Response Characteristics of Spinal Cord Dorsal Horn Neurons in Chronic Allodynic Rats After Spinal Cord Injury

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Hao, Jing-Xia, Ron C. Kupers, and Xiao-Jun Xu. Response characteristics of spinal cord dorsal horn neurons in chronic allodynic rats after spinal cord injury. J Neurophysiol 92: 1391–1399, 2004; 10.1152/jn.00121.2004. The physiological mechanisms of chronic pain in patients with spinal cord injury (SCI) are poorly understood. In the present study, we explored response characteristics of dorsal horn neurons of spinal injured rats exhibiting chronic pain (pain-like response to innocuous mechanical and cold stimulation). Several abnormalities were found in the distribution and response characteristics of dorsal horn neurons in chronic allodynic rats. First, 17% of the recorded neurons (vs. 0% in control animals) had no receptive field. Most of these units were located at or close to the lesioned spinal segment, and they discharged spontaneously at high frequencies. Allodynic rats also showed a significant decrease in the proportion of low-threshold (LT) neurons and an increase in the proportion of wide dynamic range (WDR) neurons. The rate of spontaneous activity of high-threshold (HT) neurons was significantly higher in allodynic compared with control rats. Moreover, HT neurons in allodynic animals showed increased neuronal responses to mechanical stimulation. WDR neurons responded with higher discharge rates to innocuous von Frey hair stimulation in allodynic compared with control rats. The percentage of WDR and HT neurons showing afterdischarges to noxious pinch was also significantly increased in the allodynic rats. The proportion of WDR and HT neurons responding to innocuous cold stimulation respectively increased from 53 and 25% in control rats to 91 and 75% in allodynic animals. These results suggest that the chronic pain-like behaviors in spinal injured rats may be generated and maintained by abnormalities in dorsal horn neurons.

INTRODUCTION

Many patients with spinal cord injury (SCI) or spinal cord disease suffer from chronic debilitating pain (Loeser 2000) arising from spinal levels around the site of injury (Siddall et al. 2002). This pain is often mixed with nociceptive, visceral, and neuropathic components. The neuropathic component appears to be the most challenging for clinical pain management (Siddall et al. 2002). SCI pain is difficult to treat and responds poorly to opioids and ablative neurosurgical interventions (Boivie 1994; Finnerup et al. 2002). As improved medical management of acute CNS injury has greatly increased survival rates, chronic pain has emerged as a major clinical problem in this population of patients (Casey 1991; Yezierski and Burchiel 2002).

Several models of SCI have been developed in recent years that allow the direct examination of neuronal mechanisms mediating SCI-induced pain (Christensen et al. 1996; Scheifer et al. 2002; Siddall et al. 1995; Vierck et al. 2000; Xu et al. 1992, 1994; Yezierski et al. 1998). We have established a rat model of SCI pain after spinal ischemia that in several aspects mimics chronic pain in man (Hao et al. 1996; Wiesenfeld-Hallin et al. 1997; Xu et al. 1992, 1994). The main features of the model are marked mechanical and cold allodynia and/or hyperalgesia at skin areas rostral to the dermatome of the injured spinal segments. Autotomy and/or excessive scratching behaviors directed toward the caudal part of the body were also observed and suggest the presence of spontaneous pain, itch, and/or dysesthesia.

The present study was undertaken to explore the spinal neuronal mechanisms underlying the sensory abnormalities observed in this model. Thereto, we recorded and compared the response characteristics of dorsal horn neurons in normal and SCI rats displaying chronic allodynia-like behaviors. We examined the receptive fields, rates of spontaneous activity, and the responses to noxious and innocuous mechanical and cold stimulation of these neurons. Preliminary findings have been communicated as an abstract (Kupers et al. 1997).

METHODS

Animals

Adult female Sprague-Dawley rats (B & K Universal, Stockholm, Sweden) were used. Animals were housed on a 12 h light/dark cycle. Food and water were available ad libitum. Experiments were carried out on normal control rats and on rats that had been submitted to SCI. All experiments were approved by the local research ethics committee and were in accordance with the ethical guidelines of the International Association for the Study of Pain (IASP).

