

Spinal sympathetic interneurons: Their identification and roles after spinal cord injury

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Abstract: Primary afferent neurons rarely, if ever, synapse on the sympathetic preganglionic neurons that regulate the cardiovascular system, nor do sympathetic preganglionic neurons normally exhibit spontaneous activity in the absence of excitatory inputs. Therefore, after serious spinal cord injury “spinal sympathetic interneurons” provide the sole excitatory and inhibitory inputs to sympathetic preganglionic neurons. Few studies have addressed the anatomy and physiology of spinal sympathetic interneurons, to a great extent because they are difficult to identify. Therefore, this chapter begins with descriptions of both neurophysiological and neuroanatomical criteria for identifying spinal sympathetic interneurons, and it discusses the advantages and disadvantages of each. Spinal sympathetic interneurons also have been little studied because their importance in intact animals has been unknown, whereas the roles of direct projections from the brain to sympathetic preganglionic neurons are better known. This chapter presents evidence that spinal sympathetic interneurons play only a minor role in sympathetic regulation when the spinal cord is intact. However, they play an important role after spinal cord injury, both in generating ongoing activity in sympathetic nerves and in mediating segmental and intersegmental sympathetic reflexes. The spinal sympathetic interneurons that most directly influence the activity of sympathetic preganglionic neurons after spinal cord injury are located close to their associated sympathetic preganglionic neurons, and the inputs from distant segments that mediate multisegmental reflexes are relayed to sympathetic preganglionic neurons multisynaptically via spinal sympathetic interneurons. Finally, spinal sympathetic interneurons are more likely to be excited and less likely to be inhibited by both noxious and innocuous somatic stimuli after chronic spinal transection. The onset of this hyperexcitability corresponds to morphological changes in both sympathetic preganglionic neurons and primary afferents, and it may reflect the pathophysiological processes that lead to autonomic dysreflexia and the hypertensive crises that may occur with it in people after chronic spinal injury.

Introduction

Sympathetic preganglionic neurons are the final neurons within the central nervous system that regulate sympathetic output to nearly every tissue and organ. Like somatic motoneurons, sympathetic preganglionic neurons do not exhibit pace-

maker potentials, and under ordinary circumstances their activity is determined by synaptic inputs from the brain and spinal cord (see [Laskey and Polosa, 1988](#), for review). The regulation of the activity of sympathetic preganglionic neurons by brainstem systems has been extensively investigated ([Laskey and Polosa, 1988](#); [Cabot, 1996](#); [Blessing, 1997](#)). However, less is known about the regulation of sympathetic activity after spinal cord injury, when inputs from the

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brainstem are lost. Sympathetic activity after spinal cord injury is enigmatic because it ranges from abnormally low, leading to bouts of hypotension, to abnormally high, leading to hypertensive crises (Mathias and Frankel, 1992). One characteristic upon which there appears to be little disagreement is that few, if any, spinal primary afferents synapse directly upon sympathetic preganglionic neurons (Laskey and Polosa, 1988). Therefore, by definition, after a complete spinal cord transection spinal interneurons convey all spinal inputs to sympathetic preganglionic neurons, whether these inputs are derived from primary afferents or from intraspinal sources of ongoing sympathetic activity.

For the purposes of this chapter, I define spinal interneurons as all spinal neurons other than (1) somatic motoneurons and (2) autonomic preganglionic neurons. I define spinal sympathetic interneurons as spinal interneurons with connections that can directly or indirectly affect sympathetic activity. Some spinal neurons that play no role in sources of sympathetic activity in intact spinal cords may participate in those sources after spinal cord injury. These neurons may, themselves, be under tonic descending inhibition when the spinal cord is intact, or their connections to sympathetic preganglionic neurons may be via other interneurons that are tonically inhibited. Such neurons would be classified as spinal sympathetic interneurons after, but not before, spinal cord injury.

That spinal sympathetic interneurons have been little studied is understandable for two reasons. First, as discussed below, they are unique neither in their neurotransmitters nor their morphology. Thus, they are not readily identified. Second, Miller et al. (2001) found very few spinal interneurons with activities correlated with ongoing renal sympathetic nerve activity in rats with intact spinal cords. This observation suggests that spinal sympathetic interneurons play a minor role in the regulation of sympathetic activity in animals with intact spinal cords. Therefore, these interneurons have not attracted attention in studies of normal, autonomic regulation of the circulation. In recent years, however, spinal sympathetic interneurons have attracted more attention because they may play important roles in autonomic dysfunction

after spinal cord injury and because they may play positive roles in the recovery of autonomic function or pathological roles in mediating autonomic dysfunction during spinal cord repair and regeneration.

