal cord dorsal horn nociceptive neurons (Hains et al. 2003a,b, 2004) and in higher-order neurons of the ventral posterolateral (VPL) nucleus of the thalamus (Hains et al. 2005). Most dorsal horn nociceptive neurons project rostrally to the contralateral VPL, which is involved in sensory-discriminative aspects of pain (Price and Dubner 1977; Willis and Coggeshall 2004). Pain-related changes in thalamic structure and functional properties have been documented in rats (Gerke et al. 2003), subhuman primates (Weng et al. 2000), and humans (Apkarian et al. 2004; Lenz et al. 1989, 1994; Pattany et al. 2002), with electrophysiologic changes including the emergence of burst firing (Gerke et al. 2003; Lenz 1989, 1994; Weng et al. 2000).

Factors underlying abnormal thalamic electrophysiology after SCI are only partially understood. We have recently demonstrated that in a rodent model of SCI, neurons of the VPL develop high-frequency spontaneous activity, become hyperresponsive to peripheral stimulation, produce afterdischarges, and have larger peripheral receptive fields (Hains et al. 2005). Increased spontaneous discharge activity persists after spinal cord transection rostral to the lesion, indicating that the thalamus develops independent hyperexcitability after SCI. At the same time, the Na+,1.3 sodium channel is abnormally expressed within VPL neurons after SCI, and intrathecal administration of antisense (AS) oligodeoxynucleotides generated against Na+,1.3 reverses these electrophysiologic changes. Na+,1.3 reprimers (recovers from inactivation) rapidly and produces a depolarizing current in response to small stimuli close to resting potential, thus increasing the excitability of cells in which it is expressed (Cummins and Waxman 1997; Cummins et al. 2001).

We have proposed that hyperresponsive dorsal horn neurons produce an increased spinal barrage that triggers molecular changes in thalamic nuclei that process and relay nociceptive inputs (Hains et al. 2005), that could help to maintain chronic pain after SCI, independent of spinal activity. Consistent with this hypothesis, spinal cord transection (Melzack and Loeser 1978) and conduction block (Loubser and Donovan 1991), rostral to the site of SCI, are ineffective in eliminating pain in humans after SCI, suggesting that the thalamus can serve as an abnormal central pain generator, independent of pathological drive from spinothalamic neurons (Waxman and Hains 2006).

In this study, we hypothesized that thalamic VPL neurons manifest abnormal burst firing properties after SCI. Our analysis revealed that there are changes in a number of burst features after injury and suggest that the Na+,1.3 sodium channel contributes to these phenomena.

METHODS

Animal care

Experiments were carried out in accordance with National Institutes of Health guidelines for the care and use of laboratory animals; all animal protocols were approved by the Yale University Institutional Animal Use Committee. Adult male Sprague–Dawley rats (200–225 g) were used for this study. Animals were housed under a 12-h light–dark cycle in a pathogen-free area with free access to water and food.

Address for reprint requests and other correspondence: S. G. Waxman, Department of Neurology, LCI-707, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06510 (E-mail: stephen.waxman@yale.edu).

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Spinal cord contusion injury

Rats were deeply anesthetized with ketamine/xylazine [80/5 mg/kg, administered intraperitoneally (ip)]. Spinal contusion injury (n = 22) was produced at spinal segment T9 using the MASCSI/NYU impact injury device (Gruner 1992), whereby a 10-g, 2.0-mm-diameter rod was released from a 25-mm height onto the exposed spinal cord. For sham surgery, animals (n = 7) underwent laminectomy and placement into the vertebral clips of the impactor without impact injury. After SCI or sham surgery (“intact” group), the overlying muscles and skin were closed in layers with 4–0 silk sutures and staples, respectively, and the animal was allowed to recover on a 30°C heating pad. Postoperative treatments included saline [2.0 ml, administered subcutaneously (sc)] for rehydration and Baytril (0.3 ml, 22.7 mg/ml sc, twice daily) to prevent urinary tract infection. Bladders were manually expressed twice daily until reflex bladder emptying returned, typically by 10 days postinjury. After surgery, animals were maintained under the same preoperative conditions and fed without restriction.

