Spinal Neurons That Express NK-1 Receptors Modulate Descending Controls That Project Through the Dorsolateral Funiculus

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INTRODUCTION

Hyperalgesia occurs after tissue injury and inflammation and is often difficult to manage. The mechanisms underlying the development and maintenance of hyperalgesia are not fully understood; however, it is known that hyperalgesia is mediated, at least in part, by the sensitization of nociceptive spinal neurons (Millan 1999; Treede et al. 1992), also referred to as central sensitization (Baranauskas and Nistri 1998; Woolf 1992). It is well established that substance P (SP) is involved in nociceptive processing. SP is released from nociceptive afferent fibers after noxious stimulation (Duggan 1995; Duggan et al. 1987; Schaible et al. 1990), excites nociceptive dorsal horn neurons (Henry 1976; Radhakrishnan and Henry 1995; Saltner and Henry 1991), and contributes to the development of hyperalgesia (Moohchala and Sawynok 1984). In the spinal cord, SP interacts with the SP receptor, also referred to as the NK-1 receptor.

We have previously demonstrated that NK-1-expressing neurons in the spinal cord have a unique role in nociceptive processing in that the development of hyperalgesia and central sensitization requires their intact function. Selective ablation of NK-1 positive neurons using a conjugate of SP and the ribosome-inactivating toxin saporin (SP-SAP) produced a dramatic decrease in hyperalgesia induced by capsaicin, inflammation, and nerve injury (Mantyh et al. 1997; Nichols et al. 1999). Similar results were obtained after ablation of NK-1-positive neurons using a SP-diptheria toxin conjugate (Benoliel et al. 1999). Moreover, deletion of these neurons after intrathecal SP-SAP prevented central sensitization produced by capsaicin and prevented windup in remaining nociceptive neurons (Khasabov et al. 2002). The mechanisms by which NK-1-expressing spinal neurons modulate the excitability of other spinal neurons remain to be determined. Because ablation of NK-1 possessing superficial lumbar spinal neurons by SP-SAP reduces nocifensive escape behavior (Vierck et al. 2003), an essential role of these neurons in supraspinal nociceptive processes is suggested. Because the majority of NK-1-positive neurons are projection neurons and are part of the spinohypothalamic tract (STT) and spinothalamic tracts (Marshall et al. 1996; Todd et al. 2000, 2002), one possibility is that they are part of an ascending-descending circuitry that modulates excitability of nociceptive spinal neurons.

Processing of spinal nociceptive information is under the control of descending systems from a variety of areas in the brain, including the brain stem (Millan 2002). Descending modulation was initially described as inhibitory because activation of these pathways produces antinociception (Basbaum and Fields 1978; Duggan and Morton 1988; Fields and Basbaum 1978), although descending facilitation has also been described (Fields et al. 1991; Kovelowi et al. 2000; Urban and Gebhart 1999; Vanderah et al. 2001a,b). Projections from brain stem nuclei descend to the spinal cord via the dorsolateral funiculus (DLF) and ventrolateral funiculus (VLF) (Lakke 1997; Tracey 1995).

The rostral ventromedial medulla (RVM) is a major source of descending axons that travel through the DLF (Lakke 1997; Watkins et al. 1980) and has been implicated in inhibition as well as facilitation of nociceptive transmission. For example, activation of neurons in the RVM attenuates acute pain and hyperalgesia (Basbaum and Fields 1978, 1984; Yaksh and Wilson 1979) and decreases responses of spinal neurons evoked by noxious stimulation (Fields et al. 1977; Floeter and Fields 1991). Although inhibition of STT neurons occurs after stimulation of the RVM (Beall et al. 1976; McCreery et al. 1979), hyperalgesia is blocked by inactivation or lesions of the
RVM (Morgan and Fields 1994; Watkins et al. 1998), and stimulation of the RVM can increase responses of nociceptive dorsal horn neurons (Zhuo and Gebhart 1997, 2002). These studies indicate that ascending afferent input to the supraspinal level is under the immediate control of descending brain stem modulatory systems.