The anatomy and physiology of spinal sympathetic systems have been comprehensively reviewed (Laskey and Polosa, 1988; Cabot, 1996; Weaver and Polosa, 1997). Therefore, this chapter will concentrate on recent studies of the anatomy and physiology of spinal sympathetic interneurons in rats with intact, acutely transected, and chronically transected spinal cords. I begin by reviewing the methods used to identify and characterize spinal sympathetic interneurons.

Spinal sympathetic interneurons are identified both physiologically and anatomically

Ideally, we could identify each spinal sympathetic interneuron, whether characterized physiologically or anatomically, by tracing its axon to synapses upon sympathetic preganglionic neurons. However, this is possible under only special conditions, usually *in vitro* (see, for example, Deuchars et al., 2001). In all other cases, the “sympathetic” nature of spinal sympathetic interneurons must either be inferred from their neurophysiological properties or by tracing their connections to sympathetic preganglionic neurons using specialized, trans-synaptic, retrograde labeling methods.

Gebber and colleagues pioneered neurophysiological identification of spinal sympathetic interneurons by identifying spinal neurons with discharge patterns that were correlated with the discharge patterns in either pre- or postganglionic sympathetic axons (Gebber and McCall, 1976; Barman and Gebber, 1984). These investigators cross-correlated the ongoing activity of single spinal interneurons and the ongoing activity in sympathetic nerves. Neurons with activities either positively or negatively correlated with sympathetic nerve activity were defined as spinal sympathetic interneurons. This remains the only neurophysiological method for identifying spinal sympathetic interneurons (Chau et al., 1997, 2000; Miller et al., 2001; Tang et al., 2003).

Neurophysiological identification of spinal sympathetic interneurons has two drawbacks. First,

how does one distinguish between spinal sympathetic interneurons and sympathetic preganglionic neurons? The activities of both types of neurons could be correlated with sympathetic nerve activity. Second, how does one distinguish between spinal sympathetic interneurons and other interneurons which share inputs with sympathetic preganglionic neurons but which are not involved in sympathetic processing?

Distinguishing between spinal sympathetic interneurons and sympathetic preganglionic neurons is the easier of these problems. As shown by [Gebber and McCall \(1976\)](#), sympathetic preganglionic neurons rarely discharge at rates exceeding 20 Hz. Therefore, the minimum interspike interval for sympathetic preganglionic neurons is approximately 50 ms. The discharge patterns of spinal interneurons, on the other hand, usually include bursts of action potentials with interspike intervals of 20 ms or less. Therefore, with the relatively minor risk of misidentifying some spinal sympathetic interneurons with low discharge rates, spinal sympathetic interneurons can be distinguished from sympathetic preganglionic neurons by the presence of short interspike intervals in their discharge patterns. An additional criterion for some spinal sympathetic interneurons is their dorsal location in the spinal cord. Sympathetic preganglionic neurons are never located within spinal laminae I–V. Therefore, sympathetically correlated neurons located in the dorsal laminae of the spinal cord are very likely to be spinal sympathetic interneurons.

Figure 1 illustrates the neurophysiological identification of a putative spinal sympathetic interneuron. The ongoing activity of a spinal neuron was recorded at a depth of 300 μm from the dorsal surface of the 10th thoracic (T_{10}) spinal segment of an anesthetized rat, acutely spinally transected at the 3rd cervical segment (C_3). The neuron was identified as an interneuron both by its dorsal position and by the presence of bursts of activity with interspike intervals of 10 ms. At the temporal resolution of **Fig. 1**, these bursts are visible as darker action potential indicators (upper panel, lower trace). Ongoing renal sympathetic nerve activity (**Fig. 1**, upper panel, upper trace) was recorded simultaneously with the ongoing activity of the interneuron, and the cross-correlation between