Oligodeoxynucleotide synthesis and delivery

In animals (n = 15) 28 days after SCI, under ketamine/xylazine (80/5 mg/kg, ip) anesthesia, a sterile premeasured 32G intrathecal (i.t.) catheter (ReCathCo, Allison Park, PA) was introduced through a 21 nucleotides, to the sequences for any other sodium channel or to the translation initiation site of Nav1.3 (5'-CGA TCG CGT CAT CTT TTC-3'). Search Tool (http://www.ncbi.nlm.nih.gov/BLAST/; Altschul et al. 1997). A subset of these animals. By a search using the Basic Local Alignment Search Tool (BLAST) program, we identified a potential target sequence for AS delivery. The sequence is complementary to the 3' untranslated region of Nav1.3 (5'-CGA TCG CGT CAT CTT TTC-3').

Electrophysiologic procedures

Animals from control intact, SCI, SCI + MM, and SCI + AS groups underwent extracellular single-unit recording of VPL neurons according to established methods (Hains et al. 2005). SCI animals were studied from 4 to 5 wk after injury. The activity of four to seven units/animal were recorded, yielding 28–56 units/group. Rats were initially anesthetized with halothane (4% in induction chamber) and maintained by tracheal intubation (1.1%, 2–2.5 ml tidal volume, 60–70 strokes/min). Rectal temperature was maintained at 37°C. The head was fixed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) and a skin incision was made with a minimal craniotomy to allow electrode penetration. The recording microelectrode was mounted on a hydraulic microdrive (Kopf Instruments) for accurate vertical migration and electronic display of travel distance.

Neuronal units were isolated from the ventral posterolateral (VPL) nucleus of the thalamus (stereotaxic coordinates in mm: bregma [-3.14]; lateral [2.0–3.5]; vertical [5.0–7.0]). Extracellular single-unit recordings were made with a low-impedance 5-MΩ tungsten-insulated microelectrode (A-M Systems, Carlsborg, WA). Electrical signals were amplified and filtered at 300–3,000 Hz (DAM80, World Precision Instruments, Sarasota, FL), processed by a data collection system (CED 1401+; Cambridge Instruments, Cambridge, UK), and stored on a computer (Latitude D800, Dell, Austin, TX) with Spike2 software (v5.03, Cambridge Electronic Design, Cambridge, UK). Once a cell was identified, its receptive field was mapped and it was classified as a multireceptive unit according to previously referenced methods (Hains et al. 2005). Spike2 template-matching routines and principal component analysis were used to differentiate among multiple units in waveamark records.

Records of burst firing were obtained in the absence of peripheral receptive field stimulation for periods of 600 s. Burst analysis was performed using the Spike2 bursts.txt v1.25.s k.txt (http://www.ced.co.uk/sp2/ptu.shtml#bursts) and burst events were identified by the following parameters: maximum interval signifying burst onset (6 ms), maximum interspike interval (9 ms), longest increase in interspike interval within a burst (2 ms), and minimum number of spikes within a burst (1). Burst events were examined statistically and burst duration, interspike interval, interburst interval, and events per burst versus time were computed. Histograms showing distribution of spikes/burst, burst duration, and interburst intervals were constructed. In addition, interspike interval durations were plotted against number of spikes contained within a burst and linear regression analysis was performed on the duration of first interspike intervals versus number of interspike intervals per burst from these data. Unit firing modes were categorized as silent, single-spiking, bursting, or spindle waves (identified by firing frequency [intrasequence frequency of 7–14 Hz, interspike frequency of about 0.2 Hz], waxing and waning amplitude, duration of more than 2 s, full return to baseline between spindle waves, and shape). A burst epoch was defined as a period of sustained firing of bursts for more than 2 s, where each burst was separated by <300 ms from one burst to the next burst. Phase histograms were constructed from records containing burst epochs. To analyze the relative contributions of discrete frequencies to signal, fast Fourier transformation (FFT) of waveform data into power (frequency) spectrum was performed. FFT block size was set to 1,024 (512 bins at 97.66 Hz) as determined by Nyquist criteria, with Hanning windowing of root mean square data.

