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# Ongoing and Stimulus-Evoked Activity of Sympathetically Correlated Neurons in the Intermediate Zone and Dorsal Horn of Acutely Spinalized Rats

DAVID CHAU, DEVIN G. JOHNS, AND LAWRENCE P. SCHRAMM

*Department of Biomedical Engineering, The Johns Hopkins School of Medicine, Baltimore, Maryland 21205*

**Chau, David, Devin G. Johns, and Lawrence P. Schramm.** Ongoing and stimulus-evoked activity of sympathetically correlated neurons in the intermediate zone and dorsal horn of acutely spinalized rats. *J. Neurophysiol.* 83: 2699–2707, 2000. We have shown previously that in the acutely spinalized anesthetized rat the activities of many dorsal horn interneurons (DHN) at the T<sub>10</sub> level are correlated positively with both ongoing and stimulus-evoked renal sympathetic nerve activity (RSNA) and therefore may belong to networks generating RSNA after acute, cervical, spinal transection. In the present study, we recorded from both DHN and interneurons in the intermediate zone (IZN) of the T<sub>10</sub> spinal segment in acutely C<sub>1</sub>-transected, chloralose-anesthetized, artificially respired rats. The activities of a similar percentage of IZN and DHN were correlated positively with ongoing RSNA, but the peaks of spike-triggered averages of RSNA based on the activity of IZN were larger, relative to dummy averages, than spike-triggered averages of RSNA based on the activity of DHN. Sympathetically correlated DHN and IZN differed in their responses to noxious somatic stimuli. Most correlated DHN had relatively simple somatic fields; they were excited by noxious stimulation of the T<sub>10</sub> and nearby dermatomes and inhibited by stimulation of more distal dermatomes. As we have shown previously, the excitatory and inhibitory fields of these neurons were very similar to fields that, respectively, excited and inhibited RSNA. On the other hand, the somatic fields of 50% of sympathetically correlated IZN were significantly more complex, indicating a difference between either the inputs or the processing properties of IZN and DHN. Sympathetically correlated IZN and DHN also differed in their responses to colorectal distension (CRD), a noxious visceral stimulus. CRD *increased* RSNA in 11/15 rats and *increased* the activity of most sympathetically correlated T<sub>10</sub> IZN. On the other hand, CRD *decreased* the activity of a majority of sympathetically correlated T<sub>10</sub> DHN. These observations suggest that the same stimulus may differentially affect separate, putative, sympathoexcitatory pathways, exciting one and inhibiting the other. Thus the magnitude and even the polarity of responses to a given stimulus may be determined by the modality and location of the stimulus, the degree to which multiple pathways are affected by the stimulus, and the ongoing activity of presympathetic neurons, at multiple rostrocaudal levels, before stimulation. A multipathway system may explain the variability in autonomic responses to visceral and somatic stimuli exhibited in spinally injured patients.

## INTRODUCTION

Supraspinal systems are responsible for most of the ongoing and reflex-elicited regulation of spinal sympathetic preganglionic neurons in intact mammals (Alexander 1946; Ross et al.

1983; Stornetta et al. 1989). By definition, the generators of sympathetic activity after spinal transection reside in the spinal cord, and these generators are poorly understood. Improving our knowledge of spinal sympathetic circuits is important for several reasons. First, spinal circuits may be recruited by supraspinal systems in generating ongoing and evoked sympathetic activity in intact animals. If so, our knowledge of normal modes of generating sympathetic activity would be incomplete without an understanding of these spinal systems. Second, both ongoing and evoked sympathetic activities persist in mammals, including humans, after spinal transection (Guttman and Whitteridge 1947; Sherrington 1906), and this activity has multiple consequences. For instance, ongoing sympathetic activity may play a role in defending against postural hypotension when spinally injured patients are in an upright position. On the other hand, sudden large increases in sympathetic activity elicited by somatic and visceral stimuli are rarely beneficial to spinally injured patients, for these increases may manifest autonomic dysreflexia, a potentially dangerous condition that can result in transient hypertension, stroke, and death (Mathias and Frankel 1992). Knowledge of the systems responsible for generating sympathetic activity after spinal transection may suggest new methods for preventing and treating autonomic pathology.

We have described a subset of dorsal horn neurons (DHN) in acutely cervically spinalized rats that exhibited ongoing activity that was correlated closely and positively with renal sympathetic nerve activity (RSNA) (Chau et al. 1997). In other words, increases in RSNA frequently followed single discharges and bursts of discharges in this subset of DHN. Not only were the ongoing activities of these DHN correlated with ongoing RSNA, but also their responses to noxious somatic stimulation of a wide range of somatic regions were similar to responses of RSNA. Thus noxious cutaneous stimulation of the flank of the T<sub>10</sub> and nearby dermatomes simultaneously *increased* both RSNA and the activity of correlated DHN. Noxious stimulation of more rostral and caudal regions (i.e., “distal” to T<sub>10</sub>), such as the shoulder and hip, *decreased* both RSNA and the activity of correlated DHN. On the basis of these observations, we suggested that sympathetically correlated T<sub>10</sub> DHN might be excitatory antecedents to the renal sympathetic preganglionic neurons involved in the generation of ongoing RSNA. Further, we suggested that these DHN also might play a role in mediating somato-sympathetic reflexes after spinal cord transection.

In spinally transected, chronically maintained experimental animals and patients, pelvic visceral stimulation activates sym-

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pathetic preganglionic neurons at many spinal levels even though the great majority of primary afferents mediating this response enter the spinal cord only as far rostral as caudal thoracic levels (Ness and Gebhart 1988b). Thus in rats with spinal cords chronically transected above T<sub>5</sub>, distension of the bladder (Osborn et al. 1990) or colorectal distension (Krasnioukov and Weaver 1995) elicits pressor responses that manifest widespread vasoconstriction. What neurons convey excitation from relatively localized afferents to sympathetic preganglionic neurons at widespread levels of the spinal cord? Transsynaptic retrograde tracing experiments (Cabot et al. 1994; Schramm et al. 1993) indicate the presence of putative presympathetic intermediate zone neurons (IZN) and DHN that, either in parallel or in series, could receive primary or propriospinal inputs and mediate multisegmental increases in sympathetic activity. However, the relationship between the activities of IZN and DHN and sympathetic nerve activity has not previously been studied in the same animals after spinal transection.