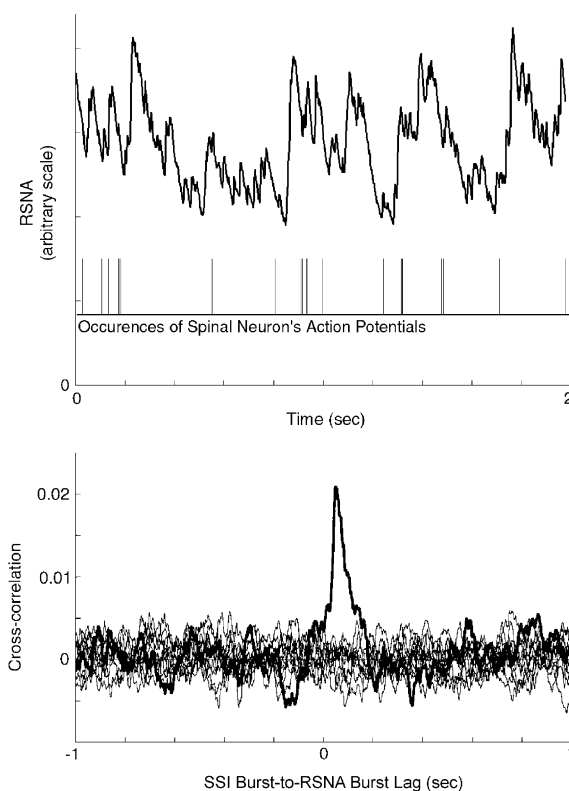


Fig. 1. Neurophysiological identification of spinal sympathetic interneurons by cross-correlation. Upper panel: renal sympathetic nerve activity (RSNA, upper trace) and simultaneously recorded occurrences of action potentials in a spinal interneuron (lower trace). Lower panel: cross-correlation between a 10 min recording of spinal neuronal action potentials and simultaneously recorded RSNA. From [Krassioukov et al. \(2002\)](#), with permission.

these activities was calculated (**Fig. 1**, lower panel, dark trace). Zero time on the correlogram was the instant at which the interneuron began a burst of activity. The sharp positive peak in the correlogram, approximately 75 ms after the onset of the burst, indicated that bursts of renal sympathetic nerve activity regularly lagged the onset of bursts of activity of the interneuron by 75 ms. To gauge the significance of the correlation, the interdischarge intervals of the interneuron's ongoing activity were shuffled 10 times to generate 10 “dummy” cross-correlations with renal sympathetic nerve activity (**Fig. 1**, lower panel, lighter traces). The positive correlation between the interneuron's actual ongoing activity and ongoing renal sympathetic

nerve activity was so much larger than the envelope of the 10 dummy correlations that the probability that this relationship could have occurred by chance was very small.

A more difficult problem than distinguishing spinal sympathetic interneurons from sympathetic preganglionic neurons is distinguishing spinal sympathetic interneurons from interneurons that are only coincidentally correlated with sympathetic nerve activity. An example of such a coincidence would be the case in which both sympathetic preganglionic neurons and interneurons were driven by a common synaptic input. Indeed, this distinction cannot be made unambiguously using neurophysiological techniques. Nevertheless, confidence in identifying spinal sympathetic interneurons is possible when (1) bursts of ongoing or evoked activity of a sympathetically correlated interneuron usually lead ongoing or evoked bursts of sympathetic nerve activity by an interval consistent with the calculated conduction time from the interneuron to the recording site on the sympathetic nerve and (2) evoked excitatory and inhibitory responses of interneurons to applied stimuli are correlated with responses in sympathetic nerve activity to the same stimuli.

Figure 1 illustrates a case in which the first of these criteria was met. The 75 ms lag between bursts of ongoing activity of the interneuron and bursts of ongoing sympathetic nerve activity represents an aggregate conduction velocity of approximately 0.5 m/s, which is consistent with the expected conduction velocity of the largely unmyelinated axons of the renal sympathetic nerve. Subsequently, this neuron also met the second criterion. Pinch of the left flank, within the region of the T₁₀ dermatome, excited both the activity of this neuron and renal sympathetic nerve activity, whereas pinch of the left hip and left shoulder caused decreases in both this neuron's activity and renal sympathetic nerve activity (data not shown).

Chau et al. (1997) found that the polarities (direction in which firing frequency changed, up or down) of somatically evoked responses of the majority of interneurons with ongoing activities positively correlated with renal sympathetic nerve activity matched the polarities of simultaneously evoked responses in renal sympathetic nerve

activity. The polarities of somatically evoked responses of uncorrelated interneurons were much less likely to match those of simultaneous responses in renal sympathetic nerve activity. Furthermore, the excitatory fields of uncorrelated neurons were significantly larger than those of correlated neurons, and they were often larger than the excitatory fields for renal sympathetic nerve activity. Excitatory fields are defined as the area of body surface from which stimulation of sensory receptors evoked excitation of the neuron.

