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Research report

Locations and morphologies of sympathetically correlated neurons in the T_{10} spinal segment of the rat

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Abstract

We precisely localized and morphologically characterized sympathetically correlated neurons in the acutely transected spinal cord of the rat. We have shown that these neurons are likely members of the spinal networks that generate sympathetic activity after spinal cord transection. In humans with injured spinal cords, these networks are responsible for hypertensive crises that occur in response to ordinarily innocuous stimuli. We recorded from neurons in the dorsal horn of the T_{10} spinal segment of anesthetized rats after acute spinal cord transection at C_2. Neurons with activities closely correlated to renal sympathetic nerve activity (RSNA) were considered to be putative components of spinal sympathetic systems. These neurons had receptive fields on the left flank and abdomen. After characterizing their ongoing activities, receptive fields, and degrees of correlation with RSNA, we juxtacellularly labeled neurons with biotinamide and subsequently reconstructed their somas and dendrites histologically. Confirming our earlier studies, sympathetically correlated neurons were found in dorsal horn laminae III, IV, and V. For the first time, we also identified sympathetically correlated neurons in laminae I and II. The dendrites of all sympathetically correlated neurons projected to multiple lamina. By virtue of the positions of their somas and the broad projections of their dendrites, we concluded that sympathetically correlated neurons may receive direct input both from supraspinal systems and from nociceptive and non-nociceptive primary afferents.

Theme: Endocrine and autonomic regulation

Topic: Cardiovascular regulation

Keywords: Autonomic dysreflexia; Hypertensive crisis; Spinal sympathetic interneuron; Renal sympathetic nerve activity

1. Introduction

Although in intact animals the source of excitatory drive for sympathetic activity is in the brain, especially the medulla (see Ref. [9] for review), after both acute and chronic spinal transection spinal systems located caudal to the lesion are capable of generating ongoing activity in some sympathetic nerves [16,17]. In chronically spinally injured patients, large, paroxysmal increases in sympathetic activity (autonomic dysreflexias), causing dangerous increases in arterial pressure (hypertensive crises), are triggered by both noxious and innocuous somatic and visceral stimuli. Like spinally injured patients, both acutely and chronically spinalized animals also exhibit exaggerated sympathetic responses to somatic and visceral stimuli [11,12,17]. The goal of this study was to expand our knowledge of networks of spinal interneurons that presumably are responsible for sympathetic activity after spinal injury. This information could play an important role in developing methods for preventing or ameliorating hypertensive crises.

Previously, we have identified interneurons with activities closely correlated with renal sympathetic nerve activity (RSNA) in laminae III–V of the dorsal horns of both acutely and chronically spinally transected rats [3,4,8]. Not only were the ongoing activities of these neurons correlated with ongoing RSNA, but the excitatory and inhibitory responses of most of these neurons to somatic and visceral stimuli closely corresponded to simultaneous responses in RSNA. Furthermore, after

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chronic spinal cord transection sympathetically correlated neurons were excited by mechanical stimulation of an enlarged region of the body wall [8]. These observations led us to suggest that sympathetically correlated spinal neurons are putative members of the spinal networks that are responsible for both ongoing sympathetic activity and stimulus-evoked responses in sympathetic activity after spinal transection.

Although we have characterized spinal sympathetically correlated neurons electrophysiologically, until the present experiments we had not been able to identify precisely their somas, nor were we able to study their dendritic fields. Therefore, we were not able to determine whether spinal sympathetically correlated neurons are more likely to receive information from many sources or only a few, nor were we able to determine whether, by virtue of the locations of their somas and dendritic trees, sympathetically correlated neurons were likely to receive substantial direct input from descending pathways from the brain or direct input from primary afferents.

We have concluded from previous studies that spinal sympathetic networks are under substantial tonic descending inhibition in spinally intact rats [13,21]. The likelihood of descending modulatory pathways from the brain synapsing *directly* on spinal sympathetically correlated neurons would be greater if the somas or dendrites of these neurons were in regions in which these pathways are thought to descend. The likelihood of primary afferents synapsing *directly* on spinal sympathetically correlated neurons would be greater if the somas and dendrites of these neurons were in regions of the dorsal horn known to receive relevant primary afferent input.

To determine these relationships, the somas and dendrites of sympathetically correlated neurons were labeled juxtacellularly in rats after acute spinal transection. The somas of these neurons were located histologically, and their dendritic fields were reconstructed. We then correlated the locations of these somas and dendrites with the locations of known spinal primary afferents and intraspinal descending modulatory brainstem systems.