One approach to studying descending modulation of nociceptive transmission is to disrupt the DLF and assess the changes in responses of nociceptive dorsal horn neurons. Under normal conditions, transection of the DLF increases spontaneous and evoked activity of nociceptive spinal neurons (Li et al. 1998; Pubols et al. 1991), leading to a state of facilitation. The present study investigated whether NK-1-positive spinal neurons modulated descending systems that travel in the DLF. We examined responses of dorsal horn neurons before and after removal of descending influences through the DLF in untreated animals and in those pretreated with intrathecal SP-SAP.

METHODS

Subjects

Forty-six male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 290–470 g were used. Animals were housed on a 12-h light-dark schedule and had access to food and water ad libitum. Studies were approved by the Animal Care Committee at the University of Minnesota and were conducted according to the guidelines set forth by the International Association for the Study of Pain.

Intrathecal injection

Animals were anesthetized by intramuscular injection of ketamine (100 mg/kg) and acepromazine (45 mg/kg). Rats were placed into a stereotaxic frame, and an incision was made in atlanto-occipital membrane. A polyethylene catheter (Intramedic; 0.28 mm ID and 0.61 mm OD) was inserted into the intrathecal space to the area of lumbar enlargement for single injection. Rats received a single intrathecal injection of either vehicle (0.9% NaCl, 10 μl; n = 24) or SP-SAP (5 × 10⁻⁵ M in 0.9% NaCl, 10 μl; n = 22) followed by a 5-μl flush with saline. After injection, the catheter was removed, the incision was closed by suture, and rats were returned to their cages for recovery.

Electrophysiological recordings

Experiments were performed 28–30 days after rats received intrathecal injection. The rats were anesthetized by intramuscular injection of ketamine (100 mg/kg) and acepromazine (45 mg/kg). The trachea was cannulated, and a catheter was placed in the external jugular vein to provide supplemental anesthesia with sodium pentobarbital (10 mg·kg⁻¹·h⁻¹). The carotid artery was cannulated, and blood pressure was monitored continuously with a pressure transducer (World Precision Instruments). Experiments were terminated if mean pressure dropped <60 mmHg. The lumbar enlargement and upper thoracic segments were exposed by laminectomies, and animals were placed in a stereotaxic apparatus and secured in a spinal frame. The spinal cord was continually bathed in a pool of warm (37°C) mineral oil. Core body temperature was maintained at 37°C by a feedback-controlled heating pad.

Extracellular recordings of dorsal horn neurons were obtained using stainless steel microelectrodes (Frederick Haer, Brunswick, ME; 10 mΩ). Recording electrodes were lowered into the spinal cord in 5-μm steps at the L₄ and L₅ segments using an electronic microdrive (Burleigh). Recordings were made only from single neurons the amplitude of which was easily discriminated. Electrophysiological activity was amplified using an AC amplifier (World Precision Instruments, Model No. DAM80), audio-monitored (Grass AM8 audio-monitor), and displayed on a storage oscilloscope before being sent to a computer for data collection using a customized version of Lab View software (National Instruments, Austin, TX) that enabled storage of raw data, discriminated impulses, and stimulus temperature.

Functional classification of spinal neurons

Nociceptive neurons the receptor fields (RFs) of which were located on the plantar surface of the hindpaw were studied. Search stimuli consisted of mechanical stimulation (stoking the skin and mild pinching with the experimenter’s fingers) of the hindpaw. The RFs of isolated neurons were mapped with a suprathreshold von Frey monofilament. Each neuron was characterized functionally according to its response to graded intensities of mechanical stimuli applied to the RF. Innoxious stimuli consisted of stroking the skin with a cotton swab. Noxious stimulation included mild pinching with the experimenter’s fingers and with serrated forceps. Neurons were classed as low threshold (LT) if they were excited maximally by innocuous stimulation, wide dynamic range (WDR) if they responded in a graded fashion to increasing intensities of stimulation, and high-threshold (HT) if responses were evoked by noxious stimulation only. Also, some neurons responded preferentially to the movement of paw joints and deep tissues. In addition to mechanical stimuli, heating the skin to 45–46°C for 5 s was used to determine whether the neuron was sensitive to heat stimuli. Only WDR and HT neurons that responded to both mechanical and heat stimuli were studied.