The present experiments accomplished three major goals. First, we determined whether sympathetically correlated neurons resided in the T<sub>10</sub> intermediate zone. Once we had determined that correlated IZN existed, we searched for similarities and differences between their ongoing activities and those of T<sub>10</sub> DHN. We also compared the relationships among the activities of IZN, DHN, and RSNA in the same rats. Second, we compared the responses of IZN and DHN, grouped according to their relationships to RSNA, to their responses to noxious somatic stimulation. Finally, we studied the responses of IZN and DHN to colorectal distension (CRD), a noxious and clinically relevant stimulus. The purpose of these experiments was to determine whether the responses of either sympathetically correlated IZN or DHN were implicated in mediating the cardiovascular responses to this stimulus.

## METHODS

### *Surgery*

All procedures were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine (protocols 94 M242 and 97 M182). Adult, male, Sprague-Dawley rats (Taconic Farms), weighing 200–300 g were used in these experiments. Pretreatment with atropine (0.05 mg/kg sc) reduced nasal and tracheal mucus. Anesthesia was induced by ether inhalation and continued by  $\alpha$ -chloralose (SIGMA,  $\beta$ -isomer < 7.5%) via the right femoral vein. Unconscious rats were shaved for later testing of somatic fields. They then were placed under a lamp and on a heating pad to maintain body temperature between 35 and 37°C, monitored with a probe inserted through an abdominal incision. The trachea was intubated for artificial respiration with 100% oxygen. Once secured in a stereotaxic frame, rats were paralyzed with gallamine triethiodide (40 mg/kg iv). The depth of anesthesia and paralysis, as indicated by the level and variability of RSNA and arterial pressure or corneal reflexes, was maintained with supplements of  $\alpha$ -chloralose and gallamine triethiodide.

Spinal dorsal processes C<sub>1</sub>–C<sub>2</sub> and the underlying dura were removed to expose the spinal cord for a complete transection. In some rats, an injection of 1 ml of human serum albumin (Baxter Healthcare, 25% solution, 12.5 g/50 ml iv) was administered within 1 min of spinal transection to return arterial pressure to >90 mmHg. A unilateral laminectomy made at spinal process T<sub>9</sub> permitted the recording of neurons in the left T<sub>10</sub> spinal segment. The exposed spinal cord was kept moist with warm mineral oil. A bilateral pneumothoracotomy

was performed to reduce respiratory movements. The left kidney was approached via a left flank laparotomy and retracted. The adrenal gland, the fat covering the psoas muscle, and the paraspinal muscles were deflected from the renal nerve, which typically is located at the junction of the aorta and the renal artery or is found traversing the aorta. Further spinal cord and body stabilization was accomplished by suspending rats by two dorsal processes. Finally, the renal nerve was dissected from the surrounding tissues and mounted on a hook electrode.

### *Extracellular and renal nerve recording*

The extracellular recordings of single neurons were made using single-barrel, carbon fiber microelectrodes (impedance 2–5 M $\Omega$ ) via a high-impedance probe (Grass HIP5). The resulting signal then was filtered (300–3,000 Hz half-power) and amplified 50,000 times (GRASS P5 AC amplifier). The single neurons' action potentials were discriminated using a dual-window discriminator (BAK Electronics). All neurons selected for recording had ongoing discharges >0.5 action potentials/s (for nonbursting neurons) or >0.5 bursts/s (for bursting neurons). This rate criterion reduced the required simultaneous recordings of the ongoing activities of neurons and renal sympathetic nerves to <10 min (see *Spike-triggered averaging*) and permitted estimation of the onsets of neurons' inhibitory responses to stimulation.

The recorded neurons very likely represented interneurons in the dorsal horn and intermediate zone rather than sympathetic preganglionic neurons or the axons of primary afferents for the following reasons. First, the duration of their 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. Second, the <50-ms intraburst interspike intervals in most of these neurons were shorter than those reported for sympathetic preganglionic neurons in rats (Gilbey et al. 1983) or cats (Gebber and McCall 1976). In approximately half of our experiments, we recorded from all neurons that met the preceding criteria that we were able to isolate in each electrode track. To prevent oversampling dorsal horn neurons, in the remaining experiments we lowered the electrode to a depth of ~650  $\mu$ m, recording from each neuron that we isolated ventral to this depth until we estimated that we had traversed lamina VII at depths >1,200  $\mu$ m. For each track, a lesion was made at the most ventral recording site. Recording sites located more dorsally were reconstructed using these lesions and readings from the microdrive.

RSNA was recorded with bipolar electrodes constructed from 60- $\mu$ m stainless steel wires. The signal detected at the electrode was amplified 10,000 times and filtered (100–3,000 Hz half-power; GRASS P15 AC and GRASS P5 AC amplifiers). At the end of each experiment, conduction in the renal nerve was abolished by crushing or transecting of the nerve's proximal end to determine the zero level of efferent renal sympathetic activity. In all reported experiments, the resulting signal contained negligible levels of renal afferent activity. Arterial pressure, RSNA, single-neuron activity, and a cutaneous/colorectal stimulation indicator were recorded on VHS tape for later analysis.

### *Somatic and colorectal 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 left forelimb, shoulder, flank, abdomen, hip, and hindlimb. A change in RSNA or single-neuron activity was considered significant if it represented a >10% difference from the prestimulus control level. Neurons' somatic fields were reconstructed later based on the significant responses.

Classification of the afferent modalities of T<sub>10</sub> spinal cord neurons was based on the neurons' responses to innocuous and noxious stimulation of the left flank. Wide-dynamic-range (WDR) neurons

were excited by brush and excited more by pinch. High-threshold (HT) neurons were unresponsive to brush but excited by pinch within their fields. Neurons inhibited by either brush or pinch of their fields or that were excited by brush and inhibited by pinch were classified as "OTHER." Responses of RSNA to these stimuli were characterized using the same criteria.

A silicon Foley catheter (Bard) with an inflatable, 1.5-cm-diam balloon was inserted 2–3 cm through the rat's anus. Water was infused (0.15 ml/s) into the balloon over 10 s. The infusion created a rigid 1.4-cm-diam sphere (2–3 times the size of a large fecal bolus) inside the colon. The distension was maintained for 3–4 min, after which the balloon was steadily deflated over 10 s.