Induction of ischemic SCI

A photochemically induced ischemic SCI was produced as described previously (Hao et al. 1996; Xu et al. 1992). In brief, rats weighing 200–220 g were anesthetized with chloral hydrate (300 mg/kg ip), and one jugular vein was cannulated. A midline incision was made on the skin overlaying vertebral segments T13–L1. The animals were positioned beneath an argon laser beam and irradiated for 10 min with the beam directed toward vertebral segment T13, or T13 (spinal segments L3–5). Immediately prior to and 5 min after the start of the irradiation, erythrosin B (Red N°3, Aldrich-Chemie, Steinheim, Germany) dissolved in 0.9% saline was injected intravenously at a dose of 32.5 mg/kg. A tunable argon ion laser (Innova model 70, Coherent Laser Product Division, Palo Alto, CA) operating at 520 nm and 15 mW was used. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
at 514 nm was used. The average beam output power was 160 mW. The beam covers the entire width of the vertebra and the length is \( \sim 1-2 \) mm. The penetration of the laser for the 10-min duration of irradiation is confined to dorsal spinal cord as estimated from the resulting injury. During irradiation, the animal’s temperature was maintained between 37 and 38°C. The wound was then closed in layers and the rats were returned to their home cages. The bladder was emptied manually for \( \sim 1 \) wk until normal function was regained.

**Selection of animals**

Responses to mechanical (von Frey hairs) and cold (ethyl chloride spray) stimuli were tested approximately once a week after irradiation. Signs indicating the presence of chronic mechanical and cold allodynia had variable onset latencies (2 wk to \( \leq 3 \) mo postirradiation). Once developed, the allodynia-like behavior remained stable with no signs of spontaneous recovery. The rats used in the present study were irradiated 3–9 mo previously, and all exhibited robust allodynia-like responses to mechanical stimuli for \( \leq 2 \) mo prior to the electrophysiological investigations.

The presence of mechanical allodynia was determined by examining the vocalization threshold to mechanical stimulation applied by a set of calibrated von Frey hairs (ranging from 0.04 to 0.2155 mN). The rat was gently held by the experimenter, and a von Frey hair was repeatedly pressed to the skin until bending. Stimulation frequency was \( \sim 0.5 \) Hz and each hair was applied 5–10 times at different areas on the flanks and back. For normal rats, the vocalization threshold was usually between 715 and 930 mN. The SCI rats were considered to be allodynic when they consistently exhibited vocalization thresholds \(<70.5\) mN. The presence of cold allodynia was tested with ethyl chloride spray. The fur was shaved for cold test. Whereas normal animals responded with a simple orientation reaction, allodynic rats vocalized and showed escape behaviors after application of cold spray to the allodynic area. When sprayed on the hands of normal human volunteers, ethyl chloride produces a distinct but nonpainful sensation of cold.

**Surgical preparation for recording**

Rats were anesthetized with urethan (1.2 g/kg ip). A tracheotomy was performed, and animals were artificially ventilated. The right carotid artery was catheterized, and rats were paralyzed with pancuronium bromide (Pavulon, Organon, 2.5 mg/kg ip). The animals were artificially ventilated. The right carotid artery was catheterized, and rats were paralyzed with pancuronium bromide (Pavulon, Organon, 2.5 mg/kg ip). The animals were anesthetized with urethan (1.2 g/kg ip). A tracheotomy was performed, and animals were artificially ventilated. The right carotid artery was catheterized, and rats were paralyzed with pancuronium bromide (Pavulon, Organon, 2.5 mg/kg ip). The animals were anesthetized with urethan (1.2 g/kg ip). A tracheotomy was performed, and animals were artificially ventilated. The right carotid artery was catheterized, and rats were paralyzed with pancuronium bromide (Pavulon, Organon, 2.5 mg/kg ip).

