AN ELECTROPHYSIOLOGICAL STUDY ON THE ORGANIZATION OF SPINAL SYMPATHETIC PATHWAYS

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ABSTRACT

AN ELECTROPHYSIOLOGICAL STUDY ON THE ORGANIZATION OF SPINAL SYMPATHETIC PATHWAYS

by

Robert Bradford McCall

The purpose of this investigation was to study the organization of spinal sympathetic pathways. The zona intermedia of the cat thoracic spinal cord was explored with microelectrodes in an attempt to locate interneurons within sympathoexcitatory and sympathoinhibitory pathways. Preganglionic neurons (PSNs) were identified by antidromic activation of their axons located in the cervical sympathetic nerve. Post-R wave time interval analysis was employed to determine the probability of unitary discharge with respect to the phases of the cardiac cycle.

The data indicated that non-antidromically activated units in the intermediolateral (IML) cell column whose discharges were correlated in time with the R wave were interneurons interposed between the terminals of reticulospinal fibers and PSNs. First, the positive relationship between the probability of unitary discharge and the phases of the cardiac cycle indicated that these neurons were contained within a sympathetic pathway. Second, the discharge patterns of these cells were distinctly different from those of PSNs. Sympathetic interneurons discharged spontaneously in bursts with short interspike intervals (<20 msec). In contrast, PSNs usually discharged only once during a

cardiac cycle. Third, sympathetic interneurons in the IML nucleus were activated by medullary pressor region stimulation and inhibited by stimulation of intramedullary components of the baroreceptor reflex arc. Finally, similarities in the conduction velocity from medullary sites to sympathetic interneurons and PSNs in the IML nucleus indicated that these two spinal sympathetic elements were closely adjacent and interconnected components of the same sympathoexcitatory pathway.

A number of observations indicated that bulbospinal projections of the baroreceptor reflex arc terminate on and excite interneurons located in the vicinity of the intermediomedial (IMM) nucleus of the thoracic spinal cord. First, the spontaneous discharges of 29 spinal units were interrupted during bilateral occlusion of the common carotid arteries. Second, the same neurons were activated by single shocks applied to depressor sites in the nucleus of the tractus solitarius (NTS). Third, components of the spontaneous discharges of certain neurons in the NTS and in the vicinity of IMM showed similar patterns of R wave locking. These observations indicate the existence of connections between the nucleus of baroreceptor fiber termination and interneurons in the spinal cord.

The value (11±3 msec) for the onset of inhibition of sympathoexcitatory elements in the IML nucleus evoked by medullary depressor region stimulation was close to that (8±1 msec) for the latency of activation of interneurons in the IMM by NTS stimulation. The difference (3 msec) is consistent with the possibility that spinal sympathoinhibition was mediated by the interneurons in the vicinity of the IMM nucleus. The data indicate that IMM interneurons terminate directly on sympathoexcitatory interneurons rather than on PSNs.

The convergence of rhythmically-active inputs to PSNs was also studied. A direct relationship existed between the degree of cardiac- and respiratory-related discharges of PSNs. In addition, the discharges of some PSNs exhibited both a 3 c/sec and a 10 c/sec periodicity. These observations indicate that individual PSNs serve as the final common path for the 3 c/sec, 10 c/sec, and respiratory-related sympathetic rhythm generating networks.

Finally, the level of the neuraxis responsible for the synchronization of SND into 10 c/sec slow waves was determined. Renal nerve discharge was synchronized into slow waves with periods approaching 100 msec in spinal cats. This observation indicates that the discharges of sympathetic nerve bundles are synchronized into 10 c/sec slow waves at the level of the spinal cord.

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By

Robert Bradford McCall

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Affectionately dedicated to my wife, Barb, whose presence was an inspiring force.

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INTRODUCTION

The purpose of the present investigation was to study the organization of spinal sympathetic pathways. To put this study in perspective, I feel it is necessary to describe the organization of several well defined spinal networks. This is not meant as a global review, rather the purpose is to illustrate the crucial role played by interneurons in the integration of neural activity in sensory and somatomotor pathways. In the first section of the Introduction I will discuss transmission in sensory pathways of the dorsal horn. Attention will be focused on Mendell and Wall's gate control theory of pain, since this theory has provided a great deal of impetus for much of the recent work involving dorsal horn mechanisms. In the second section, the reciprocal Ia and recurrent inhibitory pathways in the ventral horn will be discussed. Finally the discharge patterns of sympathetic nerves will be reviewed.

A. Organization of the Spinal Grey

1. Organization of Sensory Pathway in the the Dorsal Horn

a. Anatomy of the Dorsal Horn

Before discussing the electrophysiology of dorsal horn cells I will briefly review the anatomical works of Rexed (1952), Kerr (1975a,b) and others. As the dorsal root fibers approach the point of entry into the spinal cord, the large afferent fibers arrange themselves medially while the smaller fibers move to a lateral position.

The fine afferent fibers continue in their lateral position until they merge with the medial portion of the tract of Lissauer. The small fibers then bifurcate into a long ascending and a shorter descending branch. From each of these branches a large number of collaterals leave at right angles to enter and distribute in the marginal zone (lamina I) and the substantia gelatinosa (laminae II and III). The course and termination of the large primary afferents differ markedly from those of the fine fibers. The large fibers run ventrally along the medial surface of the dorsal horn and, at varying depths, turn laterally and then dorsally to approach the substantia gelatinosa from its ventral aspect. When these fibers reach the ventral border of the substantia gelatinosa, they bifurcate into ascending and descending branches. From these branches numerous brushes of terminal endings penetrate and distribute throughout the substantia gelatinosa (Kerr, 1975a,b).

The substantia gelatinosa cells constitute the vast majority of interneurons in the dorsal horn and tend to be arranged in radial sheets between the large primary afferent endings. The axons of substantia gelatinosa neurons either remain within lamina II or III, or travel in Lissauer's tract for distances up to 5 or 6 segments before re-entering the substantia gelatinosa. The substantia gelatinosa is characterized by an unusually large number of axo-axonic synapses as well axo-dendritic synapses (Dykes, 1975). The dendrites of the large marginal cells of lamina I and the medium sized cells in lamina IV project into the dorsal and ventral aspects of the substantia gelatinosa, respectively.

Laminae IV, V, and VI are characterized by the presence of large numbers of myelinated fibers between which large and medium sized neurons are interspersed. A portion of the myelinated fibers originate from collaterals of the large primary afferents which travel to the substantia gelatinosa, and at least some of these have been shown to be monosynaptically connected to the neurons in laminae IV, V, and VI (Pomeranz et al., 1968; Hillman and Wall, 1969; Wagman and Price, 1969; Handwerker et al., 1975; Kerr, 1975a). Wall (1967,1973) has extensively studied the cells in these laminae and has shown that there is a progressive neuronal convergence from the superficial to the deeper layers, so that receptive fields become progressively larger and individual cells respond to a greater diversity of primary afferent inputs. This very brief description of the anatomy of the dorsal horn is sufficient to indicate the tremendous potential for neuronal interactions throughout this region of the spinal cord.

b. Dorsal Horn Electrophysiology

Matthews (1934) and Barron and Matthews (1938) found that dorsal root volleys produced a long lasting depolarization in the intraspinal segments of both the fibers conducting impulses and those of adjacent dorsal roots. Barron and Matthews (1938) considered that the negative dorsal root potential (DRP) produced by dorsal root volleys was associated with an inhibitory process, since it was temporally and spatially related to a depression of flexor reflex pathways. Frank and Fuortes (1957) found that muscle afferent volleys depressed the size of excitatory postsynaptic potentials (EPSPs) elicited monosynaptically in alpha motoneurons by stimulation of Ia afferents. They showed that the depression of a monosynaptically

elicited EPSP was not associated with an inhibitory post-synaptic potential (IPSP). Frank and Fuortes (1957) concluded that the diminution of the EPSPs in motoneurons resulted from a decreased excitatory action of the presynaptic impulses and termed this phenomenon presynaptic inhibition. However, Frank (1959) offered an alternative explanation to these data which attributed the depressed EPSP to an inhibitory action of the afferent volley on the most distal dendrites of a motoneuron. In this case, no trace of an IPSP would be detected in the soma of the motoneuron (Eccles, 1964).

Extensive investigations by Eccles and his co-workers conclusively demonstrated the existence of presynaptic inhibitory mechanisms. Eccles et al. (1961) found that monosynaptically elicited EPSPs in motoneurons were depressed by impulses in Ia afferent fibers. The time course of this depression was similar to the inhibition of monosynaptic reflex discharges in the ventral roots elicited by stimulation of la afferents (Eccles et al., 1962). Furthermore, by recording the intracellular potentials of afferent fibers in the cord dorsum, it was determined that Ia afferent volleys produced a depolarization in these fibers. The time course of this primary afferent depolarization (PAD) was in good agreement with that of the depression of the monosynaptically evoked EPSPs in motoneurons (Eccles et al., 1962). The excitability of primary afferent fibers during a volley of afferent impulses was tested by applying brief pulses of current to the cord dorsum and recording the antidromic activation of afferent nerve bundles. It was determined that the time course of enhanced excitability in the afferent nerve bundle following a single afferent volley was similar to that of the PAD and the depression of monosynaptically evoked EPSPs in motoneurons (Eccles et al., 1962). These results

indicated that the depression of monosynaptically elicited EPSPs in motoneurons produced by an afferent volley resulted from the depolarization of afferent fibers near their terminals. Depolarization of the primary afferent is presumed to decrease the release of transmitter when the terminal is invaded by an action potential (Wall, 1958; Eccles, 1964).

Eccles et al. (1963) recognized that presynaptic inhibitory mechanisms exert a powerful action on all types of large afferents entering the spinal cord. They showed that the terminals of muscle spindle and tendon organ afferents were depolarized by stimulation of other muscle afferents. Large cutaneous afferents were depolarized by cutaneous and muscle afferent volleys. In general the time course of depolarization was similar in all types of afferent interactions. The central delay of afferent depolarization varied from 2-4 msec. This observation led Eccles (1964) to suggest that the central delay of presynaptic inhibition is a result of transmission through an interneuronal chain.

Wall (1962) attempted to define the area of the cord in which the interneuronal chain responsible for the origin of the DRP was located. He mapped the extracellular potential changes in intact and de-afferentated spinal segments produced by dorsal root volleys. A current source-sink distribution in the cord was then calculated. This technique locates the presence of sinks of current and indicates the location of active cells. Wall (1962) found that the time course of the distribution of sinks in the substantia gelatinosa following afferent volleys was equivalent to that of the DRP. Furthermore, discrete lesions in Lissauer's tract reduced or eliminated the DRP.

Wall concluded that following an afferent volley the substantia gelatinosa generates within itself prolonged activity which spreads to other neurons in the substantia gelatinosa via Lissauer's tract. The activity of these cells then reflect back onto the terminals of afferent fibers to produce the DRP.

Mendell and Wall (1964) examined the possibility that small diameter cutaneous afferent fibers might affect presynaptic control mechanisms. These investigators found that stimulation of myelinated and unmyelinated cutaneous afferents elicited a DRP which was characterized by the usual prolonged negative wave as well as a later positive wave which had two components. Using anodal polarization to selectively block large fibers it was demonstrated that A delta and C fibers were responsible for the two components of the positive DRP. The positive DRP was blocked by barbiturate anesthesia. Mendell and Wall (1964) also showed that a decrease in excitability of A fiber afferent terminals was associated with the positive DRP. Furthermore, the ventral root reflex elicited by A fibers was potentiated by a C fiber volley. C fiber volleys alone produced no observable ventral root reflex. These results indicated that cutaneous A delta and C fibers hyperpolarize afferent terminals and cause a facilitation of reflex transmission in the cord. Mendell and Wall (1964) suggested that the hyperpolarization of afferent terminals following A delta and C fiber activation is produced by inhibition of neurons which tonically depolarize the afferent fibers.

The difference in the action of large and small diameter afferent fibers within the spinal cord led Melzack and Wall (1965)

to postulate the existence of a gate-control mechanism of pain perception in the dorsal horn of the cord. According to this hypothesis, large diameter A alpha fiber impulses have a brief excitatory effect on neurons transmitting impulses to supraspinal levels (T cells), but then "close the synaptic gate" by presynaptcially inhibiting transmission from A alpha, A delta, and C fibers. This action is mediated by small interneurons in the substantia gelatinosa (Wall, 1962). The small diameter A delta and C fibers however block the activation of those interneurons in the substantia gelatinosa which normally close the gate (Mendell and Wall, 1964). Thus, the A delta and C fibers, which are essential for pain, "open the synaptic gate" and increase excitatory input to the T cells. When the output of the T cells reaches a critical level the neural areas that underlie pain perception are activated. Melzack and Wall (1965) further proposed that the entire gating mechanism is modulated by impulses descending from supraspinal levels.

When Melzack and Wall (1965) proposed the gate control theory of pain the existence of T cells was hypothetical. More recent studies by Wall and co-workers, as well as others, suggest that neurons with the postulated properties of T cells exist in lamina V of the dorsal horn. Pomeranz et al. (1968) found a population of lamina V neurons which were excited by electrical stimulation of small diameter afferents from viscera, muscle, and skin. Central delays indicated that these cells were activated through mono- and polysynaptic pathways. Price and Browe (1975) also found convergence of different afferents onto lamina V cells. Neurons responded to electrical stimulation of A alpha and C cutaneous afferents as well as

noxious temperature stimuli (activation of C fiber thermal nociceptors). Furthermore, graded intensities of mechanical stimuli (i.e., touch, pressure, pinch) resulted in a progressive increase in the frequency of discharge of these units. Albe-Fessard and Fessard (1975) described a similar convergence of noxious and non-noxious inputs onto lamina V neurons.

Hillman and Wall (1969) provided additional evidence to support the existence of T cells in lamina V. They found that the frequency of discharge of lamina V neurons increased when afferent volleys contained a large proportion of impulses in small fibers. In contrast, when the afferent volleys contained a high proportion of impulses in larger low threshold afferents, the discharges of lamina V neurons were inhibited. Finally it was demonstrated that cold block of the spinal cord increased the firing rate of lamina V units and suppressed all the evoked inhibitory responses.

Handwerker et al. (1975) found neurons in lamina IV which also had some of the characteristics of the T cells. They showed that lamina IV neurons were excited by electrical stimulation of A and C cutaneous fibers, as well as noxious temperature stimuli applied to the animal's foot pad. Activity evoked by noxious thermal stimuli was suppressed by electrical stimulation of low threshold fibers in the medial and lateral plantar nerves. Finally, it was found that the background activity of these cells was under the influence of a strong inhibitory influence arising from supraspinal levels.

The gate control theory of Melzack and Wall (1965) has generated much controversy and, as a result, is responsible for a renewed interest in neural mechanisms in the dorsal horn (Schmidt, 1972;

Nathan, 1976). In Wall's own words, "the least, and perhaps the best, that can be said for the 1965 paper is that it provoked discussion and experiment" (Wall, 1973). The theory has been most often attacked concerning the existence of a positive DRP (Mendell and Wall, 1964). Zimmerman (1968) and Jänig and Zimmerman (1971) failed to find any evidence for hyperpolarization of afferent nerve terminals. Using anodal current to preferentially block large afferent fibers, Zimmerman (1968) found that cutaneous C fiber stimulation evoked a negative rather than a positive DRP. Similar results were reported by Franz and Iggo (1968). Jänig and Zimmerman (1971) found that C fiber input produced PAD in cutaneous and articular afferents. In addition, the PAD produced by a C fiber volley was occluded by an A fiber volley. It was concluded that the pathways involved in the generation of PAD produced by A and C fiber volleys exhibited convergence. It should be noted that in attempting to block A fiber volleys Zimmerman (1968) and Jänig and Zimmerman (1971) placed the cathode of the blocking electrode closest to the cord. Dawson et al. (1970) have demonstrated that this method of block actually generates A fiber volleys at the cathode pole of the electrode. Thus, a negative DRP produced by insidious A fiber volleys may have obscured a positive DRP.

Gregor and Zimmerman (1972) examined the effect of cutaneous A and C fiber volleys on dorsal horn neurons. Over half of the units which were excited by stimulation of A fibers were also activated by C fibers. In agreement with the gate control theory they found the responses to C fiber volleys were partially or totally suppressed by a preceding discharge of the neuron in response to an A fiber volley. However, C fiber volleys gave rise to presynaptic

depolarization in a large fraction of myelinated fibers. It should be noted that the lack of evidence for presynaptic facilitatory effects is not surprising since these experiments were performed in barbiturate anesthetized animals (see Mendell and Wall, 1964).

Wall and his associates have re-examined the question of the existence of the positive DRP. Dawson et al. (1970) repeated the experiments of Mendell and Wall (1964) and obtained similar results. Mendell (1970) eliminated the need to block afferent nerve volleys by stimulating muscle rather than cutaneous afferents. In this regard volleys in large myelinated muscle afferents produce a smaller, briefer negative DRP than that in cutaneous nerves (Eccles et al., 1964). Using this preparation Mendell clearly observed positive DRPs following stimulation of A delta and C fibers.

Hodge (1972) recorded the intracellular responses of large diameter afferent fiber terminals elicited by stimulation of both large and small diameter afferent nerves. Three types of responses were found. Some afferent fibers displayed neither a PAD or a PAH in response to any afferent volley. Other fibers exhibited a PAD following stimulation of large diameter afferents but were unaffected by a C afferent volley. Finally, fibers were found which responded with a PAD following a large diameter afferent volley and exhibited a PAH in response to A delta and C fiber stimulation. The time course of the PAH corresponded to that of the positive DRP. In addition the PAH was associated with a facilitation of the monosynaptic mass discharge in the spinocervical tract elicited by stimulation of the superficial peritoneal nerve. Apart from providing evidence for the existence of presynaptic facilitation, this study demonstrated the lack of presynaptic interactions among some afferents.