*Spike-triggered averaging*

Spike-triggered averaging (see Chau et al. 1997 for more detailed methods) was used to identify the degree of correlation between spinal neurons' activities and RSNA. Spike-triggered averages of RSNA were generated for all neurons. Each average was computed from 400 1-s epochs of RSNA. Each epoch began 300 ms before the occurrence of an action potential and lasted 700 ms after the action potential. Triggering was suspended after each triggering action potential until  $\geq 1$  s after the end of each epoch to prevent overlapping of epochs. We also calculated a control or "dummy" average for the same RSNA, using as a trigger a pseudorandom pulse train with an average frequency approximately equal to that of the recorded neuron.

A "correlation index" was calculated for each neuron as the ratio of 1) the largest peak-to-peak value in the spike-triggered average occurring  $< 100$  ms after the onset of the neuron's action potentials to 2) the largest peak-to-peak deflection occurring at any time in the respective dummy average. For purpose of classification, neurons were considered correlated to RSNA (henceforth "sympathetically correlated") if their correlation indexes  $> 2.0$ .

*Histology*

At the end of experiments, rats were perfused transcardially with buffered saline (pH 7.4), followed by phosphate-buffered formalin solution (3.7% formaldehyde in 0.1 M  $\text{PO}_4$ , pH 7.4). The  $T_{10}$  segment of the spinal cord was removed and stored in sucrose-formaldehyde solution (30% sucrose, 3.7% formaldehyde in 10% phosphate-buffered formaldehyde, pH 7.4) for 2–5 days. After cryoprotection, transverse 40- $\mu\text{m}$  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. Statistical analyses employed either a Fisher's exact test (2-tailed) for contingency tables, a Student's *t*-test (2-tailed, unpaired) for comparing pairs of populations, or one-way ANOVA (with Tukey post hoc tests) for comparing larger numbers of populations. Values of  $P < 0.05$  were considered significant.

RESULTS

*Location and ongoing activity of interneurons in the dorsal horn and intermediate zone*

The relationship between the ongoing activities of 64  $T_{10}$  interneurons and the activities of renal sympathetic nerves of 15 rats were analyzed using spike-triggered averaging. The ongoing activity of 27 neurons (42%) exhibited correlation indices  $\geq 2.0$  and therefore were considered to be correlated with RSNA (see METHODS). The activity of one IZN was strongly correlated with *decreases* in RSNA, with a correlation index of 5.8. The significance of the small incidence of nega-

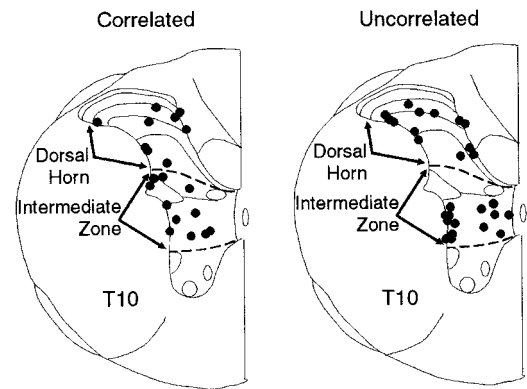


FIG. 1. Locations of histologically recovered sympathetically correlated and sympathetically uncorrelated neurons recorded in the  $T_{10}$  segment of the rat spinal cord after acute transection at the  $C_1$  segment.

tively correlated neurons is discussed in DISCUSSION. Unless noted, only the data from the 26 interneurons correlated with increases in RSNA are described.

Sympathetically correlated neurons were distributed approximately equally between the dorsal horn (14 of 31 DHN correlated) and the intermediate zone (12 of 32 IZN correlated). Histological location of 20 correlated neurons and 27 uncorrelated IZN in lamina VII, correlated and uncorrelated DHN and IZN were similarly distributed anatomically (Fig. 1). Among the histologically located uncorrelated neurons, none were located in the intermediolateral column.

Comparisons were made between the spike-triggered averages of DHN and IZN based on the magnitudes of their correlation indices (METHODS) and the latencies between action potentials and maximum changes in averaged activity (see, for example, comparison in Fig. 2). The spike triggered averages illustrated in Fig. 2 are representative of those for the sympathetically correlated IZN and DHN populations, respectively. As suggested by that figure, the average magnitude of correlation indices for correlated IZN ( $3.1 \pm 0.2$ ) was significantly larger than that for correlated DHN ( $2.4 \pm 0.20$ ,  $P = 0.007$ ). Agreeing with our previous report (Chau et al. 1997), action potentials of correlated DHN, on average, preceded bursts of RSNA by  $66 \pm 2$  ms (Fig. 2). A similar latency was observed for correlated IZN ( $63 \pm 3$  ms,  $P = 0.36$  for difference between spike-triggered average latencies of IZN and DHN).

The average firing rates of correlated IZN and DHN were not significantly different ( $4.4 \pm 0.9$  and  $3.7 \pm 0.7$  Hz, respectively,  $P = 0.48$ ). However, generation of interspike interval histograms (Fig. 3) revealed that the modal interspike intervals of many, but not all, correlated DHN were substantially shorter than those for correlated IZN. To better quantify this difference, we calculated the percentage of intervals  $\leq 5$  ms (corresponding to action potential bursts of  $\geq 200$  Hz). This value was greater for sympathetically correlated DHN ( $32 \pm 6\%$ ) than for sympathetically correlated IZN ( $12 \pm 5\%$ ,  $P < 0.05$ ). This calculation confirmed our auditory impression that the activity of DHN was much more likely to be exhibit high-frequency bursts of several action potentials than was the activity of IZN. We do not imply that only rapidly "bursting" neurons were correlated with RSNA. Indeed, correlated neurons with discharge patterns that ranged from very bursty to relatively regular were found among both DHN and IZN.

