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# Sympathetically Correlated Activity of Dorsal Horn Neurons in Spinally Transected Rats

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**Chau, David, Namjin Kim, and Lawrence P. Schramm.** Sympathetically correlated activity of dorsal horn neurons in spinally transected rats. *J. Neurophysiol.* 77: 2966–2974, 1997. In mammals with an intact neuraxis, most sympathetic nerve activity is generated by brain stem systems. Therefore these systems have attracted much more attention than spinal systems that generate excitatory inputs to sympathetic preganglionic neurons. The purpose of this study was to determine whether, within hours of C<sub>1</sub> spinal cord transection, spinal dorsal horn neurons (DHNs) play a role in generating sympathetic nerve activity. Experiments were conducted in chloralose-anesthetized rats. We recorded renal sympathetic nerve activity (RSNA) in the left renal nerve, and we recorded the activity of neurons located in the left dorsal horn at T<sub>2</sub>, T<sub>8</sub>, T<sub>10</sub>, T<sub>13</sub>, and L<sub>2</sub>. We also recorded the activity of neurons in the right dorsal horn at T<sub>10</sub>. The somatic fields and cutaneous modalities of most neurons were determined. Spike-triggered averaging was used to determine relationships between the ongoing activity of DHNs and ongoing RSNA. In the left dorsal horn, bursts of ongoing activity of 16% of DHNs at T<sub>8</sub> and 43% of DHNs at T<sub>10</sub> were positively correlated with bursts of ongoing RSNA at latencies of  $59 \pm 8$  (SE) ms. At no other level on the left side, nor in the T<sub>10</sub> segment on the right side, was the activity of DHNs correlated with RSNA. DHNs with activity correlated with RSNA were located only in dorsal horn laminae III–V. Deeper laminae were not investigated in these experiments. The activity of all sympathetically correlated DHNs exhibited bursts of action potentials with interspike intervals of <10 ms. All but one of the sympathetically correlated DHNs exhibited wide-dynamic-range modalities. The modalities of sympathetically uncorrelated neurons were more heterogeneous. Brief (5–10 s) noxious cutaneous stimulation of mid- and lower thoracic dermatomes on the left side excited all sympathetically correlated DHNs and simultaneously increased RSNA. The excitatory cutaneous fields of sympathetically correlated neurons were circumscribed by the excitatory fields for RSNA. The excitatory cutaneous fields of some sympathetically uncorrelated DHNs extended beyond the excitatory fields for RSNA. Noxious cutaneous stimulation of the extremities on the left side that decreased RSNA simultaneously decreased the activity of all sympathetically correlated DHNs. These data provide electrophysiological evidence that, in spinally transected rats, a population of DHNs may generate or convey excitatory input to renal sympathetic preganglionic neurons.

## INTRODUCTION

Supraspinal systems generate most sympathetic activity in spinally intact mammals (Alexander 1946). Simultaneous recordings from brain stem neurons and sympathetic nerves have provided the most comprehensive neurophysiological characterization of these supraspinal systems. Employing the techniques of spike-triggered averaging, spectral analysis,

and linear coherence, neurons have been identified in the brain stem with ongoing activity that is highly correlated to rhythms of simultaneously recorded, ongoing, peripheral sympathetic activity (Barman and Gebber 1981; Barman et al. 1995; Gebber et al. 1995; Zhong et al. 1993, 1995). These sympathetically correlated neurons are generally considered to be putative “presympathetic neurons,” 1) members of networks that generate or inhibit sympathetic activity or 2) neurons projecting brain stem-generated, sympathoexcitatory or sympathoinhibitory information to spinal levels.

Spinal systems that affect sympathetic activity have received much less attention than brain stem sympathetic systems. Indeed, this laboratory has conducted the only simultaneous recordings of sympathetic nerve activity and the activity of spinal interneurons in spinally transected animals (Poree and Schramm 1992). Spinal sympathetic systems, however, deserve detailed investigation, for they may generate significant levels of sympathetic nerve activity in intact animals (Taylor and Weaver 1993), and, more importantly, they are responsible for all of the sympathetic nerve activity that is generated caudal to serious spinal cord injuries (Mathias and Frankel 1983).

Sympathetic preganglionic neurons do not themselves generate ongoing sympathetic activity in vivo (Dembowsky et al. 1985; McLachlan and Hirst 1980). Therefore sympathetic activity must be generated by the synaptic antecedents of preganglionic neurons. Logically, after spinal cord transection these antecedents reside only in the spinal cord. We anatomically identified candidates for spinal presympathetic neurons in experiments in which we injected pseudorabies virus into the kidneys of rats (Schramm et al. 1993). Dorsal horn neurons (DHNs) became infected via retrograde transport of the virus across their synapses either on renal sympathetic preganglionic neurons or on other synaptic antecedents of renal sympathetic preganglionic neurons (Schramm et al. 1993).

The segmental distribution of infected DHNs corresponded exactly to that of infected renal sympathetic preganglionic neurons, and the modes for both distributions were located in the T<sub>10</sub> spinal segment. The close correlation between the longitudinal distributions of infected renal sympathetic preganglionic neurons and infected DHNs suggested the hypothesis that the infected subset of DHNs might play a role in the generation of renal sympathetic activity, especially after spinal cord transection. By analogy with experiments conducted on brain stem generators of sympathetic activity, we reasoned that this hypothesis would be supported

if the ongoing activity of a subpopulation of DHNs was closely correlated with simultaneously recorded, ongoing renal sympathetic nerve activity (RSNA). Further, we reasoned that if these neurons played a role in generating or conveying input to sympathetic preganglionic neurons, then maneuvers that altered their activity should concurrently alter RSNA.

We identified a population of DHNs, restricted to ipsilateral, caudal thoracic segments, whose ongoing activity was strongly correlated with ongoing bursts of RSNA. Somatic stimulation that increased the activity of these neurons synchronously increased RSNA. Somatic stimulation that decreased the activity of most of these neurons simultaneously decreased RSNA. Taken together, these data provide the first electrophysiological evidence for a subpopulation of DHNs that consists of excitatory synaptic antecedents to renal sympathetic preganglionic neurons.

## METHODS

### *Surgery*

Adult male Sprague-Dawley rats (Taconic Farms and Charles River), each weighing 250–350 g, were used in these experiments. All procedures for these experiments were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine (protocol 94M242). Pretreatment with atropine (0.05 mg/kg sc) reduced nasal and tracheal mucus secretion. Anesthesia was induced by ether inhalation and continued by  $\alpha$ -chloralose (100 mg/kg iv) delivered via the right femoral vein. Rats were shaved and marked with a reference grid for mapping responses to somatic stimulation (Fig. 6). The right femoral artery was cannulated for measuring arterial pressure. The trachea was intubated for artificial respiration. Rats were mounted in a stereotaxic frame and paralyzed with gallamine triethiodide (Flaxedil, 40 mg/kg iv). Rats recovered from the effects of Flaxedil every 30–60 min, permitting an assessment of the depth of anesthesia. Anesthetic ( $\alpha$ -chloralose) was supplemented (25 mg/kg) as necessary to keep rats at a surgical plane throughout the experiments. Body temperature was monitored with a rectal probe and maintained at 37°C with a heating pad and heat lamp.