The experiments described above have demonstrated that presynaptic facilitatory and inhibitory mechanisms have an important modulating effect on afferent impulses arriving in the spinal cord. However, the role of presynaptic mechanisms in pain perception has not been demonstrated. In this regard the study of Burke et al. (1971) is particularly relevant. They elicited DRPs using noxious thermal stimuli applied to the foot pads of spinal cats. Foot pad heat pulses consistently evoked negative DRPs in preparations in which positive DRPs could be elicited by electrical stimulation of both cutaneous and muscle afferent nerves. In addition, it was demonstrated that an increase in excitability of both large cutaneous and group Ib muscle afferent terminals was associated with the negative DRP. Similar results were observed when conduction in large myelinated afferents was blocked by cooling the nerve. These observations indicated that the noxious thermal stimulus was associated with depolarization of large diameter afferent fibers. These results are the opposite of those predicted by the gate control theory. That is, painful stimuli should produce hyperpolarization of large diameter afferent fibers according to this theory.

A tenet of the gate control theory is the supposed antagonistic relationship between the effects of input in the large and small diameter afferent fibers. Therefore, the relationship between these fibers has been extensively studied. Wagman and Price (1969) and Price and Wagman (1973) recorded the discharges of lamina IV and V neurons and the DRPs elicited simultaneously by stimulation of cutaneous A and C fiber afferents. In agreement with others

(Pomeranz et al., 1968; Hillman and Wall, 1969; Handwerker et al., 1975), Wagman and Price (1969) found that cutaneous A and C fibers converged on lamina IV and V neurons. Stimulation of large diameter A fibers evoked a short latency burst of spikes in these neurons which was followed by a period of depressed firing and a final phase of prolonged discharge. C fiber stimulation elicited a similar response although the onset of activation was longer. The DRPs evoked by A and C fiber volleys were diphasic. That is, a negative DRP was followed by a positive DRP. In both cases the negative and positive DRPs were temporally related to the depressed and prolonged discharge phases of lamina IV and V neurons, respectively. This suggested that presynaptic mechanisms are at least partially responsible for both the inhibitory and prolonged excitatory responses in dorsal horn neurons. In addition, the study indicated that both large and small diameter afferent fibers have qualitatively similar central effects.

The same conclusion must be drawn from the work of Mendell (1972,1973). He studied the polarization of presynaptic terminals of afferent fibers by recording DRPs, intracellular changes in polarization of single preterminal axons, and changes in excitability of populations of preterminal axons elicited by afferent volleys. Presynaptic hyperpolarization (positive DRP-PAH) was evoked by stimulation of muscle group III fibers and cutaneous A beta, A delta and C afferents. Mendell demonstrated that volleys in these afferents could also produce presynaptic depolarization (negative DRP-PAD). The type of response of individual fibers was dependent on the frequency of afferent stimulation. PAH was observed during low frequency stimulation while PAD was elicited by high frequency

stimulation. Thus, both large and small diameter fiber volleys elicited PAD and PAH. Following spinal transection the amplitude of the positive and negative DRPs increased. In addition, Mendell determined that the amplitude of a test negative DRP was diminished during a conditioning positive DRP. Furthermore, picrotoxin, which has been shown to block the negative DRP (Eccles, 1964), was found to also block the positive DRP. These observations support the contention of Mendell and Wall (1964) that the positive DRP results from inhibition of those tonically active interneurons which generate the negative DRP.

Rudomin and co-workers (1974) measured changes in the excitability of 1a afferent terminals following cutaneous afferent volleys. Observations using graded intensities of stimulation indicated that hyperpolarization of afferent fiber terminals was produced by the largest cutaneous fibers. Similar results were obtained by Hodge (1972). The monosynaptic Ia evoked EPSPs in motoneurons were enhanced during the time course of the hyperpolarization of afferent nerve terminals. Thus, the PAH generated by cutaneous nerve volleys was not restricted to A delta and C fibers as suggested by Mendell and Wall (1964). Furthermore, these data indicate that presynaptic facilitation mechanisms may not be specifically related to pain perception as proposed by Melzack and Wall (1965).

The studies described above indicate that an antagonistic relationship between the effects of large and small diameter afferent fibers does not always exist. This conclusion casts serious doubts concerning the validity of the gate control theory. However, it is important to realize that the concepts upon which the theory was

based do exist. Thus, presynaptic mechanisms have been shown to markedly affect afferent impulses which enter the spinal cord. In addition, descending pathways from supraspinal levels have a modulating effect on presynaptic mechanisms and thus are capable of altering afferent inputs presynaptically.

The relationship between visceral and somatic inputs in the dorsal horn has been investigated by Selzer and Spencer (1969a,b). They found that many lamina V neurons were activated by stimulation of both visceral afferents in the abdominal sympathetic chain and cutaneous fibers in the femoral nerve. Similar patterns of convergence could be recorded from fibers in the ventrolateral and ventral columns of the cord. It was suggested that the convergence of visceral and cutaneous fibers onto lamina V interneurons might provide a possible basis for visceral pain referral. Selzer and Spencer (1969b) found that conditioning stimuli applied to either visceral or cutaneous afferents inhibited the excitatory actions of the other nerve on lamina V neurons. The duration of the reciprocal depression was greater than 100 msec. By recording the DRP in visceral or cutaneous nerves generated by an afferent volley in the other nerve, reciprocal primary afferent terminal depolarizing actions between visceral and cutaneous afferent was demonstrated. In addition, an increased excitability of visceral or cutaneous afferent terminals was found following a conditioning volley in the other nerve. These observations indicated that the mutual inhibitory effect of visceral and cutaneous afferents resulted from presynaptic interactions. Similar observations were made by Hancock et al. (1970).

Presynaptic inhibition and facilitation has been discussed at length as it relates to the gate control theory of pain. Studies described above indicate that although presynaptic mechanisms are probably involved in pain perception, presynaptic interactions are much more complicated than the schema forcasted originally by the gate control theory. Therefore, it is of interest to briefly discuss the views of investigators regarding the functional significance of presynaptic mechanisms. One of the most remarkable features of segmental presynaptic inhibition is its ability to function as a negative feedback on the inflow of sensory inputs to the spinal cord. Muscle and cutaneous afferents have been shown to preferentially activate those reflex paths leading to the afferent terminals of their own sensory modality (Eccles, 1963; Schmidt, 1973). The advantages of this type of negative feedback system include both a central input adjustment to the stimulus intensity level, and the automatic suppression of trivial inputs (Schmidt, 1973). Mendell (1972) noted that presynaptic facilitation can be evoked by activity in group III muscle fibers and in A beta, A delta, and C cutaneous afferents. The common feature of these afferents is that their stimulation evokes the flexor reflex. Thus, Mendell (1972) suggested that presynaptic facilitation may play a role in enhancing proprioceptive inputs. Presynaptic inhibition has been suggested as a mechanism of surround inhibition. Schmidt et al. (1967) found that mechanical stimuli exerted their greatest depolarizing influence onto the afferents of those receptors which were nearest to the point of stimulation, and the PAD decreased when the conditioning stimulus was moved away from the receptive field of the fiber under study. This surround arrangement of presynaptic

inhibition may play a role in the spatial contrast and localization of a stimulation pattern (Schmidt, 1972). As described above (Pomeranz et al., 1968; Mendell, 1972; Handwerker et al., 1975), supraspinal areas tonically affect presynaptic mechanisms in the spinal cord. This may provide a mechanism for "focussing attention" on important sensory inputs. Thus, the sensitivity of the cord to incoming sensory information can be increased or decreased through PAH and PAD, respectively.

2. Organization of the Ventral Horn

a. Reciprocal Ia Inhibitory Pathway

Lloyd (1941,1946) established that electrical stimulation of large muscle afferents (group Ia from the annulospiral endings) evoked not only monosynaptic excitation of motoneurons innervating the same and synergistic muscles, but also short latency reciprocal inhibition of motoneurons which innervate muscles acting as antagonists at the same joint. Lloyd (1943) demonstrated that the excitatory two neuron reflex pathway formed the neural basis for the stretch reflex described by Liddell and Sherrington (1924). Lloyd (1946) found that the inhibition and excitation of motoneurons produced by Ia afferent volleys had nearly identical central latencies. It was concluded that the reciprocal Ia inhibitory pathway was monosynaptic.

Lloyd determined the central latency of inhibition of motoneurons indirectly by relating the interval between the Ia excitatory and inhibitory volleys entering the cord to the amount of inhibitory action on the monosynaptic reflex discharge. Eccles et al. (1956) recorded the intracellular potentials of motoneurons in order to more accurately determine the central delay in the reciprocal Ia

inhibitory pathway. It was found that the latency of IPSPs evoked by Ia afferent volleys was almost a millisecond longer than the latency of monosynaptically-evoked EPSP. Furthermore it was shown that this delay could not result from differences in the longitudinal condition time of inhibitory volleys in the cord or delay time in the synaptic mechanisms concerned in producing IPSPs and EPSPs. On the basis of central delays it was concluded that an interneuron must be interposed in the Ia inhibitory pathway. Additional evidence for a Ia inhibitory interneuron was provided by Eccles and Lundberg (1958). They demonstrated that summation of several simultaneous Ia afferent impulses was necessary to produce an IPSP in motoneurons. Finally, Eccles et al. (1960) found that Ia impulses excited interneurons in the intermediate nucleus of the cord. Ia afferent volleys evoked a high frequency response in these interneurons with an onset of activation which was 0.5 msec less than the onset of IPSPs recorded from motoneurons. These experiments all indicate the existence of an interneuron interposed within the Ia inhibitory pathway.

As reviewed by Lundberg (1969) and Jankowska (1975), great caution should be exercised in ascribing interneurons to a specific functional pathway. Thus, the fact that interneurons in the intermediate nucleus can be activated by Ia volleys does not necessarily indicate that they mediate transmission through the reciprocal Ia inhibitory pathway to motoneurons. A second approach used to characterize interneurons in the Ia inhibitory pathway is to define the pattern of convergence of fiber systems onto these interneurons. An indirect method to reveal convergence at the interneuronal level is to record the intracellular potential changes in motoneurons following

test and condition stimuli applied to Ia afferents and other fiber systems, respectively (see Jankowska, 1975). Using this method it has been shown that cutaneous, Ib, and group III muscle afferents as well as corticospinal, rubrospinal, and vestibulospinal fibers converge onto Ia inhibitory interneurons to facilitate transmission in this pathway, while flexor reflex afferents depress or facilitate the reflex at the level of the interneuron (Lundberg and Voorhoeve, 1962; Hongo et al., 1965; Lundberg, 1969; Jankowska, 1975). Hongo et al. (1965) determined the pattern of convergence directly by recording the intracellular potentials of interneurons in the intermediate nucleus. They found a large population of interneurons which were monosynaptically excited by stimulation of Ia muscle afferents. EPSPs could also be elicited in these interneurons by stimulation of cutaneous, Ib, and group III muscle afferents. Furthermore, stimulation of flexor reflex afferents evoked either EPSPs or IPSPs in these interneurons. Finally, stimulation of the corticospinal and rubrospinal tracts have been shown to evoke EPSPs in intermediate interneurons excited by Ia afferent volleys (Lundberg, 1969).

More recently Hultborn et al. (1971a) studied the effects of impulses in recurrent motor axon collaterals on reflex transmission in the Ia inhibitory pathway. They showed that IPSPs in motoneurons evoked by volleys in Ia muscle afferents could be effectively decreased when preceded by an antidromic stimulation of the ventral roots. The depression of the IPSPs by antidromic volleys was not associated with conductance changes in the motoneurons or depolarization of Ia afferent terminals. It was concluded that the effect on the Ia elicited IPSPs was due to post-synaptic inhibition of the Ia

inhibitory interneurons, evoked through α-motor axon collaterals and Renshaw cells (see following section). Hultborn et al. (1971b) determined changes in the intracellular potential of interneurons following stimulation of the ventral roots. Stimulation of motor axon collaterals elicited IPSPs in some interneurons monosynaptically excited by Ia muscle afferents. The latency of the IPSPs (1.2-2.0 msec) suggested the inhibitory effect was transmitted in a disynaptic pathway via Renshaw cells. The interneurons which exhibited convergence of monosynaptic Ia excitatory influences and motor axon collateral inhibitory influences were found in the ventral horn dorsomedial to motor nuclei. EPSPs produced by stimulation of cutaneous, Ib and group III muscle afferents, and fibers from the corticospinal, rubrospinal, and vestibulospinal tracts could be evoked in these cells (also see Jankowska, 1975). IPSPs could occasionally be evoked from flexor reflex afferents. In contrast interneurons in the intermediate nucleus which were monosynaptically excited by stimulation of Ia muscle afferents were unaffected by ventral root volleys. These findings cast doubt on the original hypothesis that inhibition from Ia afferents to motoneurons is mediated by Ia activated interneurons in the intermediate nucleus (Eccles, 1964). Rather, it was concluded that more ventral Ia activated interneurons mediate the reciprocal inhibition to motoneurons.

This contention was conclusively proven by the elegant experiments of Jankowska and Roberts (1972a,b). Jankowska and Roberts (1972a) attempted to show that the interneurons studied by Hultborn et al. (1971a,b) were interposed between the terminals of Ia muscle afferents and motoneurons. Jankowska and Roberts found that these

interneurons could be activated antidromically by microelectrode stimulation of motor nuclei. The location of the axons and the extent of their branching were reconstructed by comparing the latencies of the responses and the thresholds (0.1-5 μA) for antidromic activation of single interneurons from electrode positions in a number of different tracts. It was found that most interneurons excited from group Ia afferents in the quadriceps could be antidromically activated from a number of sites in the antagonistic motor nucleus (biceps-semitendinosus). Interneurons excited from Ia afferents in the biceps-semitendinosus nerve were antidromically activated from the motor nucleus of the quadriceps. In addition axons were shown to travel in the ventral or lateral funiculi before entering the motor nuclei. These data suggested that interneurons located dorsomedial to the motor nuclei were responsible for reciprocal Ia inhibition of motoneurons. Jankowska and Roberts (1972b) provided additional evidence in support of this hypothesis. They recorded PSPs in motoneurons following glutamate-induced spike activity of the interneurons described by Hultborn et al. (1971b). Cross-correlation analysis revealed that the discharges of Ia excited interneurons located in the ventral horn were followed by monosynaptic IPSPs in motoneurons. The IPSPs followed the spike potentials of the interneurons with the time delay expected for the interneurons mediating the reciprocal inhibition. These data indicate that the inhibition of motoneurons evoked from group Ia afferents in antagonist muscles is mediated by interneurons located in the ventral horn rather than in the intermediate nucleus. Furthermore, these investigators have estimated that a single interneuron monosynaptically excited by afferents in the quadriceps may synapse on

as many as 20% of the total population of antagonistic biceps-semitendinosus motoneurons. Thus, there appears to be a tremendous amount of divergence of Ia inhibitory interneurons.

The physiological identification of Ia inhibitory interneurons (Hultborn et al., 1971b; Jankowska and Roberts, 1972a,b) allowed Jankowska and Lindström (1972) and Jankowska (1975) to study the morphology of these interneurons using the technique of intracellular staining with Procion Yellow. The somas of the stained cells were found in lamina VII, just dorsal or dorsomedial to the motor nuclei. Their size was approximately 30x20 μm. The cells had four or five slender branching dendrites, and the total extension of their dendritic trees was about 600 μm dorsoventrally, 400 μm mediolaterally, and 300 μm rostrocaudally. Their axons were myelinated with an external diameter of about 6-14 μm and projected to either the ipsilateral ventral or lateral funiculi. Unfortunately, the axonal staining was not sufficient to reconstruct the course of these axons within the funiculi.

Hultborn et al. (1976a,b,c) re-examined the convergence of inputs onto interneurons mediating the reciprocal Ia inhibition of motoneurons. They found that the Ia inhibitory interneurons exhibited disynaptic IPSPs following Ia afferent stimulation. Furthermore, the pattern of inhibition of motoneurons and Ia inhibitory interneurons evoked by Ia afferent volleys displayed striking similarities. It was concluded that Ia inhibitory interneurons monosynaptically connected to antagonistic muscles mutually inhibit one another.

The studies cited above reveal an extensive convergence of fibers from segmental and descending sources onto interneurons

which mediate Ia inhibition of motoneurons. Lundberg (1971) has suggested that supraspinal centers receive feedback information from Ia inhibitory interneurons in order to achieve an accurate control of movement and has postulated that the ventral spinocerebellar tract (VSCT) may conduct this feedback information. Evidence to support this contention has been presented by Gustafsson and Lindström (1973). These investigators compared the pattern of synaptic convergence onto Ia inhibitory interneurons, VSCT neurons, and motoneurons. It was found that stimulation of Ia muscle afferents evoked a disynaptic IPSP in VSCT neurons and motoneurons. Furthermore, these disynaptic IPSPs could be depressed by stimulation of motor axons. The depression of the disynaptic IPSPs occurred without direct recurrent inhibitory effects on the VSCT neurons. The segmental latency and time course of the depression of disynaptic IPSPs recorded in VSCT neurons and motoneurons were similar. This suggested that the depression was due to postsynaptic inhibition of Ia inhibitory interneurons, mediated via the Renshaw cells. In addition, it was shown that disynaptic IPSPs in VSCT neurons evoked by stimulation of the vestibulospinal tract or flexor reflex afferents also were depressed by ventral root volleys. These IPSPs were found only in those VSCT cells which exhibited disynaptic IPSPs following Ia muscles afferent stimulation. On the basis of the above data it was concluded that the IPSPs in VSCT neurons are evoked through collateral connections from interneurons which mediate Ia reciprocal inhibition to motoneurons. Thus, it appears that the VSCT conveys information about the complex integration of activity in the Ia inhibitory pathway to supraspinal structures.

b. Recurrent Inhibitory Pathway

Recurrent collaterals from axons of motoneurons were identified as early as 1890 by Camillo Golgi (cf. Scheibel and Scheibel, 1971). However, the physiological significance of this finding was not realized until Renshaw (1941) showed that stimulation of motor axons was associated with a central inhibitory process. Renshaw found that antidromic stimulation of motoneuron axons resulted in an inhibition of dorsal root evoked reflex activity in motoneurons supplying the same muscles and their synergists. Inhibition was maximum when the conditioning stimulus occurred 2-4 msec before the test dorsal root volley, and was still observed when the condition test interval was as large as 50 msec. Facilitation of the test reflex was occasionally observed in cases in which volleys in extensor motor axons were used to condition reflexes in flexor motorneurons.