**Electrophysiological recording**

Extracellular recordings were made using low-impedance (2–4 mΩ at 1 kHz; Frederick Haer), glass-coated, tungsten microelectrodes which were inserted into the left spinal cord by means of a hydraulic microdrive (David Kopf). In normal rats, recordings in the rostrocaudal direction ranged from spinal segments T12–L5. The exact recording level was verified by identifying the dorsal roots. In SCI rats, the lesion site could easily be visualized. In these rats, recordings were made from the edge of the lesion up to three spinal segments rostrally. At each spinal segment, three recording tracts were explored in the mediolateral direction. As search stimuli, we used gentle tapping, stroking of the skin with a cotton tip, and light squeezing of the skin with the finger tips or a serrated forceps. Action potentials were amplified, filtered and displayed on an oscilloscope. They were also led into a window discriminator (level set at 50% above the noise level) connected to a loudspeaker and fed into a Macintosh computer through a 1401 interface (CED, Cambridge, UK) for data acquisition. Peristimulus time histograms (bin width: 1 s) were displayed on-line. Data were analyzed off-line with Spike 2 software (CED). The pattern of ongoing activity was analyzed from interspike interval histogram and by a Spike2 script “burst analysis.”

**Stimulation procedures**

Single units were isolated from background activity and their receptive fields (RF) to mechanical stimuli were mapped. The rate of spontaneous activity and the neuron’s responsiveness to thermal and mechanical stimulation were quantified. First, baseline spontaneous firing rate was measured until it had reached a steady firing rate. Then the neuron’s response to brushing the RF with a cotton swab for \( \sim 5 \) s was tested. This was followed by squeezing the skin in the center of the RF with a serrated forceps (for \( \sim 5 \) s). When the spontaneous firing rate had returned to its baseline level, an ascending series of five von Frey hairs (0.7, 6.8, 24.1, 44.7, and 69.2 mN) was applied in the center of the neuron’s RF. Stimulation was applied either dynamically at a frequency of 1 Hz (5 applications over a 5-s period) or statically whereby tonic pressure was applied for 5 s. Finally, ethyl chloride was briefly \( (\sim 1 \) s) sprayed onto the receptive field.

**Analysis of neuronal responses and classification of the units**

Evoked responses were calculated by subtracting the background firing rate from the mean discharge frequency during stimulation. An afterdischarge was considered to be present when the mean firing rate of the cell stayed \( \geq 30\% \) above baseline firing rate after stopping the stimulation. Afterdischarges were calculated for the first 5-s period after stopping the stimulus and in subsequent 10-s epochs.

Cells were classified with respect to their responses to mechanical stimulation (Willis and Coggeshall 1991). The same classification scheme as described by Chung et al. (1986) was used. Cells responding best to brush stimuli were classified as low-threshold (LT) cells. Cells responding maximally to pinch, but with a brush response \( >10\% \) of the maximal response, were classified as wide dynamic range (WDR) cells. Neurons with the largest response during pinch and with either no brush response or a brush response \( <10\% \) of the maximal response, were considered as high-threshold (HT) cells. Cells for which no peripheral receptive field could be detected were classified as NoRF (no receptive field) cells.

**Statistical analysis**

The results are expressed as the means \( \pm SE \). Statistical analysis was done by means of a one-way ANOVA, followed by Fisher PLSD test, unpaired Student’s \( t \)-test or the \( \chi^2 \) test. Values of \( P < 0.05 \) were considered as statistically significant.

**Results**

**Behavioral testing**

Before starting the recording sessions, rats were again tested for the presence of allodynia. The average vocalization threshold to von Frey hair stimulation for the nine control rats was 832.5 \( \pm 37.9 \) mN. The 11 allodynic rats used had an average vocalization threshold of 7.3 \( \pm 0.8 \) mN (range: 0.7–9.2 mN). Whereas none of the control rats exhibited abnormal reactions to cold stimulation, the SCI rats showed marked pain-like reactions to ethyl chloride spray. The allodynic body areas covered the caudal back, trunk, or lower abdomen. Regions of mechanical and cold allodynia were usually overlapping.
General electrophysiological findings and recording depths

The data presented include recordings from a total of 100 units among which 53 were recorded in allodynic and 47 in normal rats. Units with input from joints were excluded. Because mechanical stimulation was used as a search stimulus, all the neurons were mechanoresponsive except for those without a receptive field (see following text). Average recording depths did not differ between allodynic (494 ± 32 μm) and control (438 ± 30 μm) rats.