At the conclusion of recording, a direct current (1 μA for 20 s) was passed through the recording electrode to identify the location of unit recording sites. Recording sites were plotted from three to four animals in each group: intact (n = 3), SCI (n = 4), and SCI + AS (n = 3). The brain was removed and fixed in 4% cold buffered paraformaldehyde in PBS for 48 h at 4°C in 30% sucrose before frozen sectioning at 20 μm. Sections were mounted on gelatin/potassium chromium sulfate–coated slides and stained with cresyl violet (0.1%) for visualization and photomicroscopy.

Statistical analysis

All statistical tests were performed at the alpha level of significance of 0.05 by two-tailed analyses using parametric tests. Data were tested for significance using one-way ANOVA to determine degree of variability within a sample and whether there was a difference between groups among the obtained means, followed by Bonferroni post hoc analysis. Tests of factors including pairwise comparisons were performed with either the paired Student’s t-test for before–after comparisons or the two-sample Student’s t-test to compare two groups. Data management and statistical analyses were performed using SAS (1992) statistical procedures with SigmaStat (v1.0) and graphed using SigmaPlot (v7.0) as mean ± SD.

RESULTS

Recording sites

Coronal histological sectioning through the ventrobasal nucleus complex of the thalamus corresponding to bregma -3.14 mm confirmed that the tip of the recording electrode was located in the ventral posterolateral (VPL) nucleus of the thalamus.
within the VPL. A typical electrode penetration is shown in Fig. 1A. The track of the electrode passed through the hippocampus and VPM and, in this case, ended in the VPL. Superimposed on a schematic diagram of the ventrobasal complex of the thalamus (Paxinos and Watson 1998) is the distribution of histologically identified electrode lesions marking 10 recording sites (Fig. 1B). All units analyzed were contained within the atlas boundaries of the VPL. Aberrant expression of the Nav1.3 sodium channel (which is not detectable within the thalamus of intact animals) within VPL neurons after SCI is documented in Hains et al. (2005) and is not illustrated here.

**Burst epochs**

In intact animals, epoch firing was observed in 13% of units sampled, compared with 33% of units sampled in animals with SCI. In units from intact animals, the mean epoch duration was 4.9 ± 2.3 s (n = 15 units) (Fig. 2C). Interepoch intervals ranged from 4.6 to 8.0 s (mean 6.5 ± 1.2 s). In SCI animals, the mean epoch duration was 8.7 ± 2.8 s (n = 18 units) (Fig. 2C), and interepoch intervals ranged from 2.3 to 5.4 s (mean 4.0 ± 1.1 s). A typical epoch pattern in a unit after SCI is shown in Fig. 2A, with epochs of different lengths labeled a and b. In this case, each epoch had a unique duration. Phase histograms of two different units (Fig. 2B) demonstrate epochs of variable length in records of 366 s (Fig. 2Ba) and 420 s (Fig. 2Bb) lengths. Spinal administration of Na,1.3 AS to SCI animals did not significantly change the proportion of units that displayed epochs (22% of sampled units), epoch duration (7.5 ± 3.0 s), or interepoch interval range 2.4–12.5 s (mean 7.7 ± 3.2 s, n = 18 units) (Fig. 2C).

**Burst analysis**

In units from SCI animals, the rate of single-spike events was significantly elevated (6.5 ± 1.5 spikes/s, n = 18 units analyzed) compared with units from intact animals (2.9 ± 1.0 spikes/s, n = 19 units analyzed) as shown previously (Hains et al. 2005). Burst firing was present in both groups. To illustrate the differences in single-spike events and burst event firing patterns, representative traces are shown in Fig. 3. In this example, burst activity was random in intact animals (Fig. 3A). In contrast, after SCI, burst events exhibited a rhythmic (oscillatory) firing pattern (Fig. 3B). Na,1.3 AS treatment disrupted this oscillatory pattern (Fig. 3C). Expanded sample traces of individual burst events for units from intact, SCI, and SCI + AS animals are shown in Fig. 3, A, B, and C, respectively.