The early studies of Renshaw (1941) were extended by Renshaw (1946) and Eccles et al. (1954). These investigators surveyed the ventral horn for neurons which could be orthodromically activated by stimulation of the axons of motoneurons. It was found that a single stimulus applied to a set of motor axons caused a repetitive discharge in some ventral horn interneurons. The burst discharge lasted about 30-50 msec in anesthetized preparations and was characterized by an initial high frequency component (up to 1500 impulses/sec for the first spikes). Eccles named these cells after Renshaw following his tragic death. Renshaw (1946) and Eccles et al. (1954) determined that the earliest central latency of the first spike was 0.5-0.7 msec, which suggested the existence of a single synapse between the collaterals of motor axons and Renshaw cells. It was

found that gradation of the size of the antidromic volley caused a decrease in the latency of the initial spike as well as an increase in the initial burst frequency. In addition a stimulus which activated the gamma as well as the alpha motor axons had no greater effect upon Renshaw cell discharge than a stimulus which fired only the alpha motor axons. Furthermore, a single Renshaw cell was activated by stimulation of motor axons in as many as 7 different peripheral nerves (Eccles et al., 1961). On the basis of the distribution of potential during the synchronous discharge of a large population of Renshaw cells, Eccles et al. (1954) suggested that these neurons were located in the ventral portion of lamina VII. More recent studies in which the positions of recording sites adjacent to individual Renshaw cells have been histologically verified confirms the fact that these cells are located in the ventral portion of lamina VII (Willis, 1969; Jankowska and Lindström, 1971).

Intracellular recordings have revealed that the EPSPs of Renshaw cells consist initially of a large potential which then slowly decreases over a time period of about 60 msec (Eccles et al., 1961). The spike-like portion of the EPSP was associated with the high frequency discharge of the first spikes in the burst discharge, while the slow component was related to the later part of the burst which diminished gradually in frequency. In addition a graded EPSP has been observed in Renshaw cells following motor axon volleys of varying intensity.

Eccles et al. (1954) determined that the IPSP in a motoneuron elicited by motor axon volleys had precisely the latency and time course that would be predicted if it were generated by

discharges of Renshaw cells. They found that the central delay of these IPSPs was as brief as 1.1 msec and occurred 0.5 msec after the initiation of the earliest spike in a Renshaw cell. The IPSPs recorded from motoneurons had a prolonged time course which was similar to the duration of the repetitive Renshaw cell discharge. The duration of the initial burst discharge of Renshaw cells elicited by motor axon volleys was greatly reduced by administration of dihydro-β-erythroidine and prolonged by eserine. Dihydro-β-erythroidine was found to decrease and shorten recurrent IPSPs recorded in motoneurons following motor axon volleys, while eserine increased and prolonged these IPSPs. These facts led Eccles and co-workers to conclude that the inhibition of motoneurons following a motor axon volley is mediated exclusively through Renshaw cells. In agreement with Renshaw (1941,1946), these investigators also found that the greatest recurrent inhibitory effects tend to be between motoneurons located ipsilaterally in the same spinal segment.

From the work of Renshaw, Eccles, and others the view evolved that Renshaw cells were most likely Golgi type II neurons with axons confined to the grey matter of the spinal cord. However, the anatomical studies of Scheibel and Scheibel (1969,1971) do not support the existence of such neurons in the ventral portion of lamina VII. From an extensive investigation of Golgi stained spinal cord preparations, the Scheibels concluded that: 1) no short-axoned cells exist anywhere in the ventral horn nor in the internuncial pools of the overlying grey; 2) the great majority of neurons in the ventral medial portion of lamina VII are contralaterally projecting elements; 3) internuncial cells of the central and lateral portions of lamina

VII are funicular neurons and may extend for a distance of one or more spinal segments; 4) only about 40-60% of the axons of large motoneurons appear to have collaterals; 5) the terminals of these collaterals appear within one-half segment of the parent soma and are widely distributed in laminae VII, VIII, and IX of the spinal cord; and, 6) the collaterals terminate on soma and dendrites of motoneurons (including the parent motoneuron) and on interneurons. On the basis of these observations the Scheibels suggested that long-axoned funicular interneurons in the ventral horn may be the substrate for Renshaw inhibition (Scheibel and Scheibel, 1969) or, alternatively, the dendrites of motoneurons may actually serve as the "Renshaw elements" (Scheibel and Scheibel, 1971; cf. Willis, 1971).

Renshaw (1941) found that motor axon volleys most commonly resulted in an inhibition of reflex transmission in motorneurons, although facilitation was sometimes observed. Wilson and Burgess (1962) demonstrated that the recurrent facilitation resulted from a process of disinhibition. Intracellular recordings showed that ventral root volleys caused a small depolarization in some motoneurons. The depolarization was termed the recurrent facilitatory potential. In addition, antidromic conditioning increased the magnitude of subthreshold EPSPs in motoneurons elicited by dorsal root stimulation and increased the excitability of the same cells to a stimulus applied through the microelectrode. The recurrent facilitatory potential was not an EPSP, since the depolarization was diminished or reversed by a hyperpolarizing current applied through the microelectorde. Wilson and Burgess (1962) concluded that the facilitation was due to removal of background inhibition of motoneurons by the action of the recurrent pathway upon inhibitory interneurons in the spinal cord.

One source of inhibition of motoneurons are Ia inhibitory interneurons. As described above, Hultborn et al. (1971a,b,c) demonstrated that the discharges of Ia inhibitory interneurons are susceptible to recurrent Renshaw inhibition. They observed a reduction in the size of orthodromically-elicited Ia IPSPs in motoneurons upon antidromic activation of the appropriate ventral root. The strongest depression of orthodromically-elicited Ia IPSPs was evoked from motor fibers to muscles whose Ia afferents produced the IPSPs. A marked parallelism existed between recurrent inhibitory effects to motoneurons and to interneurons receiving the same Ia excitatory input. This observation led Jankowska and co-workers to suggest that the same population of Renshaw cells mediate inhibition of motoneurons and Ia inhibitory interneurons. In addition, it was found that Ia inhibitory interneurons were inhibited by antidromic volleys in three ventral roots. This observation indicates that Renshaw cell axons project to more than one spinal segment and supports the contention of the Scheibels (1969) that Renshaw cells may be funicular interneurons. Furthermore, the observation that Ia inhibitory interneurons were susceptible to recurrent Renshaw inhibition provides a mechanism for recurrent facilitation (disinhibition) of motoneurons as suggested by Wilson and Burgess (1962). Finally, these observations suggested that the operation of the Renshaw loop may subserve complex movements or postures involving a co-contraction of antagonistic muscles.

Ryall and co-workers demonstrated that the discharges of Renshaw cells are inhibited by activity in other Renshaw neurons (Ryall, 1970; Ryall et al., 1971). This was most clearly observed in a few instances when the discharges of spontaneously active Renshaw

cells were inhibited in the absence of prior excitation by motor axon volleys. In addition, Renshaw cell discharges induced by the iontophoretic application of acetylcholine were depressed by motor axon volleys. The inhibition was abolished by intravenous injection of dihydro-β-erythroidine. Furthermore, it was found that the test response of a Renshaw cell elicited by a ventral root volley was depressed by conditioning stimuli applied to certain motor axons. The central latency of inhibition of Renshaw discharge was calculated to be 2.2 msec. The time course of inhibition was similar to the duration of the burst discharge of a Renshaw cell. These observations led Ryall (1970) to conclude that Renshaw cells inhibit one another through a direct monosynaptic pathway. Ryall et al. (1971) showed that Renshaw cells activated by an antidromic volley in the ventral root were able to inhibit other Renshaw cells located at least 5.5 mm away. This observation indicates that Renshaw cell axons project to more than one spinal segment and supports the contention of the Scheibels (1969) that Renshaw cells may be funicular interneurons. Renshaw cell mediated inhibition of other Renshaw cells is also thought to participate in recurrent facilitation.

Ellaway (1971) has reported recurrent inhibition of gamma motoneuron fibers elicited by alpha motor axon volleys. The inhibition had a mean central delay of 2.3 msec which is consistent with transmission in a disynaptic pathway. The duration of inhibition was variable and lasted anywhere from 10-100 msec. Eserine, which facilitates Renshaw cell mediated inhibition of alpha motoneurons, potentiated the inhibition of gamma motoneurons following motor axon volleys. Strychnine depressed Renshaw cell mediated inhibition of

alpha motoneurons and reduced the inhibition of gamma motoneurons following an antidromic volley. Similar observations were made by Grillner (1969). Both investigators concluded that antidromic inhibition of gamma motoneurons is mediated via recurrent collaterals and Renshaw interneurons.

The morphology and projections of Renshaw cells have been studied by Jankowska and co-workers (Jankowska and Lindström, 1971; Jankowska and Smith, 1973). Using intracellular injections of Procion Yellow, Renshaw cells were morphologically identified by Jankowska and Lindström (1971). The soma of Renshaw cells were found to have a diameter of 10-15 μm and were located in the ventral portion of lamina VII. The dendrites extended to a radius of about 100-150 μm. The axons of Renshaw cells had several collateral branches, some of which were followed to the border of the ventral-medial funiculus. This indicates that Renshaw cells are funicular neurons as suggested by the Scheibels (1969) and agrees with the intersegmental inhibitory actions of the Renshaw cells on other Renshaw cells in neighboring segments (Ryall, 1970; Ryall et al., 1971) and on Ia inhibitory interneurons (Hultborn et al., 1971a,b,c). Jankowska and Smith (1973) studied the axonal projections of Renshaw cells by antidromically activating these neurons following microelectrode stimulation of the spinal cord. The location of axons and the extent of their branching were reconstructed by comparing the latencies of the responses and thresholds (0.1-5 μA) for antidromic activation of single interneurons from electrode positions in a number of different tracts. It was found that Renshaw cell axons: 1) project to distances over 12 mm, 2) run in the ventral funiculus, 3) terminate both within motor

nuclei (mainly at short distances) and adjoining dorsomedial regions which contain Renshaw cells and Ia inhibitory interneurons, and 4) have a maximal conduction velocity of 30 m/sec.

In addition to receiving excitatory input from stimulation of a variety of motor axons, Renshaw cells may be excited or inhibited upon the activation of a number of other neuronal systems. Ipsilateral dorsal root volleys polysynaptically excite Renshaw cells (Piercy and Goldfarb, 1974). Stimulation of contralateral muscle or cutaneous afferent nerves produce inhibition or sometimes weak excitation of Renshaw cell discharges (Wilson et al., 1964). Finally, the discharges of Renshaw cells are inhibited by stimulation of the contralateral medullary reticular formation (Haase and Van der Meulen, 1961).

B. Properties of Sympathetic Nervous Discharge (SND)

The studies described above illustrated the crucial role played by interneurons in the integration of neural activity in spinal sensory and somatomotor pathways. In contrast, knowledge of the organization of spinal sympathetic pathways is very limited. Some features of sympathetic discharge are introduced in this section. Three distinct periodic wave forms of multiunit sympathetic nerve activity are described and the origins of these oscillations are discussed. The discharge patterns of single pre- and postganglionic sympathetic neurons are characterized. Finally, studies employing the use of computer aided techniques to identify central sympathetic neurons are discussed.

1. Sympathetic Discharges Recorded from Multiunit Preparations

Adrian, Bronk, and Phillips (1932) were the first to demonstrate that the cervical and abdominal sympathetic nerves of the decerebrate or urethane anesthetized cat and rabbit exhibited a continuously fluctuating electrical discharge which emanated from the central nervous system. In the majority of animals oscillations of electrical activity were found to be temporally related to the respiratory and/or cardiac cycles. In the naturally breathing rabbit, the amplitude of the respiratory-locked oscillation of SND was maximal at the end of inspiration. These rhythmic waveforms were thought to reflect the synchronized discharge of a large number of contributing neural elements. The experiments of Adrian et al. (1932) were repeated by Bronk and Ferguson (1932), and essentially the same observations were made on the inferior cardiac nerve of urethane anesthetized cats. In addition it was found that the average level of nerve activity was increased and decreased by procedures which lowered and raised arterial blood pressure, respectively.

Bronk and coworkers (1936,1940) continued the study of the centrally emanating spontaneous discharges in the inferior cardiac nerve. In addition to the previously described cardiac and respiratory oscillations of SND, these investigators recorded rhythmically occurring waveforms which varied between 5-20 Hz. Irregularly occurring wavelets of SND were also observed. A central synchronizing mechanism was postulated to account for the marked respiratory- and cardiac-related periodicities of SND. An in-depth discussion concerning the origin of the cardiac and respiratory related discharges in sympathetic whole nerves is presented in the following section.

Since these pioneer investigations there have been a large number of studies devoted to the description and analysis of SND recorded from multifiber preparations of the cervical sympathetic nerves (Alexander, 1946; Joels and Samueloff, 1956; Biscoe and Purves, 1967a,b; Biscoe and Sampson, 1968; Koizumi et al., 1971; Gebber et al., 1973; Preiss et al., 1975; Gebber, 1976; Barman and Gebber, 1976), thoracic nerves (Downing and Siegel, 1963; Green and Heffron, 1967a,b,1968; Koizumi et al., 1971; Ninomiya et al., 1971), abdominal nerves (Tang et al., 1957; Millar and Biscoe, 1965; Kedzi and Geller, 1968; Illert and Seller, 1969; Cohen and Gootman, 1969,1970; Gootman and Cohen, 1970,1971; Taylor and Gebber, 1975; McCall and Gebber, 1976; Gebber, 1976), and lumbar nerves (Koizumi et al., 1971). From these studies a number of observations pertinent to the present investigation were made.

1. Experiments involving serial transections of the brain stem indicated that the integrity of the medulla and the caudal 1/3 of the pons was necessary for the generation of tonic SND (Alexander, 1946).

2. Cardiac and respiratory related rhythms are present in the background electrical activity of sympathetic pre- and postganglionic nerves, regardless of the species of the experimental animal or type of anesthesia used.

3. Hagbarth and Vallbo (1968), using themselves as subjects, recorded the spontaneously occurring discharges from sympathetic postganglionic nerve bundles. Cardiac related bursts of SND were temporally related to the phases of the naturally occurring respiratory cycle. Sympathetic activity was greatest during late inspiration

and early expiration and least during late expiration and early inspiration.

4. The temporal relationships between sympathetic and phrenic nerve discharges in vagotomized animals is variable. Cohen and Gootman (1970) and Gootman and Cohen (1974) described the temporal relations between computer-summed splanchnic sympathetic and phrenic nerve discharges as phase spanning. SND increased during late expiration and early inspiration, became maximal in midinspiration, and then declined to a minimum in early expiration. In contrast Koizumi et al. (1971) found that SND was phase locked to the inspiratory portion of the respiratory cycle.

5. Cardiac periodicity is most prominent in the discharges of sympathetic nerve bundles that contain a high percentage of vasoconstrictor fibers. Cardiac modulation, which is prominent in the renal postganglionic nerves, is often diminished in the preganglionic splanchnic nerve and absent in the white ramus (Koizumi et al., 1971).

6. High frequency stimulation (50 Hz) of the dorsolateral reticular formation of the medulla produced an increase in splanchnic nerve discharge and was accompanied by a rise in arterial blood pressure (Gootman and Cohen, 1970). Although total SND increased during the stimulation period, the cardiac and respiratory periodicities of SND disappeared (i.e., desynchronization of sympathetic nerve activity). Similar observations have been reported by Scherrer (1962) in the rat during high frequency stimulation of the posterior hypothalamus.

7. Using computer averaging techniques, Gootman and Cohen (1971) characterized the evoked response recorded in the splanchnic

nerve following single shock stimulation of medullary pressor sites. The evoked response in the splanchnic nerve had a modal onset latency of 40 msec. The conduction velocity in the descending sympathoexcitatory pathway was calculated to be 4 m/sec. Gebber et al. (1973) studied the responses evoked in the external carotid postganglionic sympathetic nerve by single shocks and trains of stimuli applied to pressor sites in the medulla. Using an average-response computer two distinct systems of sympathetic pathways were identified. The first was a slowly conducting system of pathways (conduction velocity = 2.7 m/sec) which was sensitive to baroreceptor reflex inhibition. Postganglionic potentials evoked from the second, more rapidly conducting system (conduction velocity = 5.4 m/sec), were not blocked during baroreceptor reflex activation. Both systems could be activated by stimulation of pressor sites in the hypothalamus, midbrain, medulla, and cervical spinal cord. It was concluded that sympathetic outflow from the brain to the external carotid nerve is organized into two systems of parallel pathways, each of which is related differently to the baroreceptor reflex arc. In addition to these studies, Foreman and Wurster (1973) reported conduction velocity in the descending spinal sympathoexcitatory pathway to be 6 m/sec.

8. Stimulation of cutaneous, visceral, and muscle afferent nerves evoked both spinal and supraspinal reflexes in sympathetic nerve bundles. The late supraspinal component of the reflex was follwed by a prolonged period of quiescence (Koizumi and McC. Brooks, 1972).

9. Sympathetic nerve activity was immediately inhibited by a rapid sustained rise in carotid sinus pressure (Koizumi et al., 1971),

during the rise in arterial pressure produced by an intravenous injection of norepinephrine (McCall and Gebber, 1976), and by high frequency (30-50 Hz) stimulation of medullary depressor sites (Gootman and Cohen, 1970).

10. Severe hemorrhage of an animal resulted in the disappearance of the cardiac related periodicity in splanchnic nerve activity and the appearance of higher frequency components of SND (Taylor and Gebber, 1975).