Receptive fields

The 47 neurons recorded in normal rats all had an identifiable cutaneous RF on the left hindpaw, hindlimb, or flank area. The size of the RFs varied from very small (e.g., a single toe) to large (e.g., several square centimeters on the flank). The neurons recorded from lumbar segments had RFs mostly on the paw, limb, or hip, whereas neurons recorded from low thoracic segments usually had their RFs on the flank.

In contrast to the neurons recorded in control rats, 10 of the 53 units in allodynic rats had no peripheral RFs. The mean recording depth of these units was 482 ± 79 μm (range: 50–853), and the majority of these neurons were clustered around the edge of the injured spinal segment. They had a high rate of spontaneous activity (see following text). The remaining 43 neurons had identifiable cutaneous RFs. As the injury site was located at spinal segments L2–4 and recordings were made one to three segments rostral to the lesion, the explored segments were in the high lumbar and low thoracic region. Consequently, the receptive fields were most often located on the hip and flank area with only few units with a RF on the hindlimb. No major changes were observed in terms of size and continuity of RFs between chronic allodynic and control rats.

Classification of neurons

The relative proportion of LT, WDR, and HT neurons differed significantly between normal and allodynic rats (Table 1). The most striking differences were the higher proportion of WDR and the smaller proportion of LT units in the allodynic rats (Table 1). Unlike in normal rats, the distribution of different types of neurons covaried with the rostrocaudal recording level in the allodynic rats (Table 1). This was primarily because the neurons without RF were mostly found close to the edge of the lesion. However, the HT neurons also tended to be located in the same region, with seven of nine HT neurons recorded one segment rostral to the lesion (Table 1).

Spontaneous activity (SA)

The majority of neurons in control rats had either no or a background SA <1 Hz (Fig. 1). There was no significant difference in the levels of SA among the three types of neurons (Fig. 1A). In the allodynic rats, the majority of neurons had a SA >1 Hz. The neurons without RF usually spontaneously discharged at high frequencies. All but one had a SA >5 Hz. HT neurons in allodynic rats also exhibited higher level of SA with three units showing a SA >15 Hz. In allodynic rats, average firing rates for HT or neurons without RF were significantly higher than that of LT or WDR neurons (Fig. 1A). Furthermore, SA rate of HT neurons was significantly higher in allodynic compared with control rats. No differences in SA were found for LT and WDR neurons between the two groups (Fig. 1A).

When the neurons in the allodynic rats were classified with respect to recording level, it appeared that there was an increase in SA in neurons situated close to the lesion site. As shown in Fig. 1B, the number of units with a SA of >5 Hz was largest at recording sites close to the lesion. This is largely attributable to the presence of neurons with no RF in this area. In contrast, we found no rostrocaudal gradient in the level of SA in control rats (Fig. 1B).

We also analyzed the spontaneous firing pattern of the NoRF neurons. Only two of these neurons had a regular firing pattern, whereas the remaining neurons fired in a burst-like manner. Only in one neuron, the SA was of very short interspike interval. Further analysis of patterns of background activity using a Spike2 script burst analysis also confirmed the presence of burst-like activity in the majority of the neurons without RF.

Responses to mechanical stimulation

BRUSH STIMULATION. LT or WDR neurons in control and allodynic rats did not differ with respect to their responses to brush (Fig. 2). Similarly, the brush responses of the WDR neurons in the two groups also did not differ significantly from each other (Fig. 2).

VON FREY STIMULATION. LT neurons in both groups responded to von Frey hair stimulation especially when stimulation was applied dynamically. Allodynic and control rats did not differ with respect to their discharge patterns of LT neurons to von Frey stimulation (data not shown). HT neurons in both groups responded poorly to von Frey stimulation with only a few neurons responding to the highest strength tested (69.2 mN).