Once identified neurophysiologically, spinal sympathetic interneurons can be anatomically located and morphologically characterized either by intracellular labeling (Deuchars et al., 2001) or by the juxtacellular labeling method (Pinault, 1996; Schreihofer and Guyenet, 1997; Tang et al., 2003). The juxtacellular method involves approaching the soma or proximal dendrites of a spinal sympathetic interneuron very closely and passing positive current pulses into it through a biocytin-filled electrode. The current apparently electroporates (generates temporary pores in) the neuron's membrane and carries biocytin into the cell. Biocytin rapidly diffuses throughout the neuron's soma and dendrites. Labeled neurons are identified and reconstructed histologically after treatment with a streptavidin-conjugated chromogen. Although this method has been used to visualize spinal sympathetic interneurons (Tang et al., 2003), it suffers from two drawbacks. First, respiratory and vascular movements of the spinal cord often prevent one from approaching interneurons closely enough to label them without injuring them. Second, although the somas and dendrites of labeled spinal sympathetic interneurons are well demonstrated by juxtacellular labeling, axons are never observed. Axons of spinal sympathetic interneurons can be demonstrated by intracellular labeling. To date, however, intracellular labeling of spinal sympathetic interneurons has been accomplished only in vitro (Deuchars et al., 2001).

Although neurophysiological studies permit functional characterization of spinal sympathetic interneurons, correlation methods, alone, cannot unequivocally identify spinal sympathetic interneurons. Spinal sympathetic interneurons can be more definitively identified by retrograde, trans-synaptic

tracing from sympathetic preganglionic neurons. In an ingenious series of experiments, Cabot and colleagues (1994) simultaneously injected the beta subunit of cholera toxin (cholera toxin B) and wheat germ agglutinin into the superior cervical ganglion of rats. Both the cholera toxin B and the wheat germ agglutinin were transported from the ganglion to the somas and dendrites of sympathetic preganglionic neurons with synapses in that ganglion. However, only the wheat germ agglutinin was transported further in the retrograde direction, across the synapses made by spinal sympathetic interneurons on sympathetic preganglionic neurons, thereby labeling the spinal sympathetic interneurons. Thus, sympathetic preganglionic neurons were identified by their combined labeling with cholera toxin B and wheat germ agglutinin. Spinal sympathetic interneurons were identified by their labeling with wheat germ agglutinin but not cholera toxin B. Although these were landmark experiments, they were hampered by faint labeling of spinal sympathetic interneurons, due in large part to restricted, retrograde, trans-synaptic transport of wheat germ agglutinin from sympathetic preganglionic neurons.

More recently, spinal sympathetic interneurons have been identified by the retrograde, trans-synaptic transport of herpes viruses (Strack et al., 1989a, b; Schramm et al., 1993; Clarke et al., 1998; Tang et al., 2004). Herpes simplex and pseudorabies virus are two herpes viruses that are rapidly taken up by the axons of sympathetic postganglionic neurons and by the axons of preganglionic neurons projecting to the adrenal medulla. Virus is transported back to the somas of these neurons where it replicates and moves trans-synaptically to the neurons' synaptic antecedents. Thus, virus taken up from a peripheral organ or tissue by sympathetic postganglionic neurons infects the sympathetic preganglionic neurons that synapse on those neurons. Virus replicates in the sympathetic preganglionic neurons, and spinal and brainstem interneurons that synapse on infected sympathetic preganglionic neurons are infected by further retrograde, trans-synaptic movement of virus. Antibodies to the viruses are used to label infected neurons. The approximate number of synapses traversed by the virus can be controlled by the interval between the

infection and the perfusion of the animal. This interval usually ranges between 3 and 6 days. For identification of spinal sympathetic interneurons, infected rats are kept for approximately 72 h before perfusion. Rats kept for this time manifest no visible symptoms of the infection.

When virus is injected into the adrenal gland, both preganglionic neurons projecting directly to adrenal chromaffin cells and postganglionic neurons projecting to both adrenal medullary and adrenal cortical blood vessels are infected. Therefore, the spinal sympathetic interneurons infected by injection of virus into the adrenal gland may belong to at least two classes of interneurons, neurons involved in overall metabolic regulation and neurons involved in the regulation of the adrenal circulation. As discussed below, the distinction between these classes of interneurons may be of limited importance because it is likely that neither play an important role in animals with intact spinal cords. After spinal cord lesions, most stimuli that activate one class of adrenal spinal sympathetic interneurons are likely to activate both.

As in the case of the cholera toxin B and wheat germ agglutinin experiments described above, viral methods also require distinguishing between sympathetic preganglionic neurons and spinal sympathetic interneurons. Sympathetic preganglionic neurons can be identified because, in addition to being immunohistochemically labeled for the virus, they also label positively for choline acetyltransferase, a synthetic enzyme for acetyl choline found in relatively few spinal neurons other than sympathetic preganglionic neurons and somatic motoneurons. Thus, spinal neurons that co-label for virus and choline acetyltransferase can be identified as sympathetic preganglionic neurons, and neurons that are infected but do not co-label for choline acetyltransferase can be identified as spinal sympathetic interneurons. Sympathetic preganglionic neurons and somatic motoneurons can be distinguished by their differential, dorsoventral locations.