11. Spontaneously occurring SND was observed in spinal animals during periods of anoxia (Alexander, 1945).

12. Oscillations of SND often occurred with a frequency of 10 c/sec (Green and Heffron, 1967b). As described more fully in the following section, Cohen and Gootman (1969,1970) demonstrated that the 10 c/sec rhythm of SND is often phase locked in a 3:1 relationship to the cardiac cycle.

From the above discussion it is evident that the discharges of sympathetic nerve bundles are most often characterized by prominent respiratory- and cardiac-related periodicities. In addition a 10 c/sec periodicity of SND has been reported. These oscillations of neural activity must reflect the synchronized discharge of a large number of individual sympathetic neurons. The respiratory- and cardiac-related rhythms of SND are classically thought to be extrinsically imposed on central sympathetic pathways by a component of the respiratory oscillator and the baroreceptor reflexes, respectively (Cohen and Gootman, 1970). However, if one, or all, of these waveforms are representative of sympathetic rhythms of central origin, then they may provide valuable information concerning the fundamental organization

of central sympathetic networks. For this reason it is important to discuss the origin of sympathetic nervous rhythms.

2. Origin of Cardiac and Respiratory Related Oscillations of SND

a. Origin of the Cardiac Related Discharges of Sympathetic Nerve Bundles

As described above, oscillations of SND (≃3 c/sec) locked in a 1:1 relation to the cardiac cycle are classically thought to result from the waxing and waning of baroreceptor nerve discharge associated respectively with the systolic and diastolic phases of the arterial pulse. That is, it is thought that randomly generated SND is periodically inhibited by baroreceptor afferent discharges and this molds sympathetic outflow into pulse synchronous waveforms. The basis for this hypothesis rests in the fact that interruption of baroreceptor afferent nerve discharges abolished the cardiac locked component of SND recorded from the cervical sympathetic nerve (Adrian et al., 1932), the inferior cardiac nerve (Bronk et al., 1936; Downing and Siegel, 1963; Green and Heffron, 1968b), the splanchnic nerve (Adrian et al., 1932; Koizumi et al., 1971; see also Cohen and Gootman, 1970), and sympathetic fibers innervating the lungs (Widdicombe, 1966).

Taylor and Gebber (1975) have recently challenged the traditional view of the formation of the cardiac locked component (≃ 3 c/sec) of SND. They noted that while some investigators (Bronk et al., 1936; Aström and Crafoord, 1968) reported that SND appeared essentially random in character after elimination of baroreceptor activity; others (Alexander, 1945; Green and Heffron, 1968b; Koizumi et al., 1971) observed irregularly occurring oscillations of SND which were no longer in phase with the cardiac cycle. These reports suggested

to Taylor and Gebber (1975) the possibility that oscillations of SND which were locked in a 1:1 relation to the cardiac cycle may be formed by mechanisms intrinsic to the central sympathetic centers and regulated by the baroreceptor reflexes. To test this possiblity Taylor and Gebber (1975) observed the effect of baroreceptor denervation on splanchnic and renal SND using a wide preamplifier bandpass (1-1000 Hz) to more accurately characterize the duration of slow waves of SND. They noted that while bilateral section of the carotid sinus, aortic depressor, and vagus nerves unlocked the phase relations between SND and the cardiac cycle, the 3 c/sec periodic component persisted. In addition it was demonstrated that one complete oscillation of SND could be aborted by a stimulus delivered to the baroreceptor reflex arc during a time span which accounted for less than 1% of the cardiac cycle. This inhibition was mediated at the level of the brain stem. It was concluded that the 3 c/sec periodic component of SND is representative of a sympathetic rhythm of brain stem origin which is entrained to the cardiac cycle by the baroreceptor reflexes.

Gebber (1976) has extended the observations of Taylor and Gebber (1975) by studying the phase relations between the cardiac cycle and splanchnic SND using an average-response computer. Peak amplitude of the cardiac locked slow wave of SND occurred during early diastole at heart rates of between 3 and 4 beats per second. Dramatic shifts in the phase relations between SND and the cardiac cycle accompanied the decrease in heart rate produced by stimulation of the distal end of the cut right vagus nerve. The point of maximum SND was shifted from early diastole to near peak systole and then into the late diastolic phase of the preceding cardiac cycle as the heart rate

was progressively lowered to 2 beats per second. These observations again indicated that the cardiac locked slow wave of SND is not the simple consequence of the waxing and waning of baroreceptor nerve activity, but rather represents a sympathetic rhythm of central origin which is entrained to the cardiac cycle by the barorceptor reflexes (see also Gebber et al., 1976). In addition Gebber (1976) found that the ≈200-300 msec slow waves were generated aperiodically at frequencies ranging from 3 to 5 cycles per second when the heart rate was lowered below 2 beats per second. This suggests that the baroreceptor reflexes entrain the centrally generated slow wave in a 1:1 relation to the cardiac cycle in order to control the periodicity of SND.

Green and Heffron (1967b) and Cohen and Gootman (1969) have observed a prominent 10 c/sec periodic waveform in the discharges of the inferior cardiac and splanchnic nerves, respectively. Green and Heffron (1967b) noted that this rapid rhythm was approximately three times faster than the rate of the cardiac cycle. The 10 c/sec rhythm of SND was most often elicited during procedures which reduced or eliminated baroreceptor nerve discharge (i.e., occlusion of the ascending aorta, pulmonary aorta, or inferior vena cava). However, large vessel occlusion may not only decrease baroreceptor reflex activity but also increase chemoreceptor activity and produce cerebral asphyxia. Therefore, two possibilities exist regarding the augmented production of 10 c/sec waves of SND: 1) diminished baroreceptor afferent discharges, and 2) enhanced chemoreceptor activity. More recently McCall and Gebber (1976) have shown that the 3 and 10 c/sec components of SND are differentially affected by the baroreceptor reflexes. It was demonstrated that the 10 Hz component is more

sensitive to neural inhibition of baroreceptor origin than the 3 Hz component of SND.

Using the R wave of the ECG to trigger an averaging computer, Cohen and Gootman (1970) found that the 10 c/sec rhythm of SND was often, but not always, phase locked in a 3:1 relation to the cardiac cycle. In addition, single shock stimulation of a medullary pressor site elicited an evoked response in splanchnic SND which was followed by damped 10 c/sec oscillations of SND locked to the stimulus (Gootman and Cohen, 1971). Due to the ubiquity of occurrence of the 10 c/sec wave in their preparations, Cohen and Gootman (1970) suggested that this rhythm reflects the fundamental organization of central sympathetic networks. More recently Gootman and Cohen (1973) demonstrated that the spontaneous discharge of sympathetic nerves at different segmental levels have closely coupled 10 c/sec periodic components. Thus, the 10 c/sec periodicity in different sympathetic nerves normally is dependent on supraspinal driving inputs. On the basis of this observation it was concluded that the 10 c/sec rhythm of SND reflects the fundamental organization of brain stem sympathetic networks. However, these experiments do not preclude the possibility that a spinal mechanism was responsible for the synchronization of brain stem outflow into slow waves with a period approaching 100 msec.

b. Origin of the Repiratory Related Discharges of Sympathetic Nerve Bundles

The naturally occurring discharges of sympathetic nerve bundles exhibit a slow rhythmic component with a period of the respiratory cycle (Adrian et al., 1932; Bronk et al., 1936; Cohen and Gootman, 1970; Gootman and Cohen, 1974; Barman and Gebber, 1976). In addition

to the sympathoinhibitory effects associated with activation of vagal inflation afferents (Bronk et al., 1936; Daly et al., 1967; Okada and Fox, 1967), it is generally agreed that some form of direct coupling between the brain stem respiratory oscillator and the central sympathetic network is responsible for the slow respiratory related rhythm of SND (Cohen and Gootman, 1970; Koizumi et al., 1971; Gootman and Cohen, 1974; Preiss et al., 1975). Evidence for this statement comes from the fact that in vagotomized animals, SND often oscillates with period of the respiratory cycle (Adrian et al., 1932; Tang et al., 1957; Joels and Samueloff, 1956). In addition Millar and Biscoe (1965) observed a prominent respiratory related periodicity in the splanchnic SND of paralyzed rabbits and this rhythm was maintained after the respirator was shut off. Hagbarth and Vallbo (1968) found that the respiratory waves of SND in man persisted during breath-holding.

It is generally assumed that the respiratory related periodicity of SND is extrinsically imposed on central sympathetic networks by elements of the brain stem respirator oscillator (Cohen and Gootman, 1970; Koizumi et al., 1971; Preiss et al., 1975). However, a number of observations made by Barman and Gebber (1976) in vagotomized, paralyzed and artifically ventilated cats contradicts this view. First, spontaneous changes in the central respiratory rate were accompanied by dramatic shifts in the phase relations between sympathetic and phrenic nerve discharge. Second, the slow oscillations of sympathetic and phrenic nerve discharge were not always locked in a 1:1 relation. Third, and perhaps most important, it was found that the slow sympathetic rhythm persisted when respiratory

rhythmicity (as monitored by phrenic nerve discharge) disappeared during hyperventillation. In this regard Cohen (1968) reported that hypocapnia led to a disappearance of the rhythmic components of the discharges of brain stem respiratory neurons. The work of Barman and Gebber (1976) makes it difficult to attribute the generation of the slow respiratory related rhythm of SND to a direct connection between the brain stem respiratory oscillator and the central sympathetic networks. It was concluded that the slow periodic components of sympathetic and phrenic nerve discharges are generated by independent oscillators that normally are entrained to each other. Entrainment might result from direct connections between the two independent oscillators or, alternatively, both oscillators might receive common inputs from a distinct brain stem synchronizing mechanism.

A primary objective in the study of sympathetic nervous system is to define the functional interrelationships between those elements which constitute the brain stem and spinal sympathetic networks. As described above, transection and stimulation of the central nervous system, as well as a detailed analysis of the periodic components of SND, have yielded much valuable information concerning the organization of central sympathetic centers. However, it is clear that the intrinsic organization of central sympathetic networks must ultimately be resolved by single unit nerve recording of elements contained within this system. Therefore, the following section will discuss some of the characteristics of medullary "sympathetic" units and spinal sympathetic preganglionic neurons.

3. Spontaneous Discharges Occurring in Single Sympathetic Neurons

Analysis of tonic firing of single sympathetic neurons has been accomplished by recording the discharges from single axons of sympathetic preganglionic neurons (PSNs) and by positioning microelectrodes near the soma of medullary or spinal sympathetic neurons in order to record their extracellular spike potentials.

a. "Sympathetic" Neurons in the Medulla

Great difficulty has been encountered in identifying medullary neurons which regulate the discharges of preganglionic sympathetic nerves. Since efferent sympathetic activity recorded in nerve bundles is most often locked in a 1:1 fashion to the cardiac cycle, many investigators have attempted to locate neurons which discharge in a time locked fashion to a phase of each cardiac cycle (Smith and Pearce, 1961; Salmoiraghi, 1962; Humphrey, 1967; Przybyla and Wang, 1967). In these studies medullary units with such a cardiac rhythm were located only in the area of the nucleus tractus solitarius and were most likely first or second order afferents of the baroreceptor reflex arc (see below).

A second approach used to identify medullary sympathetic neurons has been based on the alteration of unitary discharges produced by reflex changes in arterial blood pressure (Salmoiraghi, 1962; Preobrazhenskii, 1966; Przybyla and Wang, 1967; Weiss and Kastella, 1972; Hukuhara and Takeda, 1975; Putnam and Manning, 1977). Salmoiraghi identified 41 medullary units which he considered to be sympathetic neurons based on the following criteria: 1) unitary activity was increased or decreased during blood pressure changes produced by vasodilator and vasoconstrictor agents, respectively;

2) unitary activity was synchronized with spontaneously occurring slow fluctuations in blood pressure; and 3) unitary activity increased during carotid occlusion. Similarly, Preobrazhenskii (1966) found medullary "cardiovascular" units whose spontaneous discharges were reflexly altered during changes in carotid sinus pressure and during the pressor effect produced by the intravenous injection of epinephrine. Pryzbyla and Wang (1967) identified medullary "cardiovascular" neurons as ones which exhibited not less than a 30% decrease in discharge frequency during the rise in blood pressure produced an intravenous injection of norepinephrine. Fourteen such neurons were found in the dorsolateral reticular formation.

In these studies it was assumed that neurons which were depressed during baroreceptor reflex activation were contained within central sympathetic networks. The difficulty with this approach is that not only the sympathetic nervous system, but also respiration, motor systems, and the degree of EEG synchronization are affected by the baroreceptor reflex (Moruzzi, 1964; Jouvet, 1967; Koepchen, 1974; Henatsch, 1974). For this reason an alteration of a neuron's discharge pattern produced by blood pressure change is not an adequate criterion for the classification of a neuron as sympathetic in function. Thus, until recently attempts to locate individual sympathetic neurons whose processes are contained solely within the central nervous system have failed (see below).

b. Sympathetic Neurons in the Spinal Cord

It is generally agreed that the vast majority of PSN lie within the intermediolateral cell column of the thoracic and upper lumbar segments of the spinal cord (Cummings, 1969; Henry and Calaresu,

1972; Petras and Cummings, 1972; Réthelyi, 1972; Chung et al., 1975). In addition, anatomical evidence indicates that a relative small number of PSN are also found in the spinal intermediomedial nucleus (Szenthágothai, 1966; Petras and Cummings, 1972; Chung et al., 1975). Embryologically and phylogentically the intermediomedial and intermediolateral cell columns constitute a single cell mass which appears first dorsal and dorsolateral to the central canal. Part of the cells retain a position near the central canal of the adult cord in the intermediomedial (IMM) cell column. Part of them migrate laterally to form an extension of the grey matter, the intermediolateral (IML) cell column. Scattered PSN throughout lamina VII of the spinal cord mark the course of this lateral migration (Crosby et al., 1962). Henry and Calaresu (1972) have estimated that there are nearly 40,000 PSN in the cat IML cell column. The highest concentrations were found in the first and second thoracic segments. Drastic fluctuations in the number of cells in the IML nucleus from one cross-section to the next have been reported (Cummings, 1969; Henry and Calaresu, 1972). In a horse radish peroxidase study, Chung et al. (1975) found that the longitudinal axis of the soma of PSNs ranged from 9.2 to 36.8 μm in the cat. Henry and Calaresu (1972) have estimated the modal cross-sectional area of the soma of PSNs to be 290 μm^2. In an exhaustive study, Petras and Cummings (1972) reported at least three cell types in the IML nucleus: 1) triangular or multipolar neurons, varying in size from 28x24 μm to 48x30 μm; 2) fusiform, sizes ranging from 22x19 μm up to as large as 58x18 μm; and 3) round neurons which range in size from 18 μm to 29 μm in diameter. Réthelyi (1972) found the perikarya of IML neurons were elongated and oriented longitudinally,

with a long diameter of 25-35 μm. The dendrites of PSN generally originated from the rostral and caudal ends of the perikarya and immediately oriented themselves into a longitudinal direction. In addition some transversely positioned dendrites were observed. Axons of PSN emerged with a conspicuous axon-hillock from the soma or more frequently from one of the dendrites. Axons were traced ventrally following the border between the ventral horn grey matter and the lateral funiculus before joining the ventral rootlets. No initial collateral branches were ever observed along the entire intraspinal course of PSN axons. Réthelyi (1972) also noted that presynaptic fibers appeared to approach the IML nucleus from the lateral funiculus in small bundles which quickly dispersed and coursed longitudinally so that the axons were oriented in a parallel fashion with PSN dendrites. Axo-dendritic and axo-somatic synapses were observed in the IML cell column.

Cummings (1969) has described a diffuse transverse band of PSN extending from the IML to the IMM nucleus. Cells were found to be spindle shaped (20x40 μm) or round (20x20 μm). The IMM nucleus is located lateral and dorsolateral to the central canal of the spinal cord. PSN within the nucleus are usually oval (30x22 μm) or round (25x25 μm). Following dorsal rhizotomy there is a massive terminal degeneration within the IMM nucleus. In contrast no degeneration is seen within the IML nucleus following dorsal root section (Szentágothai, 1966; Petras and Cummings, 1972). In addition fibers from the IMM nucleus have been shown to project towards the IML nucleus. These observations have led Szentágothai (1966) and Petras and Cummings (1972) to suggest that neurons within the IMM nucleus function as

interneurons connecting dorsal root afferents fibers to PSN in the IML cell column.

In an extensive study of the upper thoracic spinal segments, Fernandez de Molina and coworkers (1965) characterized extra- and intracellular PSN potentials evoked antidromically by cervical sympathetic nerve stimulation. It was found that the contour of the extracellular and intracellular records of PSN exhibited initial segment (IS) and soma-dendritic (SD) components of depolarization similar to those seen in lumbosacral motoneuron spikes (Eccles, 1964). A third component appeared in association with the soma-dendritic phase and was thought to represent electrotonic recording from impulses slowly conducted in short dendrites. In a few successful intracellular penetrations, a hyperpolarizing phase followed the SD response and lasted 20-60 msec. Extracellular and intracellular spike durations were 7.2 and 7.1 msec, respectively. These values are much longer than durations of PSN spikes (1-2 msec) reported by other investigators (Hongo and Ryall, 1966a,b; DeGroat and Ryall, 1967; Taylor and Gebber, 1973; however see Polosa, 1968). In agreement with the anatomical studies of Réthyeli (1972), Fernandez de Molina et al. (1965) found no evidence to support the possibility of recurrent inhibition of PSNs by axon collaterals of these neurons.