In normal rats, von Frey stimulation of increasing strength produced proportional increases in firing rate of WDR neurons (Fig. 3B, 4). On the other hand, WDR neurons in allodynic rats often failed to encode stimulus intensity (Fig. 3C, 4). These neurons already responded maximally to the lowest intensity without further increasing their firing rate at higher stimulus...
FIG. 1. A: the average rate of spontaneous activity (SA) recorded from control (○) and allodynic (□) rats, classified by cell types. The data are expressed as means ± SE, and the number of neurons studied is indicated under the columns. ANOVA indicated a significant difference in the rate of SA among different types of neurons in allodynic \( F(3,47) = 8.3, P < 0.001 \) but not in normal \( F(2,45) = 1.8, P > 0.05 \) rats. The Fisher PLSD test was used for post hoc analysis. *\( P < 0.01 \) and ***\( P < 0.001 \) compared with either low-threshold (LT) or wide dynamic range (WDR) neurons in the allodynic rats. †\( P < 0.05 \) compared with high-threshold (HT) neurons in normal rats (unpaired \( t \)-test).

B: percentage of neurons exhibiting SA = 1 Hz (○), between 1 and 5 Hz (□) or >5 Hz (■) as classified by the rostrocaudal recording level. The number of neurons included in each group is indicated under the columns. The \( \chi^2 \) test shows that in control rats the degree of SA is not influenced by the recording location (\( \chi^2 = 1.4, df = 4, P > 0.05 \)). In contrast, SA varies significantly according to recording location in allodynic rats (\( \chi^2 = 9.9, df = 4, P < 0.05 \)).

FIG. 2. Average increases in discharge rates over baseline activity in dorsal horn neurons during a 5-s brush (○) or pinch (□) in control and allodynic rats. The type and number of neurons are indicated under the columns. No difference between control and allodynic rats was seen for any of the 3 neuronal cell groups (unpaired \( t \)-test).
intensities (Fig. 3C). WDR neurons in allodynic rats had significantly lower static von Frey hair thresholds compared with control rats (Fig. 4). They also responded with higher spike frequencies to von Frey stimulation than control rats, although the difference did not reach statistical significance for any of the stimulus intensities examined (Fig. 4). The difference between allodynic and control rats was most evident for the lower stimulation intensities and tended to fade off at higher intensities (Fig. 4).

PINCH STIMULATION. Control and allodynic rats did not differ with respect to their responses of WDR and HT neurons to pinch (Fig. 2).

AFTERDISCHARGES. Table 2 shows the incidence of afterdischarges to brush and pinch stimulation for LT, WDR, and HT neurons in normal and allodynic rats. Only one LT neuron in the control groups showed an afterdischarge to pinch. Afterdischarges were more frequently observed in WDR and HT neurons of control rats. The proportion of WDR and HT neurons exhibiting afterdischarges to pinching was significantly higher in allodynic compared with control rats (Table 2). In addition, three WDR neurons also showed afterdischarges to innocuous brushing. Figure 5 shows examples of prolonged afterdischarges of a WDR neuron to brushing and of a HT neuron to pinching in allodynic rats.

Responses to cold stimulation

Because our cold stimulus (ethyl chloride spray) involves an initial innocuous mechanical component, WDR or LT neurons were classified as cold responsive only if their response to the cold spray exceeded 5 s. In control rats, a substantial portion of LT or WDR neurons were cold-responsive, whereas the majority of HT neurons were not (Table 3). The magnitude and duration of the cold response varied across neurons.

The proportion of LT cells responding to cold spray was not significantly altered in the allodynic rats (Table 3). In contrast, the proportion of WDR or HT neurons responding to cold spray was significantly higher in the allodynic rats (Table 3). Figure 6 shows the average discharge frequencies of LT, WDR, and HT cells in normal and allodynic rats after cold stimulation. There is a shift toward higher discharge rates in
FIG. 4. Average increase in discharge rate over baseline SA of WDR neurons during 5-s von Frey hair application in either dynamic (A) or static mode (B) in control (●) and allodynic (●) rats. The data are expressed as means ± SE. Stimulation intensity is expressed on a log scale. C, response thresholds of individual dorsal horn WDR neurons to von Frey stimulation applied either in dynamic or static mode. Each neuron is represented by a circle and the median value for each group is indicated by the height of the columns. *P < 0.05 between normal and alldynic rats with Mann-Whitney U test. Stimulation intensity is expressed on a log scale.