Response measures and experimental design

After identification and functional classification of a neuron, the RF was mapped by stroking and mildly pinching with forceps and was outlined on the skin with a felt-tip pen. Mechanical threshold (mN) was determined using calibrated von Frey monofilaments applied to the most sensitive area of the RF. To obtain responses evoked by mechanical stimuli before and after transection of the DLF, four test sites within the RF were marked on the skin and stimulated with a standard von Frey monofilament (178 mN bending force applied for 2 s). Each test site was stimulated three times with a 10-s interval between stimuli.

After responses to mechanical stimulation were obtained, sensitivity to heat stimuli was assessed. Stimuli of 35–51°C were delivered from a base temperature of 32°C using a Peltier thermode (contact area of 1 cm²) and were applied in ascending order of 2°C increments with an interstimulus interval of 60 s. Stimuli were 4–5 s duration and were delivered at a ramp rate of 18°C/s.

After the initial responses of the neuron were determined, the DLF on the ipsilateral side to the recording cell was transected at the upper thoracic level (∼T₂). The mineral oil bathing the thoracic laminae was removed and transection was performed just ventral to the entry zone of the thoracic dorsal root 1.5–2 mm deep into the white matter using microsurgical scissors. To confirm that the effects of transection were not due to mechanical stimulation of descending axons, in four experiments, 4% lidocaine was applied to the cut ends of the spinal cord of vehicle-treated rats for a period of 20 min using filter paper. The test stimuli were repeated as described above at 1 h after transection.

Histological localization of recording sites and DLF transection

At the end of the experiment, the recording site was marked by passing current (10 μA for 15 s) through the recording electrode. Animals were perfused with normal saline followed by 10% Formalin containing 1% potassium ferrocyanide. Serial transverse

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sections (50 μm) were cut using a freezing microtome and were stained with neutral red. The localization of recording sites were identified by Prussian Blue marks or by small lesions. Similarly, the thoracic spinal cord that contained the DLF transection was removed, cut into transverse sections, and stained with neutral red. Sections that contained the transection were traced to determine the spatial extent of the lesion.

Immunohistochemistry studies

Rats received an intrathecal injection of vehicle or SP-SAP (n = 5 per group). At 28–30 days later, they were deeply anesthetized with pentobarbital sodium (50 mg/kg ip) and perfused intracardially with 50 ml of 0.1 M PBS followed by 400 ml of 4% paraformaldehyde in 0.1 M PBS. Spinal cord segments of the lumbar enlargement (L4-5) and upper thoracic level (T2-3) were removed, postfixed for 2 h, and cryoprotected in 20% sucrose for 48 h. Serial frozen sections (60 μm) were cut on a sliding microtome in sagittal plane and collected in 0.1 M PBS for processing as free-floating sections and washed three times in PBS for 15 min. Tissue sections were incubated for 1 h at room temperature in the blocking solution of 10% normal donkey serum in PBS, containing 0.3% Triton X-100 and incubated overnight at room temperature in the primary rabbit anti-NK-1 receptors antiserum, raised in our laboratory. Primary antibodies were dissolved at 1:5,000 by PBS containing 1% normal donkey serum and 0.3% Triton X-100. The next day, sections were washed three times for 10 min in PBS and incubated for 2 h in donkey anti-rabbit secondary antibodies labeled by the fluorescent marker Cy3 (Jackson ImmunoResearch, West Grove, PA). Secondary antibodies were dissolved at 1:600 using the same dissolving media as for primary antibodies. Finally, sections were washed three times for 10 min in PBS, mounted on gelatin-coated slides, air dried, dehydrated via an alcohol gradient (50, 70, 80, and 100%), cleared in xylene, and coverslipped. To confirm the immunohistochemical detection specificity, controls included preabsorption of the primary antibody with the corresponding specific peptide or omission of the primary antibody.