Polosa (1967,1968) characterized the antidromically evoked and spontaneous discharge characteristics of PSNs located in the IML nucleus. By varying the interstimulus interval between pairs of antidromic stimuli, it was determined that the SD component of a PSN spike always failed before the IS component. The IS component failed when interstimulus intervals decreased below 4 msec. Polosa

found that only 21% of the antidromically identified PSNs were spontaneously active. Of these 15% exhibited a pattern consisting of 8 to 20 bursts per minute, while in most cases the discharges were continuous and regular (7%) or irregular (78%). The average frequency of discharge of these PSNs was 1.4 spikes per sec. For any given neuron the firing rate was stable over long periods, but was altered by procedures which caused changes in the arterial blood pressure. A feature consistently observed in the spike trains of spontaneously active PSN was the absence of interspike intervals less than 100 msec. Polosa has also observed spontaneously active PSNs in spinal cats (Polosa, 1968; Mannard and Polosa, 1973). The mean discharge rate of these units (0.7 spikes/sec) was less than that observed in intact preparations.

Although Fernandez de Molina et al. (1965) and Polosa (1967,1968) recorded PSNs in upper thoracic segments of the spinal cord, similar observations have been made in the IML cell column of lower thoracic and upper lumbar spinal segments. The spontaneous discharge rate of PSNs was found to be 2.3 Hz for units activated antidromically by splanchnic nerve stimulation (Hongo and Ryall, 1966a,b) and 2.1 Hz for neurons contributing axons to the cervical sympathetic nerve (DeGroat and Ryall, 1967; Wyszogrodski and Polosa, 1973). Seller (1973) observed that the spontaneous discharge frequency of single fibers in thoracic and lumbar white rami was 1.2 Hz and 1.8 Hz, respectively. Kaufman and Koizumi (1971) and Sato (1972) found the mean discharge rate of single lumbar white rami fibers to be 1.2 and 2.1 discharges/sec, respectively. Single fibers in the cervical sympathetic nerve also discharge between 1.0 and 2.0 Hz (Iggo and Vogt, 1960; Jänig and Schmidt, 1970).

Periodic bursts of spikes in phase with the respiratory cycle have been observed at the single unit level. Iggo and Vogt (1960) recorded PSNs whose discharges were phase locked to the inspiratory phase of the respiratory cycle. Tuttle (1963) observed a prominent respiratory rhythm in the discharges of single "cardiovascular" preganglionic fibers (identified by their responses to changes in arterial pressure) in the ventral roots of comatose cats. The high discharge frequency reported by Tuttle (25 spikes/sec) is not consistent with the majority of studies of PSN. It is possible that the units Tuttle studied were gamma motoneurons, or, alternatively spikes from more than one PSN might have been recorded. Preiss et al. (1975) reported that the pattern of respiratory modulation of the spontaneous discharges of PSN was phase locked to the central respiratory cycle. Unitary discharge most often was augmented during inspiration and reduced during expiration, although three units also were found that fired primarily during expiration. Autocorrelation analyses performed by Mannard and Polosa (1973) also revealed a prominent respiratory periodicity in the discharges of single PSNs.

Taylor and Gebber (1973) and Gebber (1975) have characterized the response of antidromically identified PSNs following single shock stimulation of the medullary pressor region. PSN characteristically discharged once with a variable onset latency to each shock applied to a medullary pressor site. Individual PSNs had large medullary receptive fields, suggesting extensive convergence of descending sympathoexcitatory pathways onto these neurons. Convergence was also indicated by the degree of variaiblity of discharge onset latency following single shock stimulation of medullary pressor

sites. It was concluded that the variability of the onset latency of preganglionic spike initiation reflected changing patterns of discharge in the pathways converging onto preganglionic neurons.

Recordings of single sympathetic fibers have been used to study sympathetic reflexes. Beacham and Perl (1964) demonstrated that noxious, thermal, and mechanical stimuli applied to the limbs of spinal cats elicited excitatory responses in thoracic and lumbar PSNs. A reflex discharge of PSNs was also evoked by electrical stimulation of dorsal roots, spinal nerves, and limb nerves. Analysis of reflex responses recorded from white rami has revealed that a considerable proportion of PSNs (26%) participate in both spinal and supraspinal reflexes (Kaufman and Koizumi, 1971; Sato, 1972). Other fibers were excited only by impulses from the supraspinal (46%) or spinal (28%) reflex pathways. Inhibition of the spontaneous discharges of PSNs, without prior excitation, can also be elicited by somatic afferent stimulation in spinal animals (Jänig and Schmidt, 1970; Kaufman and Koizumi, 1971; Koizumi and Sato, 1972; Wyszogrodski and Polosa, 1973; Jänig, 1975). Pagani *et al*. (1974) and Malliani *et al*. (1975) found that discrete mechanical, chemical, or electrical stimulation of cardiac and aortic receptors produced either excitation or inhibition of the spontaneous discharges of PSNs in spinal vagotomized cats. Wyszogrodski and Polosa (1973) reported that glutamate-evoked discharges of PSNs could be suppressed by stimulation of somatic afferent nerves in spinal cats. The observation that chemically induced excitation (iontophoretic application of glutamate) could be suppressed led these authors to conclude that the afferent inhibition was mediated directly on the preganglionic cell.

Several investigators have postulated the existence of spinal sympathetic interneurons. Beacham and Perl (1964) found that single shock stimulation of a dorsal root usually evoked a reflex discharge in sympathetic preganglionic fibers. The central delay time from dorsal afferent nerves to the preganglionic sympathetic ramus (after subtraction of afferent and efferent conduction time) was found to be at least 1.3 msec. Since the central delay time was considerably longer than that found in the monosynaptic flexor reflex arc (0.3-0.8 msec; Eccles, 1964), the authors suggested that the response was mediated through a polysynaptic pathway. This observation is supported by the work of Szenthágothai (1966) and Petras and Cummings (1972) who found minimal terminal degeneration in the IML nucleus following dorsal rhyzotomy.

Wyszogrodski and Polosa (1973) found units in the IML cell column which did not respond to antidromic stimulation of the cervical sympathetic nerve. These units had a higher frequency of discharge than antidromically identified PSNs. Responses to afferent nerve stimulation in nonantidromically activated and preganglionic neurons were similar. It was postulated that the high frequency, nonantidromically activated neurons in the IML nucleus were sympathetic interneurons.

Henry and Calaresu (1974c) observed short (3 msec) and long (20 msec) latency unitary responses in the IML cell column upon stimulation of cardioacceleratory sites in the medulla. The conduction velocity in the short latency pathway was 63 m/sec, a value much higher than those (2-7 m/sec) reported by others (Gootman and Cohen, 1971; Gebber *et al.*, 1973; Taylor and Gebber, 1973). It was

suggested that the long latency responses were recorded from PSNs, while the short latency responses were elicited in sympathetic interneurons interposed between rapidly conducting medullospinal fibers and preganglionic units. However, it was not determined whether either of the two spinal neuronal types could be antidromically activated.

As discussed in the following section, sympathoinhibitory pathways descend from the brain stem to the spinal cord. Stimulation of these pathways results in inhibition of the discharges of PSNs. Kirchner et al. (1975) found that intraspinal stimulation in chronic cats evoked an inhibition of preganglionic nerve discharges. The authors suggested that the descending sympathoinhibitory pathway was not a system made of long medullospinal axons which terminate directly onto PSNs, but rather is composed of a series of sympathetic inhibitory interneurons located in the spinal cord.

c. Use of Computer-aid Techniques to Identify Central Sympathetic Neurons

A distinct cardiac rhythm within the spontaneous discharges of single pre- or postganglionic sympathetic neruons has seldom been observed. Indeed the mean firing rate of preganglionic neurons almost always is reported to be less than the average heart rate (Polosa, 1968; Jänig and Schmidt, 1970; Seller, 1973; Taylor and Gebber, 1973). These observations suggest that only a small and continually changing portion of the total population of sympathetic neurons participates in each cardiac related burst of activity recorded from peripheral sympathetic nerve bundles. This assumption has prompted a number of investigators to analyze the characteristics of spike trains in order to establish whether a probabilistic relationship exists between the discharges of single sympathetic neurons and

the phases of the cardiac cycle. Widdicombe (1966) noted that single postganglionic fibers innervating the lung did not discharge with a prominent cardiac periodicity. However, in some cases an insidious cardiac rhythm became apparent after graphical averaging of the time delays between some fixed point in the cardiac cycle and the occurrence of subsequent neuronal discharges. Similarly, Green and Heffron (1968a) observed low frequency (2-3 spikes/sec), irregularly spaced, discharges in single inferior cardiac nerve fibers. For individual cardiac cycles no constant time delay between the start of the systole of the unitary potential was evident. However, when the time interval between the R wave of the ECG and the fiber discharge was plotted for a number of successive cardiac cycles (i.e., post-R wave time interval histogram; post-R wave TIH) it was found that the probability of discharge of some postganglionic fibers was greatest from 100-175 msec after the onset of the femoral arterial pulse wave. Green and Heffron (1968a) showed that the cardiac-related discharge in multifiber records from the inferior cardiac nerve displayed similar phase relations to the cardiac cycle.

Using computer analysis, Seller (1973) was successful in demonstrating a positive post-R wave relationship for the spontaneous discharges of some single sympathetic preganglionic fibers in the thoracic and lumbar white rami of the cat. The peak probability of unitary discharge in these units occurred 150-200 msec after the R wave of the ECG. However, the discharges of the majority (82%) of preganglionic neurons were not correlated in time to a phase of the cardiac cycle. That is, these units discharged randomly with respect to the R wave. Similarly, using interspike interval and autocorrelation analyses, Mannard and Polosa (1973) found that the discharges

of only 13% of the thoracic PSNs recorded in their study were related in time to the cardiac cycle. Preganglionic units whose discharges showed markedly different degrees of correlation in time with the cardiac cycle often were located in the same cat during a time span when blood pressure remained essentially unchanged.

Recently, post-R time interval and cross-correlation analysis have been used to locate brain stem sympathetic neurons (components of central autonomic pathways) involved in regulating the discharges of PSNs. Gebber (1975) and Gebber et al. (1976) identified neurons in the pressor region of the cat medulla whose probability of discharge was greatest near the peak of systole and in early diastole. The mean discharge rate of these neurons was less than 1.0 Hz, suggesting that only a small and continuously changing segment of the total population of brain stem sympathetic units participated in each cardiac related burst of activity recorded from sympathetic nerve bundles. Langhorst et al. (1975,1976) have analyzed brain stem neurons using time interval histograms triggered by the R wave of the ECG and by high amplitude slow waves of the EEG. The most significant observation in these studies was that the discharges of some units which exhibited a positive R wave relationship were also related in time to the slow waves of the EEG. No correlation between the two trigger signals existed, although a cardiac and an EEG rhythm were detectable in a single sequence of neuronal discharges. It was concluded that afferents from a delta-theta oscillator and from the cardiovascular system converge on single brain stem reticular neurons. Langhorst et al. (1975,1976) suggested that such neurons are components

of both the reticular activating and central autonomic systems. However, it should be mentioned that the post-R wave TIHs illustrated in these studies were not impressive. Peak probability of discharge of these units (with respect to the R wave) was only about 5% greater than the background activity. Gootman et al. (1975) found two neurons in the cat medulla whose discharge pattern was correlated with that of the whole preganglionic splanchnic nerve. The cross-correlogram of unit to splanchnic activity exhibited two superimposed oscillations: 1) an 8 sec oscillation, which corresponded to the period of the central respiratory cycle; and 2) a 300 msec oscillation which was equivalent to the duration of the cardiac cycle. Thus, these units exhibited both respiratory- and cardiac-related discharges. The studies described above demonstrate that it is possible to employ computer-aided techniques to identify central sympathetic neurons and indicate that these methods may be of great value in elucidating the organization of brain stem and spinal sympathetic networks.

C. Baroreceptor Reflex-Induced Inhibition of Sympathetic Nervous Discharge

This section includes a description of studies performed on the baroreceptor reflex arc and its effect on sympathetic nervous discharge. Specifically the patterns of baroreceptor afferent nerve discharge, the central sites of baroreceptor afferent nerve termination, and the proposed loci of baroreceptor-sympathetic integration will be discussed. For a discussion of the vagal components of the baroreceptor reflex arc the reader is referred to a recent review by Kirchheim (1976).

Baroreceptor afferent nerve dishcarge, transmitted in the carotid sinus and aortic depressor nerves, normally exerts tonic inhibitory effects on the discharges of sympathetic nerve bundles. This fact is best illustrated by the hypertension developed after bilateral section of the aortic depressor and carotid sinus nerves (Heymans and Neil, 1958; Iggo and Vogt, 1962). In their classic study of the impulse discharge from baroreceptors of the carotid sinus Bronk and Stella (1932,1935) recorded the action potentials from single fibers of the carotid sinus nerve during normal blood flow and while perfusing the sinus at different nonpulsatile pressures. They found that the rate of discharge of single baroreceptor afferent fibers was closely related to the level of pressure within the sinus. Fibers were quiescent when the mean static pressure was reduced below 40 mmHg. As the static sinus pressure was raised different baroreceptor fibers began to discharge, and the discharge frequency of each unit increased as pressure was elevated to about 200 mmHg. Single carotid sinus nerve fibers discharged in high frequency bursts during each arterial pulse wave. At any given arterial blood pressure some fibers discharged throughout the arterial pulse cycle, while others fired only during the systolic rise in pressure. It was noted that the point on the arterial pressure curve at which impulses ceased was higher than that at which it began. Bronk and Stella (1932) also found that units which became quiescent during diastole often discharged during systole when the systolic pressure was lowered to a level below the previous diastolic pressure. These facts were interpreted to mean that the rate of change in arterial pressure, as well as the mean pressure, determine the threshold of activation and the rate of discharge of

baroreceptor afferent nerves. This hypothesis has been more recently confirmed by a number of investigators (Ead et al., 1952; Landgren, 1952; Ninomya and Irisawa, 1967; Angell James, 1971; Kirchheim, 1976).

The central neural substrate necessary for the integration of the baroreceptor reflexes resides within the medulla. Support for this statement is derived from anatomical studies of the intramedullary terminations of baroreceptor afferents (Kerr, 1962; Cottle, 1964) and from studies indicating that the cardiovascular responses to stimulation of baroreceptor afferent nerves or carotid occlusion are abolished by medullary lesions (Douglas and Schaumann, 1956; Katz et al., 1967; Chai and Wang, 1968; Miura and Reis, 1972). Following transection of the IX and X cranial nerves, Kerr (1962) and Cottle (1964) noted that terminal degeneration was localized in the middle third of the nucleus tractus solitarius (NTS), lying at, or just rostral to the obex. Discrete lesions encompassing the NTS have been shown to abolish the reflex depressor responses produced by carotid sinus stretch and carotid sinus nerve stimulation (Chai and Wang, 1968; Miura and Reis, 1972). Using unanesthetized rats, Doba and Reis (1973) found that electrolytic lesions limited to the NTS abolished baroreceptor reflexes and resulted in an immediate elevation in arterial blood pressure. The hypertension was associated with a marked increase in total peripheral resistance, a reduction of blood flow in the abdominal aorta, and an increase in central venous pressure. More recently Nathan and Reis (1977) have produced chronic labile hypertension and loss of baroreceptor reflexes in cats by placing discrete lesions in the NTS. These studies indicate that baroreceptor afferents terminate in, or traverse through, medial medullary nuclei.

A number of electrophysiological studies have provided evidence that baroreceptor afferent fibers terminate in medial medullary nuclei. Electrical stimulation of the carotid sinus and aortic depressor nerves evoked field and unitary potentials in the NTS (Humphrey, 1967; Miura and Reis, 1968,1969; Sampson and Biscoe, 1968; Seller and Illert, 1969; Biscoe and Sampson, 1970a,b; Spyer and Wolstencroft, 1971; Lipski et al., 1972; McAllen and Spyer, 1972; Middleton et al., 1973; Lipski and Trzebski, 1975; Lipski et al., 1975; Schwaber and Schneiderman, 1975; Spyer, 1975; Lipski et al., 1976) and in the paramedian reticular nucleus (PRN; Humphrey, 1967; Miura and Reis, 1968,1969,1972; Homma et al., 1970). These responses included short latency (0.7-2.0 msec), monosynaptic potentials as well as longer latency, polysynaptic potentials. Thus, it has been proposed that mono- and polysynaptic baroreceptor pathways project to the paramedian reticular and solitary nuclei from baroreceptor afferent nerves. However it should be mentioned that several investigators have been unable to provide evidence for a projection of baroreceptor afferent fibers to the paramedian reticular nucleus (Spyer and Wolstencroft, 1971; Lipski et al., 1975; Spyer, 1975). Electrophysiological studies have also suggested that secondary projections of the baroreceptor reflex arc synapse in the raphé nuclei, the central tegmental area of the pons, the dorsolateral reticular formation of the medulla, and the spinal cord (Humphrey, 1967; Miura and Reis, 1969; Biscoe and Sampson, 1970a,b; Lipski and Trzebski, 1975; Trzebski et al., 1975).

The spontaneous activity of NTS neurons has been classically thought to exhibit a distinct cardiac related rhythm. Recordings of units in the NTS, like those from single carotid sinus nerve fibers,

have indicated that these neurons often discharge in high frequency bursts during the systolic phase of each arterial pulse wave (Humphrey, 1967; Miura and Reis, 1969, 1972; Middleton et al., 1973; Werz et al., 1974; Schwaber and Schneiderman, 1975; Lispki et al., 1976). However, as first noted by Humphrey (1967), the majority of NTS units which responded to electrical stimulation of the sinus nerve exhibited a continuous, often irregular discharge pattern, bearing no obvious relation to the arterial pulse wave. In addition, Humphrey (1967) was unable to demonstrate an insidious cardiac rhythm after graphical averaging of the time delays between some fixed point in the cardiac cycle and the occurrence of subsequent neuronal discharges. These observations suggest that the cardiac periodicity in the discharges of baroreceptor afferent nerves is often lost within the first several synapses of the baroreceptor reflex arc.