FIG. 5. Peristimulus histograms showing afterdischarges of dorsal horn neurons to mechanical stimulation (bin width: 1 s). A: a WDR neuron showing prolonged afterdischarges to brush. This neuron was recorded from spinal segment T1, 3 segments rostral to the lesion at a depth of 715 μm. The receptive field is on the flank area. B: a HT neuron with a largely prolonged response to pinch. This neuron was recorded from spinal segment L3, 1 segment rostral to the lesion, at a depth of 404 μm.

TABLE 2. Number of cells with afterdischarges after mechanical stimulation

<table>
<thead>
<tr>
<th>Control</th>
<th>Alldynic</th>
</tr>
</thead>
<tbody>
<tr>
<td>WDR</td>
<td></td>
</tr>
<tr>
<td>Brush</td>
<td>0/18</td>
</tr>
<tr>
<td>Pinch</td>
<td>5/18</td>
</tr>
<tr>
<td>HT</td>
<td></td>
</tr>
<tr>
<td>Pinch</td>
<td>3/8</td>
</tr>
</tbody>
</table>

*P < 0.05 compared to control with χ² test.

TABLE 3. Number of cells with cold responses in control and alldynic rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Alldynic</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT</td>
<td>4/16</td>
<td>3/7</td>
</tr>
<tr>
<td>WDR</td>
<td>10/19</td>
<td>18/21*</td>
</tr>
<tr>
<td>HT</td>
<td>2/8</td>
<td>7/8*</td>
</tr>
</tbody>
</table>

The χ² test indicates that the proportion of cold-responsive neurons is significantly higher for WDR and HT neurons in the alldynic rats (*P < 0.05).

DISCUSSION

In normal rats, the reported proportion of different neuronal types depends on factors such as the classification criteria used, the type of preparations, anesthesia, etc. In the present study, we used an intact rat preparation with urethan anesthesia, and we recorded from neurons with response characteristics resembling those of LT, WDR, and HT neurons (Chung et al. 1986; Willis and Coggeshall 1991). The relative proportion of these neuronal types in our sample of normal rats is similar to that reported in most previous studies in the field. In contrast, we encountered considerably more WDR neurons in the alldynic rats two to three segments above injury. In addition, we recorded from neurons without identifiable receptive field. Because the neurons in normal and alldynic rats were recorded from different animals, it is impossible to know the original phenotype of these WDR neurons. We speculate that the increased proportion of WDR neurons reflects either the appearance of novel innocuous input to HT neurons or increased responses of LT neurons to noxious input. Both possibilities imply that there is an increased excitability in sensory pathways in the spinal cord underlying the behavioral alldynia.

It is well documented that the different types of input to a given neuron are not hard-wired and can exhibit considerable plasticity under conditions such as persistent noxious stimulation, deafferentation, and disinhibition (Willis 1994; Woolf...
DORSAL HORN NEURONS IN RATS

WDR (\(\text{WDR}\)) neurons after cold stimulation in control (\(\text{H}17040\)) and HT (\(\text{HT}\)) allodynic (with sprouting of afferent sensory normal and SCI rats (Hao et al. 1992; Sorkin and Puig 1996; McMahon 1994; ; Willis 1994; Woolf 1997; Woolf et al. 1997). Such plasticity may be achieved by alterations in neuronal responsiveness, activation of silent synapses, and changes in anatomical connections between neurons (Wall and McMahon 1994; ; Willis 1994; Woolf 1997; Woolf et al. 1992). These mechanisms may contribute to the changes in the response characteristics of dorsal horn neurons in chronic allodynic rats. For instance, we and others have shown that dysfunction of the spinal inhibitory GABA and glycine system may be involved in the generation of mechanical allodynia in normal and SCI rats (Hao et al. 1992; Sorkin and Puig 1996; Yaksh 1989). Moreover, SCI has been shown to be associated with sprouting of afferent sensory fibers (Christensen and Hulsebosch 1997).