Sections were viewed through a 1-cm² eyepiece grid divided into 100 1×1-mm units. Total numbers of NK-1 immunopositive cell bodies per unit area were counted for superficial (I and II) and deep (III–V) laminae in lumbar and thoracic spinal segments of vehicle and SP-SAP-treated groups. Results are expressed as mean percentages of surviving neurons in superficial and deep laminae in SP-SAP-treated rats as compared with mean numbers of NK-1-positive neurons in corresponding laminae of vehicle-treated rats.

Data analyses

The numbers of impulses evoked by heat and mechanical stimuli before and after the DLF transection were compared between groups using ANOVA and Bonferroni post hoc comparisons. Evoked responses were determined by subtracting spontaneous discharge rate from the response that occurred during the stimulus. The proportion of neurons that were classed as HT and WDR neurons were compared between the groups using the χ² test. For all statistical tests, a probability value <0.05 was considered significant.

RESULTS

Effects of lumbar intrathecal injection of SP-SAP on NK-1 labeling in lumbar and upper thoracic spinal segments

The mean number of NK-1-positive neurons in the superficial dorsal horn of lumbar enlargement was decreased 28–30 days after SP-SAP treatment. The proportion of NK-1 expressing cells in the superficial dorsal horn was only 15.8 ± 6.1% of that found in vehicle-treated animals (84.4% decrease, P < 0.001). However, NK-1-positive neurons in deeper laminae (III–V) were unaffected by SP-SAP (Fig. 1A). Lumbar infusion of SP-SAP did not alter the labeling of NK-1 neurons at the thoracic level of the spinal cord. In SP-SAP-treated animals, the number of NK-1-expressing neurons in the thoracic spinal cord was 98.6 ± 4.8% (superficial dorsal horn) and 98.9 ± 4.1% (deep dorsal horn) of that found in vehicle-treated animals (Fig. 1B).

General characteristics of noiceptive neurons in vehicle- and SP-SAP-treated animals

Recordings were made from 27 neurons in vehicle-treated animals and from 25 neurons in animals pretreated with SP-SAP. The RF of all neurons included the plantar surface of the hindpaw. The distribution of recording sites from both groups of animals was similar and recording sites identified histologically were located in both the superficial and deep dorsal horn (Fig. 2). Of the neurons studied in the vehicle-treated group, 78% (21 cells) were classified functionally as WDR, whereas 22% (6 neurons) were classified as HT. The proportion of WDR and HT neurons encountered differed in animals that received SP-SAP: 96% (24) were WDR neurons and only 4% (1 neuron) were HT (χ² test, P < 0.05). Mechanical thresholds of each class of neurons were similar in vehicle and SP-SAP-treated groups. Mean response thresholds of WDR neurons were 12.0 ± 2.3 and 13.0 ± 2.6 mN in vehicle and SP-SAP-treated groups, respectively. For HT cells, mean thresholds in vehicle and SP-SAP-treated animals were 116.1 ± 18.3 and 97.8 mN, respectively. The proportion of all neurons in vehicle and
SP-SAP-treated groups that exhibited spontaneous activity was 74% (20 neurons) and 72% (18 neurons), respectively, and did not differ. Similarly, mean spontaneous activity did not differ between the control (4.2 ± 1.5 imp/s) and SP-SAP (4.3 ± 1.7 imp/s) groups.

Responses evoked by the standard mechanical stimulus, a von Frey monofilament with a bending force of 178 mN applied for the duration of 2 s did not differ between the groups. The mean number of impulses evoked by the mechanical stimulus was 28.5 ± 6.6 and 32.2 ± 7.0 for vehicle- and SP-SAP-treated groups, respectively.

Responses evoked by heat stimuli also did not differ between neurons recorded from control and SP-SAP-treated animals. Mean response thresholds were 42.2 ± 0.5°C for neurons in vehicle-treated animals and 42.0 ± 0.7°C in animals that received SP-SAP. As illustrated in Fig. 3, stimulus-response curves that plot the mean number of impulses evoked by each stimulus temperature did not differ between the groups (2-way ANOVA with repeated measures). Also, the mean cumulative number of impulses evoked by all the heat stimuli was 1,056.1 ± 208.0 imp for neurons recorded from the vehicle group and 1,068 ± 297 from those recorded from the SP-SAP group and did not differ significantly (t-test). Thus although the proportion of HT and WDR neurons differed between vehicle- and SP-SAP-treated animals, spontaneous activity and responses to acute mechanical and heat stimuli of remaining nociceptive neurons were not altered by pretreatment with SP-SAP.