The predominant spike configuration [88% of sampled bursts (n = 50)] in our records from units from SCI animals was consistent with high-threshold spikes; characterized by discharges without a preburst silent period, an increased number of burst events occurring during epochs of increased firing, and the presence of oscillatory frequencies at 5–10 Hz. In some cases (12%), low threshold spikes characterized by very long preburst interspike intervals were observed.
Power spectrum analysis showed greater oscillatory burst activity in units from SCI and SCI/AS groups, compared with units from the intact group (n = 6 units/group analyzed). In units from intact animals, power amplitudes were dominant at lower frequencies (Fig. 4A) and discrete peaks were present at frequencies within the power spectrum corresponding to spontaneous discharge frequencies (2.3 ± 1.3 Hz, Fig. 4A). In units from SCI (Fig. 4B) and SCI + AS (Fig. 4C) groups, increases in power amplitudes occurred at higher frequencies, indicative of burst activity (2.1 ± 1.1 and 5.0 ± 1.8 Hz, and broad peak from 4.3 to 9.7 Hz, Fig. 4B). Delivery of Na\textsubscript{v}1.3 AS did not significantly decrease peak power amplitude or area, and increases in power amplitudes were also observed at 4.9 ± 1.8 Hz (Fig. 4C), although the broad peak was less prominent, suggesting a trend toward reversal to an irregular firing mode.

Burst features are shown in Fig. 5. Representative interval duration histograms showed that in units from intact animals (n = 89 bursts analyzed) (Fig. 5A), spike events occurred with variable interval durations. Higher spike counts were present within several domains on the histogram, the first of which is 0–10 ms, and the second 10–35 ms. After SCI (n = 114 bursts analyzed), spike intervals became oscillatory, as evidenced by the high spike count within a single short interval duration period (0–10 ms), and not at any other time period (Fig. 5B). Treatment with Na\textsubscript{v}1.3 AS (n = 124 bursts analyzed) caused spike events to assume an irregularly distributed firing pattern (Fig. 5C), similar to the pattern in units from intact animals. There was no difference between SCI and SCI + MM groups (data not shown).

Individual spike events per burst were significantly reduced in units from SCI animals (5.1 ± 1.4 spikes/burst) (Fig. 5Ba) compared with those in intact animals (9.2 ± 1.1 spikes/burst) (Fig. 5Aa). After Na\textsubscript{v}1.3 AS treatment, the number of spike events within bursts (8.0 ± 1.5 spikes/burst) was significantly greater compared with SCI (Fig. 5Ca). Mean burst duration was not significantly different in units from SCI animals (15.9 ± 8.0 ms) (Fig. 5Ab), compared with units from SCI animals (11.1 ± 5.1 ms) (Fig. 5Bb), or after Na\textsubscript{v}1.3 AS administration (12.6 ± 7.8 ms) (Fig. 5Cb). Distribution of mean interburst intervals showed no significant differences between intact (3.9 ± 0.9 ms) (Fig. 5Ac), SCI (3.6 ± 0.7 ms) (Fig. 5Bc), and SCI + AS (3.8 ± 1.4 ms) (Fig. 5Cc) groups.

Quantitative analysis of burst duration is summarized in Table 1. Compared with units from intact animals, units from animals with SCI showed significant differences in spikes/burst and burst duration for bursts that contained at least four spikes.
(a range of two to seven spikes/burst was analyzed). Nav1.3 AS, but not MM, treatment after SCI resulted in a significant reversal in spikes/burst and burst duration for bursts of three, six, and seven spikes only. In units from the SCI group, there was also a greater decrement in the duration of the first interspike interval compared with the intact group, which became statistically different after four spikes/burst. In units from all three groups of animals, interspike intervals became

![Image](https://i.imgur.com/3.png)  
**FIG. 3.** Representative records of spontaneous firing of VPL units from intact, SCI, and Nav1.3 AS–treated animals to illustrate single-spike events (SE) and burst events (BE), denoted above each trace by line deflections. In the unit from the intact group, SE and BE occurrence appeared random (A). In animals with SCI, SE appears random, but BE exhibited a rhythmic oscillatory firing pattern (B). This unit from an SCI animal treated with Nav1.3 AS showed a random pattern of SE and BE distribution (C). Individual BEs in units from intact (a), SCI (b), and SCI + AS (c) groups are shown to illustrate the differences in number of discharges per burst and burst duration. After SCI, for example, there were fewer spikes per burst and the burst duration is shorter (b).