The upper cervical spinal processes and the dura matter were removed to expose the C<sub>1</sub>–C<sub>3</sub> spinal segments for spinal cord transection. Immediately after complete spinal cord transection between C<sub>1</sub> and C<sub>2</sub>, 1 ml of human serum albumin (Baxter Healthcare) was injected (25% solution, 12.5 g/50 ml iv) to return arterial pressure to  $\geq$ 90 mmHg. A second laminectomy was performed to expose the spinal cord at the level of the neuronal recording (i.e., at either T<sub>2</sub>, T<sub>8</sub>, T<sub>10</sub>, T<sub>13</sub>, or L<sub>2</sub>). The exposed spinal cord was covered by a pool of warm mineral oil. A bilateral pneumothorax was performed to reduce respiratory movements. The spinal cord was stabilized by clamping two processes: one just rostral to the level of the neuronal recording and the other at sacral levels. The left kidney was approached via a left flank laparotomy. After the kidney was retracted, the adrenal gland and fat covering the psoas and the paraspinal muscles were deflected away from the renal nerve, which was usually located at the juncture of the aorta and the renal artery. The nerve was then dissected from the surrounding tissues, placed on a bipolar hook electrode, and covered with warm mineral oil.

### *Extracellular and renal nerve recording*

The extracellular recordings were made with single-barrel carbon fiber microelectrodes (impedance 2–4 M $\Omega$ ) connected to a high-impedance probe (Grass HIP5). The resulting signal was filtered (300–3,000 Hz half-power cutoff frequencies) and amplified

50,000 times (GRASS P5 AC amplifier). The single neurons' action potentials were discriminated by a dual-window discriminator (BAK Electronics). Neurons selected for recording were spontaneously active. We are confident that these neurons represented DHNs, rather than sympathetic preganglionic neurons or the axons of primary afferents, for the following reasons. First, we rarely recorded at depths at which sympathetic preganglionic neurons are found. Second, the intraburst interspike intervals of neurons located at even our most ventral recording sites were much shorter than those reported for sympathetic preganglionic neurons in rats (Gilbey et al. 1986) or cats (Gebber and McCall 1976). Finally, the durations of these neurons' action potentials ranged from 1 to 1.5 ms, substantially longer than the 0.3- to 0.7-ms duration of action potentials we recorded from primary afferents in Lissauer's tract, the lateral funiculus, or the dorsal columns. In most electrode tracks, the first neuron encountered was selected for recording, after which the recording site was marked with an electrolytic lesion (60–90  $\mu$ m diam) by delivery of an anodal current through the recording electrode (15  $\mu$ A for 10–15 s). In the remaining electrode tracks, all neurons encountered in a single track were recorded, and a lesion was made at the deepest recording site. For these tracks, more superficial recording sites were reconstructed from this lesion based on readings from the microdrive.

RSNA was recorded with bipolar electrodes constructed from 60- $\mu$ m stainless steel wire. Afferent renal nerve activity was minimal in most of our preparations, for the activity recorded after crush of the nerve proximal to the electrode at the end of experiments (to obtain 0 nerve activity) was negligible. Therefore the renal nerve distal to the bipolar electrode was often left intact. The observed correlations between renal nerve activity and the activity of DHNs could not have been due to the activation of DHNs by renal afferents, for correlated bursts of activity in DHNs clearly preceded activity in the renal nerve.

Renal sympathetic activity was amplified 10,000 times (GRASS P15 AC and GRASS P5 AC amplifiers). Filters were set at 100–3,000 Hz half-power cutoff frequencies on both amplifiers. At the end of each experiment, conduction in the renal nerve was abolished by crush or transection to determine the zero level of RSNA. Arterial pressure, RSNA, single-neuron activity, and a cutaneous stimulation indicator were recorded on VHS video tapes for off-line analysis.

### *Somatic stimulation*

Innocuous somatic stimulation (light brush with a cotton applicator) and noxious somatic stimulation (pinch with toothed forceps, maintained for 5–10 s) were delivered to sites delineated by the reference grid (Fig. 6). A response in either the RSNA or the single-neuron activity was considered excitatory or inhibitory if it represented an increase or decrease, respectively, of  $\geq$ 10% from the prestimulus control level.

### *Classification of neurons' afferent modalities*

Neurons were classified as low threshold (LT) if they were excited by brush and not further affected by pinch, wide dynamic range (WDR) if they were excited by brush and excited more by pinch, and high threshold (HT) if they were excited only by pinch. Six DHNs were inhibited by either innocuous or noxious stimulation of their (normally excitatory) primary dermatomes, or they responded to neither brush nor pinch. We classified these neurons as "other".

### *Spike-triggered averaging*

Spike-triggered averages of ongoing RSNA were calculated for all recorded neurons. On detection of a neuron's action potential,

an epoch of renal nerve activity (rectified and filtered, time constant = 0.04 s) was extracted from the continuous renal nerve recording. The epoch began 300 ms before the occurrence of the action potential and lasted 700 ms after the action potential. Further unit detection was disabled for  $\geq 1$  s to avoid the overlapping of RSNA epochs. At least 300 epochs were collected and averaged for each neuron. We calculated a control or "dummy" average for the same RSNA, using as a trigger an electronically generated pseudorandom signal with a frequency approximately equal to that of the recorded neuron.

A "correlation index" was calculated from each spike-triggered average as the ratio of 1) the largest peak in the spike-triggered average occurring  $< 100$  ms after the onset of DHN action potentials to 2) the largest peak-to-peak deflection occurring at any time in the respective dummy average. For purposes of classification, neurons were considered correlated to RSNA (henceforth "sympathetically correlated") if the correlation indexes  $\geq 2$  (Fig. 2) and spike-triggered averages generated from nonoverlapping subsections of their recordings had similar peaks at similar latencies.

### Interspike interval analysis

Interspike interval histograms (ISIHS, maximum interval 1 s, bin size 10 ms) were generated for all neurons. The histograms were computed using either 4 min of recordings of ongoing neuronal discharges or 1,000 action potentials, whichever occurred first. The degree of skewness (Sokal and Rohlf 1969) and the mode of each ISIH were computed as measures of the degree to which neurons' action potentials occurred in bursts.

### Histology

At the end of experiments, rats were perfused transcardially with buffered saline, followed by 10% buffered formaldehyde. The relevant spinal cord segments were removed and stored in sucrose-formaldehyde solution (30% sucrose in 10% phosphate-buffered formaldehyde, pH 7.4) for 2–5 days. Transverse 40- $\mu$ m sections were cut on a sliding microtome, mounted on gelatin-coated glass slides, and air dried. The sites of electrolytic lesions were identified microscopically.

### Data presentation and statistical analysis

Data are expressed as means  $\pm$  SE. All statistical analyses employed either a one-tailed Fisher's exact test or a  $\chi^2$  test. Values of  $P \leq 0.05$  were considered significant.