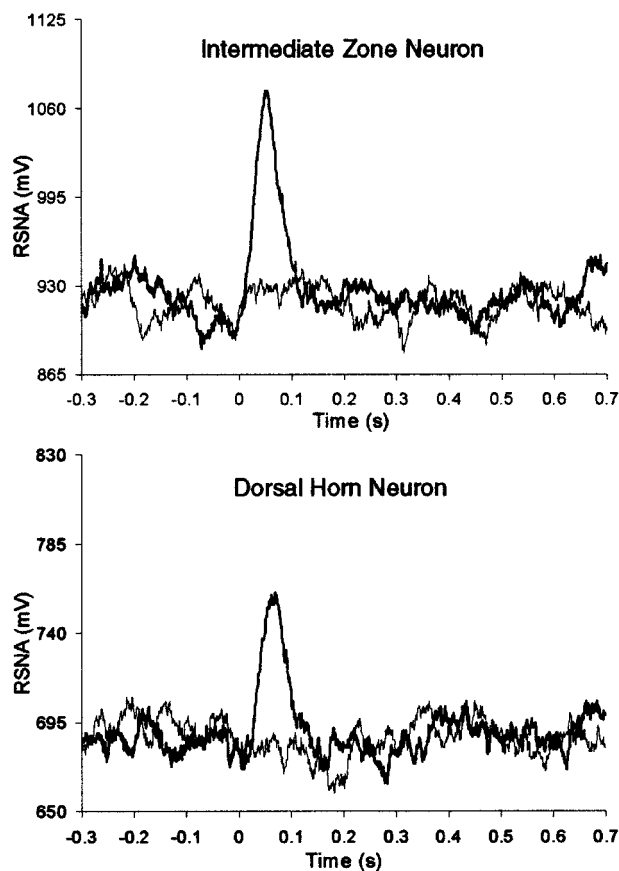


FIG. 2. **Bold traces:** spike-triggered averages of renal sympathetic nerve activity (RSNA) related to representative action potentials of a neuron in the intermediate zone (*top*) and a neuron in the dorsal horn (*bottom*). Both examples are averages of 400 action potential-related epochs. **Light traces:** dummy spike-triggered averages using random events as triggers and the same segments of RSNA used for generating actual averages. Correlation indices (see METHODS) for interneurons in the intermediate zone (IZN) and dorsal horn neurons (DHN), 3.6 and 2.2, respectively.

*Correlated neurons in both the intermediate zone and the dorsal horn were more likely to exhibit wide dynamic range modalities than were uncorrelated neurons*

We characterized the modalities of 45 neurons as WDR, HT, and OTHER (METHODS). The modalities of a majority of both correlated IZN (WDR = 9, HT = 0, OTHER = 2) and correlated DHN (WDR = 8, HT = 0, OTHER = 1) were categorized as WDR. Indicating that correlated and uncorrelated neurons received or processed inputs independently, the somatic fields of uncorrelated neurons were far more likely to exhibit high-threshold and OTHER fields than were the fields of correlated neurons.

*Somatic fields of intermediate zone neurons were more heterogeneous than those of dorsal horn neurons*

We determined the responses of 11 correlated  $T_{10}$  DHN and 14 correlated  $T_{10}$  IZN to brushing and pinching the skin of the left flank, forelimb, shoulder, hip, and hindlimb. The ongoing activity of 82% (9/11) of correlated DHN was increased by brushing the left flank in the estimated region of the  $T_{10}$  dermatome, increased more by pinch of the same region, and decreased by pinch of “distal” regions, such as the left fore-

limb, shoulder, hip, and hindlimb. In contrast, the ongoing activity of only 50% (7/14) of correlated IZN shared the type of response exhibited by the majority of DHN. The discharge rate of the remaining 50% of the correlated IZN was *decreased* by noxious stimulation within the estimated  $T_{10}$  dermatome, *increased* by stimulation of the hip and elbow, or uniformly increased or decreased by stimulation within a entire quadrant or more of the body wall.

*Responses of individual IZN and DHN to pinch and brush were related to concurrent responses in RSNA*

Responses of renal sympathetic nerve activity were, of course, recorded during all responses of DHN and IZN. We classified the RSNA responses to pinching the skin of the left flank in a manner identical to that used for single neurons. Complete recordings in which concurrent responses of DHN or IZN and RSNA were clear and artifact-free for both brush and pinch were obtained for a total of 19 sympathetically correlated DHN and IZN in 11 rats. The agreement between responses in RSNA and responses in interneurons was good (Table 1). When rats exhibited either WDR or HT responses in RSNA, all correlated neurons exhibited WDR fields. Eleven neurons recorded during HT responses in RSNA exhibited lower thresholds than did RSNA (HT column and WDR row). No interneurons exhibited higher thresholds to stimuli than RSNA (WDR column and HT row). Agreement extended to OTHER responses in RSNA and responses of interneurons. The only two correlated interneurons whose rates of discharge were *decreased* by pinching the flank were recorded in rats in which pinching the flank *decreased* RSNA. Both of these neurons

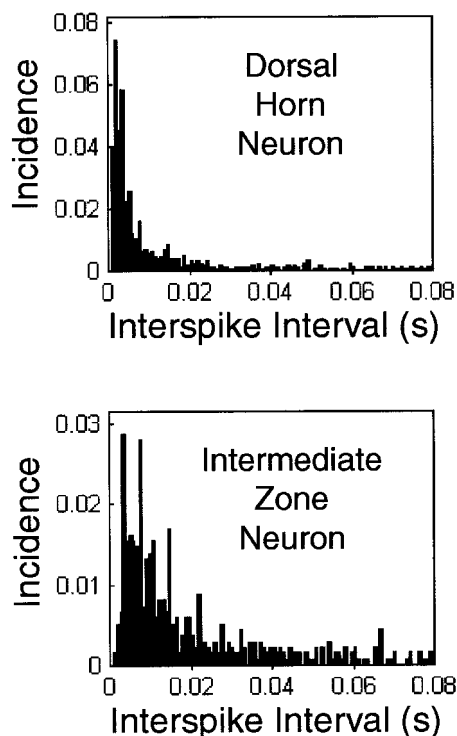


FIG. 3. Interspike interval histograms of representative DHN and IZN. Percentage of discharges (“incidence”) is plotted for each interspike interval between 1 and 80 ms. “Incidence” axis is magnified 3 times for the IZN histogram. Although the histograms for both neurons exhibit short intervals, the incidence of these intervals for the DHN is much greater than for the IZN.

TABLE 1. Numbers of sympathetically correlated interneurons responding in each of three categories during concurrent responses of renal sympathetic nerve activity in each of three categories

Responses in DHN and IZN	Responses in RSNA			Totals
	WDR	HT	Other	
WDR	6	11	0	17
HT	0	0	0	0
Other	0	0	2	2
Totals	6	11	2	19

DHN, correlated dorsal horn neuron; IZN, correlated intermediate zone neuron; WDR, wide dynamic range; HT, high threshold; Other, inhibited by pinch of flank.

were located in the intermediate zone. The thresholds for RSNA responses to flank stimulation sometimes changed during the experiment. This resulted in changes of classification of responses from WDR to HT (as thresholds increased) or from HT to WDR (as thresholds decreased) in the same rat. Unfortunately, because we moved as quickly as possible through our stimulus paradigm, we did not have the opportunity to determine whether responses in a single neuron changed as the thresholds for responses in RSNA changed.