An alternative method for identifying sympathetic preganglionic neurons depends on their propensity for transporting retrograde tracers from the circulation to their somas and dendrites (Fig. 2). In this method, a large quantity (8–12 mg/kg) of a conventional retrograde tracer such as Fluorogold[®]

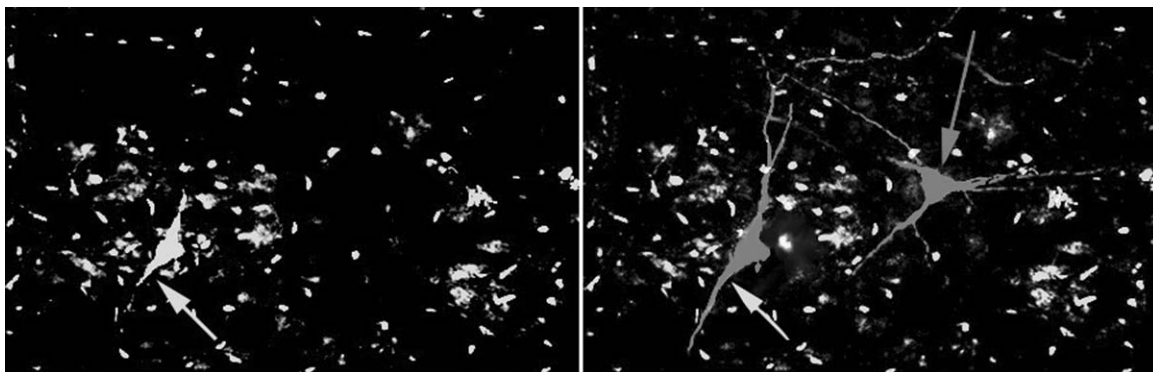


Fig. 2. Anatomical identification of interneurons using pseudorabies virus and Fluorogold[®]. Left panel: ultraviolet illumination. Sympathetic preganglionic neuron (white arrow) identified by fluorescence of intraperitoneally injected Fluorogold[®]. Right panel: under illumination for the chromogen used to identify pseudorabies virus, both the sympathetic preganglionic neuron (white arrow) and a spinal sympathetic interneuron (gray arrow) are visible as gray neurons. The spinal sympathetic interneuron is definitively identified by its absence under ultraviolet illumination. After Tang et al. (2004), with permission.

is injected either intraperitoneally or subcutaneously (Anderson and Edwards, 1994). Approximately 1 week post-injection, most peripherally projecting neurons (such as autonomic preganglionic neurons and somatic motoneurons) are labeled with the tracer and can be detected under ultraviolet illumination. Although the labeling of somatic motoneurons is highly variable by tracers administered intraperitoneally, the labeling of autonomic preganglionic neurons is more uniform. A potential drawback of this method is that freshly administered Fluorogold[®] appears to interfere with some viral tracing methods (Strack and Loewy; Schramm, unpublished data). In our hands, however, pseudorabies virus can be safely injected 1 week after treatment with this retrograde tracer.

The major drawback of the viral tracing methods is that infection by the virus may be capricious. Within a population of identically treated, virus-injected animals, some may not exhibit any infection, some may exhibit infections that appear highly specific (infecting only sympathetic preganglionic neurons and spinal sympathetic interneurons) and some may exhibit infections that destroy many neurons. A second drawback is that the number of synapses retrogradely traversed by the virus can only be estimated from the survival time. Finally, the viral infection often initiates an immune response that, itself, could alter the further transport of the virus.

Spinal interneurons play a more important role in generating sympathetic activity after spinal cord lesions in rats

Although most investigators have found that activity is reduced in sympathetic nerves of unanesthetized people (Wallin, 1986) and rats (Krassioukov and Weaver, 1995; Randall et al., 2005) after spinal cord transection, many investigators report that detectable levels of ongoing activity remain in some nerves. Therefore, spinal sympathetic interneurons must provide ongoing excitatory input to sympathetic preganglionic neurons in the absence of pathways from the brainstem sympatho-excitatory systems. Although in anesthetized, surgically prepared rats with acute spinal transections, sympathetic activity is substantially reduced in some nerves, it is maintained or even increased in others (Meckler and Weaver, 1985; Taylor and Schramm, 1987).