Effect of DLF transection on spontaneous activity

Acute transection of the DLF at the thoracic level produced an immediate and long-lasting increase in spontaneous activity of all 27 lumbar nociceptive neurons (21 WDR and 6 HT) in vehicle-treated animals (Fig. 4A). The peak increase in discharge rate occurred 20–45 min after transection. At 1 h after transection of the DLF, the mean discharge rate for all neurons increased from 4.2 ± 1.5 imp/s before transection to 13.4 ± 2 imp/s after, an increase of 233% (paired t-test, P < 0.01; Fig. 5). To avoid the possibility that the increase in spontaneous activity was due to activation of descending axons resulting from trauma, 4% lidocaine was applied to the transected area (n = 4). Local anesthesia of the area of transection did not alter the increase in spontaneous activity. For these four neurons (2 WDR and 2 HT), spontaneous activity increased 240% at 1 h after transection. This suggests that the increase in spontaneous

![Fig. 2](image2.png)

FIG. 2. Histologically identified recording sites in the spinal cord of animals pretreated with vehicle and SP-SAP. Neurons recorded from both groups of animals were distributed in the superficial and deep laminae.

![Fig. 3](image3.png)

FIG. 3. The means ± SE number of impulses evoked by heat stimuli in vehicle and SP-SAP-treated groups prior to transection of the dorsolateral funiculus (DLF). Responses evoked by stimulus intensities of 35–51°C did not differ between the groups.

![Fig. 4](image4.png)

FIG. 4. Effect of DLF transection on spontaneous activity of dorsal horn neurons in animals pretreated with vehicle and SP-SAP. A: representative example of spontaneous activity for 1 cell after vehicle-pretreatment before and at 1 h after transection. This cell was not spontaneously active prior to transection of the DLF but developed spontaneous activity after transection. Also illustrated is the maximal extent of the DLF lesion (left) in this animal and the recording site in the spinal cord for this neuron (right). B: example of spontaneous activity for 1 cell after SP-SAP pretreatment before and at 1 h after transection. This cell exhibited spontaneous activity before transection of the DLF that did not change after transection. The maximal extent of the DLF lesion (left) in this animal and the recording site in the spinal cord for this neuron (right) are also illustrated.
activity after DLF transection results from the interruption of descending axons that contribute to tonic inhibition.

In contrast, transection of the DLF had no affect on the spontaneous activity of nociceptive neurons in animals treated with SP-SAP (Fig. 4B). Only 3 of 24 neurons exhibited a small mean increase (34%) in the rate of spontaneous discharge after transection, whereas the rate of spontaneous activity either did not change or decreased in the remaining 21 neurons. The mean rate of spontaneous activity for all neurons was 4.3 ± 1.7 imp/s before and 4.4 ± 2.0 imp/s after DLF transection, which did not differ (t-test; Fig. 5).

**DLF transection and responses evoked by heat stimuli**

In animals pretreated with vehicle, transection of the DLF significantly increased responses of nociceptive dorsal horn neurons to heat stimuli. A representative example of the increased response to heat for one neuron is provided in Figure 6. At 1 h after DLF transection, the mean number of impulses evoked by heat stimuli of 35–51°C for all neurons increased (ANOVA and Bonferroni t-test; Figure 7A), and the mean heat response threshold (Fig. 7B) decreased 3.4°C (from 42.2 ± 0.5 to 38.9 ± 0.5°C; paired t-test, P < 0.001). In addition, the mean cumulative number of impulses evoked by all intensities of heat stimuli was increased by 100% at 1 h after transection (from 1,056.1 ± 208.0 imp before to 2,115.3 ± 393.3 imp after transection; paired t-test, P < 0.01; Fig. 7C). Although responses of WDR and HT neurons to heat before and after transection did not differ, the effect of DLF transection was more pronounced in HT neurons. Thus at 1 h after transection the mean cumulative number of impulses evoked in HT neurons increased 133% (from 1,360.5 ± 961.8 imp before transection to 3,167.6 ± 1,910.2 imp after, paired t-test, P < 0.05), whereas responses of WDR neurons increased 91% at this time (from 1,002.4 ± 197.1 imp to 1,918.9 ± 335.7 imp, paired t-test, P < 0.01).