![Image](https://i.imgur.com/4.png)  
**FIG. 4.** Power spectrum analysis revealed random burst cycling in units from intact animals, whereas oscillatory burst activity was present in units after SCI and after administration of Nav1.3 AS. Representative spectra are shown. In this unit from an intact animal, power amplitudes were dominant at lower frequencies (arrows, A). Discrete peaks were observed within the power spectrum, corresponding to frequencies of spontaneous discharges, but power amplitude was elevated throughout the record. In representative units from SCI (B) and SCI + AS (C) groups, increases in power amplitudes were also observed at relatively higher frequencies (arrowheads), indicative of a greater degree of spontaneous and burst firing. Delivery of Nav1.3 AS did not significantly decrease peak power amplitude or power area, although the broad peak suggesting disorganized firing became less prominent.
FIG. 5. Features of burst firing in units from intact, SCI, and SCI animals treated with Na1.3 AS are shown in representative interval-duration histograms. In a unit from an intact animal, spike events occurred at irregular intervals (A). After SCI, intervals became more regular, as evidenced by the high spike count within a single interval-duration period (0–10 ms), and not at any other duration period (B). After treatment with Na1.3 AS, spike events became irregularly distributed (C). Wavemark overdraws are shown for each histogram to confirm analysis of single units. Within bursts, units from intact animals exhibited a significantly (*P < 0.05) greater number of individual spike events per burst (As) when compared with SCI (Bs). In units from SCI animals receiving Na1.3 AS, the number of spike events within bursts was significantly (‡P < 0.05) reduced (Cs). Units from intact animals (AB) did not have a significantly longer burst duration than after SCI (BB). Na1.3 AS administration did not result in a significant increase in burst duration (CB). Similarly, there were no significant differences in interburst interval in units from intact (Ac), SCI (Bc), and SCI + AS (Cc) groups.

longer with each successive spike in a burst (Fig. 6). For bursts containing increasingly higher numbers of spike events, further interspike interval lengthening was associated with higher burst ordinal, and a deceleration in firing rate throughout the burst was observed. In all groups, an inverse relationship between the first interspike interval duration and the number of spike events per burst was observed; as the number of spike events per burst increased, the duration of the first interspike interval was reduced. Plotting the duration of the first interspike interval against number of intervals per burst illustrated a linear relationship in all groups. The slope function in units from SCI animals was significantly greater (y = −0.69x, \( r^2 = 0.91 \)) (Fig. 6, C and D) compared with units from intact animals (y = −0.46x, \( r^2 = 0.90 \)) (Fig. 6, A and B). Administration of Na1.3 AS to SCI animals did not significantly change slope function compared with units from the intact group (y = −0.38, \( r^2 = 0.72 \), \( P = 0.06 \)) (Fig. 6, E and F).

Firing modes

A number of sampled units exhibited the ability to spontaneously switch between several unique firing modes (silent, single-spike, burst, spindle waves).

Population analysis of units from intact (n = 25 units), SCI (n = 32 units), and SCI + AS (n = 35 units) groups showed different trends in firing mode distribution. In all groups, discharge activity occurred primarily in single-spike and burst modes. In units from intact animals, where 16% of the units analyzed exhibited alternating firing modes, percentage of time spent in silent, single-spike, burst, and spindle wave modes was 8.3, 47.6, 31.7, and 12.4%, respectively. In units from the SCI group, where 20% of the units analyzed exhibited alternating firing modes, percentage of time spent in silent, single-spike, burst, and spindle wave modes was 3.0, 27.5, 53.8, and 15.1%, respectively. Therefore after SCI, units spent a significantly higher percentage of time in the burst mode and less time in single-spike mode, compared with intact animals. Na1.3 AS did not significantly reverse the shift to a higher percentage of time in burst mode after SCI. In units from SCI + AS animals, where 20% of the units analyzed exhibited