Several investigators used post-R wave TIH analysis to accurately study the temporal relationship between the cardiac cycle and discharges in NTS units (Middleton et al., 1973; Werz et al., 1974; Schwaber and Schneiderman, 1975). NTS neurons which discharged with a prominent cardiac periodicity had a peak probability of discharge 70-90 msec after the R wave of the ECG (Middleton et al., 1973; Werz et al., 1974). The temporal relationship between this peak and the R wave is in good agreement with the start of the pulse synchronous component of carotid sinus nerve discharge which begins about 65-70 msec after the R wave (Paintal, 1972; Gebber, 1976). Middleton et al. (1973) also described NTS units whose peak probability of discharge began about 35 msec after the R wave and lasted for a period of 100 msec. A second and third period of discharge occurred 250 and 300

msec after the R wave, respectively. Schwaber and Schneiderman (1975) described three NTS units which exhibited multimodal post-R wave TIHs. Peak probability of discharge of these neurons occurred 40, 50 and 60-75 msec following the R wave. A fourth unit displayed two peaks in the histogram occurring 25 and 70 msec after the R wave. Werz et al. (1974) described similar histograms. In addition, the post-R wave TIHs of some NTS neurons were unimodal with a peak probability of discharge occurring 30 msec after the R wave. As discussed by Middleton et al. (1973) and Werz et al. (1974), peaks in the post-R wave TIHs of NTS units which are inconsistent with the start of the pulse synchronous component of carotid sinus nerve discharge (65-70 msec after the R wave) might reflect the fact that these units received pulse synchronous afferent input from sources other than the carotid sinus and aortic depressor nerves. However, no attempts have been made to define the sources of the proposed auxilliary afferent input.

Integration of inhibitory influences of baroreceptor origin and intrinsic excitatory components of the sympathetic nervous system have been postulated to occur at medullary and spinal levels. With regard to the medulla, several investigators have found that the discharges of many medullary neurons were inhibited during baroreceptor reflex activation (Salmoiraghi, 1962; Przybyla and Wang, 1967; Biscoe and Sampson, 1970a,b). More direct evidence for baroreceptor-mediated sympathoinhibition of medullary origin has been presented by Koizumi et al. (1971). Recording from sympathetic white rami, they reported that spinal reflex discharges evoked by stimulation of somatic afferents were not affected during carotid sinus distension. However, reflex discharges mediated over supraspinal pathways were inhibited

during baroreceptor reflex activation. On the basis of these observations, they suggested that sympathoinhibition of baroreceptor origin occurred at a medullary locus.

In contrast to the study of Koizumi et al. (1971), Kirchner et al. (1971) and Coote and Macleod (1974b) described inhibition of the spinal, as well as supraspinal, components of the somato-sympathetic reflex during carotid sinus distension or intravenous administration of pressor doses of norepinephrine. This observation suggests that sympathoinhibition of baroreceptor reflex origin may be mediated at spinal as well as supraspinal levels. In addition, Coote and MacLeod (1974a,b) localized two distinct descending sympathoinhibitory pathways in the dorsolateral and ventrolateral funiculi of the cord, respectively. On the basis of lesion experiments they suggested that the pathway in the dorsolateral funiculus was responsible for the spinal component of baroreceptor induced sympathoinhibition. However, it should be noted that this area also includes descending sympathoexcitatory pathways (Taylor and Brody, 1976), and the lack of inhibition following a lesion in the dorsolateral funiclus may be due, in part, to the removal of excitatory inputs to PSNs.

Gebber and coworkers have demonstrated that baroreceptor induced sympathoinhibition is mediated at brain stem and spinal levels. Taylor and Gebber (1975) described the time course of computer-summed inhibition of splanchnic nerve activity evoked by stimulation of the carotid sinus nerve, aortic depressor nerve, and the paramedian reticular nucleus. They found that trains of pulses applied to these sites elicited both an early and a late phase of positivity (i.e., inhibition) of splanchnic nerve activity. Splanchnic nerve discharge

evoked from descending spinal tracts was depressed by stimulation of the paramedian reticular nucleus during the time course of the early but not the late inhibition. In addition, it was shown that mid-collicular transection did not eliminate either phase of inhibition. These observations indicate that the early positivity monitored baroreceptor-induced sympathoinhibition at a spinal site, while the late positivity reflected sympathoinhibition at a brain stem locus. A spinal component of the baroreceptor reflex arc was also suggested by the finding that baroreceptor reflex activation resulted in the depression of sympathetic responses evoked by spinal stimulation of descending sympathoexcitatory fibers (Gebber et al., 1973; Snyder and Gebber, 1973).

STATEMENT OF PURPOSE

The purpose of the present investigation was to study the organization of spinal sympathetic pathways. Unlike other systems in the spinal cord, the organization of spinal sympathetic pathways is not well understood. Virtually no experimental evidence exists to suggest that sympathetic interneurons are interposed between the terminals of reticulospinal systems and PSNs. Implicit with this statement is the fact that spinal sympathetic pathways have only a limited capacity to integrate neuronal inputs. Thus, the general view is that spinal sympathetic pathways are rather simply organized. Our lack of knowledge concerning these pathways results from an inability to identify sympathetic neurons whose processes are contained solely within the central nervous system. Recently studies have demonstrated that it is possible to employ computer-aided techniques to identify central sympathetic neurons (Gebber, 1975; Gootman et al., 1975; Langhorst et al., 1975,1976). The first portion of this study was designed to take advantage of such techniques in order to answer a number of questions concerning the organization of spinal sympathetic pathways. First, are interneurons interposed between the terminals of reticulospinal systems and PSNs? If so, can the discharge patterns of the interneurons be distinguished from those of preganglionic neurons? Are the interneurons contained within both sympathoexcitatory and sympathoinhibitory pathways; and if so, can these two populations of interneurons

be distinguished? What are the functional interrelationships between these sympathetic elements and PSNs. Finally, the question of the locus of spinal sympathetic inhibition (interneuronal or preganglionic) was investigated.

The spontaneous discharges of sympathetic nerve bundles exhibit several periodic components in the vagotomized cat. The cardiac- (3-5 c/sec) and respiratory-related periodicities of SND are representative of brain stem rhythmogenic mechanism which are normally entrained respectively to the cardiac and respiratory cycles by extrinsic influences (Taylor and Gebber, 1975; Barman and Gebber, 1976). In addition, a third and more rapid sympathetic periodicity (≈10 c/sec) has been described (Cohen and Gootman, 1970). Although recordings made from sympathetic nerve bundles often reveal mixtures of the three periodicities of SND, it is not known whether they are mediated by the same or different populations of PSNs. The second portion of this study was designed to differentiate between these alternative possibilities. The rhythmic components of PSN discharge were studied using time interval and autocorrelation analysis.

The 10 c/sec periodicity in SND is normally dependent upon supraspinal driving inputs (Gootman and Cohen, 1973). However, the level of the neuraxis at which sympathetic activity is synchronized into 10 c/sec slow waves is unknown. Therefore, the final portion of this investigation was designed to determine the origin of the 10 c/sec periodicity of SND. Specifically, patterns of renal SND were compared in spinal and intact cats.

METHODS

Cats of either sex, weighing between 2.0 and 4.0 kg were used in this study. The majority of animals were anesthetized by the intraperitoneal injection of a mixture of sodium diallybarbiturate (60 mg/kg), urethan (240 mg/kg), and monoethylurea (240 mg/kg). The remaining experiments were performed on unanesthetized high spinal cats. Rectal temperature was maintained between 36 and 38°C with a heat lamp. Blood pressure was monitored from the lumbar aorta (via a femoral cathether) with a Statham transducer (model P23AC) and displayed on a Grass polygraph. Tracheal pressure was measured in some experiments. All drugs were administered through a cannula inserted into the femoral vein. The electrocardiogram (ECG, lead II) also was recorded.

In the majority of experiments animals were immobilized with gallamine triethiodide (4 mg/kg; i.v.) and artifically respired. The volume of the respirator (Harvard, model 607) was adjusted between 35-60 ml/stroke at 12-16 strokes/min depending on the weight of the cat. Bilateral pneumothoracotomy was performed to minimize movements associated with artifical respiration. Supplemental doses (2 mg/kg, i.v.) of gallamine were administered, as required, during the course of the experiment. The doses of gallamine employed failed to affect spontaneously occurring SND recorded from either peripheral nerve bundles or single sympathetic neurons. In some experiments the animals were allowed to respire spontaneously.

A. Experiments Characterizing Spontaneous Discharges of Sympathetic Nerve Bundles

The experiments were performed on intact anesthetized cats or unanesthetized spinal animals. Spinal transection at the first cervical segment was performed under halothane-nitrous oxide anesthesia. Animals were placed in a David Kopf Instruments stereotaxic apparatus and the overlying connective tissue, muscles and dorsal arch of the first and second cervical segments were removed, thereby exposing the spinal cord. After removal of a portion of the dura mater, the spinal cord was quickly sectioned using an iris scissors. Nerve recordings were made not less than 3 hours after spinal transection and discontinuation of the anesthesia. The completeness of the spinal transection was visually evaluated at the end of each experiment.

1. Nerve Recording and Data Analysis

The left postganglionic sympathetic renal nerve was exposed via a retroperitoneal approach. One of the branches was ligated with a saline-soaked silk thread and sectioned between the tie and its entrance into the kidney. A 5 mm diameter loop was tied in the thread and the proximal portion of the renal nerve bundle was positioned on one of two platinum electrodes. The silk loop was placed on the indifferent platinum electrode in order to record the efferent discharges of the renal nerve monophasically. The abdominal skin flaps were secured to a specially constructed frame in order to form a pool, subsequently filled with warm mineral oil (Fisher Scientific Co., Paraffin O-120). The oil pool was sufficiently deep to cover the nerve and recording electrodes.

Nerve potentials were amplified with a capacity coupled preamplifier (high and low pass filter settings at 1 and 1,000 Hz,

respectively). Nerve activity was stored on magnetic tape and simultaneously displayed on a polygraph and oscilloscope. The autocorrelation function of SND was analyzed using a Nicolet 1070 series computer. This type of analysis was used to accurately assess the periodicity of sympathetic nervous activity. The period of the autocorrelogram approximates the duration of sympathetic waves over many trials. If the occurrence of sympathetic oscillations is random with respect to one another, the autocorrelation function approaches at straight line.

In some experiments the temporal relationship between sympathetic and phrenic nerve discharge was studied. The left phrenic nerve was exposed via a ventral approach in the neck following reflection of a portion of the trachea and esophagus. Nerve potentials were recorded under oil with bipolar platinum electrodes after capacity-coupled preamplification (high and low pass filter settings at 10 and 1,000 Hz, respectively). Following RC integration (time constant, 0.05 sec), phrenic nerve potentials were simultaneously displayed on a polygraph and oscilloscope. The vagus nerves were sectioned bilaterally in these experiments.

2. Neural Stimulation

Pressor sites in the cervical spinal cord were stimulated in order to evoke activity in the renal nerves of spinal cats. The fourth cervical spinal segment was exposed using the method described above, and a portion of the dura mater was removed. The dorsal roots were sectioned at the dorsolateral sulcus. The dorsolateral sulcus and the cord surface were used as the reference points for lateral and vertical orientation. To locate descending spinal pressor tracts, the

electrodes were always positioned lateral to the dorsolateral sulcus, usually in the dorsolateral portion of the white column. Mineral oil was intermittently poured over the cord to prevent the nervous tissue from drying. Sites in the cord were stimulated with square wave pulses passed from a Grass S88 stimulator through a stimulus isolation unit (Grass, model SIU-5) to bipolar, concentric, stainless steel electrodes (David Kopf Instruments, model SNE-100). The center lead of the electrode and shaft (outer contact) were exposed 0.25 mm. The distance between the two leads was 0.50 mm. Sites were stimulated continuously for 30 sec periods at 6-12 v, 0.5 msec, and 20-50 Hz. Stimulation was performed ipsilateral to the recording electrodes.

B. Experiments Characterizing the Discharges of Single Sympathetic Units

1. Sympathetic Unit Recording

a. Cervical Sympathetic Fiber Recording

Cats were placed in a David Kopf Instruments stereotaxic apparatus. The right cervical sympathetic nerve was exposed via a ventral approach after reflection of a portion of the trachea and esophagus. Following section near its entrance into the superior cervical ganglion, the nerve was placed under oil on a small laryngeal mirror and the epineural sheath was removed using a fine dissecting needle. The cervical sympathetic nerve was then split into fine branches under a dissection microsope. The branches were layed on a bipolar platinum electrode and unitary discharges were amplified with a capacitance-coupled Grass P511 preamplifier (low and high half-amplitude responses at 100 and 1,000 Hz, respectively). Recordings were made only from those branches in which spontaneously active

fibers could easily be distinguished from one another. A single unit was indicated by the constancy of shape and amplitude of spontaneously occurring action potentials during a high speed sweep on a oscilloscope.

b. Spinal Sympathetic Unit Recording

Animals were placed in a David Kopf Instruments stereotaxic apparatus and spinal investigation unit. The spinal cord was exposed from the first to the fourth thoracic segments. The dura mater was removed. The fifth thoracic spinous process was clamped to hold the spinal cord rigidly in place. Platinum-coated stainless steel microelectrodes (Frederick Haer and Co.) with 1 μm tip diameters and exposed tip lengths of 20 μm were used to monitor unit discharges in the spinal cord. Electrode tip resistance ranged from 1-3 megohms. Unit discharges were recorded extracellularly between the microelectrode tip and an indifferent gold electrode which was placed on the frontal bone. Unitary discharges were amplified with a capacitance-coupled Grass P511 preamplifier (low and high half-amplitude responses at 300 Hz and 3,000 Hz, respectively). In experiments in which the IML cell column was explored, the dorsal roots were sectioned and tip of the microelectrode was positioned on or within 100 μm of the dorsolateral fissure (point of entry of dorsal roots). In other experiments the dorsal median sulcus and/or dorsolateral sulcus were used for orientation in the lateral plane. The position of the microelectrode was controlled by a David Kopf Instruments stepping hydraulic microdrive. Recordings were always made on the left side of the spinal cord. Permanent records of unitary activity were made on 35 mm film. In addition, unit discharges were passed through a window

discriminator (F. Haer and Co., Model 74-45-1), the output (5 v pulses) of which was recorded on a grass polygraph. Electrode positions were determined histologically as described below.

c. Medullary Unit Recordings

In several experiments the occipital bone and a portion of the cerebellum were removed to expose the caudal medulla. The microelectrode was placed into the NTS using the obex as a surface landmark and the stereotaxic coordinates of Berman (1968). Recording techniques were identical to those described above. Electrode positions were determined histologically.

2. Electrical Stimulation

a. Stimulation of Sympathetic Nerve Bundles

The left preganglionic cervical sympathetic nerve was isolated at the level of the third cervical vertebra, sectioned, and submerged in a pool of warm mineral oil. Stimulation of the central end of the nerve with bipolar platinum electrodes was used for the antidromic identification of preganglionic neurons lying in thoracic spinal segments. The parameters of the square wave applied to the cervical sympathetic nerve were 3-10 v and 0.1-0.5 msec.

Spinal and medullary units were activated orthodromically by stimulation of afferent fibers in the left inferior cardiac nerve. The nerve was isolated following removal of a portion of the head of the second rib, and was stimulated, under oil, at its site of exit from the stellate ganglion. The parameters of stimulation were 2-7 v and 0.05-0.2 msec.

b. Brain Stem Stimulation

The skin, muscle, occipital bone, and meninges overlying the cerebellum were removed. Electrical pulses were applied to selected sites in the medulla oblongata by means of a Grass S88 square-wave stimulator, the output of which was passed through a stimulus-isolated unit to bipolar concentric stainless steel electrodes (David Kopf Instruments, model SNE-100). The electrodes were stereotaxically positioned according to the coordinates of Berman (1968). Pressor and depressor sites at a level 2-3 mm rostral to the obex were identified by high frequency (10 v; 0.5 msec; 50 Hz) stimulation for 10 second periods. The effects produced on spinal units by single shocks (10 v; 0.5 msec) or 5 msec trains of 3 pulses (10 v; 0.5 msec; 600 Hz) applied to these sites were observed. Stimulation always was performed on the side ipsilateral to the recording microelectrode.

3. Data Analysis

Unit discharges, blood and tracheal pressure, and timing pulses coincident with the R wave of the ECG, the onset of expiration, or stimuli applied to the medulla and cervical sympathetic or inferior cardiac nerves were recorded on magnetic tape. The data on tape were analyzed by Nicolet 1070 or Med-80 computers. Unit recordings were subjected to window discrimination before presentation to the computer. The memory content of either computer was displayed in analog form on an oscilloscope or X-Y recorder. In addition, digital printouts were obtained from the Nicolet Med-80 computer. The following methods of analysis were employed. 1) Post-R wave time interval histogram (post-R wave TIH). The probability of spontaneously occurring unitary discharge during the phases of the cardiac cycle was established

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by triggering the sweep of the computer with the timing pulse derived from the R wave of the ECG. The arterial pulse wave was simultaneously averaged in some experiments. 2) Post-expiratory time interval histogram (post-expiratory TIH). This histogram was constructed by triggering the sweep of the computer with the timing pulse derived from the expiratory phase of the tracheal pressure recording. The histogram was used to depict the probability of spontaneously occurring unitary discharge during the phases of the central respiratory cycle. 3) Autocorrelograms. Spontaneous unitary discharges were used to trigger the sweep of the computer. The post-spike histogram described the autocorrelation function of a unit and was used to define periodicities within unitary discharges. 4) Interspike interval histogram (ISIH). This histogram depicted the probability distribution of intervals between spontaneously occurring unitary spikes. 5) Post-stimulus histogram (PSH). PSH depicted the distribution of occurrence of unitary discharge following a stimulus applied to pressor or depressor sites in the medulla or to the cervical sympathetic and inferior cardiac nerves. 6) First-order latency histogram (LH). This histogram was used to define the distributions of intervals from the electrical stimulus to the first discharge in the resulting spike train.

4. Histology

The medulla and/or thoracic spinal segments were removed and fixed in formalin following most experiments. The formalin-fixed tissue was cut in sections of 30 μm thickness with a cryostat-microtome (Lipshaw Cryotome, model 1500). Sections were cut in the frontal

plane to allow for the identification of electrode tracts and lesions. Sections were stained with cresyl violet for cell bodies according to a modified method of Powers and Clark (1955). Shrinkage of 15% was taken into account before the position of stimulating or recording sites were determined.