2. Methods

All procedures were approved by the Johns Hopkins Committee on Animal Care and Use (protocol number RA99M217). Male Sprague–Dawley rats (Charles River and Taconic Farms), weighing between 300 and 450 g were anesthetized with alpha-chloralose (100 mg/kg) after induction of anesthesia with halothane (5%). Some rats were anesthetized throughout the experiment with halothane (5% induction, 0.75–1.5% maintenance). Rats were paralyzed with gallamine triethiodide and artificially respired. Arterial and venous catheters were placed in the right femoral artery and femoral vein for measurements of arterial pressure and infusions of drugs, respectively. Before supplemental doses of gallamine were administered, the level of anesthesia was gauged using the corneal reflex, and chloralose was supplemented or the concentration of halothane was adjusted as necessary. Arterial blood gases were monitored throughout the experiment, and temperature was controlled between 35 and 37 °C.

The spinal cord was transected at the C_2 level using dura scissors and suction. The left renal nerve was approached via a left lateral laparotomy, crushed or cut peripherally, and placed on hook electrodes. RSNA was amplified at a bandpass of 30–1000 Hz, recorded on magnetic tape, and acquired by computer at a sampling rate of 2000 Hz.

The T_{10} segment of the spinal cord was approached by removal of the spinus process of the T_9 vertebra. After retracting the dura, the spinal cord was covered with mineral oil. Recordings of spinal neurons were made with electrodes fabricated from 2-mm capillary tubing (World Precision Instruments, Sarasota, FL, USA; M1B200F-4) and filled with 6% biotinamide (Sigma, St. Louis, MO, USA) dissolved in a solution of 0.5 M sodium acetate. Once filled with the recording electrolyte, these electrodes were broken back to impedances between 13 and 30 MΩ and mounted in either a piezoelectric or hydraulic microdrive. Recordings were made with a bridge amplifier (8700 Cell Explorer, Dagan Corporation, Minneapolis, MN, USA), and signals were acquired both by computer and on magnetic tape. Neurons with ongoing activity were identified as the electrode penetrated the left side of the spinal cord. Receptive fields to innocuous cutaneous stimulation (brushing) were determined for all neurons. Noxious cutaneous stimulation usually caused loss of (or damage to) neurons, and it was rarely attempted.

The ongoing activity of each neuron was used to trigger averaged RSNA as described in detail previously [3,4]. Only neurons that exhibited substantial positive peaks in spike-triggered averaged RSNA between 40 and 100 ms after their action potentials were characterized further. Upon completion of experiments, relationships between the action potentials of spinal neurons and RSNA were determined by true cross correlation [13]. All of the neurons reported in the present study exhibited cross correlations at least 50% larger than the magnitude of the envelope of 20 ‘dummy’ correlations. The dummy correlations were generated by cross correlating an identical segment of RSNA with a scrambled train of action potentials with interdischarge intervals identical to those in the original recording (Fig. 1A).

After characterizing their ongoing discharge properties and determining their somatic fields, we approached sympathetically correlated neurons until their recorded action potentials were large and well isolated. We used pulses of anodal current (1–8 nA, 200 ms on–200 ms off) to entrain the neurons [18,22,23], thereby introducing biotinamide into the neurons (Fig. 1B). One to three neurons were electrophysiologically characterized and