### TABLE 1. ISI burst analysis

<table>
<thead>
<tr>
<th>Property</th>
<th>Group</th>
<th>INTACT (n = 89)</th>
<th>SCI (n = 114)</th>
<th>SCI + AS (n = 124)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burst duration, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 spikes</td>
<td></td>
<td>4.9 ± 0.2</td>
<td>8.9 ± 0.5</td>
<td>13.7 ± 0.6</td>
</tr>
<tr>
<td>3 spikes</td>
<td></td>
<td>9.3 ± 0.7</td>
<td>9.5 ± 0.8*</td>
<td>12.2 ± 1.1</td>
</tr>
<tr>
<td>4 spikes</td>
<td></td>
<td>13.7 ± 0.6</td>
<td>11.2 ± 0.7*</td>
<td>21.9 ± 1.4</td>
</tr>
<tr>
<td>5 spikes</td>
<td></td>
<td>18.2 ± 0.8</td>
<td>19.4 ± 2.2*</td>
<td>26.8 ± 1.7</td>
</tr>
<tr>
<td>6 spikes</td>
<td></td>
<td>21.9 ± 1.4</td>
<td>13.1 ± 1.9*</td>
<td></td>
</tr>
<tr>
<td>7 spikes</td>
<td></td>
<td>26.8 ± 1.7</td>
<td>19.4 ± 2.2*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. *Statistically significant difference between intact and SCI groups (P < 0.05). †Statistically significant difference between SCI and SCI + AS groups (P < 0.05).
alternating firing modes, component percentage of silent, single-spike, burst, and spindle wave modes was 6.2, 44.2, 40.3, and 9.3%, respectively. There was no significant difference between SCI and SCI + MM groups (data not shown).

**DISCUSSION**

Dorsal horn nociceptive neurons receive input from the periphery by the dorsal root ganglia and project rostrally to third-order neurons of the ventral posterolateral (VPL) nucleus of the thalamus. This pathway makes up the spinothalamic tract, which mediates pain and thermal signaling (Willis and Coggeshall 2004). Spinal cord injury alters nociceptive processing within both the spinal cord dorsal horn and the VPL, contributing to the development of chronic neuropathic pain in both animal models and humans.

In the current study, we characterized burst firing of VPL neurons after SCI. We report that SCI induces oscillatory burst activity, changes in discrete burst properties, and shifts in the amount of time spent in burst firing mode, concomitant with upregulated expression of the Na\(_{\text{a}}\)1.3 sodium channel. Intrathecal delivery of Na\(_{\text{a}}\)1.3 AS can partially restore thalamic burst firing properties in SCI animals. These results show that alterations in burst firing properties of VPL neurons after SCI, and arguably nociceptive processing, can be partially reversed by knockdown of abnormal expression of Na\(_{\text{a}}\)1.3.

A number of studies have implicated upregulated Na\(_{\text{a}}\)1.3 in the hyperexcitability of dorsal root ganglion neurons after injury (Black et al. 1999; Cummins and Waxman 1997; Kim et al. 2001), although one recent study questions the role of Na\(_{\text{a}}\)1.3 in pain after nerve injury (Lindia et al. 2005). Our results are not directly comparable with the results of this study, however, because of differences in pain models, Na\(_{\text{a}}\)1.3 AS sequences and tissue penetrability, and Na\(_{\text{a}}\)1.3 antibodies used for protein detection. We previously showed that hypersensitivity of lumbar dorsal horn nociceptive neurons is associated with pain-related behavior after SCI and demonstrated the contribution of injury-induced upregulated expression of Na\(_{\text{a}}\)1.3 (Hains et al. 2003b). In a study on pathological changes within the thalamus after SCI, more recently we showed that VPL neurons become hyperresponsive and produce afterdischarges to peripheral stimulation, develop larger peripheral receptive fields, and produce high rates of spontaneous activity that are independent of ascending spinal barrage; furthermore, we observed that these changes are associated with upregulated Na\(_{\text{a}}\)1.3 (Hains et al. 2005).

Burst activity of VPL neurons was observed both in intact animals and in animals after SCI, but occurred more frequently after SCI. It has been hypothesized that bursts are involved in normal and pathological perceptual processing (for review, see Steriade 2004); however, rhythmic oscillation of burst firing is observed in pathophysiological conditions, and it has been suggested that abnormal thalamic activity may contribute to the perception of chronic pain (Jeanmonod et al. 1993; Lenz et al. 1989, 1994; see McCormick 1999).