C. Drugs

The following drugs were used: gallamine triethiodide, hexamethonium chloride, and norepinephrine bitartrate. All doses are expressed in terms of the salt.

D. Statistical Analysis

Statistical analysis was performed with the Student's *t*-test for unpaired data. P values of less than 0.05 were considered to indicate statistical significance. Values are expressed as means ± standard error. The Pearson correlation test also was used.

RESULTS

I. Identification and Discharge Patterns of Spinal Sympathetic Interneurons

Great difficulty has been encountered in identifying central neurons involved in regulating the discharges of preganglionic sympathetic neurons (PSNs). Recently it has been shown that the identification of sympathetic neurons whose processes lie within the central nervous system requires methods that present a picture of the probability of unitary discharge during the phases of the cardiac cycle or of the discharge pattern of whole sympathetic nerves (Gebber, 1975; Gootman et al., 1975). One of these methods, the post-R wave time interval histogram (post-R wave TIH) was employed in the present study to identify spinal interneurons contained within sympathoexcitatory and sympathoinhibitory pathways. In addition, a test for antidromic activation was used to differentiate between PSNs and spinal interneurons whose discharges were temporally related to the R wave of the ECG. Finally, comparison of the response patterns of PSNs and sympathetic interneurons to stimulation of medullary pressor and depressor sites was used to determine the relationship between spinal sympathetic elements. Nerve cells interposed between the terminals of reticulospinal systems and preganglionic units will be referred to as spinal sympathetic interneurons (SIN) in the present study.

A. Spinal Interneurons Contained Within Sympathoexcitatory Pathways

1. Non-antidromically Activated Units in the Recording Field of Preganglionic Neurons

In the first series of experiments the intermediolateral cell column of the thoracic spinal cord was explored with microelectrodes in order to record unitary discharges. Figure 1 shows the discharges of two units recorded 1.47 mm below the dorsolateral fissure of the second thoracic spinal segment (within the intermediolateral cell column). Using the criteria of Hongo and Ryall (1966b), Polosa (1967), and Taylor and Gebber (1973), the larger spike monitored the antidromic activation of a preganglionic unit since: 1) spike onset latency and amplitude remained essentially constant to each shock (1/sec) applied to the cervical sympathetic nerve; 2) the response was all or none and exhibited a sharp threshold; and 3) the IS component of the spike followed frequencies of cervical sympathetic nerve stimulation in excess of 50 Hz. The preganglionic unit in Figure 1 was not spontaneously active. The smaller unitary spike appearing in panels B, E, and F of Figure 1 did not show a time-locked relation to the stimulus applied to the cervical sympathetic nerve. Thus, this neuron was spontaneously active but could not be antidromically excited. Wyszogrodski and Polosa (1973) also noted spontaneously active units which could not be antidromically excited in the recording field of identified thoracic preganglionic neurons of the cat. They suggested that such units might be spinal sympathetic interneurons lying within or near the intermediolateral cell column. This possibility was vigorously tested in the present series of experiments.

Figure 1. Neuronal types within thoracic intermediolateral cell column of a cat. Large preganglionic unitary spike (A-F) was elicited by antidromic stimulation (10 V; 0.25 msec; 1 Hz) of cervical sympathetic nerve. Negativity is recorded as an upward deflection in this and all subsequent figures and spikes were retouched. Smaller spontaneously occurring unitary spike (B,E,F) was elicited from a cell that was not antidromically activated by stimulation of cervical sympathetic nerve. Vertical calibration is 100 μV. Horizontal calibration is 20 msec.

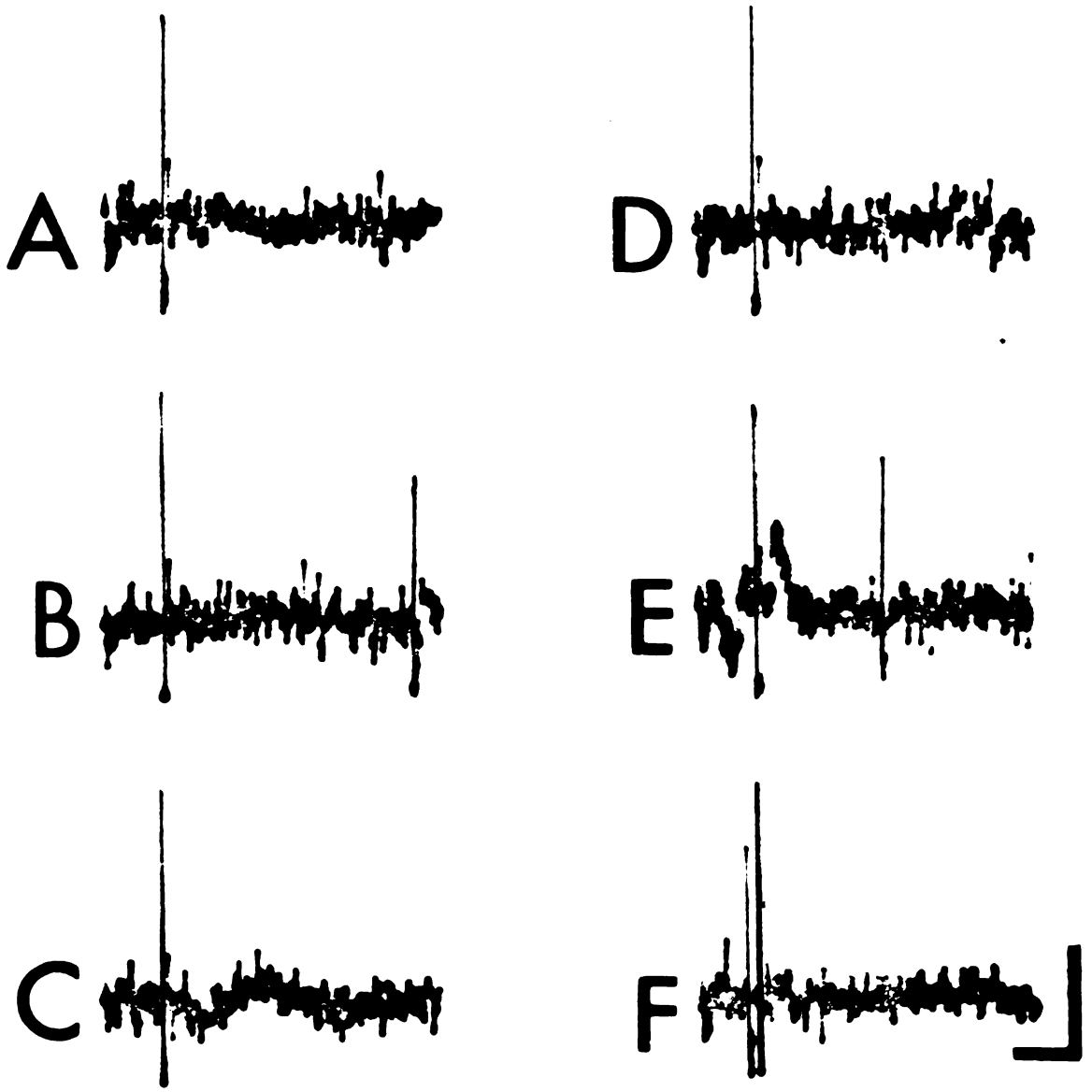

Figure 1

Figure 2 compares the spontaneous discharge patterns of antidromically and nonantidromically excited units lying in the region of the intermediolateral cell column. Fifty-four of 150 antidromically identified preganglionic units located from 1.2-2.0 mm below the dorsolateral fissure were spontaneously active. Their axonal conduction velocities ranged from 2-14 m/sec. As reported by others (Jänig and Schmidt, 1970; Mannard and Polosa, 1973; Seller, 1973; Taylor and Gebber, 1973), the spontaneous discharges of these units did not exhibit an obvious cardiac rhythm (Figure 2A-C). That is, preganglionic units missed firing in a significant number of cardiac cycles although single discharges in successive heart beats often were observed (Figure 2A,B). Four units were found which occasionally discharged twice during a cardiac cycle (Figure 2B).

Non-antidromically activated cells (n=43) in the region of the intermediolateral cell column fired in bursts with some very short (<20 msec) interspike intervals (Figure 2D-F). Four of these cells discharged during each cardiac cycle (Figure 2D). The majority, however, missed firing during a significant number of cardiac cycles (Figure 2E,F). This observation argues against the possibility that the high frequency discharge pattern resulted from mechanical irritation produced by arterial pulse-related movement of the recording electrode. Figure 2F shows recordings from a nonantidromically activated unit which fired in couplets with interspike intervals as short as 2 msec.

Extreme care was taken to insure that the high frequency discharge pattern (short interspike intervals) was recorded from one neuron rather than from multiple units with equivalent spike

Figure 2. Spontaneous discharge patterns of antidromically identified preganglionic neurons (PSN) and non-antidromically excited neurons (sympathetic interneurons; SIN) located in vicinity of intermediolateral cell column. A-C: PSN. D-F: SIN. Top trace in each panel is blood pressure. Bottom trace shows unitary discharges. Blood pressure was in millimeters of mercury: 160/120 (A); 170/120 (B); 125/70 (C); 160/125 (D); 185/145 (E); 165/115 (F). Vertical calibration is 200 μV for panel A, 100 μV for panels B-E, and 400 μV for panel F. Horizontal calibration is 220 msec for panels A-E and 110 msec for panel F.

heights. First, the constancy of the positive and negative components of each spike in a train was ascertained on an oscilloscope with a sweep speed of 1-5 msec/cm. Second, the microelectrode was moved 25-50 μm first in a dorsal direction and then in a ventral direction from the point of maximal spike amplitude. Discharges were considered unitary if movement of the electrode failed to reveal differences between the contour and amplitude of individual spikes within a train. Maximum spike height of the non-antidromically excited cells usually was observed about 75-100 μm ventral to the point where spike amplitude of preganglionic units in the same recording field was greatest. In some cases, the preganglionic unitary spike(s) disappeared from the recording field when the electrode was positioned to record maximum spike amplitude of the non-antidromically excited cell.

2. Computer-aided Analysis of Spontaneous Discharge Patterns of Preganglionic and Non-antidromically Activated Spinal Neurons

Post-R wave TIH and interspike interval histograms (ISIH) of spontaneously occurring unitary discharge were constructed to test the possibility that non-antidromically activated units located in the region of the intermediolateral cell column were sympathetic interneurons interposed between the terminals of reticulospinal fibers and preganglionic neurons. Only those units which could be easily separated from all others in the recording field by window discrimination were subjected to computer analysis.

a. Post-R Wave TIH

It was reasoned that a unit could be classified as sympathetic in function if its probability of spontaneous discharge was related in time with the R wave of the ECG (via baroreceptor

reflex mechanisms). Post-R wave TIH for preganglionic cells and closely adjacent non-antidromically activated neurons are shown in Figure 3. The probability of discharge of 15 non-antidromically activated neurons was strongly correlated in time with the R wave. Peak probability of discharge occurred 159±7 msec after the R wave. Examples are shown in Figure 3A-C. With respect to the lumbar aortic pulse wave, the probability of discharge of these neurons was greatest during systole and early diastole. It should be noted, that the falling phase of the pulse-synchronous component of carotid sinus nerve discharge precedes peak systole recorded from the lumbar aorta (Gebber, 1976). The discharge pattern of 28 additional non-antidromically activated units located near or in the recording field of preganglionic neurons failed to show a positive R wave relationship.

Typical examples of the time relations between the spontaneous discharges of antidromically-identified preganglionic neurons and the lumbar aortic pulse wave are shown in Figure 3D-F. R wave locking of spontaneous preganglionic unitary discharge was present in 23 cells (panels D and E) and absent in 14 cells (panel F). Peak probability of discharge occurred 177±10 msec after the R wave for the preganglionic units which showed a positive R wave relationship. The negative post-R wave relationship for the unitary discharges shown in panel F was not indicative of malfunctioning or inoperative baroreceptor reflexes, since strong R wave locking was observed for the discharges of a non-antidromically activated cell (panel C) located in the same recording field. Indeed, as described above, preganglionic units whose discharges showed markedly different degrees of correlation in time with the R wave often were located in

Figure 3. Phase relations between averaged arterial pulse wave and post-R wave TIH of spinal unitary discharge. A-C: sympathetic interneurons (SIN). D-F: antidromically identified preganglionic units (PSN). Units A and D were recorded from same cat, units B and E from another cat, and units C and F from a third animal. Sample run was 128 sec for panels A-D, 292 sec for E, and 217 sec for F. Address bin was 4 msec for all panels. Blood pressure was in millimteres of mercury: 140/100 (A); 160/105 (B); 160/100 (C); 140/100 (D); 160/100 (E); 160/100 (F).

the same cat during a time span when blood pressure remained essentially unchanged.

The spontaneous discharges of the majority of antidromically identified preganglionic neurons were inhibited during the pressor action (50-80 mmHg) produced by the intravenous injection of norepinephrine (i.e., baroreceptor reflex activation (Gebber et al., 1973; Taylor and Gebber, 1973)). Each of the 15 non-antidromically activated neurons in the IML cell column whose spontaneous discharges were correlated in time with the R wave was inhibited during the pressor action of norepinephrine. An example is shown in Figure 4. In no case was the discharge rate of these neurons increased during the pressor action of norepinephrine.

b. ISIH

The ISIH of non-antidromically activated neurons whose spontaneous discharges were correlated in time with the R wave were bi- or multimodal (Figure 5). The characteristics of these histograms are summarized in Table 1. The first modal interval was usually less than 20 msec indicating that, as shown in Figure 2D-F, these units discharged in high frequency bursts. The second modal interspike interval was not significantly different from the period of the cardiac cycle (R-R wave interval). Subsequent modal intervals were separated by a period approximating the cardiac cycle. Bimodal ISIH (Figure 5) indicated that the unit discharged in bursts during each cardiac cycle (see Figure 2D). Multimodal ISIH (Figure 5B,C) indicated that unitary discharge did not occur during each cardiac cycle (see Figure 2E,F). The ISIH in Figure 5B,C show that the units missed firing for as many as 6 and 10 heart beats, respectively.

Figure 4. Inhibition of spontaneous discharges of a spinal sympathetic interneuron (SIN) during pressor action of norepinephrine (1 μg/kg, i.v.). A: top trace is blood pressure (mmHg). Bottom trace shows 5-v pulses from a window discriminator monitoring discharge of unit. Norepinephrine was injected at downard deflection of time base (1 sec/division). B: 90 sec after injection of norepinephrine.

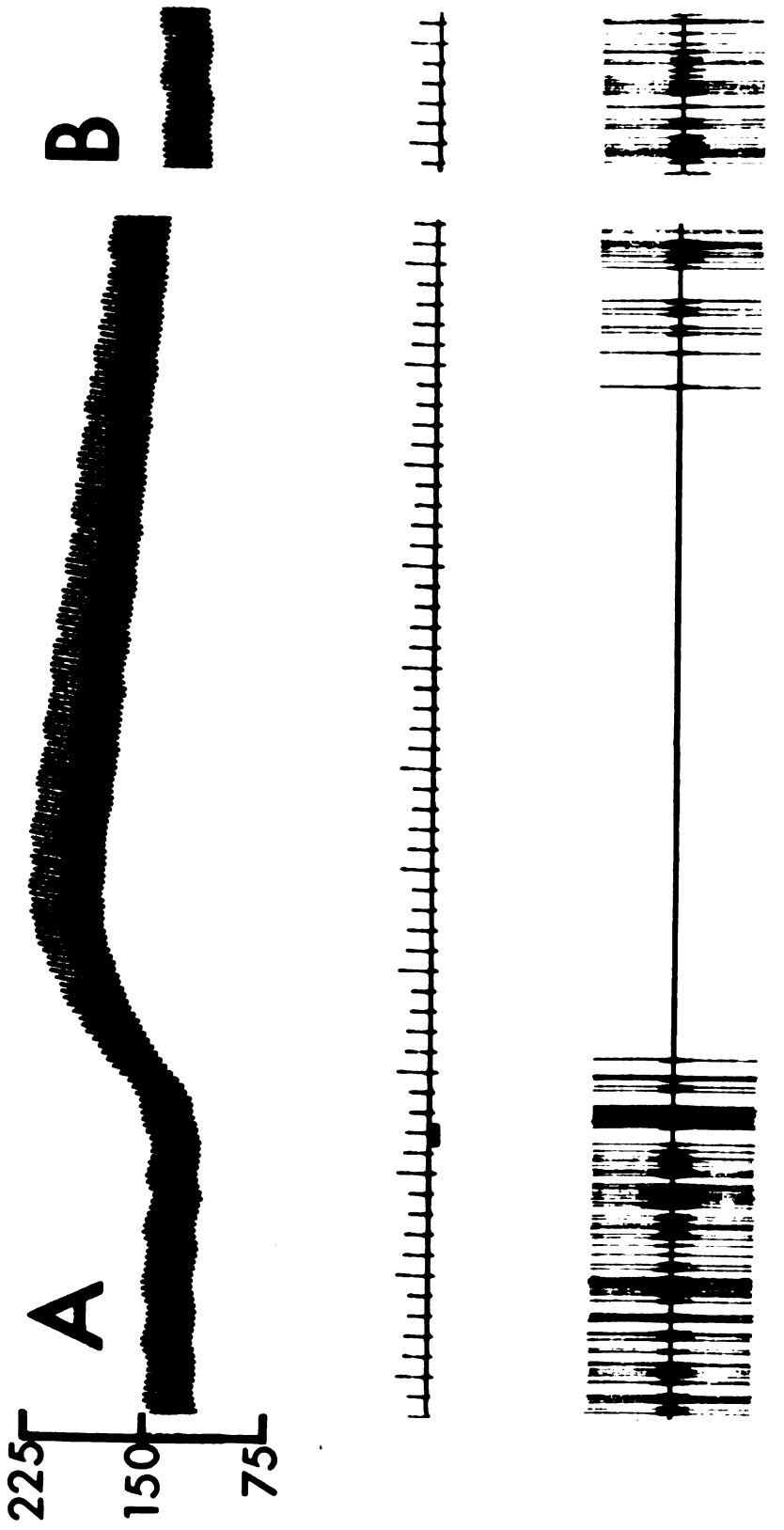

Figure 4

Figure 5. Typical interspike interval histograms (ISIH) exhibited by spinal sympathetic interneurons (SIN). ISIH for 3 different units are illustrated on the left in A-C. The phase relations between the averaged arterial pulse wave and post-R wave TIH for the same units are shown on the right side. ISIH: 640 spikes (A), 713 spikes (B), 900 spikes (C). Address bin was 10 msec. Post-R wave TIH: Sample run was 128 sec for A and B, 87 sec for C. Address bin was 4 msec for A and B, 1 msec for C. Blood pressure was in mmHg: 155/110 (A); 150/105 (B); 160/105 (C).