## RESULTS

### Sample of DHNs

In 56 spinally transected rats we simultaneously recorded the ongoing activity of the left renal sympathetic nerve and the activity of single DHNs, ipsilateral and contralateral to the renal nerve recording (Table 1). Spike-triggered averages were computed for all DHNs listed in Table 1. Afferent modalities of DHNs were based on the neurons' responses to stimulation of their estimated primary dermatomes (METHODS). The fields from which unitary responses could be elicited by noxious stimuli were completely surveyed for a subset of these neurons (Table 1, *Somatic fields fully surveyed*). The excitatory and inhibitory somatic fields of this subset of DHNs were later compared with fields that, when stimulated by pinch, increased or decreased RSNA (Fig. 6A).

TABLE 1. Numbers of dorsal horn neurons analyzed

Spinal Segments	Afferent Modalities Tested			Total Number of Neurons Recorded
	Somatic fields fully surveyed	Somatic fields not fully surveyed	Not Tested for Responses to Stimulation	
T <sub>2</sub> ipsi	21	0	0	21
T <sub>8</sub> ipsi	0	0	25	25
T <sub>10</sub> ipsi	20	9	6	35
T <sub>13</sub> ipsi	0	0	15	15
L <sub>2</sub> ipsi	25	1	0	26
T <sub>10</sub> contra	25	1	0	26
Totals	91	11	46	148

Ipsi, ipsilateral; contra, contralateral.

*After spinal cord transection, the ongoing activity of only those DHNs recorded in segments containing renal sympathetic preganglionic neurons (Schramm et al. 1993) was correlated to ongoing RSNA*

When DHN-triggered averages were compared with averages triggered by frequency-matched dummy neurons, it was clear that the action potentials of a subset of these neurons regularly preceded bursts of RSNA (Fig. 1A). The spike-triggered averages of a much larger subset of DHNs (Fig. 1B) exhibited no relationship between the firing of the spinal neurons and RSNA. A correlation index was used to measure the degree of correlation between the activity of DHNs and RSNA (METHODS). A population of DHNs with an average correlation index of  $\sim 0.9$  existed at each spinal level investigated (Fig. 2). Spike-triggered averages generated from nonoverlapping segments of the records from these neurons rarely exhibited a consistent peak at any latency. In the ipsilateral T<sub>8</sub> and T<sub>10</sub> segments, however, we observed DHNs with substantially higher correlation indexes. More neurons in the ipsilateral T<sub>10</sub> segment than in T<sub>8</sub> exhibited these higher indexes. Spike-triggered averages generated from nonoverlapping segments of the records from these neurons invariably exhibited a consistent peak  $\sim 60$  ms after action potentials of the DHNs.

For the purpose of further analyses, DHNs that had correlation indexes  $\geq 2$  and that also exhibited peaks in their spike-triggered averages at repeatable latencies in nonoverlapping segments of their records were considered correlated. Using these criteria, 43% of the neurons recorded in the ipsilateral T<sub>10</sub> segment were sympathetically correlated, compared with 16% of the neurons recorded in the T<sub>8</sub> segment. The average latency between the action potentials of correlated DHNs and the peak of their spike-triggered averages of RSNA was  $59 \pm 8$  (SE) ms. Because histologically identified, sympathetically correlated neurons were widely distributed across dorsal horn laminae, their numbers were too small to permit statistical comparisons of their laminar distributions (Fig. 3). However, the relative incidence of correlated neurons appeared to increase in the deeper laminae of ipsilateral T<sub>10</sub>.

ISIHS were computed for all ipsilateral T<sub>2</sub>, T<sub>10</sub>, L<sub>2</sub>, and contralateral T<sub>10</sub> neurons. All correlated T<sub>10</sub> ipsilateral neurons had similarly shaped ISIHS with modes that were  $< 10$

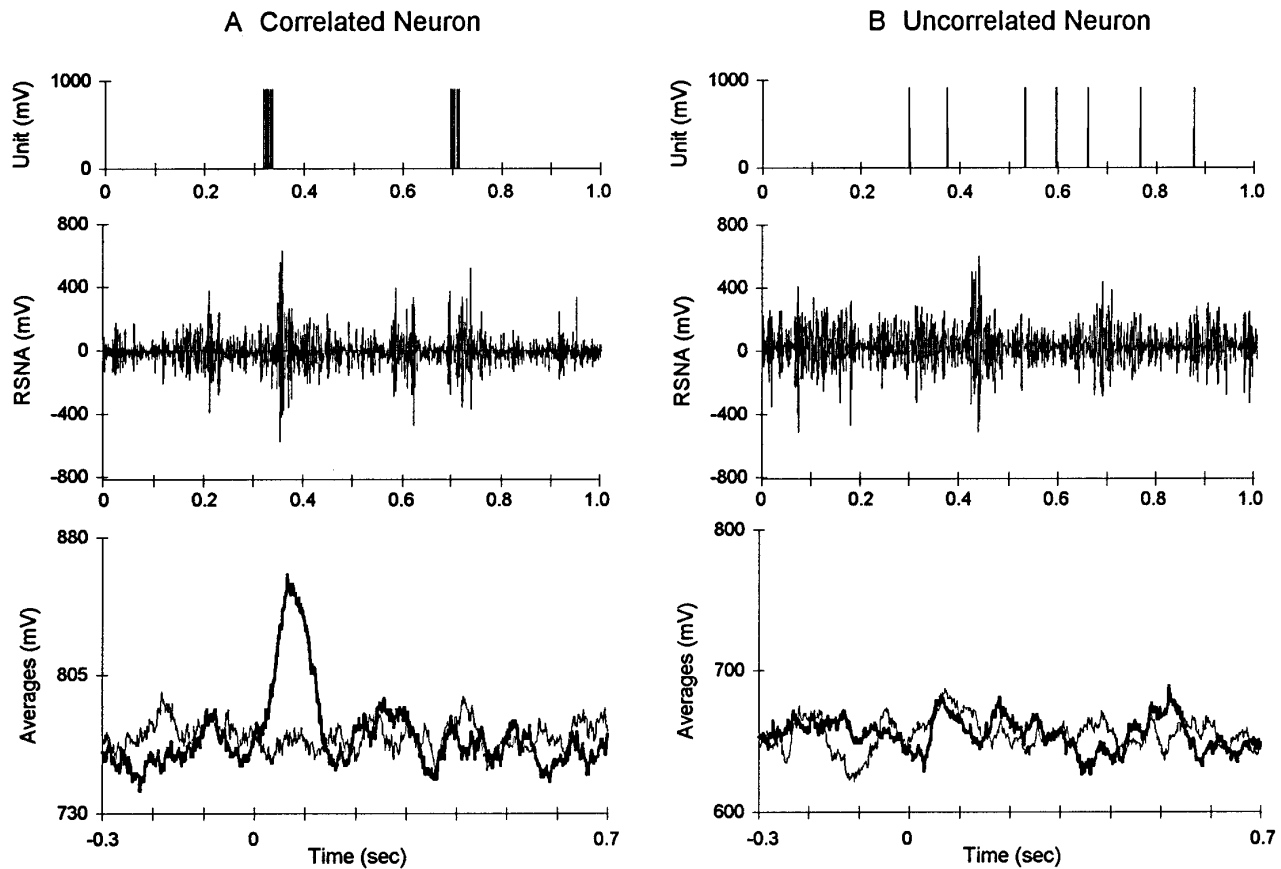


FIG. 1. Representative recordings from sympathetically correlated (A) and sympathetically uncorrelated (B) dorsal horn neurons. *Top*: discriminator output showing action potential occurrences. *Middle*: renal sympathetic nerve activity (RSNA) recorded simultaneously with dorsal horn neuron recordings. *Bottom*: bold traces, spike-triggered averages of RSNA triggered with action potentials of dorsal horn neurons (METHODS); fine traces, spike-triggered averages of RSNA triggered with "action potentials" of "dummy neurons." Occurrences of triggers: 0 s. Vertical scales for *middle* and *bottom* reflect amplification of 10,000 times (METHODS). Correlation indexes for these representative correlated and uncorrelated neurons: 2.5 and 0.6, respectively.