Our data do not allow us to know what information is contained within normal or pathological bursts, but it has been suggested that the information content of bursts is higher than that for single spikes in the visual system (Reinagel et al. 1999), and the overall probability of generating at least one postsynaptic spike is higher for bursts than for single spikes in the hippocampus (Csicsvari et al. 1998). In neurons of the ventrobasal thalamus, increased gain or “transfer ratio” induced by burst firing results in increased thalamocortical efficacy, enhancing the postsynaptic response (Swadlow and Gusev 2001). Thus it is possible that pathological burst firing after SCI may more potently activate cortical circuits involved in pain perception.

In humans with post-SCI chronic pain, thalamic neurons exhibit oscillatory burst firing characterized by high discharge rates and deceleration of firing rate throughout the burst period (Lenz et al. 1989). Neurons in anesthetic zones and zones with intact sensory fields of the human thalamus show differential bursting activity after SCI, whereby burst activity in anesthetic zones occurred at higher frequencies than zones with intact sensory fields (Lenz et al. 1994). This may be attributable in part to deafferentation of the thalamus, raising the possibility that the emergence of an imbalance between thalamic nuclei contributes to neurogenic pain (Jeanmonod et al. 1993). One report, however, showed no correlation between bursting activity of thalamic neurons and pain (Radhakrishnan et al. 1999).

**FIG. 6.** Interspike interval analysis revealed deceleration of spiking within bursts. In units from intact animals (A), burst length was significantly greater than that for units from SCI (C), and SCI animals treated with Na\(_{\text{a}}\)1.3 AS (E). After SCI, there was a greater decrement in the first interspike interval compared with the intact group. In all groups, an inverse relationship between the first interspike interval and the number of spike events per burst was observed (B, D, F). Compared with units from intact animals (B), SCI units (D) demonstrated a significantly decreased first interspike interval and slope function. Administration of Na\(_{\text{a}}\)1.3 AS (F) resulted in a slope function that more closely approximated the intact group.
Our results are similar to those presented by Gerke et al. (2003), but we extend their data to show that, after SCI, burst firing intervals become more regular, spike events are reduced within each burst, acceleration in burst duration occurs in bursts containing higher spike counts, and shifts occur among spike firing modes. Furthermore, we observed that Na\(_{\text{v}}\)1.3 returned the number of spikes/burst, burst duration, and inter-burst interval toward control levels after SCI. We cannot exclude, however, the additional potential contributions of other voltage-dependent ionic channels, such as calcium channels (Llinas and Jahnsen 1982), in configuring these electrophysiological changes.

The single-spike and burst events recorded in SCI animals are consistent with high-threshold calcium spikes (Guido et al. 1992; Jahnsen and Llinas 1984a,b; Llinas and Jahnsen 1982), although to a lesser extent, we also observed low threshold spikes. Changes in sodium current properties associated with Na\(_{\text{v}}\)1.3 expression may, in turn, influence high-threshold calcium spikes, but the present results do not permit us to firmly make this conclusion. The ability of abnormally expressed Na\(_{\text{v}}\)1.3 channels to generate rapidly inactivating currents at relatively negative potentials, as well as large ramp currents in response to slow depolarizations, suggest that neurons expressing Na\(_{\text{v}}\)1.3 may exhibit a reduced threshold and/or a relatively high frequency of firing (Cummins et al. 2001). The biophysical properties of Na\(_{\text{v}}\)1.3 may also contribute to lowered membrane potentials that configure neurons to transition from single-spike to burst firing in a voltage-dependent manner (Llinas and Jahnsen 1982).

Spindle waves, synchronized oscillations in thalamocortical and thalamic reticular systems typically observed during sleep and epilepsy, have also been associated with pain (Walker and Yaksh 1986). Damage to the spinal cord increases sleep spindle incidence in humans (Cicirata et al. 1983). Our recordings after SCI show an increased density of spindle waves compared with intact animals. Sodium currents play a role in spindle wave generation, specifically the plateau potentials and slow afterhyperpolarization that follow the cessation of each spindle wave (Kim and McCormick 1998), and it is possible that the altered kinetics conferred by Na\(_{\text{v}}\)1.3 may influence spindle wave generation. Furthermore, because corticothalamic neurons participate in the generation of spindle waves, increased cortical activity associated with SCI (Hofstetter et al. 2003; Turner et al. 2003) may result in increased drive to thalamic reticular neurons that trigger spindle waves in thalamocortical neurons. Certain anesthetics can influence the frequency of spindle wave discharges, with barbiturates producing very strong spindles and halothane producing less-frequent occurrence. We used 1.1% halothane as a maintenance concentration for all of our recordings. It is known that halothane can depress the responses of dorsal horn WDR neurons to innocuous and noxious stimuli (Namiki et al. 1980; Yamauchi et al. 2002). Despite this caveat, halothane has been used extensively as an anesthetic for electrophysiological recording, particularly in the analysis of VPL neurons in the context of pain (Guilbaud et al. 1990).