The observations of decreased activity in some nerves are easily explained by the decrease in supraspinal drive to some sympathetic preganglionic neurons after spinal cord injury. Maintenance — and even increases — in sympathetic activity after spinal cord injury are less easily explained. Very likely, sympathetic preganglionic neurons whose activity was either not diminished or was increased after spinal transection received little drive from brainstem circuits *before* transection. Alternatively,

brainstem sources of activity for these neurons were replaced by even more powerful intraspinal sources *after transection*. In either case, it also is likely that potentially excitatory *spinal inputs* to these sympathetic preganglionic neurons were under tonic inhibition from supraspinal systems. Thus, I propose that spinal transection abolishes descending excitation, either directly to sympathetic preganglionic neurons or indirectly to spinal sympathetic interneurons. However, it also abolishes descending inhibition of spinal systems with excitatory inputs to sympathetic preganglionic neurons. Ruggiero et al. (1997a, b) provided clear evidence that acute spinal transection releases the activities of many dorsal horn and intermediate zone neurons from inhibition. They found that acute cervical spinal cord transection in anesthetized rats and pigs significantly increased the number of neurons expressing the *c-fos* gene in many dorsal horn laminae and in lamina VII of the thoracic spinal cord.

Based on these observations, Miller et al. (2001) predicted that spinal neurons with ongoing activities correlated with renal sympathetic nerve activity would be relatively rare in rats with intact spinal cords because spinal circuits that might excite sympathetic preganglionic neurons would be under tonic, supraspinal inhibition. As noted above, this prediction was confirmed by their observation that the activities of only one-fifth as many spinal interneurons were correlated with renal sympathetic nerve activity in rats with intact spinal cords as were correlated in rats with acutely transected spinal cords.

The generation of ongoing sympathetic activity after spinal transection is localized to a restricted number of spinal segments

As described above, after acute spinal transection, activity persists in some sympathetic nerves. To what extent is this ongoing activity generated locally, and to what extent does it represent activity common to the entire spinal cord? Chau et al. (1997) searched the spinal cord from T₂ to the 2nd lumbar (L₂) segment for interneurons with activities correlated to renal sympathetic nerve activity in rats with acute spinal transections. Although the

ongoing activities of almost 50% of the interneurons recorded at T₁₀ were correlated to ongoing renal sympathetic nerve activity, the activities of only 16% of interneurons at T₈ were correlated with renal sympathetic nerve activity. The activities of no interneurons at T₂, T₁₃ or L₂ were correlated with renal sympathetic nerve activity. In unpublished studies (Chau and Schramm), this exploration extended to C₂ and L₅ without detecting additional interneurons correlated with renal sympathetic nerve activity. Because anatomical data indicate that the sympathetic preganglionic neurons that are most likely to generate renal sympathetic nerve activity lie in 8th through 12th thoracic segments (Tang et al., 2004), these data show that circuits in distant spinal segments play little role in generating ongoing renal sympathetic nerve activity. Whether a similar degree of longitudinal specificity exists for cardiac and pelvic sympathetic nerves remains to be determined.

Long propriospinal pathways affecting sympathetic activity are multisynaptic

Although distant spinal segments appear to play little or no role in generating ongoing sympathetic activity in a given segment after spinal transection, sympathetic reflexes can be evoked by stimulating afferents to distant segments (see Weaver and Polosa, 1997, for review). To what extent are the sympathetic reflexes elicited from distant segments mediated by monosynaptic projections to sympathetic preganglionic neurons? Cabot et al. (1994) noted that spinal sympathetic interneurons retrogradely labeled by injecting wheat germ agglutinin into the superior cervical ganglion exhibited “a strict segmental organization” with respect to their associated sympathetic preganglionic neurons. In other words, wheat germ agglutinin-labeled neurons (spinal sympathetic interneurons) were not found in segments that did not contain cholera toxin B-labeled neurons (sympathetic preganglionic neurons).

These observations were confirmed in the renal sympathetic system using the retrograde transport of pseudorabies virus. Tang et al. (2004) found that between caudal cervical and caudal lumbar segments, infected spinal sympathetic interneurons

were located only in segments of caudal thoracic and rostral lumbar segments, the segments in which infected sympathetic preganglionic neurons were also located. The first infected thoracic interneurons appear 68–72 h after injection of pseudorabies virus into the kidney. This delay is identical to that required to infect brainstem neurons that have known, monosynaptic projections to sympathetic preganglionic neurons. Apparently in this model, the time required for retrograde transport of pseudorabies virus from its uptake at a synapse to the soma of the next neuron is brief compared to the time necessary for enough replication to occur for the virus to be visible immunohistochemically in that newly infected neuron. Similarly, the transport time is brief compared to the time required for replication to increase the intracellular concentration of virus for retrograde infection of a neuron's synaptic antecedents. Because neurons as far rostral as the paraventricular nucleus of the hypothalamus can be infected in as little as 72 h, the absence of spinal sympathetic interneurons in caudal cervical, rostral thoracic and caudal lumbar spinal cord that lack infected sympathetic preganglionic neurons strongly suggests that long propriospinal inputs to sympathetic preganglionic neurons infected from renal injections are multisynaptic.