ms and degrees of skewness that were  $>0.5$  (Fig. 4, *top*). Neurons with these ISIH properties exhibited high incidences of action potentials occurring in clusters (bursts). The absence of RSNA-correlated neurons at spinal levels other than  $T_8$  and  $T_{10}$  was not due to an absence of bursting neurons at those levels, for the relative distributions of neurons with ISIH modes  $<10$  ms and degrees of skewness  $>0.5$  were not significantly different between segments ( $P = 0.674$ ,  $\chi^2$  test for independence).

RSNA-correlated  $T_{10}$  ipsilateral DHNs (12 WDR, 1 HT) were significantly more likely to exhibit WDR properties than uncorrelated  $T_{10}$  ipsilateral DHNs (1 LT, 9 WDR, 6 HT;  $P = 0.04$ , Fisher's exact test). Uncorrelated ipsilateral  $T_2$  DHNs (17 WDR, 2 HT, 2 other),  $L_2$  DHNs (1 LT, 21 WDR, 1 HT), and contralateral  $T_{10}$  DHNs (17 WDR, 8 HT, 1 other) were heterogeneous with respect to modality. Although statistical tests were impossible, correlated and uncorrelated  $T_{10}$  ipsilateral DHNs were sorted by modality and laminar location (Fig. 3). All correlated  $T_{10}$  ipsilateral DHNs found in laminae III–V exhibited WDR properties. Uncorrelated  $T_{10}$  ipsilateral DHNs found in laminae III–V were heterogeneous with respect to modality, and those found in lamina I all exhibited HT properties.

*Fields from which excitatory and inhibitory renal sympathetic nerve responses were evoked by noxious cutaneous stimulation were topographically organized*

In all rats, pinch of the left flank, back, and abdominal regions increased RSNA (Figs. 5A, *top* and 6A, *left*). The magnitude of this increase in sympathetic activity was positively related to the applied pressure. Pinch of areas on the rostral and superior boundaries of the laparotomy elicited the greatest responses. Progressively smaller responses were elicited by pinch at increasing distance from the incision. Pinch of the left shoulder and forelimb and the left hip and hindlimb elicited transient reductions in RSNA (Figs. 5B, *top* and 6A, *right*). Pinch of contralateral dermatomes occasionally decreased RSNA. The largest reductions in RSNA elicited from the contralateral side followed pinch of the right abdomen and flank.

*Fields from which excitatory and inhibitory responses in the activity of DHNs evoked by noxious cutaneous stimulation were topographically organized*

Most DHNs were excited when their primary dermatomes were stimulated (Fig. 6, B–F, *left*). These neurons were

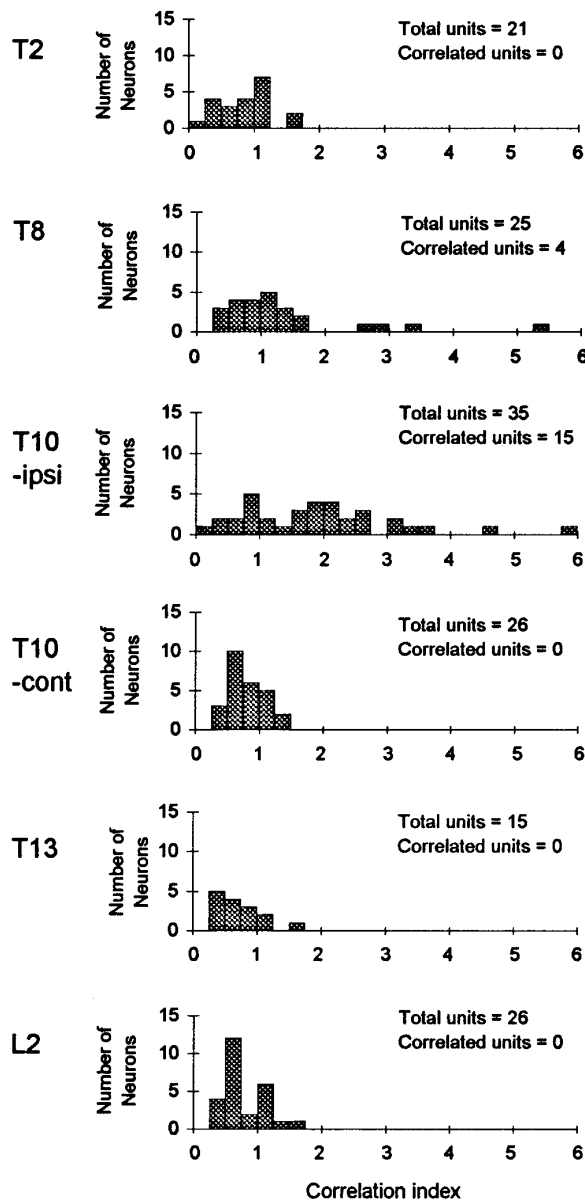


FIG. 2. Incidence of correlation indexes (METHODS) as a function of spinal segment. Note that only segments T<sub>8</sub> and T<sub>10</sub> contained neurons with correlation indexes >2. Ipsi, ipsilateral; cont, contralateral.

often inhibited when regions adjacent to or distal to their primary dermatomes were stimulated (Fig. 6, B–F, right). The cutaneous receptive fields of six DHNs were either aberrant or nonexistent, and these DHNs were assigned to the category of “others” (Fig. 3). Of these, one T<sub>2</sub> DHN (Fig. 6B, right) and one L<sub>2</sub> DHN (Fig. 6F, right) were inhibited by pinch in areas corresponding to the excitatory fields of all other neurons from the same spinal segment. One T<sub>2</sub> neuron was excited by pinch of its primary dermatome, but it was inhibited by brush of exactly the same area. Cutaneous somatic fields could not be found for one ipsilateral T<sub>10</sub> DHN and two L<sub>2</sub> DHNs.