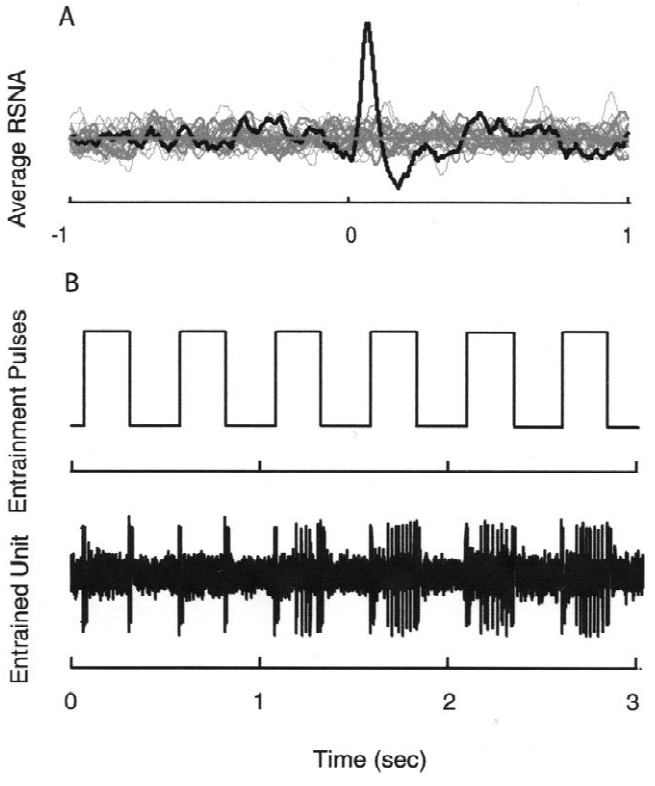

Fig. 1. (A) Dark trace: cross correlation between the incidence of discharge of a sympathetically correlated dorsal horn interneuron and simultaneously recorded RSNA. Light traces: dummy cross correlations between RSNA and discharges of a simulated neuron (see Section 2). (B) Top trace: entrainment currents applied to juxtacellularly recorded neuron. Bottom trace: onset of entrainment of action potentials of juxtacellularly recorded neuron.

labeled in successful experiments. Perhaps due to the selectivity of our electrodes (see Section 4), the incidence of sympathetically correlated neurons was significantly lower than observed in previous studies in this laboratory. Furthermore, juxtacellular labeling of spinal neurons proved to be difficult due to movement caused by arterial and venous pulsations. Many neurons were severely depolarized or lost immediately upon initiation of entrainment.

At the end of successful experiments, rats were perfused with physiological saline followed by a solution of 4% paraformaldehyde in 0.1 M phosphate buffer. Spinal cords were removed and post fixed in 4% paraformaldehyde. Forty micron sagittal sections of the T_{10} segment were cut on a vibrating microtome, incubated for 48 h in streptavidin-CY3 (Jackson ImmunoResearch Laboratory, West Grove, PA, USA) at 4 °C, rinsed, and mounted serially on slides.

The shape of each soma and the course of each dendrite was examined at 40× using fluorescent microscopy. Dendrites were tracked, photographed, and traced on a computer-linked drawing tablet in serial sagittal sections until they either ended or became too faint to reconstruct. This procedure resulted in a sagittal projection of each neuron. In addition, the mediolateral and dorsoventral extent of dendrites was recorded separately on a standardized transverse section of the T_{10} level of the spinal cord. Because photographs were taken of each segment of dendrite, prolonged observation of fluorescence was unnecessary, and reconstruction was not hampered by fading.

Average discharge rates, minimum interdischarge intervals, intervals between action potentials and peaks of cross correlograms, durations of recordings, and durations of entrainment periods are presented as arithmetic means±1 standard error of the mean.

3. Results

3.1. The sample of neurons

The activities of 97 dorsal horn neurons were positively correlated with bursts of renal sympathetic nerve activity. The reported data are from 19 sympathetically correlated neurons that we were able both to entrain and to identify unequivocally histologically. Forty-nine correlated neurons could not be entrained or were damaged by entrainment. Twenty-nine correlated neurons were successfully entrained but could not be unequivocally identified histologically. There were no significant differences between the discharge rates, somatic field characteristics, or degrees of correlation between correlated and entrained neurons that we could and could not identify histologically. All further descriptions refer to the 19 neurons that were sympathetically correlated, entrained, and subsequently identified.

3.2. Electrophysiological properties of the neurons

All neurons exhibited bursting behavior consisting of a low-to-moderate average rate of discharge (4±1 impulses/s) interspersed by short bursts of two or more action potentials with very short interdischarge intervals (average minimum interval between successive action potentials was 3±0.4 ms). These short minimum interdischarge intervals identified the labeled neurons as interneurons rather than sympathetic preganglionic neurons (see Ref. [4] for details). The average duration of recordings in all rats from which data are reported was 219±20 s, and during this period we recorded an average of 821±224 action potentials. The average latency between the discharge of spinal neurons and the peak of subsequent bursts of sympathetic activity was 56±3 ms. The duration of entrainment of the histologically recovered, sympathetically correlated neurons was 67±30 s and ranged from 2 to 900 s. For entrainments longer than 5 s, there was no consistent relationship between the duration of entrainment and the completeness of labeling or the likelihood of histologically recovering labeled neurons.