*CRD specifically excited sympathetically correlated neurons in the intermediate zone and inhibited sympathetically correlated neurons in the dorsal horn*

CRD increased the firing rates (Fig. 4) of most sympathetically correlated IZN (11 excited, 1 inhibited). Conversely, CRD decreased the ongoing activity of the majority of sympathetically correlated DHN (8 were inhibited, 3 were excited,

and 3 exhibited no response,  $P < 0.002$ , Fisher's exact test, Fig. 4). Uncorrelated DHN and IZN were not differentially affected by colorectal distension. Importantly, CRD neither significantly changed the degree of correlation between the activity of neurons and RSNA (i.e., the correlation index) nor caused uncorrelated neurons to become correlated, even when CRD elicited large and sustained increases in RSNA.

*CRD reliably increased mean arterial pressure, but it elicited less predictable changes in heart rate and RSNA*

During CRD, we recorded the changes in rats' mean arterial blood pressure (MAP), heart rate, and RSNA (Fig. 5). Three or four distensions, each separated by  $>30$  min, elicited increases in MAP of  $\geq 2$  mmHg in all rats. The average increase in MAP during all trials was  $5.7 \pm 0.6$  mmHg from an average pre-stimulus pressure of  $94 \pm 4$  mmHg. There was, however, considerable variability in the sizes of arterial pressure changes elicited by CRD, both within and between rats. Maximum increases in mean arterial pressure in each rat ranged between 7 and 21 mmHg, and the average maximum increase for all rats was  $12.5 \pm 1.1$  mmHg.

Heart rate changes during CRD ranged between very small to unmeasurable. The only detectable responses were increases in heart rate, and these were observed in only 3 of 15 rats during CRD (average change in all rats was  $2 \pm 0.2$  beats/min,  $n = 15$ , from initial rates of  $380 \pm 12$  beats/min). In all rats, CRD elicited increases or decreases in RSNA that were  $\geq 10\%$  of the magnitude of ongoing activity. Neither repeated distensions nor changes in the position of the colorectal balloon altered the *direction* of these responses. However, the magnitudes of responses varied considerably between rats and from trial to trial in some rats. Because the *polarities* (excitation or

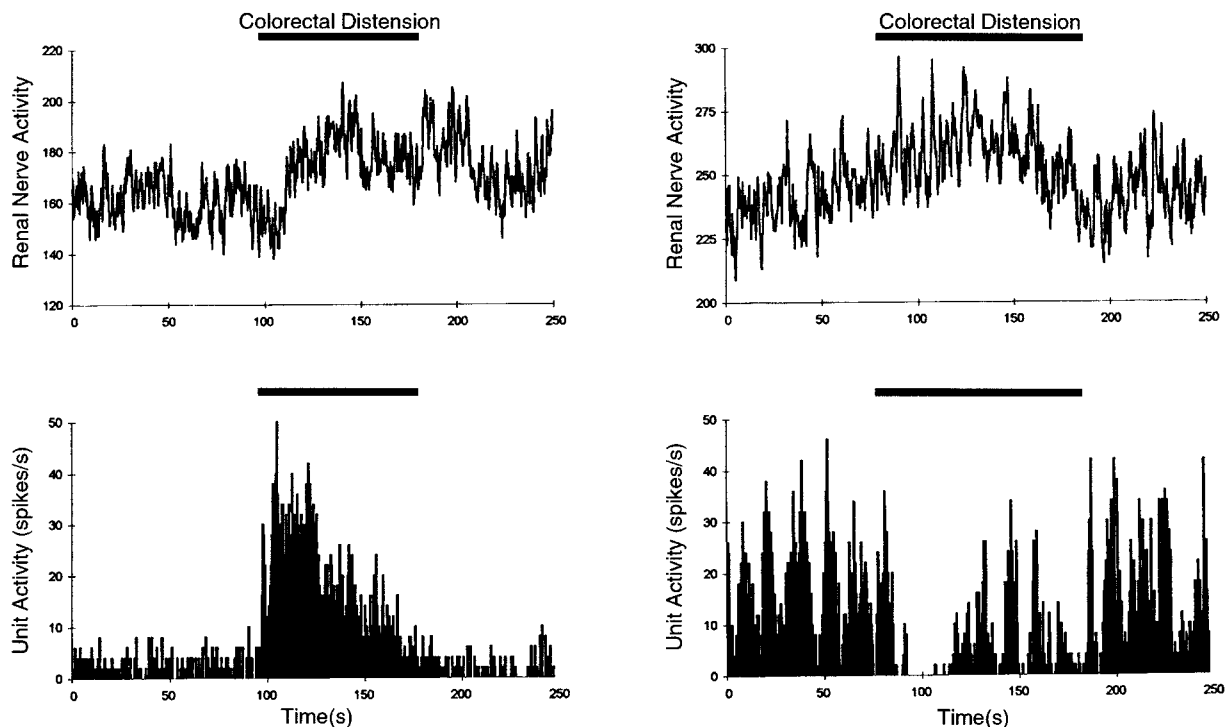


FIG. 4. Responses to colorectal distension of renal sympathetic nerve activity and 2 sympathetically correlated neurons in the T<sub>10</sub> spinal cord (left, IZN; right, DHN). The ongoing activity of the IZN was increased during colorectal distension (CRD). That of the DHN was decreased. Both distensions elicited increases in RSNA.