The majority of spinal sympathetic interneurons projecting monosynaptically to sympathetic preganglionic neurons are located either among or just dorsal to their functionally related populations of sympathetic preganglionic neurons. Not only are the longitudinal distributions of spinal sympathetic interneurons and their related sympathetic preganglionic neurons similar, but the densities of spinal sympathetic interneurons are greatest in or near the spinal laminae that contain their associated sympathetic preganglionic neurons. Thus, [Cabot et al. \(1994\)](#) localized spinal sympathetic interneurons to the sympathetic preganglionic neuron-rich lateral portion of lamina VII and the reticulated (lateral) portion of lamina V, just dorsal to the intermediolateral column. [Clarke et al. \(1998\)](#) used the retrograde transport of modified Herpes simplex virus to identify spinal sympathetic interneurons that were presynaptic to adrenal sympathetic preganglionic neurons. Infected

adrenal sympathetic preganglionic neurons were located across the entire mediolateral span of lamina VII, and spinal sympathetic interneurons were similarly distributed, usually intercalated among the sympathetic preganglionic neurons. Pseudorabies virus injected into the kidney of the rat also infected sympathetic preganglionic neurons located across the entire intermediate zone of the spinal cord between the lateral funiculus and lamina X ([Tang et al., 2004](#)). The majority of spinal sympathetic interneurons labeled in those experiments were similarly distributed.

Although most anatomically identified spinal sympathetic interneurons have been detected among, or just dorsal to, populations of sympathetic preganglionic neurons, small numbers identified after renal injections of pseudorabies virus were located (in descending order of density) in lamina IV, II, and I ([Tang et al., 2004](#)). Interestingly, spinal sympathetic interneurons identified by their positive cross-correlations with renal sympathetic nerve activity were distributed somewhat more widely than anatomically identified spinal sympathetic interneurons, for instance in the medial portions of laminae I, II, and III ([Chau et al., 2000](#); [Tang et al., 2003](#)). The wider distribution of neurophysiologically identified spinal sympathetic interneurons was not surprising. Anatomically identified spinal sympathetic interneurons were visualized using a relatively short, post-infection survival time (72 h). As discussed above, during that time, pseudorabies virus would have been unlikely to have traversed more than the two synapses between the renal sympathetic postganglionic neurons and the first spinal sympathetic interneurons presynaptic to infected sympathetic preganglionic neurons. Spinal sympathetic interneurons identified by cross-correlation, on the other hand, could have been located in spinal circuits many synapses removed from sympathetic preganglionic neurons and could, therefore, be expected to be located more remotely.

The locations of spinal sympathetic interneurons with respect to sympathetic preganglionic neurons may be more important than their locations with respect to their inputs. Histological reconstruction of spinal sympathetic interneurons ([Deuchars et al., 2001](#); [Tang et al., 2003](#)) indicated

that the dendritic trees of these neurons often extended hundreds of microns in two, and sometimes three, dimensions. Tang et al. (2004) concluded that the dendrites of some individual spinal sympathetic interneurons were so extensive that they could receive not only primary afferent inputs but inputs from a variety of descending or propriospinal pathways as well.

Spinal sympathetic interneurons in rats are more likely to be excited and less likely to be inhibited by somatic stimuli after chronic spinal cord transection

Because the severity of autonomic dysreflexia increases with time after spinal cord injury (Krassioukov and Weaver, 1995; Krassioukov et al., 2003), we have supplemented the studies of rats with acutely transected spinal cords described above with studies after chronic, T₃, spinal transection. In both chronically and acutely spinally transected rats, Krassioukov et al. (2002) identified spinal sympathetic interneurons in the T₁₀ segment by cross-correlation with renal sympathetic nerve activity. They compared the responses in activity of spinal sympathetic interneurons to somatic stimulation in those two populations. To standardize stimulation sites, the left body wall was divided into five regions, beginning at approximately the T₈ dermatome and ending at the left hip and hindlimb (Fig. 3).