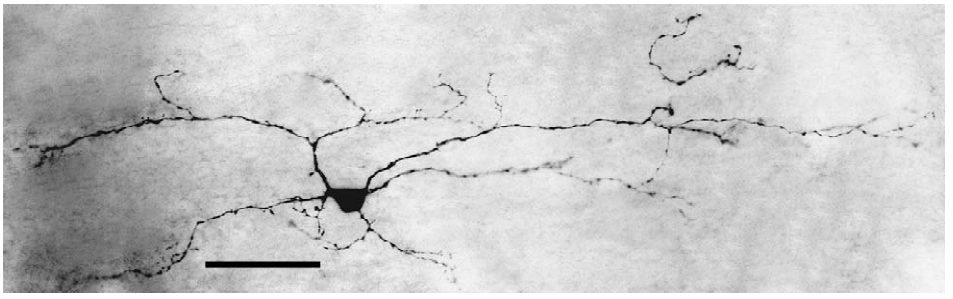

Fig. 2. Low magnification photograph of a juxtacellularly labeled, sympathetically correlated neuron histologically located in lamina IV of the dorsal horn. The location of this neuron is shown in Fig. 3A (closed circle in lamina IV). Its reconstruction is shown in Fig. 3E. Calibration bar=50 μm.

All neurons were excited by light brushing of fields that ranged in size from approximately 1 to 5 cm^2. These fields were located rostral and ventral to the lateral laparotomy or on the abdomen ventral to the laparotomy. In several cases, fields extended ventrally almost to the midline of the abdomen. In general, the more rostral and dorsal receptive fields were smaller than those more caudal and ventral.

3.3. Anatomical properties of the neurons

Of the 19 neurons in our sample, 17 were located in the gray matter of the dorsal horn: three in lamina I, two in lamina II, two in lamina III, seven in lamina IV (Fig. 2), and three in lamina V. Two neurons were located just lateral to the gray matter of the dorsal horn (Fig. 3A). One of these neurons was located in the lateral spinal nucleus, and one was located in the lateral funiculus, just lateral to lamina VII. Although the somas of our sample were distributed widely, the somas of eight neurons were located at the periphery of the dorsal horn, either in lamina I or on the lateral gray/white border of laminae III and IV.

Panels B–F of Fig. 3 show sagittal reconstructions of the best-labeled neurons from each dorsal horn lamina. Although we were not able to distinguish between the axons and dendrites of labeled neurons, based on the morphology of labeled processes, our experience, and the