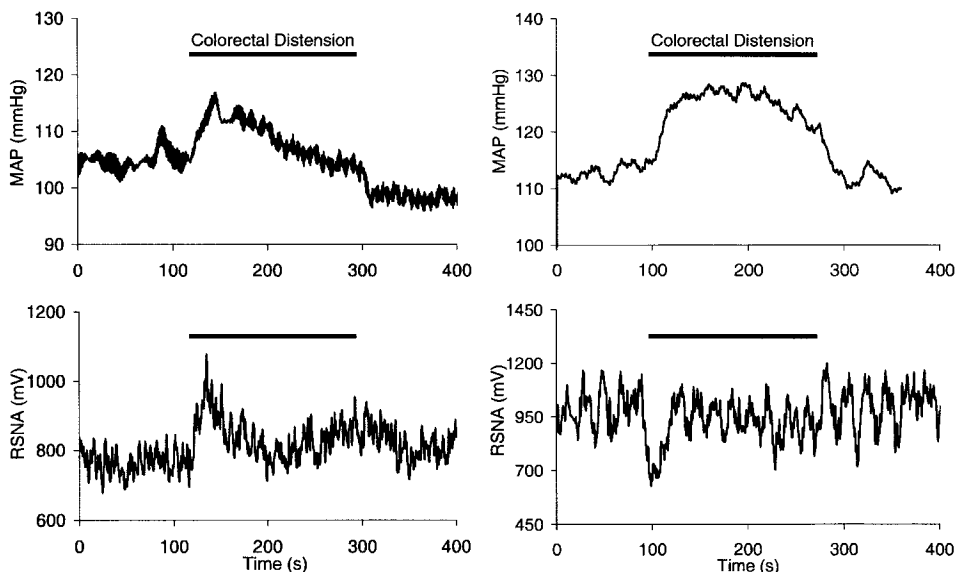


FIG. 5. Responses of mean arterial pressure (MAP) and RSNA to colorectal distension in 2 rats (*left and right*). Although arterial pressure and heart rate increased at the onset of CRD in both rats, RSNA increased in 1 rat (*left*) and decreased in the other (*right*).

inhibition) of responses in each rat were invariant and because all rats responded to CRD with either an increase or decrease in RSNA, we classified rats into two groups according to the polarities of their responses. CRD elicited a  $25 \pm 5\%$  increase in RSNA in 11 rats and a  $16 \pm 2\%$  decrease in RSNA in 4 rats. In the former group of rats, RSNA sometimes remained elevated for 10 min after cessation of CRD.

## DISCUSSION

### *Location and ongoing activity of interneurons in the dorsal horn and intermediate zone*

In a previous study, we reported that 43% of dorsal horn interneurons at T<sub>10</sub> (but substantially fewer in nearby segments) exhibited ongoing activities strongly correlated with RSNA after acute spinal transection (Chau et al. 1997). Further, noxious somatic stimuli that increased RSNA also increased the activity of most of these neurons, whereas noxious stimuli that decreased RSNA also decreased the activity of most of these neurons. We concluded that these correlated neurons might be elements of circuits that generate both ongoing and reflex-elicited sympathetic activity after spinal transection. We now report that more ventral interneurons, in the intermediate zone, also may play a role in generating RSNA after spinal transection, for the ongoing activities of 42% (27/64) of these neurons also were correlated with either increases or, in one case, a decrease in ongoing RSNA.

Although we had recorded from 35 T<sub>10</sub> DHN in our previous study (Chau et al. 1997), we chose to record again from DHN as well as IZN in the present study for several reasons. We wanted to compare DHN and IZN in the same rats with respect to degrees of correlation with RSNA, somatic fields, somatic modalities, and responses to colorectal distension. In addition, we wanted to corroborate the results of our previous study on sympathetically correlated T<sub>10</sub> DHN, which had been the first of its kind. Using this strategy, we observed that the peaks in the averages of RSNA triggered by IZN were larger, when compared with their dummy averages, than those triggered by DHN. This observation is subject to several interpretations. The most physiologically significant (and perhaps most parsimonious)

interpretation is that the RSNA is more closely correlated to preceding activity in IZN than it is to preceding activity in DHN. Several mechanisms could mediate a stronger correlation between discharges of IZN and RSNA. All of these mechanisms are interesting and potentially important, but all of them are currently speculative. Examples of several are 1) correlated DHN play a role in sensory and somatomotor processing as well as autonomic processing. Therefore correlated DHN may receive inputs related to these other functions, “diluting” their relationship to sympathetic output. 2) Different populations of sympathetic preganglionic neurons may discharge after activity in DHN and IZN, respectively. Therefore correlation indices for IZN and DHN are being calculated for two independent pathways. Recordings from single postganglionic neurons will be required to rule out this possibility. And 3) the synaptic pathway between IZN and preganglionic neurons may be shorter or more secure than that between DHN and preganglionic neurons.

Alternatively, the apparently smaller degree of correlation between DHN activity and RSNA might only manifest differences in the discharge patterns of DHN and IZN or in our method of detecting discharges. Were this the case, correlation indices might not accurately represent relationships between the discharges of DHN or IZN and RSNA. Clearly, a definitive explanation for the difference in correlation indices of IZN and DHN will require both additional experiments and, because of the complexity of the signals, extensive simulations.

### *Responses of individual IZN and DHN to pinch and brush were related to concurrent responses in RSNA*

Stimulus evoked responses in RSNA in spinally transected rats were not stereotypic. Thresholds and magnitudes of responses varied from rat to rat and within rats during the course of experiments, perhaps due to changes in the level of anesthesia, core temperature, delivery of fluids, or other variables that could not be precisely controlled. If responses in RSNA are determined by responses of presympathetic interneurons, then we would expect that during WDR responses in RSNA, most excitatory presympathetic neurons should exhibit WDR responses and few should exhibit HT responses; during HT



responses in RSNA, most excitatory presympathetic neurons should exhibit either HT or WDR responses; and OTHER responses, such as *inhibitions* of single neurons' activity elicited by pinch of the flank should be more likely to occur in rats during similar OTHER responses in RSNA. Although relatively few sympathetically correlated neurons could be characterized completely with concurrent responses in RSNA, all met the criteria specified in the preceding text. Even though we would not have been surprised to record from, for instance, an occasional correlated interneuron with a HT response during WDR responses in RSNA, none were observed. We did observe a significant number of neurons with WDR responses during HT responses in RSNA. These responses do not, however, violate our assumptions because the relatively small responses to brushing exhibited by WDR neurons may not have been sufficient to detectably excite preganglionic neurons.

*Sympathetically correlated T<sub>10</sub> DHN and IZN are affected differentially by somatic and colorectal stimulation*

Before conducting these experiments, we hypothesized that correlated IZN received much of their excitatory input from correlated DHN in T<sub>10</sub> and nearby segments. Thus the activities of many IZN should have represented a composite of the activities of DHN. As reviewed in the following text, however, most of our data support the existence of separate sources of input to sympathetically correlated DHN and IZN and independent pathways from IZN and DHN to preganglionic neurons. That sympathetically correlated neurons receive different information or process information differently from uncorrelated neurons was indicated by the observation that uncorrelated neurons much more commonly exhibited HT and OTHER fields than did correlated neurons.