Two types of stimuli were delivered to these regions, a 10-s pinch with toothed forceps (noxious) and 10 s of brushing with a cotton applicator (innocuous). Responses in the activities of spinal sympathetic interneurons and renal sympathetic nerve activity observed in acutely spinally transected rats by Krassioukov et al. (2002) corresponded closely to those reported previously in rats with acutely transected spinal cord (Chau et al., 1997, 2000). Both noxious and innocuous stimulation of somatic regions projecting to caudal thoracic spinal cord (Fig. 3, regions 1–3), increased the magnitudes of bursts in ongoing renal sympathetic nerve activity. Responses in the activities of spinal sympathetic interneurons were more variable. Nevertheless, the majority of T₁₀ spinal sympathetic interneurons were excited by stimulation of regions 1–3. Noxious and innocuous stimulation

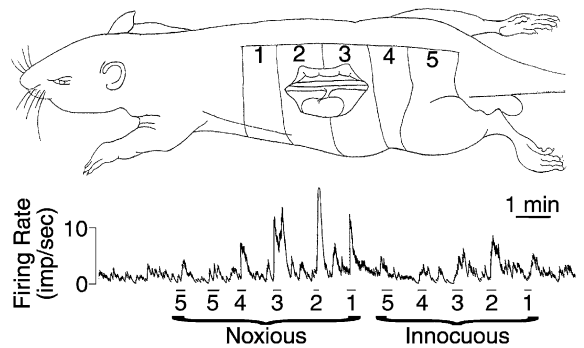


Fig. 3. Responses of spinal sympathetic interneurons to somatic stimulation 1 month after T₃ spinal cord transection. Upper panel: schematic drawing of the cutaneous regions from which responses of spinal sympathetically correlated interneurons were elicited. Lower panel: representative rate meter responses of a sympathetically correlated neuron to noxious (left) and innocuous (right) stimulation of cutaneous regions 1–5 in a rat chronically transected at T₃. From Krassioukov et al. (2002), with permission.

of somatic regions that project to caudal lumbar spinal cord (Fig. 3, regions 4 and 5) decreased ongoing renal sympathetic nerve activity. The ongoing activities of a majority of T₁₀ spinal sympathetic interneurons were inhibited by stimulation of these regions. One month after spinal cord transection, both noxious and innocuous stimulation of regions 1, 3, and 5 were significantly more likely to increase the activities of spinal sympathetic interneurons than in the acutely transected state, and innocuous stimulation of regions 1 and 5 was less likely to decrease their activities.

Although autonomic dysreflexia may occur in the acute stage of spinal cord injury (Krassioukov et al., 2003), it is far more common in the chronic stage in both humans (Mathias and Frankel, 1992) and rats (Krassioukov and Weaver, 1995). In rats, the onset of autonomic dysreflexia correlated well with morphological changes in sympathetic preganglionic neurons (Krenz and Weaver, 1998b) and with increases in sprouting of primary afferent axons (Krenz and Weaver, 1998a; Wong et al., 2000). Some of these axons appeared to synapse on neurons appropriately positioned to be spinal sympathetic interneurons (Wong et al., 2000). The electrophysiological experiments described above provided a neurophysiological correlation to both

the morphological changes in sympathetic preganglionic neurons and primary afferents exhibited in rats after spinal injury and to the increased sympathetic reactivity to somatic stimuli experienced by spinally injured patients.

Summary

Physiological data suggest that spinal sympathetic interneurons play a minimal role in generating and modulating renal sympathetic activity in rats with intact spinal cords. Similar data suggest that spinal sympathetic interneurons are released from normally occurring, descending, tonic inhibition after spinal cord injury. Spinal sympathetic interneurons then play a significant (although not a functionally adaptive) role in both generating ongoing sympathetic activity and participating in spinal sympathetic reflexes. Most spinal sympathetic interneurons are located close to their associated sympathetic preganglionic neurons, and the ongoing activity in individual sympathetic nerves appears to be generated within restricted numbers of spinal segments. Although stimulation of afferents to distant spinal segments can affect the activity of sympathetic preganglionic neurons, multisynaptic (rather than monosynaptic) pathways mediate these effects. Finally, 1 month after spinal cord transection at rostral thoracic level, spinal sympathetic interneurons at T₁₀ are more likely to be excited by both innocuous and noxious stimulation of more widespread cutaneous regions. Collectively, these observations provide anatomical and physiological substrates for both the generation of ongoing sympathetic activity and the intense, dysfunctional, sympathetic responses to both noxious and innocuous stimuli observed in humans after spinal cord injury.

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