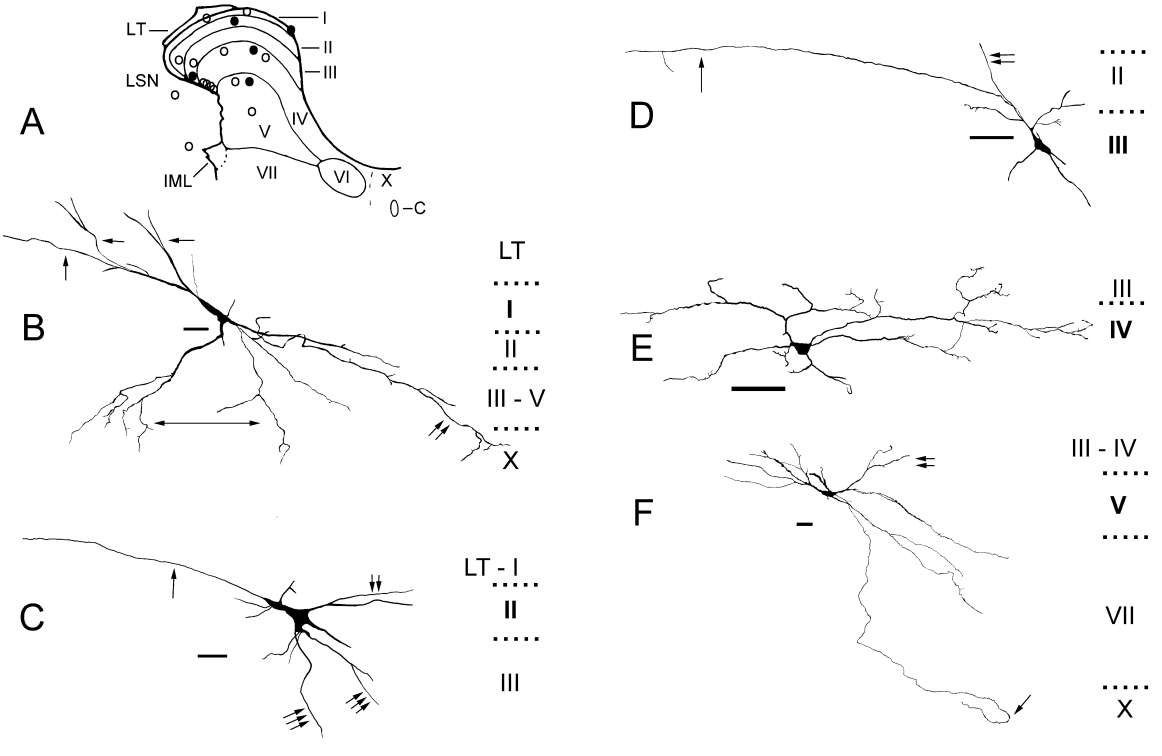

Fig. 3. Locations and reconstructions of sympathetically correlated, juxtacellularly labeled, dorsal horn neurons. (A) Diagrammatic cross section of the rat spinal cord at T_{10} (redrawn from Ref. [14]). Circles indicate the locations of sympathetically correlated, juxtacellularly labeled neurons. Closed circles are locations of representative neurons shown in B–F. (B–F) Sagittal reconstructions of juxtacellularly labeled, sympathetically correlated neurons with somas located in laminae I–V, respectively. Approximate locations of laminae are indicated on the right side of each panel. The lamina in which each soma is located is indicated to the right of each panel in boldface. Calibration lines=50 μm. C, central canal; IML, intermediolateral column; LT, Lissauer's tract; LSN, lateral spinal nucleus.

experience of other laboratories using juxtacellular labeling [22], labeled processes were most likely dendrites rather than axons. The morphology of the neurons from each lamina are described below.

3.3.1. Lamina I

The somas of the three labeled, sympathetically correlated neurons in lamina I were spindle shaped and oriented rostrocaudally. The somas of two of these neurons were exactly horizontal. The shape of the soma of the example neuron shown in Fig. 3B, was characteristic, but it was slanted at approximately 30° from the horizontal plane, perhaps due to its location at the acute angle of the medial dorsal horn. Extensive dendritic trees were labeled in two of the lamina I neurons, and the example neuron is representative of both. In addition to dendrites that projected rostrally and dorsally into Lissauer's tract (single arrows), long dendrites of both neurons projected caudally and ventromedially along the medial gray/white border, just lateral to the dorsal columns and corticospinal tracts. In the example neuron, this dendrite extended to the medial border between lamina IV and lamina X (double arrows). The ventromedially oriented dendrite of the other well labeled lamina I neuron extended to the dorsomedial portion of lamina VII. Additional dendrites from both neurons projected rostrally and ventrally into laminae III, IV and V (double headed arrow). The third neuron in lamina I was not as densely labeled. We were, however, able to identify one dendrite that extended dorsally into Lissauer's tract.

3.3.2. Lamina II

The morphologies of the two neurons labeled in lamina II were almost identical. Neither of these neurons was densely labeled. Their somas were longitudinally oriented and pyramidal in shape. The apex of each neuron faced rostrally, and a long 'apical dendrite' projected rostrally from each. In the example neuron (Fig. 3C), this dendrite could be traced for approximately 500 μm in a rostrodorsal direction (single arrow). After traversing lamina I, it appeared to course parallel to axons in Lissauer's tract. The apical dendrite of the second lamina II neuron could be traced only into lamina I. Both neurons had extensive 'basilar' dendrites that projected from their caudal poles. These dendrites could be traced for approximately 150 μm. Some coursed dorsally into more dorsal portions of lamina II (double arrow), and some projected into lamina III (triple arrow). A dendrite of one of these neurons projected laterally into the medial portion of the dorsolateral funiculus.

3.3.3. Lamina III

The two neurons labeled in lamina III were morphologically distinct. The soma of the example neuron (Fig. 3D) was flat. One dendrite projected dorsolaterally, through lamina II, into the reticular portion of the dorsal horn, just dorsal to the lateral spinal nucleus (single arrow). There, it divided into rostral and caudal branches (for clarity, not shown on the illustration). A rostrally directed dendrite projected into the dorsal portion of lamina II (double arrow). The soma of the second neuron labeled in lamina II was spindle shaped and oriented rostroventrally. Lengthy dendrites that remained in lamina III emanated from both the rostral and caudal poles of this neuron. In addition, this neuron projected a dendrite that could be traced into the dorsal portion of lamina II.

3.3.4. Lamina IV

Seven neurons, the largest number in any lamina, were labeled in lamina IV. The somas of four of these neurons were flattened mediolaterally, but otherwise unoriented. The somas of three of this flattened subset were located very laterally, near the gray/white border. All of the neurons in lamina IV, including the example neuron (Fig. 3E), had multiple rostrocaudally oriented dendrites in lamina IV, branches of which projected dorsally into lamina III and occasionally into lamina II. Two of these neurons projected dendrites ventrolaterally into the lateral funiculus, just lateral to the intermediolateral column or dorsolaterally into the dorsolateral funiculus just ventral to the lateral spinal nucleus. The dendrites of one of these neurons coursed ventrally into lamina VII.