**NOXIOUS SOMATIC STIMULATION.** Differences between the inputs to DHN and IZN can be inferred from differences between these neurons' somatic receptive fields. As reported previously (Chau et al. 1997) and observed again in these experiments, the fields of a majority of DHN were relatively simple, excited by noxious stimulation of regions in and proximal to the T<sub>10</sub> dermatome and inhibited by stimulation of more distal regions, such as the elbow and hip. Neurons with identical fields must be common in the dorsal horn, for they have been observed in many previous studies in several species (see, for instance, Bolser et al. 1991; Hobbs et al. 1992; Ness and Gebhart 1991). However, the intraspinal mechanisms that are responsible for the proximal-excitatory/distal-inhibitory fields of these neurons are only now being investigated (Miller et al. 1998; Zhang et al. 1996).

Unlike DHN, correlated IZN exhibited a greater variety of fields. Some of these fields were similar to the "conventional" fields described in the preceding text. Other neurons, however, were *inhibited* (rather than excited) by pinching skin in the T<sub>10</sub> dermatome, *excited* (rather than inhibited) by pinching the elbow and hip, or uniformly excited or uniformly inhibited from the entire ipsilateral body wall. The incidence of neurons with such fields was twice as great among IZN than among DHN, suggesting a significantly different range of inputs to IZN than to DHN.

**COLORECTAL DISTENSION.** Even more convincing evidence for independent inputs to DHN and IZN came from the differential

responses of sympathetically correlated T<sub>10</sub> DHN and IZN to CRD. CRD increased levels of RSNA in a majority of rats. Were these responses mediated by correlated T<sub>10</sub> DHN? Apparently not, for most correlated DHN were *inhibited* by CRD. On the other hand, the majority of correlated IZN were excited by CRD. That excitation and inhibition were equally common among *uncorrelated* neurons in both the dorsal horn and intermediate zone manifests a degree of specificity for the inputs to correlated and uncorrelated interneurons.

Although we cannot refute the hypothesis that sympathetic responses to CRD are mediated, in part, by nonsympathetically correlated DHN, a more parsimonious explanation is that the excitability of renal sympathetic preganglionic neurons is increased by CRD via sympathetically correlated IZN. Thus multisegmental excitation of IZN, perhaps via pathways mentioned in the following text, is more likely responsible for the multisegmental excitation of sympathetic activity by CRD than is input from segmental (i.e., T<sub>10</sub>) DHN.

Importantly, CRD decreased RSNA in 4 of 15 rats. Because CRD also decreased the ongoing activity of most correlated DHN, what might be the involvement of correlated DHN in these CRD-elicited sympathetic responses? If, as shown previously for noxious somatic stimulation (Chau et al. 1997), changes in the activity in T<sub>10</sub> DHN strongly correlate with subsequent changes in RSNA, then DHN may be excitatory antecedents of sympathetic neurons. By inhibiting these DHN, CRD may decrease DHN's excitatory drive to renal sympathetic preganglionic neurons, decreasing RSNA despite the CRD-elicited increase in activity of many of IZN. Thus CRD may differentially affect RSNA via two, independent, ordinarily sympathoexcitatory pathways (DHN → renal preganglionic neurons, and IZN → renal preganglionic neurons). If this is the case, then the magnitude and even the polarity of sympathetic responses to CRD could depend on the relative responses of DHN and IZN to CRD, the degree of convergence or divergence between DHN and IZN and renal sympathetic preganglionic neurons, and the levels of activity in DHN and IZN before stimulation. For instance, if in one animal the level of RSNA before CRD were high and largely driven by correlated T<sub>10</sub> DHN, then, because CRD substantially reduced the activity of these segmental DHN, the effects of a concomitant increase in sympathetic drive via excited IZN could be reduced or even reversed. If, in another animal, the activity of correlated DHN before CRD provided very little drive for ongoing RSNA, then CRD might elicit a relatively large increase in RSNA simply by exciting IZN. Therefore we suggest that during a visceral stimulus such as CRD, the net change in peripheral sympathetic activity is a function of the excitatory *segmental* input from correlated DHN and IZN combined with the differential effects on more remote (rostral or caudal to the stimulus) correlated DHN and IZN. These multiple pathways (via IZN and DHN) and the differential effects of stimuli may account for some of the variability in the responses of RSNA observed in these experiments. We note, however, that a more complex model than that described above will need to be developed to account for the bimodal distribution of the magnitudes of RSNA responses to CRD.

What are the pathways for the segmental and propriospinal axons that elicit responses to stimuli such as CRD? As noted in the preceding text, a small number of primary colorectal afferents enter the spinal cord at T<sub>10</sub> and nearby segments (Ness and Gebhart 1988b). These afferents will definitely excite DHN and IZN at their first synapses. More caudal, at their modal

segments of entry, L<sub>1</sub> and L<sub>6</sub>-S<sub>2</sub> (Ness and Gebhart 1988b), colorectal afferents will excite many DHN, but sympathetic preganglionic neurons reside only as far caudal as L<sub>2-3</sub>. Nevertheless, excitation of sympathetically-correlated DHN and IZN at rostral lumbar levels would very likely increase sympathetic activity to the pelvis and lower extremities

Propriospinal neurons that are affected by primary afferents at their levels of entry and that project rostrally and caudally are the sources of the secondary axons that, in our experiments, probably projected rostrally to, at least, T<sub>10</sub> and nearby segments. T<sub>10</sub> is the modal segment for renal sympathetic preganglionic neurons in the rat (Schramm et al. 1993). These, too, are the projections that resulted in the inhibition of most correlated DHN and the excitation of most correlated IZN. Although the pathways by which such propriospinal afferents might ascend are largely conjectural, recent work has shown that many pelvic afferents ascend to supraspinal levels in the dorsal quadrants of the rat spinal cord (Hubscher and Johnson 1999). These axons would be appropriately located to project collaterals into either the dorsal horn or the intermediate zone.