We conducted a further analysis, considering only the 20 T<sub>10</sub> DHNs whose somatic fields were fully surveyed (Table 1). Of these 20 DHNs, 9 exhibited ongoing activity correlated with ongoing RSNA. All nine of these correlated T<sub>10</sub>

ipsilateral neurons had nociceptive excitatory and inhibitory fields circumscribed by fields which, when stimulated, increased and decreased RSNA, respectively (compare Fig. 6, A and C). The nociceptive excitatory and inhibitory fields of individual correlated T<sub>10</sub> ipsilateral neurons were generally smaller than those for responses of RSNA. Collectively, however, they encompassed the respective excitatory and inhibitory fields for RSNA. Of the 20 fully surveyed T<sub>10</sub> DHNs, 11 exhibited ongoing activity that was not correlated with ongoing RSNA. Of these 11 DHNs, 8 (72%) had nociceptive excitatory and inhibitory fields circumscribed by fields that, when stimulated, elicited similar effects in RSNA. Three uncorrelated T<sub>10</sub> ipsilateral neurons had nociceptive excitatory fields extending into regions that, when pinched, reduced RSNA. Significantly, these three neurons had no nociceptive inhibitory fields. Noxious stimulation of the left shoulder and forelimb decreased RSNA but increased the activity of 90% (19 of 21) of T<sub>2</sub> DHNs. Similarly, noxious stimulation of the left hip and hindlimb increased the activity of 92% (23 of 25) of L<sub>2</sub> DHNs. Conversely, the inhibitory fields of many T<sub>2</sub> and L<sub>2</sub> DHNs, when present, extended into regions from which increases in RSNA could be elicited. Nociceptive excitatory fields of all (25 of 25) contralateral T<sub>10</sub> DHNs overlapped regions that, when stimulated, decreased RSNA. Pinch of the left flank, back, and abdominal regions often reduced the activity of contralateral T<sub>10</sub> DHNs.

DISCUSSION

Our major observations are 1) that the activity of DHNs in a limited range of thoracic segments is strongly correlated with bursts of simultaneously recorded RSNA and 2) that somatic stimuli that alter RSNA similarly alter the activity of these DHNs. Both of these observations support the hypothesis that a subset of DHNs consists of excitatory synaptic antecedents to renal sympathetic preganglionic neurons.

Correlations between ongoing activity in DHNs and ongoing RSNA

We began our search for sympathetically correlated neurons in the left T<sub>10</sub> segment for two reasons. First, renal injections of pseudorabies virus (Schramm et al. 1993) indicated that this segment contained the largest number of renal sympathetic preganglionic neurons, and the distribution of renally infected DHNs correlated strongly with that of renal preganglionic neurons. Second, microinjection of D,L-homocysteic acid into the intermediolateral column at T<sub>10</sub> in rats produces the largest increases in RSNA (Taylor and Weaver 1992). In many previous attempts, we failed to find renal sympathetically correlated DHNs at segments rostral to T<sub>8</sub> and caudal to T<sub>13</sub>. These failures were explained when we reinvestigated a wide, longitudinal range of segments in the current study. We found that, rostral and caudal to T<sub>10</sub>, the incidence of sympathetically correlated DHNs diminished rapidly. At T<sub>8</sub>, a segment that contains a substantial number of renal sympathetic preganglionic neurons and presumptive renal interneurons (Schramm et al. 1993), the activity of only 16% of DHNs was correlated with RSNA. At T<sub>13</sub>, a segment that contains a small number of renal sympathetic preganglionic neurons (Schramm et al. 1993) and presump-

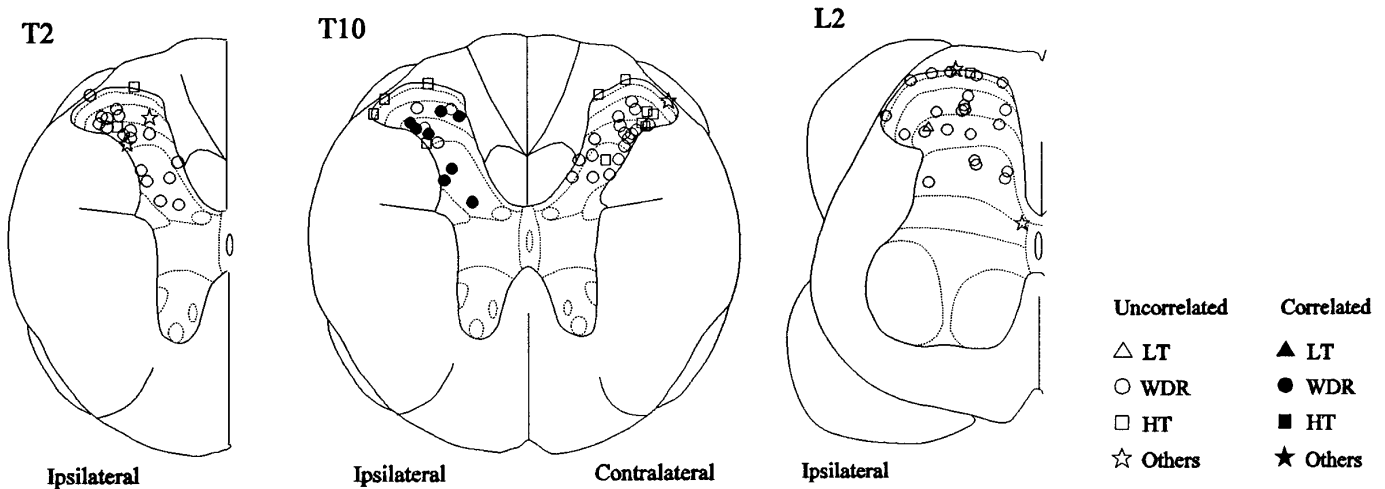


FIG. 3. Laminar distributions of histologically recovered dorsal horn neurons at 3 spinal levels. Ipsilateral neurons were located ipsilateral to the left renal nerve. See text for criteria for “correlated” neurons. LT, low threshold; WDR, wide dynamic range; HT, high threshold; “others,” none of the above (METHODS). Outlines modified from Figs. 68 and 69 of Paxinos and Watson (1982).

tive renal interneurons, we observed no sympathetically correlated DHNs.

We were unable to find any sympathetically correlated DHNs on the contralateral side of the spinal cord at T<sub>10</sub>. This observation is consistent with the results of three previous studies. First, Taylor and Weaver (1992) found that contralateral injections of D,L-homocysteic acid produced few increases in RSNA. Second, many fewer DHNs are infected contralateral to renal pseudorabies virus injections (Schramm et al. 1993). Third, Cabot et al. (1994) observed no retrograde, transynaptic transport of cholera toxin from sympathetic preganglionic neurons to neurons on the contralateral side of the spinal cord. All of these observations are consistent with the hypothesis that, in spinally transected rats, the DHNs that are excitatory antecedents to renal sym-

pathetic preganglionic neurons are both longitudinally and laterally restricted.