The somas of the remaining three neurons labeled in lamina IV were smaller, spindle shaped, and oriented horizontally and rostrocaudally. The dendrites of the more medial two neurons of this subset could be traced for short distances rostrally, caudally, dorsally and ventrally. Their dorsal dendrites were traced into lamina III, and their ventral dendrites were traced to the ventral border of lamina IV. Only a rostrally projecting dendrite was labeled in the third (lateral-most) neuron of this subset.

3.3.5. Lamina V

The somas of all three neurons labeled in lamina V were oriented rostrocaudally. All had extensive dendritic trees. Two of the neurons, including the example neuron (Fig. 3F), had long dendrites that coursed ventromedially. In the example neuron, this dendrite extended medially to the boundary between lamina VII and lamina X (single arrow). The comparable dendrite of the other neuron of this pair extended into both lamina VII and lamina X. Dorsally projecting dendrites of these neurons projected into lamina IV and lamina III (double arrow). The third neuron observed in lamina V was not as densely labeled. However, the trajectories of its proximal dendrites were similar to those of the other two lamina V neurons.

3.3.6. Lateral spinal nucleus and lateral funiculus

Neither of the two neurons observed lateral to the dorsal horn (Fig. 3A) was densely labeled, and they were not reconstructed. The somas of both were spindle shaped and longitudinally oriented. Dendrites of the neuron in the

lateral spinal nucleus could be traced medially into lamina II and laterally into the medial portion of the dorsolateral funiculus, where they divided and coursed rostrally and caudally. Dendrites coursing rostrally and caudally from the soma of this neuron could be traced for short distances. Dendrites from the neuron in the lateral funiculus projected from the rostral and caudal poles of the soma and dorsally and ventrally into the lateral and dorsolateral funiculus.

4. Discussion

4.1. The sample of neurons

The purpose of this study was to infer the degree to which spinal sympathetic networks could be affected either by direct input from supraspinal systems or by direct input from primary afferent axons, based on the locations of the somas and dendrites of sympathetically correlated neurons. In two previous studies, we used a combination of electrolytic lesions and stereotaxic coordinates to locate the somas of 17 sympathetically correlated neurons in the dorsal horn [3,4]. The 19 identified neurons in the present study, therefore, more than doubled the number of histologically localized somas of spinal sympathetically correlated neurons. Furthermore, juxtacellular labeling more precisely localized the sympathetically correlated neurons in this study and revealed the morphology of their somas and dendrites. To our knowledge, this is the first published report of juxtacellularly labeled spinal neurons.

We cannot conclude that the 19 neurons we report represented an unbiased sample. Not only was the number of neurons small, but the sampling across the laminae of the dorsal horn was variable. The larger sample of sympathetically correlated neurons recorded from the deeper laminae probably manifested a greater physical stability of recordings in those laminae, not a greater incidence of correlated neurons in those laminae.

A more serious technical concern was the difference between the incidence of sympathetically correlated neurons in this study and in earlier studies in this laboratory. Previously [3,4], approximately 40% of spontaneously active dorsal horn neurons exhibited sympathetically correlated activity after spinal transection. In the present experiments, the activities of only 10% of neurons were correlated with RSNA. A potential source of this difference is that in previous experiments we used carbon fiber electrodes with impedances of approximately 4 MΩ, and in the current experiments we used biotinamide and acetate-filled pipettes with average impedances of approximately 20 MΩ. In addition to differences in impedance, the electrodes had very different physical properties. The carbon fiber electrodes had blunt tips, and the pipettes had much sharper tips. Our recordings with pipettes were significantly more delicate (subject to movement), and we entrained and recovered no neurons with somas smaller than 20 μm among either the sympathetically correlated neurons reported or an even larger number of unreported sympathetically uncorrelated neurons. These observations suggest that, in the present experiments, we may have sampled only the larger of the sympathetically correlated neurons.

4.2. Electrophysiological properties of the neurons

The electrophysiological properties of the sympathetically correlated neurons reported in this study were very similar to those reported in previous studies from this laboratory [3,4,13] with respect to average ongoing discharge rate, minimum interdischarge interval, and the latency between their discharges and the maximum cross correlation between those discharges and RSNA. Furthermore, the locations and sizes of the somatic fields of correlated neurons were similar to those reported previously [3].