In the allodynic sample, we encountered neurons without identifiable peripheral RF. This is in line with results from studies showing spontaneously active neurons without RF in spinal cord dorsal horn or sensory thalamus after peripheral nerve, dorsal root, or spinal cord injury (Hoheisel et al. 2003; Laird and Bennett 1993; Lenz 1991; Lenz et al. 1994; Loeser and Ward 1967; Sotgiu 1993). Although we do not know the original phenotype of these neurons, it is unlikely that they would have joint input as SA in the majority of NoRF neurons is not regular. It is therefore more reasonable to assume that the normal cutaneous afferent input (either direct or indirect) to these neurons has been interrupted by the spinal cord injury, resulting in a lack of a RF. Moreover, because the neurons without RF were recorded in the vicinity of the lesion, the input to these neurons is likely to come from spinal segments at or caudal to the lesion. Thus the original RFs of these neurons are likely to be at skin areas rendered anesthetic by the injury; this implies that abnormal spontaneous discharges of neurons that project to higher centers may give rise to dysesthesia or pain referred to the anesthetic skin area.

Dysesthetic pain felt caudal to the level of a spinal lesion presents a major component of the pain syndrome in patients with spinal cord injury, often defined as below-level pain (Davidoff and Roth 1991; Finnerup et al. 2003; Siddall et al. 2002; Yezierski 1996). In our SCI rats, autotomy behavior directed toward the hind paws and tail (Xu et al. 1992) and excessive scratching leading to skin damage (J.-X. Hao, unpublished observation) also suggests that the animals suffer chronic pain below the level of the lesion. Excessive scratching has also been reported in other models of SCI (Hoheisel et al. 2003; Scheifer et al. 2002; Yezierski et al. 1998). It is thus possible that some of the behaviors and underlying sensory experiences may be caused by the abnormal discharges of the neurons without receptive field. Electric microstimulation of these neurons produced sensations that were referred to the anesthetic body parts (Lenz et al. 1994). These authors suggested that the presence of these neurons may be the physiological basis for phantom sensation and phantom pain (Lenz et al. 1994).

Allodynic rats had a larger proportion of neurons with high spontaneous activity. These neurons were located near the edge of the lesion. This is similar to the results of Hoheisel et al. (2003), who also found that neurons close to the spinal contusion exhibited increased level of ongoing activity. This is likely to be due to a clustering of HT neurons and neurons without RFs in this area. Interspike interval analysis indicated that the discharges are irregular and burst-like in the majority of these SA neurons. In patients, ongoing pain is known to fluctuate, which may be related to the presence of ongoing activity and its irregular nature. In the allodynic rats, the rate of average spontaneous activity was significantly higher in HT compared with WDR or LT neurons. In addition, SA of HT neurons was significantly higher in the allodynic compared with the normal rats. Because HT neurons receive input from nociceptors and are involved in pain signaling (Chung et al. 1986), the high rate of SA in HT neurons may give rise to spontaneous pain sensation at the level around sensory loss.

Systemic lidocaine suppresses SA in SCI rats (J.-X. Hao and R. Kupers, unpublished observations), indicating the possible involvement of sodium channels. The ectopic discharges generated in the neurona and DRG cells after peripheral nerve injury are also related to activation of sodium channels (Devor 1991; Devor et al. 1993), and it has been reported recently that the sodium channel NaV1.3 was upregulated in the spinal cord after spinal contusion, and antisense directed against this channel reduced hyperexcitability of dorsal horn neurons in spinally

**Fig. 6.** Average increase in discharge rates over baseline SA in DT (A), WDR (B), and HT (C) neurons after cold stimulation in control (C) and allodynic (B) rats. The responses are shown for the time periods 0–5, 6–10, 11–20, and 21–30 s after cold application, and are expressed as means ± SE. *P < 0.01 compared with normal rats with nonpaired t-test.
injured rats (Hains et al. 2003). Clinically, drugs with sodium channel blocking properties are to some extent useful in treating SCI pain (Finnerup et al. 2002).