All sympathetically correlated DHNs exhibited bursting patterns of ongoing activity, and bursts of ongoing renal sympathetic activity were usually correlated with bursts of (rather than single) DHN action potentials. This observation is consistent with the hypothesis that temporal summation plays an important role in determining the strength of coupling between renal sympathetic preganglionic neurons and their synaptic antecedents. Nevertheless, this observation does not preclude a role for steady, nonbursting patterns of action potentials in the generation of sympathetic activity. Single action potentials may, on a longer time scale, affect the overall level of depolarization of sympathetic preganglionic neurons and thereby affect the average level of sympathetic nerve activity without being correlated with identifiable bursts of ongoing activity. Therefore we cannot yet exclude the possibility that apparently uncorrelated DHNs, in any segment or on either side of the spinal cord, could affect levels of ongoing RSNA.

These experiments were feasible only because anesthetized rats, unlike cats (and other mammals), exhibit ongoing RSNA immediately after acute spinal cord transection (Kimura et al. 1995; Osborn et al. 1987; Taylor and Weaver 1993). The potential sources of ongoing renal sympathetic activity after spinal transection in rats have been thoroughly reviewed by Taylor and Weaver (1993). Ongoing activity could be attributed to drive provided by primary afferents. Alternatively, the source of ongoing sympathetic activity could be spinal networks that are independent of afferent drive. After spinal transection, extensive dorsal rhizotomy decreases renal sympathetic activity by 25%, suggesting a role for tonic afferent drive in generating renal sympathetic activity (Taylor and Weaver 1993). On the other hand, the fact that 75% of RSNA (and 100% of mesenteric nerve sympathetic activity) survives these rhizotomies supports the existence of endogenous spinal sympathetic generators. Although the present experiments are the first to suggest a role for DHNs in either the generation or transmission of ongoing

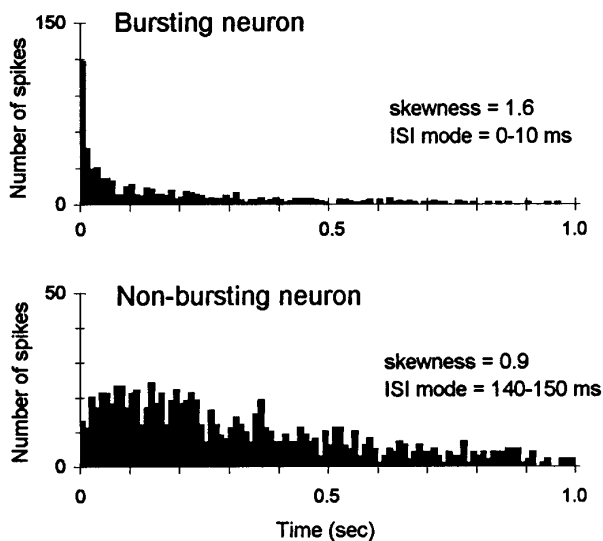


FIG. 4. Interspike interval (ISI) histograms of a bursting dorsal horn neuron (top) and a nonbursting dorsal horn neuron (bottom). Bin size: 10 ms. Number of action potentials for bursting and nonbursting neurons: 600 and 900, respectively.

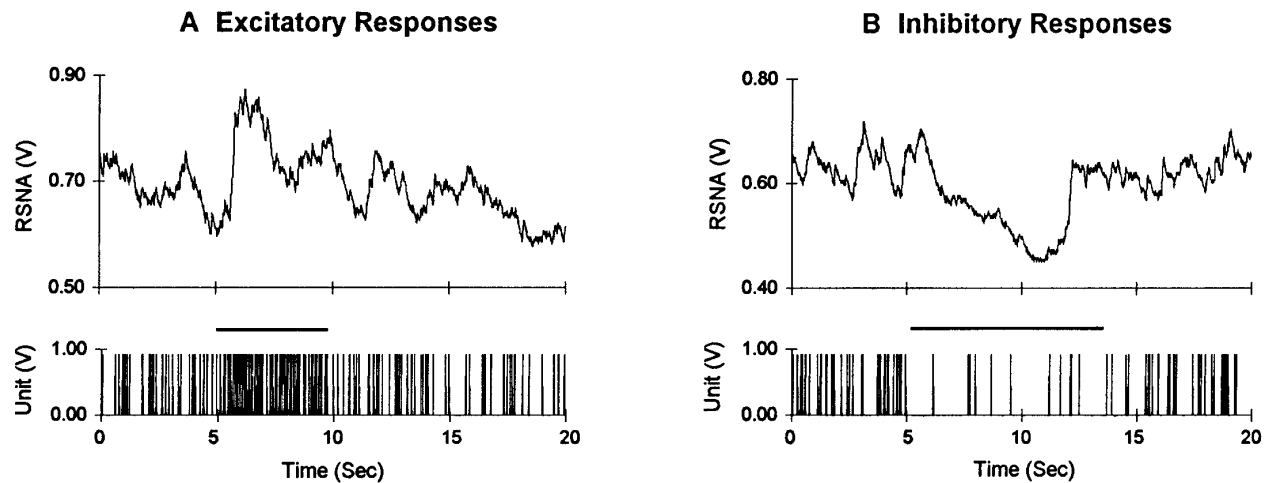


FIG. 5. Representative renal sympathetic nerve responses (*top*) and dorsal horn neuronal responses (*bottom*) to noxious cutaneous stimulation of the left flank (A) and left hip (B). Responses in A and B were recorded from same neuron and sympathetic nerve. Stimuli were maintained for durations indicated by horizontal bars. Vertical scales for *top* reflect amplification of 10,000 times (METHODS).

excitatory drive to sympathetic preganglionic neurons, we are not able to conclude which of these roles, generation or transmission, is manifested by the observed correlations. It should be noted, however, that some portion of the ongoing RSNA and some of the ongoing activity in DHNs observed in these acutely spinalized rats may have been generated by the surgery and anesthesia necessary to perform our experiments. Maiorov et al. (1997) have recently shown that, in the absence of anesthesia, acute surgery, or other somatic or visceral stimuli, chronically transected rats exhibit relatively little ongoing RSNA. The role of DHNs in generating this small, residual RSNA after chronic spinal transection is conjectural. On the basis of the data from present experiments, however, it is plausible that DHNs play a role in generating the temporal patterns of stimulus-driven RSNA in the chronic spinal mammal.

#### *Relationships between reflex responses in renal sympathetic activity and reflex responses of DHNs*

Our observation that noxious mechanical stimulation of the caudal flank, back, and abdomen of spinally transected rats increased RSNA, confirms previous data from this and other laboratories (Kimura et al. 1995; Poree and Schramm 1992). In our earlier experiments, we showed for the first time that noxious thermal stimulation of these regions not only increased RSNA, but synchronously increased the activity of caudal thoracic DHNs (Poree and Schramm 1992). On the basis of those results, we tentatively hypothesized that thoracic DHNs may mediate homotopic somatosympathetic and viscerosympathetic reflex responses to noxious stimuli by increasing excitatory input to sympathetic preganglionic neurons.