A difference between this and our previous studies was that we were unable to test neurons for their responses to noxious stimuli. The loss or destruction of neurons during noxious mechanical stimulation (see Section 2) was not surprising because the impedance of our electrodes was as much as 10-fold greater in the present experiments. Nevertheless, we believe that had we been able to study responses to noxious stimuli, our results would have been comparable to those reported previously, for we have never observed purely low threshold, sympathetically correlated, neurons, and we have only rarely observed neurons that responded only to noxious stimulation. Therefore, the majority of the neurons reported herein were likely to have been wide dynamic range neurons, the most common category of sympathetically correlated neuron in our previous experiments [3,4].

4.3. Anatomical properties of the neurons

Although the largest percentage of correlated neurons was found in lamina IV, neurons were found in all of the lamina of the dorsal horn. Our previous studies indicated that a substantial number of sympathetically correlated neurons were located at the dorsomedial and lateral borders of the dorsal horn, excluding, however, laminae I and II [3]. The present study confirmed this concentration of peripherally located neurons. In addition, for the first time we also identified sympathetically correlated neurons in laminae I and II, thus providing evidence for a sheet of sympathetically correlated neurons on the surface of the dorsal horn.

We noted that the dendritic fields of our most completely labeled neurons were substantially less complex than those of dorsal horn neurons labeled intracellularly by others in previous studies [5,27]. Although this difference in apparent dendritic complexity might have represented an actual morphological property of sympathetically corre-

lated neurons, we consider it more likely a manifestation of incomplete filling of dendrites. This conclusion is supported by our observation (unpublished) that the complexity of dendritic trees of juxtacellularly labeled, *uncorrelated* spinal neurons was no more complex than that of correlated neurons.

Because previous studies have shown that the functional properties of neurons are more likely to be related to the locations of their somas and to the distribution of their dendrites than to the shapes of their somas [10,28], we have concentrated our analysis on those morphological characteristics. Furthermore, because of the importance of both descending supraspinal pathways and primary afferents to the activity of spinal sympathetic circuits, we have concentrated our analysis on potential relationships between these systems and the morphology of sympathetically correlated neurons.

What is the relationship between supraspinal projections to the dorsal horn and the locations and morphology of sympathetically correlated neurons? Experiments in this laboratory indicated that pathways descending from the brain tonically inhibit most spinal sympathetically correlated neurons [13]. This inhibition, then, permits brainstem systems to exercise control over sympathetic nerve activity. This inhibition also probably reduces the importance of spinal systems in the generation of sympathetic activity in rats with intact spinal cords. We have suggested that the loss of this descending inhibition may provide one mechanism for autonomic dysreflexias [3,4,13].

The anatomy of these descending sympathomodulatory systems has not been thoroughly studied. However, existing evidence indicates that they course in the dorsolateral funiculus and the medial portions of the lateral funiculus [20] and that they closely correspond to descending systems that tonically inhibit afferent excitation of dorsal horn neurons [1,19]. The axons of afferent modulatory systems leave the dorsolateral funiculus and ramify widely in all lamina of the dorsal horn [6]. Thus, sympathetically correlated neurons in all lamina are very likely to be affected either by direct inhibitory input from these descending systems or by inhibitory spinal interneurons that are excited by descending systems.

What is the relationship between the terminations of primary afferent axons and the locations and morphology of spinal sympathetically correlated neurons? Historically, primary afferents mediating nociception were thought to synapse almost exclusively in the superficial lamina of the dorsal horn, and afferents mediating discriminative touch were thought to synapse in the deeper lamina. According to this scheme, it would seem unlikely, for instance, that the responses to light brushing of sympathetically correlated neurons located in superficial laminae could have resulted from direct input from non-nociceptive primary afferents or that neurons in deep laminae could have received direct input from nociceptive primary afferents. Is now clear, however, that many exceptions to this pattern exist. For instance, superficial laminae receive input from primary afferents that do not mediate nociception [26] and putative nociceptor primary afferents project collaterals to deep laminae [24].

The relationship between the modalities of sympathetically correlated dorsal horn neurons and their morphologies is further confounded by their extensive dendritic trees. Indeed, regardless of the location of their somas, the dendrites of all labeled sympathetically correlated neurons traversed regions in which they could have come in contact with both nociceptive and non-nociceptive primary afferents. For instance, many neurons projected dendrites to either Lissauer's tract or laminae I and II, regions densely populated with nociceptive primary afferents. Those neurons that did not project axons to superficial lamina projected dendrites into or close to lamina X, another site of termination of nociceptive primary afferents [15,25]. Similarly, most neurons projected dendrites into deeper lamina of the spinal cord, where they could have contacted non-nociceptive primary afferents. However, it is now clear that even dendrites in superficial lamina could have been contacted by non-nociceptive primary afferents [26]. Therefore, both the widespread projections of primary afferent axons mediating noxious and innocuous sensory input and the extensive dendritic trees of labeled neurons predict that sympathetically correlated neurons could receive direct input from afferent systems mediating a wide range of modalities. These anatomical observations correlate well with our observation that a majority of spinal sympathetically correlated neurons exhibit wide dynamic range properties [3,4].