In animals pretreated with SP-SAP, transection of the DLF did not alter responses of dorsal horn neurons to heat (Fig. 8). The mean number of impulses evoked by each heat stimulus was similar before and after transection (Fig. 9A), and mean response thresholds (Fig. 9B) were not altered (42.0 ± 0.7°C before transection and 42.3 ± 0.8°C after). Furthermore, the mean cumulative numbers of impulses evoked by all heat stimuli were 1,068.0 ± 297.6 before transection and 1,095.2 ± 361.0 after (Fig. 9C) and did not differ significantly.

**DLF transection and responses to mechanical stimuli**

As illustrated by the representative example in Fig. 10A, transection of the DLF in animals pretreated with vehicle produced an increase in responses of dorsal horn neurons to mechanical stimuli, and responses were typically increased throughout the RF. The mean number of impulses evoked by a single application of the von Frey monofilament for all neurons
monofilament for all neurons was 32.2 ± 11 A. The mean numbers of impulses evoked by the von Frey mechanical stimuli in animals pretreated with SP-SAP (Fig. 33.9 ± 9.1 imp, paired t-test, P < 0.01; Fig. 10 B). As was found for responses to heat stimuli, transection of the DLF resulted in a greater increase in mechanically evoked responses of HT neurons as compared with WDR neurons. At 1 h after transection, the mean number of impulses for HT neurons increased 1,026% (from 5.8 ± 1.8 to 65.3 ± 24.1 imp, paired t-test, P < 0.05), whereas responses of WDR neurons increased 43% (from 34.6 ± 7.6 to 49.5 ± 9.1 imp, paired t-test, P < 0.01).

Transection of the DLF had no effect on responses to mechanical stimuli in animals pretreated with SP-SAP (Fig. 11 A). The mean numbers of impulses evoked by the von Frey monofilament for all neurons was 32.2 ± 7.0 imp before and 33.9 ± 6.9 imp after transection (Fig. 11 B).

In earlier studies, we found that ablation of spinal NK-1-expressing neurons in the superficial dorsal horn resulted in a dramatic decrease in nocifensive behaviors and hyperalgesia produced by intraplantar injection of capsaicin, inflammation, peripheral nerve injury (Mantyh et al. 1997; Nichols et al. 1999), and spinal cord injury (Yezierski et al. 2004). After SP-SAP, the remaining nociceptive neurons in the dorsal horn responded weakly to injection of capsaicin and lost their ability to become sensitized (Khasabov et al. 2002). The lack of central sensitization did not result from a decrease in the initial excitation evoked by capsaicin because injection of a high dose of capsaicin, which elicited discharge similar to that evoked in control animals, also did not result in sensitization (Khasabov et al. 2002). Consequently, we hypothesized that the development of central sensitization is dependent, at least in part, on the regulation of descending modulatory pathways by ascending NK-1-positive spinal neurons. This hypothesis is supported by the present study in which loss of NK-1-positive neurons re-

![Figure 8](http://jn.physiology.org/)

**FIG. 8.** Effect of DLF transection on heat-evoked responses of dorsal horn neurons in animals pretreated with SP-SAP. A: representative example of responses of a single neuron to stimuli of 39, 43, and 51°C before (top) and at 1 h after transection (bottom). Responses of this neuron to heat were not altered after transection. A digitized trace of each temperature and a time scale are provided. B: the maximal extent of the DLF lesion in the animal from which this neuron was recorded. C: reconstruction of the recording site in the dorsal horn for this cell.