This is the first study that examines abnormal thalamic activity after SCI in relation to the expression of Na\(_{\text{v}}\)1.3, and the mitigation of firing abnormalities by knockdown of Na\(_{\text{v}}\)1.3. Because the thalamus is involved in not only relaying but also processing incoming information from the spinal cord on route to the cortex (see Lenz et al. 2004; Sherman and Guillery 2002), injury-induced changes in spinal pain generator circuitry may feed aberrant signals into the injured thalamus, which further processes and amplifies the signals before relay to supratthalamic structures (Waxman and Hains 2006). Abnormal processing at thalamic levels would then be expected to further exaggerate abnormal firing patterns from the spinal cord after SCI. Our observations of increased primary burst firing activity and reduced silence in VPL neurons after SCI lead us to suggest that, after SCI, an increased level of abnormal afferent firing is being forwarded to cortical structures involved in interpreting pain. To further discriminate between dorsal horn and VPL effects, intracerebroventricular or direct administration of Na\(_{\text{v}}\)1.3 AS into the VPL could be valuable.

Interruption of thalamic afferents has been suggested to contribute to changes in thalamic firing properties and chronic pain (Faggii et al. 1997; Jain et al. 1998; Weng et al. 2000), but thalamic abnormalities observed in our study cannot be explained solely by deafferentation of ascending inputs. In all of our analyzed units, the ability to elicit evoked responses by stimulation of the neuron’s peripheral receptive field was a necessary requirement. It is quite possible, however, that in addition to Na\(_{\text{v}}\)1.3 upregulation, reconfiguration of inputs or disinhibition of previously silenced inputs could contribute to penetration of subthreshold inputs that might alter burst firing properties (Ferreira-Gomes et al. 2004). Rhythmic network oscillation in the thalamus is accessible to external synaptic input and it has been shown that somatosensory afferents interact with other ascending pathways and corticothalamic projections (Muthuswamy et al. 1999). During sensory processing, \(\gamma\)-aminobutyric acid type B (GABA\(_B\)) receptor-mediated inhibition seems to be important in modulating receptive field size of thalamic neurons, therefore regulating the efficacy of the sensory input (Lee et al. 1994). \(\gamma\)-aminobutyric acid type B receptor-mediated inhibition seems to be important in modulating receptive field size of thalamic neurons, therefore regulating the efficacy of the sensory input (Lee et al. 1994). \(\gamma\)-aminobutyric acid type B receptor-mediated inhibition seems to be important in modulating receptive field size of thalamic neurons, therefore regulating the efficacy of the sensory input (Lee et al. 1994). In conclusion, our data show that after SCI, neurons of the VPL that possess well-defined peripheral receptive fields undergo alterations in a number of burst firing properties, and that Na\(_{\text{v}}\)1.3 sodium channel is associated with these changes. We suggest that the abnormal burst firing of VPL neurons contributes to chronic neuropathic pain after SCI. It is unclear what effects abnormal burst firing have on higher-order cortical structures involved in pain perception. Further study of burst properties using techniques such as information transfer analysis and stimulus reconstruction (Reinagel and Reid 2000; Reinagel et al. 1999) could be helpful in deciphering the information content and significance of burst firing after SCI.

Acknowledgments

The authors thank Dr. Joel Black for valuable experimental advice and B. Toftness for technical assistance.

Grants

This work was supported in part by grants from the Medical Research Service and Rehabilitation Research Service, Department of Veterans Affairs, and the National Multiple Sclerosis Society. The Center for Neuroscience and Regeneration Research is a collaboration of the Paralyzed Veterans of America and the United Spinal Association. B. C. Hains was funded by The Christopher

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