Previous experiments by several groups have concentrated on anatomically or physiologically characterizing spinal sympathetic interneurons. In a landmark study, Cabot et al. [2] identified putative sympathetic interneurons using transneuronal retrograde transport of wheat germ agglutinin (WGA) injected into the superior cervical ganglion. In the dorsal horn, the heaviest concentration of labeled interneurons was located in the lateral (reticulated) portion of lamina V. Interneurons were also seen at the border between lamina IV and the lateral funiculus. No interneurons were observed in more superficial laminae.

Although we observed many fewer interneurons, there are a number of points of agreement between our study and that of Cabot et al. [2]. First, sympathetically correlated neurons were identified only in the lateral portion lamina V although we explored thoroughly the medial portion of that lamina. Second, we also observed a clustering of sympathetically correlated interneurons in the most lateral portion of lamina IV. A major difference between the work of Cabot et al. [2] and the present study is that we were electrophysiologically identified sympathetically correlated neurons in the medial portions of lamina IV as well as in laminae I–III, regions in which Cabot et al. [2] did not observed labeled interneurons. The wider anatomical distribution of physiologically identified

sympathetic interneurons is not surprising. It is likely that transneuronally transported WGA could be visualized only in neurons that synapsed directly on sympathetic preganglionic neurons. On the other hand, neurons separated from sympathetic preganglionic neurons by several synapses may have been identified by their cross correlations with renal sympathetic nerve activity. Although detailed morphological characterization of sympathetic interneurons was not a major goal in the study by Cabot et al. [2], the sizes and shapes of many of the interneurons they observed were similar to those observed in the present study.

Weaver et al. [7] identified putative renal sympathetic interneurons using transneuronal infection by herpes simplex virus type 1 (HSV-1) injected into the adrenal glands of hamsters. In agreement with the results of Cabot et al. [2], in the dorsal horn putative interneurons were most heavily concentrated in lamina V, dorsal to the intermediolateral column. The morphology of the neurons infected with HSV-1 in the hamster differed considerably from those most commonly labeled with WGA in the study of Cabot et al. [2] and from those juxtacellularly labeled in the present study. The putative interneurons identified by Cabot et al. [2] and by us were often large and clearly multipolar, and they exhibited large dendrites. The somas of HSV-1 infected neurons tended to be relatively small and round or oval. The dendrites of these neurons, although long, tended to be very fine. It is not clear whether these morphological differences can be attributed to differences in species or to differences in the selectivity of the labeling methods used.

Deuchars et al. [5] anatomically identified and electrophysiologically and pharmacologically characterized a population of putative sympathetic interneurons in the intermediolateral cell column of the preweanling rat. These neurons differed dramatically from sympathetic preganglionic neurons in their morphology, their action potentials, and their expression of the Kv3.1b potassium channel. Morphologically, the somas and proximal dendritic trees of these neurons resembled those of the juxtacellularly labeled sympathetically correlated neurons observed in the present study. The major morphological difference was that the juxtacellularly labeled neurons were more likely to have longitudinally oriented dendritic trees while the interneurons in the study by Deuchars et al. [5] appeared to be more transversely oriented. Whether this difference is attributable to the orientation of the spinal slices in which Deuchars et al. [5] recorded their neurons is not clear. An important finding in the study by Deuchars et al. [5] was that putative sympathetic interneurons surrounded—but were rarely found within—clusters of sympathetic preganglionic neurons. This distribution appears to agree well with that of putative sympathetic interneurons described by Cabot et al. [2] and it is consistent with our failure to localize sympathetically correlated interneurons within the intermediolateral cell column.

In summary, we have shown that the somas of sympathetically correlated neurons in the T_{10} segment of the spinal cord are widely distributed but include a significant population located in lamina I and on the lateral surface of laminae II–IV. Several correlated neurons were also located slightly lateral to the dorsal horn in closely related clusters of neurons in the lateral spinal nucleus and lateral funiculus. Either (or both) the somas and the dendrites of all labeled sympathetically correlated neurons were in regions that receive both direct descending input from supraspinal systems and direct input from both nociceptive and non-nociceptive spinal primary afferents. Definitive demonstration that sympathetic interneurons actually receive these direct inputs will require further, more direct, anatomical studies.

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