![Figure 9](http://jn.physiology.org/)

**FIG. 9.** Responses to heat for all neurons before and at 1 h after transection of the DLF in animals pretreated with SP-SAP. A: mean ± SE number of impulses evoked by heat stimuli of 35–51°C before and after the DLF transection. Responses to heat were not altered after transection. B: mean ± SE response threshold (°C) for all neurons before and after DLF transection. Mean response thresholds were not changed after transection. C: the cumulative number of impulses evoked by all heat stimuli. The sum of impulses (impms) across all stimuli remained constant after transection.

![Figure 10](http://jn.physiology.org/)

**FIG. 10.** Enhanced responses of dorsal horn neurons to mechanical stimuli (178 mN bending force) at 1 h after transection of the DLF. A: representative examples of evoked responses of a single neuron to mechanical stimuli at various locations within the RF before and after transection. The RF is indicated by the stippled area and test sites for mechanical stimulation are indicated by the dots within the RF. Arrows point to specific test sites where pairs of responses (before and after transection) were obtained. Horizontal bars between pairs of responses represent period of stimulation (2 s). B: the mean ± SE number of impulses evoked by a single mechanical stimulus before and after the DLF transection. Responses were averaged across all test sites. **, a significant difference after transection (P < 0.01).

**DISCUSSION**

In earlier studies, we found that ablation of spinal NK-1-expressing neurons in the superficial dorsal horn resulted in a dramatic decrease in nocifensive behaviors and hyperalgesia produced by intraplantar injection of capsaicin, inflammation, peripheral nerve injury (Mantyh et al. 1997; Nichols et al. 1999), and spinal cord injury (Yezierski et al. 2004). After SP-SAP, the remaining nociceptive neurons in the dorsal horn responded weakly to injection of capsaicin and lost their ability to become sensitized (Khasabov et al. 2002). The lack of central sensitization did not result from a decrease in the initial excitation evoked by capsaicin because injection of a high dose of capsaicin, which elicited discharge similar to that evoked in control animals, also did not result in sensitization (Khasabov et al. 2002). Consequently, we hypothesized that the development of central sensitization is dependent, at least in part, on the regulation of descending modulatory pathways by ascending NK-1-positive spinal neurons. This hypothesis is supported by the present study in which loss of NK-1-positive neurons re-

![Figure 11](http://jn.physiology.org/)

**FIG. 11.** The effect of DLF transection on responses of dorsal horn neurons to mechanical stimuli (178 mN bending force) at 1 h after transection of the DLF. A: examples of mechanically evoked responses of a single neuron before and at 1 h after transection. Format is the same as in Fig. 9. Responses after transection were similar to those obtained before transection at all test sites within the RF. B: mean ± SE number of impulses evoked by a single application of the von Frey monofilament before and after the DLF transection. Responses were averaged across all test sites and were not changed after transection.
sulted in a dramatic loss of the facilitation of spinal neurons that normally follows DLF transection. Consistent with these results, it has been shown that mice lacking the NK-1 receptor also exhibited a decrease in descending control of nociceptive transmission (Bester et al. 2001).

**Descending modulation via the DLF**

Although there are several descending projections through the DLF (Watkins et al. 1981), those originating from the RVM are a main source of the projections through this pathway (Watkins et al. 1980). Two types of RVM neurons directly related to nociceptive stimulation have been identified according to their responses evoked by noxious stimuli and the correlation between their responses and withdrawal reflex activity (Basbaum and Fields 1978; Fields et al. 1991; Hirakawa et al. 2000). ON cells exhibit a burst-like increase in their discharge that is correlated with a nociceptive withdrawal reflex, and prolonged noxious thermal stimulation causes prolonged activation of these neurons (Morgan and Fields 1994). Ablation of ON cells decreased hyperalgesia (Porreca et al. 2001). OFF cells appear to inhibit nociceptive transmission because their discharge rate decreases or pauses during noxious stimulation. The notion that ON cells facilitate and OFF cell inhibit nociceptive transmission is supported by the activity of these cells during opioid analgesia. ON cells are inhibited and OFF cells are excited by opioids. Furthermore, the antinociceptive effects of morphine were blocked with selective blockade of OFF cell activity (Heinricher et al. 1999). It has been proposed that either OFF or ON cell activity within descending systems can be dominant and result in a state of decreased nociceptive transmission and analgesia (e.g., after opioid administration) or enhanced nociceptive transmission and hyperalgesia (e.g., prolonged noxious stimulation) (Fields 2004). Indeed persistent nociceptive input appears to be necessary for the development of neuronal plasticity in descending systems (Pertovaara 2000; Ren and Dubner 2002).