The detailed mapping of the somatic fields of DHNs conducted in the present experiments further supports this hypothesis by showing that the excitatory and inhibitory somatic fields of sympathetically correlated  $T_{10}$  DHNs corresponded more closely to the excitatory and inhibitory fields for RSNA than did the excitatory and inhibitory fields of sympathetically uncorrelated  $T_{10}$  DHNs. The conjunction of

the excitatory somatic fields of the sympathetically correlated  $T_{10}$  DHNs was nearly identical to the conjunction of the excitatory somatic fields for RSNA (compare Fig. 6, A, *left* with C, *left*). The excitatory fields of some sympathetically uncorrelated DHNs, however, extended further rostrally and caudally, well beyond the borders of the excitatory somatic fields for RSNA (compare Fig. 6, A, *left* with D, *left*). It is not surprising that the excitatory fields of some sympathetically uncorrelated DHNs were very similar in extent to excitatory fields for RSNA, because many DHNs that play no role in the generation or transmission of drive to renal sympathetic preganglionic neurons would be expected to be excited by stimulation of these fields.

Detailed mapping of inhibitory fields for RSNA and the activity of  $T_{10}$  DHNs provided additional support for a role of DHNs in sympathetic processing. Stimuli that transiently decreased RSNA, i.e., pinch of the ipsilateral fore- and hindlimbs and the contralateral flank, synchronously reduced the ongoing activity of ipsilateral sympathetically correlated  $T_{10}$  DHNs (Figs. 5B and 6C, *right*). With one exception, inhibitory fields of ipsilateral uncorrelated  $T_{10}$  DHNs also matched inhibitory fields for RSNA (Fig. 6D, *right*). Again, it is not surprising that the inhibitory fields of many sympathetically uncorrelated DHNs were nearly identical to inhibitory fields for RSNA because widespread inhibition of DHNs by heterotopic noxious stimulation, both before and after spinal cord transection, has been well documented (see, for example, Cadden et al. 1983). That separate propriospinal mechanisms might mediate inhibition of DHNs and inhibition of sympathetic preganglionic neurons is possible. It is more likely, however, that noxious stimulation of ipsilateral fore- and hindlimb dermatomes and the contralateral flank reduces the activity of many lower thoracic DHNs. Reduction of the activity of those DHNs that are excitatory synaptic antecedents to renal sympathetic preganglionic neurons would be expected to reduce RSNA.

Despite reports of significant intersegmental sympathetic reflexes (Laskey et al. 1979; Weaver et al. 1983), most authors emphasize the segmental character of sympathetic reflexes after

**Excitatory Fields**

**Inhibitory Fields**

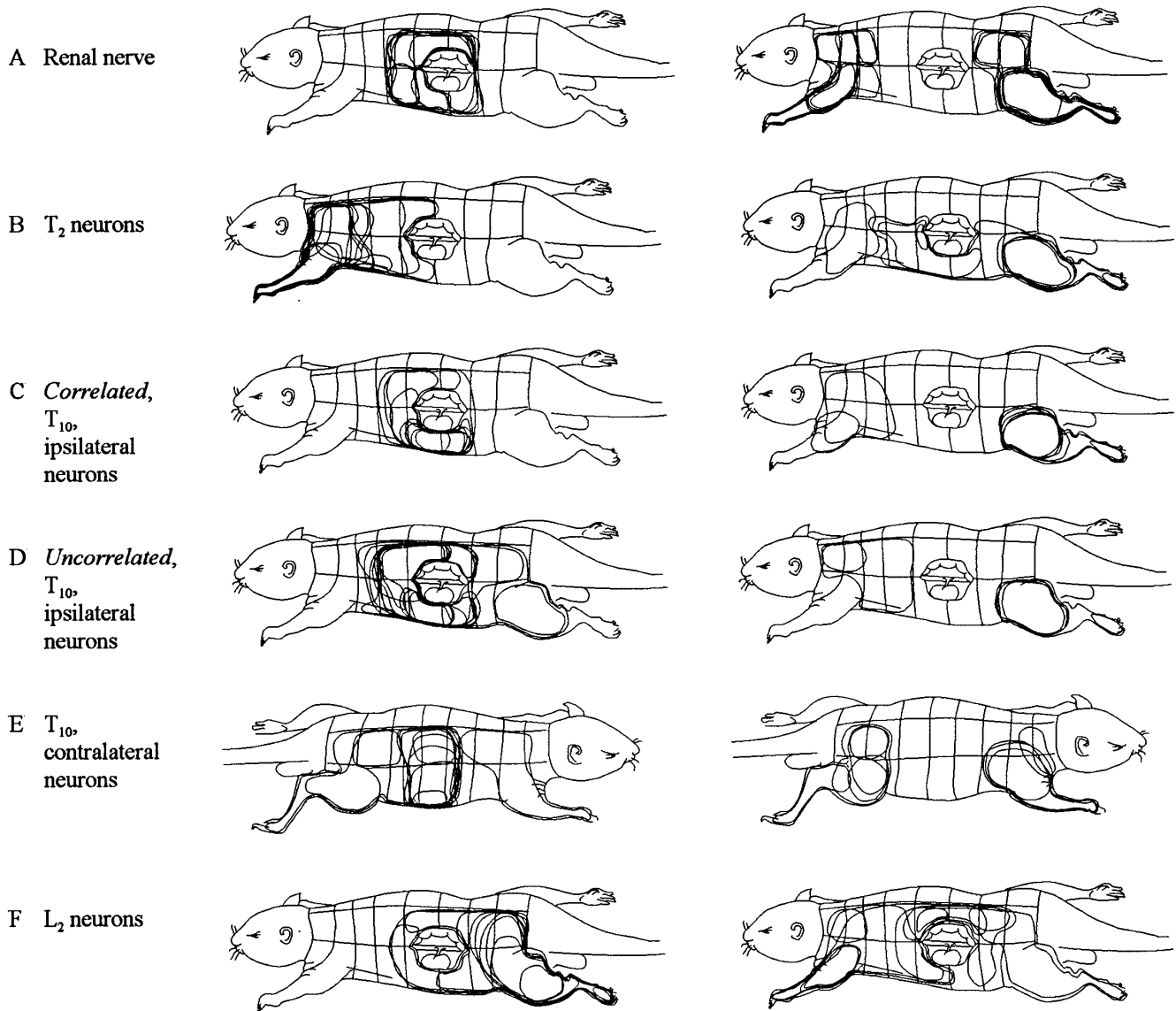


FIG. 6. Cutaneous fields for noxious stimulation (METHODS) that excited (A, left) and inhibited (A, right) RSNA or excited (B–F, left) and inhibited (B–F, right) dorsal horn neurons in the spinal segments indicated. Note that fields for sympathetically correlated and uncorrelated ipsilateral T<sub>10</sub> dorsal horn neurons are shown separately (C and D). For clarity, only representative fields are illustrated.

spinal cord transection (see Kimura et al. 1995 for review). The reduction of RSNA by noxious stimulation of the extremities and the inhibition of most DHNs at T<sub>2</sub>, T<sub>10</sub>, and L<sub>2</sub> by heterosegmental, noxious stimulation are important, however, because they demonstrate the existence of functional, intersegmental regulation of the activity of DHNs and sympathetic activity after spinal transection.