In a sample of ~200 sympathetically correlated neurons recorded in spinally transected rats (Chau et al. 1997 and subsequent unpublished experiments), the activities of only 2 have been correlated with *decreases* in RSNA. Both of these negatively correlated neurons were located at depths consistent with the intermediate zone, but the location of neither was confirmed histologically. The significance of this paucity of negatively correlated neurons in our preparations is unclear. However, one interesting implication is that, in the anesthetized spinally transected rat, decreases in RSNA are more likely to be mediated by dysfacilitation than by active inhibition of either sympathetic preganglionic neurons or excitatory presympathetic neurons.

#### Effects of CRD on arterial pressure

Because CRD always increased systemic arterial pressure but elicited variable changes in heart rate, we believe it likely that CRD always elicited a net increase in total peripheral resistance. In this sense, the cardiovascular responses in the anesthetized rat model of "dysreflexia" are similar to those observed in chronically transected humans in whom vasoconstriction plays the predominant role in posttransection hypertensive crises (Mathias and Frankel 1992). However, because the CRD-elicited changes in RSNA were highly variable from rat to rat, we hypothesize that this stimulus elicited different patterns of excitation and inhibition of correlated IZN and DHN (and, therefore different patterns of vasoconstriction in different rats). We conducted extensive post hoc statistical examinations of our data in an attempt to test this hypothesis. For instance, we attempted to determine whether the ongoing activities of correlated DHN were higher in those rats in which CRD decreased, rather than increased, RSNA. However, our samples of neurons were substantially smaller than necessary to adequately answer such questions.

Although CRD reliably increased arterial pressure in our rats, these increases were modest and variable. We suspect that the magnitudes of these pressor responses were reduced by anesthesia, for Ness and Gebhart reported that the cardiovascular responses to CRD were substantially reduced (or even reversed) by many anesthetics, including chloralose, in intact

rats (Ness and Gebhart 1988a). The technical difficulties of recording both spinal neurons and renal sympathetic activity in the present experiments, however, required the use of anesthetized (rather than decerebrate) rats.

Interestingly, CRD did not cause previously uncorrelated IZN to become correlated nor did it significantly change the degree of correlation between interneurons and RSNA. This observation has several implications. First, it demonstrates that, after spinal transection, the degree of correlation between a neuron's activity and RSNA is not simply a function of afferent drive. Second, it warns that the processes and pathways that mediate the correlations detected by spike-triggered averages may be distinct from those that mediate the effects of transient afferent input on sympathetic activity. Additional experiments will be needed to clarify this second point.

#### Synthesis

The *average* effects of stimuli on mean arterial blood pressure in spinally injured patients are not large. Mathias et al. (1979) measured the arterial pressure responses to increased bladder pressure in six chronic tetraplegic patients. This stimulus increased mean arterial pressure from  $73 \pm 4.3$  to  $109 \pm 36.6$  mmHg. On the other hand, the magnitude of these responses was highly variable, as manifested by the large standard error for the mean arterial pressure during bladder stimulation (36.6 mmHg), and the increase in average *systolic* pressure during stimulation from 107 to 164 mmHg.

What mechanisms might account for variations in the overall circulatory effect of somatic or visceral stimuli from one experimental preparation to another or from one patient to another after spinal transection? This study has demonstrated three potential mechanisms: differential effects of some stimuli on sympathetically correlated DHN and IZN, dependence of the effects on DHN and IZN of their segmental distance from stimuli, and apparently independent convergence of DHN and IZN on sympathetic preganglionic neurons. Logically, the pre-stimulus activity of sympathetically correlated DHN and IZN at different spinal levels is another factor that could affect the magnitude and direction of responses. However, we have no evidence either to support or refute this mechanism. Interactions between some of these factors are shown in Table 2.

For example, referring to row one of Table 2, we attribute the increase in RSNA in response to noxious T<sub>10</sub> somatic stimulation to the increased activity of putative presympathetic (correlated) DHN and IZN.

TABLE 2. Summary of effects of noxious stimuli on the rates of discharge of sympathetically correlated DHN, IZN, and renal nerve fibers

Noxious Stimulus	Discharge Rate		
	DHN	IZN	RSNA
Pinch T10 dermatome	▲*	▲	▲
Pinch hip	▼	▲	▼
Colorectal distension	▼	▲	▲†

\* Arrows indicate the direction of the most common responses of the discharge rates of the neurons (columns) to the noxious stimuli (rows). † RSNA increased in 11 rats and decreased in 4 rats in response to colorectal distension (see text).

Noxious T<sub>10</sub> stimulation is “proximal” or “segmental” with respect to renal presympathetic interneurons and renal sympathetic preganglionic neurons. However, it is “distal” with respect to presympathetic interneurons and sympathetic preganglionic neurons controlling more rostral and caudal tissues and organs. Therefore based on our data, noxious somatic stimulation at T<sub>10</sub>, while exciting nearly all sympathetically correlated interneurons at T<sub>10</sub>, would be expected to *inhibit* correlated DHN and excite correlated IZN at these more distal levels. For instance in our experiments, stimulation of the hip (which is distal to T<sub>10</sub>) inhibits sympathetically correlated DHN and RSNA despite exciting correlated IZN (Table 2, row 2). A stimulus-evoked increase in RSNA could be either ameliorated or exacerbated depending on the strength of the excitation of segmental sympathetic systems, the relative inhibition and excitation of *distal* correlated DHN and IZN, the relative strength of the inputs of these differentially affected interneurons to sympathetic preganglionic neurons, and the relative prestimulus activities of interneurons at levels proximal and distal to T<sub>10</sub>. CRD, like noxious cutaneous stimulation of the hip, inhibited sympathetically correlated DHN and excited correlated IZN (Table 2, row 3). The balance of the complex response to CRD, however, appeared to vary considerably from rat to rat. In 11 rats, this balance favored an increase in net RSNA, and in 4 rats it favored a decrease in net RSNA.

Given the complex nature of the intraspinal reflexes described above and our incomplete understanding of those reflexes, neither scientists nor clinicians are able to accurately predict cardiovascular responses to a given stimulus after spinal cord transection. A future challenge for neurophysiology, then, will be to better characterize these interactions, first, to aid in designing tests that will more accurately predict responses in individual patients and, second, to aid in designing methods for ameliorating dangerous cardiovascular responses.

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Address for reprint requests: L. P. Schramm, The Johns Hopkins University School of Medicine, 720 Rutland Ave., 606 Traylor Bldg., Baltimore, MD 21205-2196.

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