Several abnormalities in response to mechanical stimulation were observed in the allodynic rats, including increased responses to pinch in HT neurons, increased afterdischarges to mechanical stimulation, and reduction of response threshold to von Frey hairs for WDR neurons. These data suggest that the responses of dorsal horn neurons to mechanical input are enhanced in allodynic rats, which may be reflected in the behavioral changes. Significantly increased response levels of dorsal horn neurons to mechanical stimulation have been reported after neurochemical or contusion injury (Drew et al. 2001; Yezierski and Park 1993), while Hoheisel and colleagues (2003) found a nonsignificant trend toward lower von-Frey hair thresholds in LT mechanosensitive neurons after SCI. WDR neurons have been suggested to play a key role in intensity coding of noxious stimulation (Bushnell et al. 1984; Dubner et al. 1989). Thus a reduction in their response threshold and an increase in their response to low-intensity mechanical stimulation may underlie the mechanical allodynia.

Prolonged afterdischarges of dorsal horn neurons to mechanical stimulation has been shown in rats after partial sciatic nerve injury (Laird and Bennett 1993; Palecek et al. 1992; Sotgiu et al. 1995) or spinal cord injury (Drew et al. 2001). Similar phenomena have been encountered in the present study. These prolonged discharges, together with an increased response to pinch stimulation, may account for the exaggerated behavioral reactions of rats to mechanical stimulation. Although not systematically studied, the phenomenon of mechanical hyperalgesia also exists in SCI rats. Also the painful behavioral response pattern to light mechanical stimulation in allodynic rats is more intense than the response pattern to strong mechanical stimulation in normal rats.

Enhanced responsiveness to cold is one of the hallmarks of our SCI pain model. In the present study, we used ethyl chloride spray as cold stimulus. In normal rats, a substantial portion of LT and WDR neurons react to this type of cold stimulation, possibly because of the mechanical component of the stimulus. To minimize the potential confounding effect of mechanical activation produced by the cold spray, we limited our data analysis to those cells showing a response duration exceeding 5 s. The high percentage of cold-responsive neurons encountered compared with earlier studies (Laird and Bennett 1993; Pertovaara et al. 1997) may be due to the fact that we used a strong cold stimulus applied over a relatively large skin area.

A higher percentage of WDR and LT neurons in the allodynic rats reacted to cold stimulation. In addition, HT neurons, which normally react poorly to cold, showed clear cold responses. It is unlikely that the presence of SCI influenced the properties of the skin receptors. Our results therefore suggest that the normal input from cold receptors to HT neurons is inhibited under normal conditions and that the reduction of this inhibition by SCI may give rise to increased cold responses. The increased incidence and magnitude of cold responses of HT neurons may be primarily responsible for the cold allodynia or hyperalgesia observed in this model. Interestingly, this hypothesis fits well with results from our previous study showing that treatment with resiniferatoxin, an ultrapotent capsaicin analogue, totally abolishes cold but not mechanical allodynia. This suggests that the enhanced cold responses are mediated by capsaicin-sensitive C-afferent input (Hao et al. 1996).

In conclusion, the present study documents a series of abnormal electrophysiological properties of dorsal horn neurons in rats with chronic pain-related behaviors after SCI. Based on the similarities between the neuronal and behavioral abnormalities, it is suggested that the behavioral symptoms in SCI rats may be explained by neuronal changes in the dorsal horn around the level of injury. The neuronal response pattern in SCI rats varied according to the segmental recording level, indicating that they are caused by the spinal lesion and subsequent plasticity. Part of the abnormalities are most likely the result of deafferentation in the zone immediately rostral to the spinal lesion. The abnormalities observed in the segmental area most rostral to the lesion may be sustained in part by the abnormal input from the more caudal segments. Moreover, SCI induces complex neurochemical changes in areas rostral to the lesion, and this may also contribute to the neuronal abnormalities.

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Sensory function in spinal cord injury patients with and without central pain.