We found that descending systems that travel via the DLF are regulated by spinal NK-1-expressing neurons, the majority of which project to supraspinal structures (Marshall et al. 1996; Todd et al. 2000, 2002). In SP-SAP-treated animals, transection of the DLF did not increase spontaneous activity of remaining dorsal horn neurons and did not enhance their responses to mechanical and heat stimuli. These results demonstrate that descending modulatory mechanisms, perhaps originating in the RVM, are altered after the ablation of spinal NK-1-positive neurons. Results of the present study are in agreement with an earlier study (Suzuki et al. 2002) demonstrating that NK-1 expressing spinal neurons activate descending systems to control neuronal excitability in the spinal cord. In that study, loss of NK-1-expressing neurons in the superficial dorsal horn using SP-SAP attenuated serotonin-dependent descending facilitation during persistent inflammatory nociception. Because the majority of NK-1-positive spinal neurons project to supraspinal structures, it is suggested that spinal neurons possessing the NK-1 receptor play a pivotal role in driving descending pathways, including those that travel through the DLF. Transection of the DLF interrupted descending projections to the spinal cord but not ascending nociceptive pathways because these ascend primarily via the contralateral ventrolateral funiculus (Tracey 1995). Transection of the DLF in naïve animals increased spontaneous activity of nociceptive dorsal horn neurons and enhanced their responses to heat and mechanical stimuli (Li et al. 1998; Pubols et al. 1991). It is well established that the DLF-dependent descending inhibition of spinal neurons results from activity in the RVM (Basbaum and Fields 1984; Li et al. 1998; Pubols et al. 1991). Injection of local anesthetic into the RVM eliminated descending inhibition of spinal nociceptive neurons (Li et al. 1998) and inhibited the antinociceptive effect produced by stimulation of the periaqueductal gray (Sandkuhler and Gebhart 1984). Complete transection of the spinal cord did not enhance the inhibition of dorsal horn neuronal responses produced by transection of the DLF alone (Li et al. 1998), suggesting that the RVM, with its descending axons in the DLF, is the primary source of descending inhibition.

**Potential mechanisms by which NK-1-expressing spinal neurons alter descending modulation**

The mechanisms by which NK-1 positive ascending neurons affect descending modulation from supraspinal structures are unclear. Although the increased responses of spinal neurons after DLF transection were abolished by SP-SAP, it is unlikely that NK-1-positive neurons drive descending inhibition because animals that received intrathecal SP-SAP exhibited normal behavioral responses to noxious stimuli (Mantyh et al. 1997; Nichols et al. 1999) and responses of nociceptive dorsal horn neurons to acute stimuli were unchanged (Khasabov et al. 1997; Nichols et al. 1999) and responses of nociceptive dorsal horn neurons to acute stimuli were unchanged (Khasabov et al. 1997; Nichols et al. 1999) and responses of nociceptive dorsal horn neurons to acute stimuli were unchanged (Khasabov et al. 1997; Nichols et al. 1999). Injection of local anesthetic into the RVM eliminates the antinociceptive effect produced by transection of the DLF alone (Li et al. 1998), suggesting that the RVM, with its descending axons in the DLF, is the primary source of descending inhibition.

**Conclusions**

Results of the present study demonstrate that NK-1-positive spinal neurons are part of circuitry that includes rostral medullary structures involved in descending modulation of nociceptive transmission in the spinal cord. As more becomes known about the anatomical organization of NK-1-positive ascending neurons and brain stem descending modulatory neurons, it will be important to determine the transmitter systems and receptors involved at various levels within this circuitry. These studies will provide new information on the mechanisms by which descending systems modulate excitability of dorsal horn neurons.

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