The hypothesis that some DHNs are excitatory synaptic antecedents to renal sympathetic preganglionic neurons is supported by observations from previous experiments in this laboratory. Schramm and Livingstone (1987) found that, after C<sub>1</sub> spinal cord transection in rats, electrical or chemical stimulation of the dorsolateral surface of the cervical spinal cord substantially

reduced RSNA, very likely by exciting part of a descending brain stem inhibitory pathway or a propriospinal inhibitory pathway. Poree and Schramm (1992), recording simultaneously from thoracic DHNs and the renal sympathetic nerves, showed that electrical and chemical cervical stimulation, similar to that described by Schramm and Livingstone, synchronously reduced ongoing DHN activity and ongoing RSNA. Further, cervical stimulation reduced responses in both RSNA and DHNs to noxious stimuli applied to cutaneous excitatory fields on the left flank.

In summary, the activity of a subpopulation of thoracic DHNs is closely correlated with bursts of RSNA. Somatic stimulation that increases and decreases RSNA excites and

inhibits these DHNs. Finally, in previous experiments we showed that cervical stimulation that decreases RSNA decreases the activity of thoracic DHNs and reduces somatically elicited responses in both DHNs and renal nerves. Taken together, these observations suggest that a subpopulation of DHNs constitutes excitatory antecedents to renal sympathetic preganglionic neurons.

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## REFERENCES

- ALEXANDER, R. S. Tonic and reflex functions of medullary sympathetic cardiovascular centers. *J. Neurophysiol.* 9: 205–217, 1946.
- BARMAN, S. M. AND GEBBER, G. L. Brain stem neuronal types with activity patterns related to sympathetic nerve discharge. *Am. J. Physiol.* 240 (*Regulatory Integrative Comp. Physiol.* 9): R335–R347, 1981.
- BARMAN, S. M., ORER, H. S., AND GEBBER, G. L. A 10-Hz rhythm reflects the organization of a brainstem network that specifically governs sympathetic nerve discharge. *Brain Res.* 671: 345–350, 1995.
- CABOT, J.H.B., ALESSI, V., CARROLL, J., AND LIGORIO, M. Spinal cord lamina V and lamina VII interneuronal projections to sympathetic preganglionic neurons. *J. Comp. Neurol.* 347: 515–530, 1994.
- CADDEN, S. W., VILLANEUVA, L., CHITOUR, D., AND LEBARS, D. Depression of activities of dorsal horn convergent neurones by propriospinal mechanisms triggered by noxious inputs; comparison with diffuse noxious inhibitory controls (DNIC). *Brain Res.* 275: 1–11, 1983.
- DEMBOWSKY, K., CZACHURSKI, J., AND SELLER, H. An intracellular study of the synaptic input to sympathetic preganglionic neurones in the third thoracic segment of the cat. *J. Auton. Nerv. Syst.* 13: 201–244, 1985.
- GEBBER, G. L. AND MCCALL, R. B. Identification and discharge patterns of spinal sympathetic interneurons. *Am. J. Physiol.* 231: 722–733, 1976.
- GEBBER, G. L., ZHONG, S., AND BARMAN, S. M. The functional significance of the 10-Hz sympathetic rhythm: a hypothesis. *Clin. Exp. Hypertens.* 17: 181–195, 1995.
- GILBEY, M. P., NUMAO, Y., AND SPYER, K. M. Discharge patterns of cervical sympathetic preganglionic neurones related to central respiratory drive in the rat. *J. Physiol. Lond.* 378: 253–265, 1986.
- KIMURA, A., OHSAWA, H., SATO, A., AND SATO, U. Somatocardiovascular reflexes in anesthetized rats with the central nervous system intact or acutely spinalized at the cervical level. *Neurosci. Res.* 22: 297–305, 1995.
- LASKEY, W., SCHONDORF, R., AND POLOSA, C. Intersegmental connections and interactions of myelinated somatic and visceral afferents with sympathetic preganglionic neurons in the unanesthetized spinal cat. *J. Auton. Nerv. Syst.* 1: 69–76, 1979.
- MAIOROV, D. N., WEAVER, L. C., AND KRASSIOUKOV, A. V. Relationship between sympathetic activity and arterial pressure in conscious spinal rats. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H625–H631, 1997.
- MATHIAS, C. J. AND FRANKEL, H. L. Clinical manifestations of malfunctioning sympathetic mechanisms in tetraplegia. *J. Auton. Nerv. Syst.* 7: 303–312, 1983.
- MCLACHLAN, E. M. AND HIRST, G.D.S. Some properties of preganglionic neurons in upper thoracic spinal cord of the cat. *J. Neurophysiol.* 43: 1251–1265, 1980.
- OSBORN, J. W., LIVINGSTONE, S. R., AND SCHRAMM, L. P. Elevated renal nerve activity after spinal transection: its effect on renal function. *Am. J. Physiol.* 253 (*Regulatory Integrative Comp. Physiol.* 22): R619–R625, 1987.
- PAXINOS, G. AND WATSON, C. *The Rat Brain in Stereotaxic Coordinates*. Sydney, Australia: Academic, 1982.
- POREE, L. R. AND SCHRAMM, L. P. Role of cervical neurons in propriospinal inhibition of thoracic dorsal horn neurons. *Brain Res.* 599: 302–308, 1992.
- SCHRAMM, L. P. AND LIVINGSTONE, R. H. Inhibition of renal nerve sympathetic activity by spinal stimulation in rat. *Am. J. Physiol.* 252 (*Regulatory Integrative Comp. Physiol.* 21): R514–R525, 1987.
- SCHRAMM, L. P., STRACK, A. M., PLATT, K. B., AND LOEWY, A. D. Peripheral and central pathways regulating the kidney: a study using pseudorabies virus. *Brain Res.* 616: 251–262, 1993.
- SOKAL, R. R. AND ROHLF, F. J. *Biometry*. San Francisco, CA: Freeman, 1969, p. 112–113.
- TAYLOR, R. B. AND WEAVER, L. C. Spinal stimulation to locate preganglionic neurons controlling the kidney, spleen or intestine. *Am. J. Physiol.* 263 (*Heart Circ. Physiol.* 32): H1026–H1033, 1992.
- TAYLOR, R. B. AND WEAVER, L. C. Dorsal root afferent influences on tonic firing of renal and mesenteric sympathetic nerves in rats. *Am. J. Physiol.* 264 (*Regulatory Integrative Comp. Physiol.* 33): R1193–R1199, 1993.
- WEAVER, L. C., FRY, H. K., MECKLER, R. L., AND OEHL, R. S. Multisegmental spinal sympathetic reflexes originating from the heart. *Am. J. Physiol.* 245 (*Regulatory Integrative Comp. Physiol.* 14): R345–R352, 1983.
- ZHONG, S., GEBBER, G. L., AND BARMAN, S. M. Differential relationships among 10-Hz rhythmic discharges of sympathetic nerves with different targets depend on supraspinal rather than spinal mechanisms. *Brain Res.* 670: 329–332, 1995.
- ZHONG, S., HUANG, Z. S., GEBBER, G. L., AND BARMAN, S. M. Role of the brain stem in generating the 2- to 6-Hz oscillation in sympathetic nerve discharge. *Am. J. Physiol.* 265 (*Regulatory Integrative Comp. Physiol.* 34): R1026–R1035, 1993.