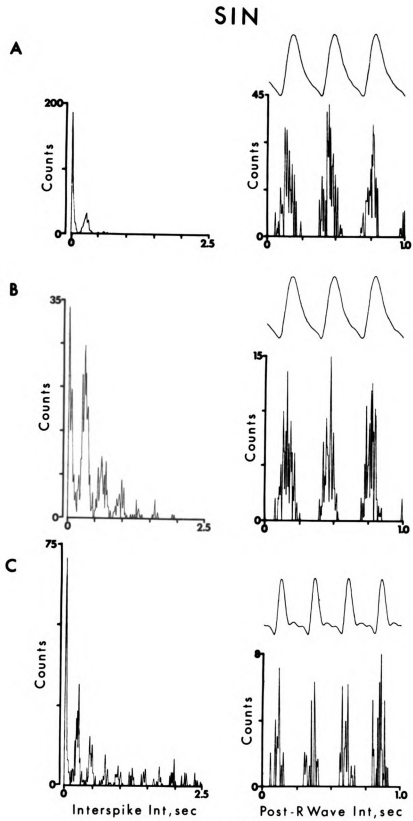
SIN
A
B
C
Counts
200
0
2.5
45
1.0
35
15
75
8
Interspike Int, sec
Post-R Wave Int, sec

Figure 5

TABLE 1

Characteristics of Spontaneous Discharge Patterns of Spinal Sympathetic Interneurons (SIN) and Preganglionic Units (PSN)

Characteristics	ISIH TYPE			
	Unimodal	Bimodal	Multimodal	
	PSN n=7	SIN n=4	SIN n=8	PSN n=8
Mean Discharge freq., spikes/ sec	1.0±0.2 (0.3-1.4)	8.6±2.1* (5.8-15.0)	4.0±0.8* (1.9-8.0)	2.1±0.2 (1.4-3.4)
First modal interval, msec	593±62 (400-780)	13±3 (10-20)	21±5 (10-40)	328±12 (3160-360) 117±3[a] (115-125)
Second modal interval, msec	---	340±53 (240-500)	281±8 (250-300)	622±23 (560-670) 351±31[a] (260-395)
Third modal interval, msec	---	---	573±15 (510-610)	933±19 (880-960) 661±64[a] (470-735)
Fourth modal interval, msec	---	---	880±28 (750-980)	1212±45 (1110 1320)
R-R wave interval, msec	342±29 (275-470)	376±31 (300-450)	288±10 (245-320)	321±17 (250-380)

Values are mean ± standard error with ranges in parenthesis. n = number of cells. *Significantly different ($P<0.05$) than values for PSN. [a]The first modal interval of 117 msec reflected the ability of 4 PSN to occasionally discharge twice during a cardiac cycle. ISIH of those 4 cells were trimodal.

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Third modal interval, msec	---	---	573±15 (510-610)	933±19 (880-960) 661±64[a] (470-735)
Fourth modal interval, msec	---	---	880±28 (750-980)	1212±45 (1110 1320)
R-R wave interval, msec	342±29 (275-470)	376±31 (300-450)	288±10 (245-320)	321±17 (250-380)

Values are mean ± standard error with ranges in parenthesis. n = number of cells. *Significantly different ($P<0.05$) than values for PSN. [a]The first modal interval of 117 msec reflected the ability of 4 PSN to occasionally discharge twice during a cardiac cycle. ISIH of those 4 cells were trimodal.

The characteristic which most clearly distinguished the ISIH of antidromically identified preganglionic and nonantidromically activated units was the absence of the 10-20 msec modal interspike interval for preganglionic cells (Figure 6). This observation indicated that preganglionic units did not discharge in high frequency bursts (see Figure 2A-C). The characteristics of preganglionic ISIH are summarized in Table 1. The ISIH of units whose spontaneous discharges were strongly correlated in time with the R wave were multimodal. With four exceptions, the first modal interspike interval for these preganglionic cells was not significantly different from the R-R wave interval (Figure 6A). Subsequent modal intervals were separated by the period of the cardiac cycle. The first modal interval in the ISIH for the remaining 4 cells averaged 117 msec (Figure 6B). This value could be related to the ability of these 4 preganglionic units to discharge occasionally twice during a cardiac cycle (see Figure 2B). The ISIH of preganglionic units whose discharges showed no relationship or only a vague correlation in time with the R wave were essentially unimodal and positively skewed (Figure 6C). The modal interspike interval ranged from 400 to 780 msec. These histograms exhibited the characteristics of those described by Mannard and Polosa (1973) for irregularly firing preganglionic neurons in the cat. Mannard and Polosa also reported multimodal ISIH for preganglionic neurons which were similar to those presented in this study.

Figure 6. Typical ISIH exhibited by antidromically identified preganglionic neurons (PSN). ISIH for 3 different units are illustrated on the left A-C. The phase relations between the averaged arterial pulse wave and post-R wave TIH for the same units are shown on the right side. ISIH: 548 spikes (A), 458 spikes (B), 435 spikes (C). Address bin was 10 msec in A and B, 20 msec in C. Post-R wave TIH: Sample run was 256 sec (A), 194 sec (B) and 1000 sec (C). Address bin was 4msec for A and B, 2 msec for C. Blood pressure was in mmHg: 160/125 (A); 135/90 (B); 175/125 (C).

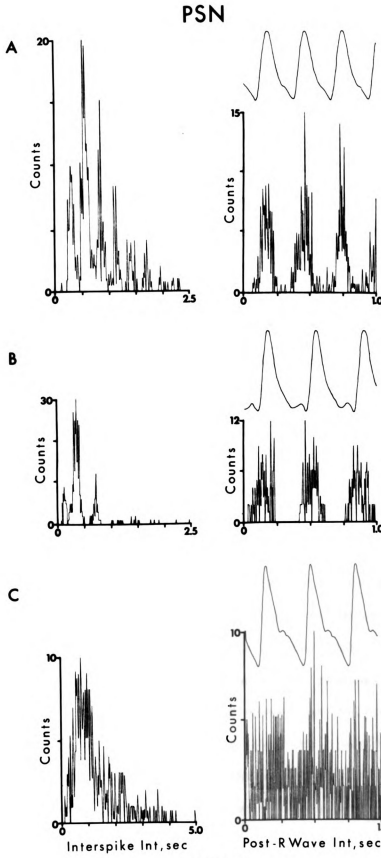
PSN
A
B
C
Counts
20
15
30
12
10
0
2.5
1.0
5.0
Interspike Int, sec
Post-R Wave Int, sec

Figure 6

3. Responses of Spinal Units to Stimulation of Medulla

a. Response Patterns Produced by Stimulation of Pressor Sites

Preganglionic units and non-antidromically activated neurons also could be distinguished by their response patterns to single shocks (10 v; 0.5 msec) applied to pressor sites within nucleus reticularis parvocellularis and nucleus reticularis ventralis of the lateral medulla (Figure 7). These nuclei form part of the classical medullary pressor region (Alexander, 1946; Gebber et al., 1973). The responses in the first column of Figure 7 are typical of those of non-antidromically activated spinal units whose spontaneous discharges were strongly correlated in time with the R wave. These neurons discharged repetively with some very short interspike intervals (<20 msec) when single shocks were applied once every 2 sec to a medullary pressor site. As shown in the post-stimulus histogram (PSH) depicted in Figure 8A, the spike train elicited in non-antidromically activated units by medullary stimulation was followed by a "silent period" lasting several hundred msec.

Antidromically identified preganglionic units usually discharged only once to each shock applied to the medullary pressor region. This characteristic is shown in the second column of Figure 7. As previously reported by Taylor and Gebber (1973) and Gebber (1975), the onset latency of the single preganglionic spike was quite variable. The PSH in Figure 8B shows that the probability of unitary discharge exceeded background level for a period from 42-100 msec following the shock applied to the medullary pressor site. In contrast, and as would be expected, the discharge onset latency of the same preganglionic unit to antidromic stimulation of the cervical

Figure 7. Responses of spinal sympathetic interneuron (SIN) and antidromically identified preganglionic cell (PSN) to stimulation of medullary pressor region. SIN and PSN discharges were recorded from 2 different cats. Single shocks (10 v; 0.5 msec) were applied once every 2 sec to sites in nucleus reticularis ventralis at a level 2 mm rostral to the obex. Vertical calibration is 100 μV. Horizontal calibration is 45 msec for SIN and 20 msec for PSN.

Figure 8. Post-stimulus histograms (PSH) of discharges elicited in a sympathetic inteneuron (SIN) and a preganglionic unit (PSN) in the same recording field. A: SIN response pattern to single shocks (10 v; 0.5 msec) applied once every 2 sec to a medullary pressor site in nucleus reticularis ventralis (100 trials). B: PSN response pattern produced by stimulation of same pressor site (100 trials). C: PSN response pattern (same cell as in B) to antidromic activation (10 v; 0.25 msec; 1 Hz) of cervical sympathetic nerve (100 trials). Address bin was 4 msec for each PSH. A delay of 2 msec for PSH in A and B was used to eliminate stimulus artifact from the trace.

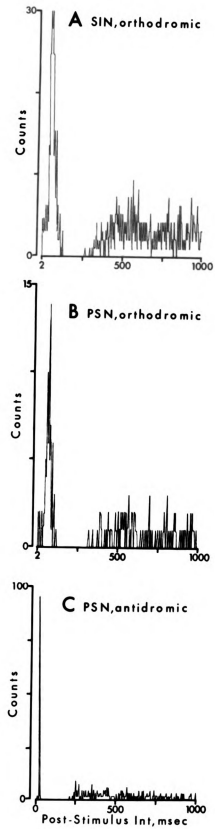
A SIN, orthodromic
B PSN, orthodromic
C PSN, antidromic
Counts
30
15
100
0
2
500
1000
Post-Stimulus Int, msec

Figure 8

sympathetic nerve was essentially constant (Figure 8C). The period of post-excitatory depression following orthodromic or antidromic activation of the preganglionic cell (Figure 8B,C) was equivalent in duration to the "silent period" following the spike train elicited in the non-antidromically exicted unit (Figure 8A). The data shown in Figure 8 were obtained from 2 units in the same recording field.

The characteristics of the response patterns of preganglionic and non-antidromically activated neurons produced by stimulation of medullary pressor sites are summarized in Table 2. Conduction velocity from the stimulating to the recording sites was not significantly different for the two neuronal types. This observation suggests that the preganglionic and non-antidromically activated cells were closely adjacent and interconnected components of the same sympathetic pathway. First order latency histograms (LH) showed that the discharge onset latency of the first spike in the train elicited in non-antidromically activated neurons upon medullary stimulation was almost as variable as that for the single discharge elicited in preganglionic units (Table 2). The first order LH for the non-antidromically activated unit whose PSH appeared in Figure 8A is shown in Figure 9.

b. Response Patterns Produced by Stimulation of Depressor Sites

PSH depicting the time course of inhibition of spontaneously occurring spinal unitary discharge produced by 5 msec trains of 3 pulses applied to depressor sites in the paramedian reticular nucleus are shown in Figure 10. The paramedian nucleus lies within the classical medullary depressor region; and is purported to function as a relay station in the baroreceptor reflex arc (Humphrey,

TABLE 2

Characteristics of Response Patterns Elicited in 7 SIN and 11 PSN by Single Shocks Applied to Medullary Pressor Region

Characteristic	SIN	PSN
Earliest discharge onset latency[a], msec	31±3 (24-45)	41±6 (16-80)
Maximum conduction velocity[b], m/sec	3.3±0.3 (2.2-4.2)	3.1±0.6 (1.3-6.3)
Modal onset latency, msec	53±4 (38-64)	61±7 (28-87)
Modal conduction velocity[c], m/sec	1.9±0.2 (1.6-2.6)	1.9±0.3 (1.2-3.6)
Duration of spike train, msec	53±8 (29-92)	---[d]
Variability of onset latency of first spike, msec	48±4[e] (42-52)	67±8 (37-128)
Duration of silent period, msec	317±41 (204-540)	227±39 (103-428)

Value are mean ± standard error with ranges in parenthesis. [a]Based on first count above background. [b]Calculated on basis of shortest discharge onset latency. [c]Calculated on basis of modal onset latency. [d]PSN responded only once to each shock applied to pressor region of medulla. [e]Based on first order LH of 3 cells.

Figure 9. First order latency histogram (LH) for SIN whose PSH appeared in Figure 8A. Site and parameters of medullary pressor stimulation are the same as described in Figure 8A. LH depicts variability of onset latency of the first discharge in the SIN spike train elicited by stimulation of medullary pressor site (100 trials). Address bin was 4 msec.

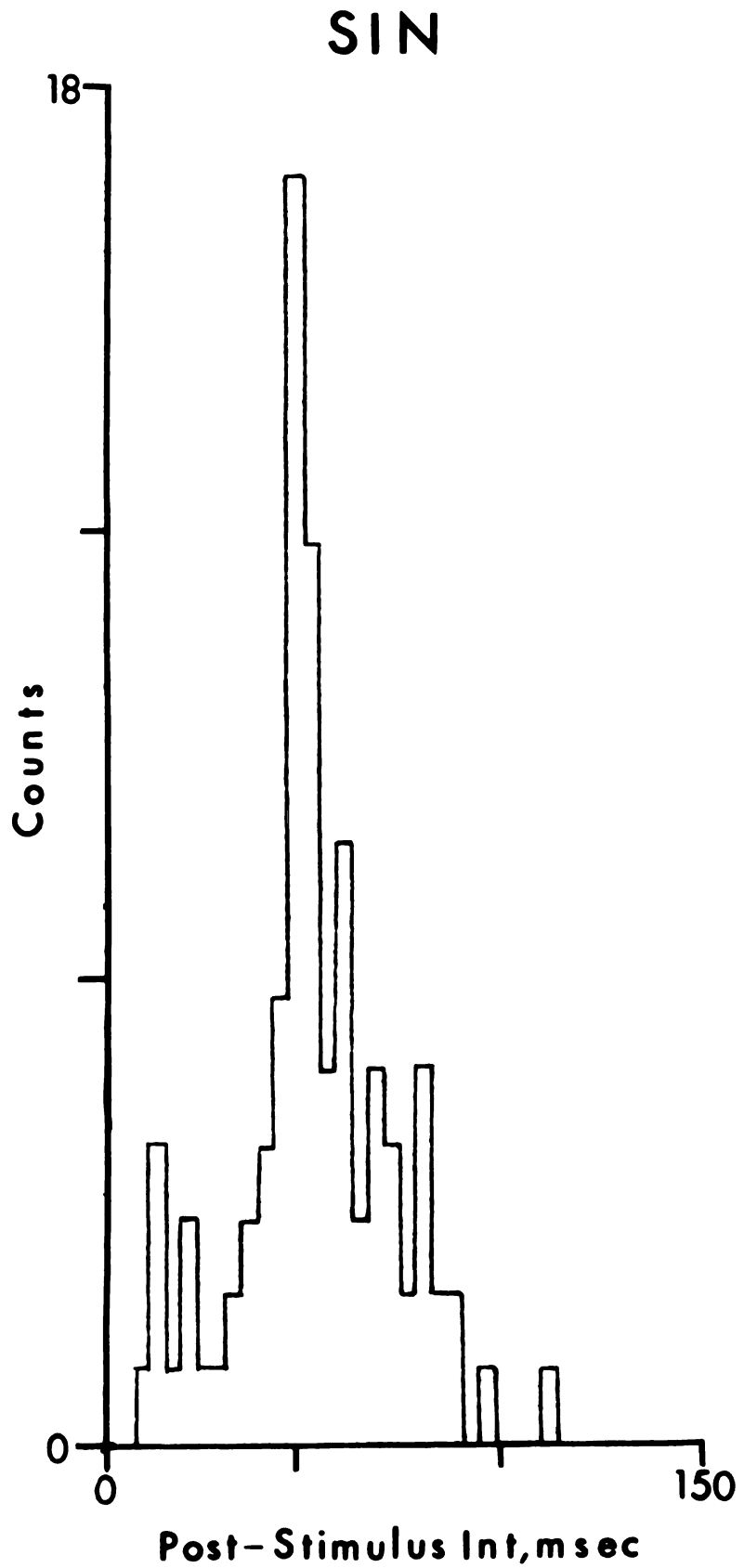

Figure 9

Figure 10. Post-stimulus histograms (PSH) depicting early and late periods of medullary-induced inhibition of spontaneously occurring discharges of a spinal sympathetic interneuron (SIN) and an antidromically identified preganglionic cell (PSN). SIN and PSN were from same cat. Inhibition of unitary discharges was produced by 5 msec trains of 3 pulses (10 v; 0.5 msec; 600 Hz) applied once every 2 sec to a depressor site in the paramedian reticular nucleus. Bottom graphs show portion of same PSH on an expanded time base. PSH for SIN based on 100 trials and an address bin of 4 msec. PSH for PSN based on 121 trials and an address bin of 4 msec. Delays of 6 msec (SIN) and 5 msec (PSN) were used to eliminate stimulus artifacts from the graphs.

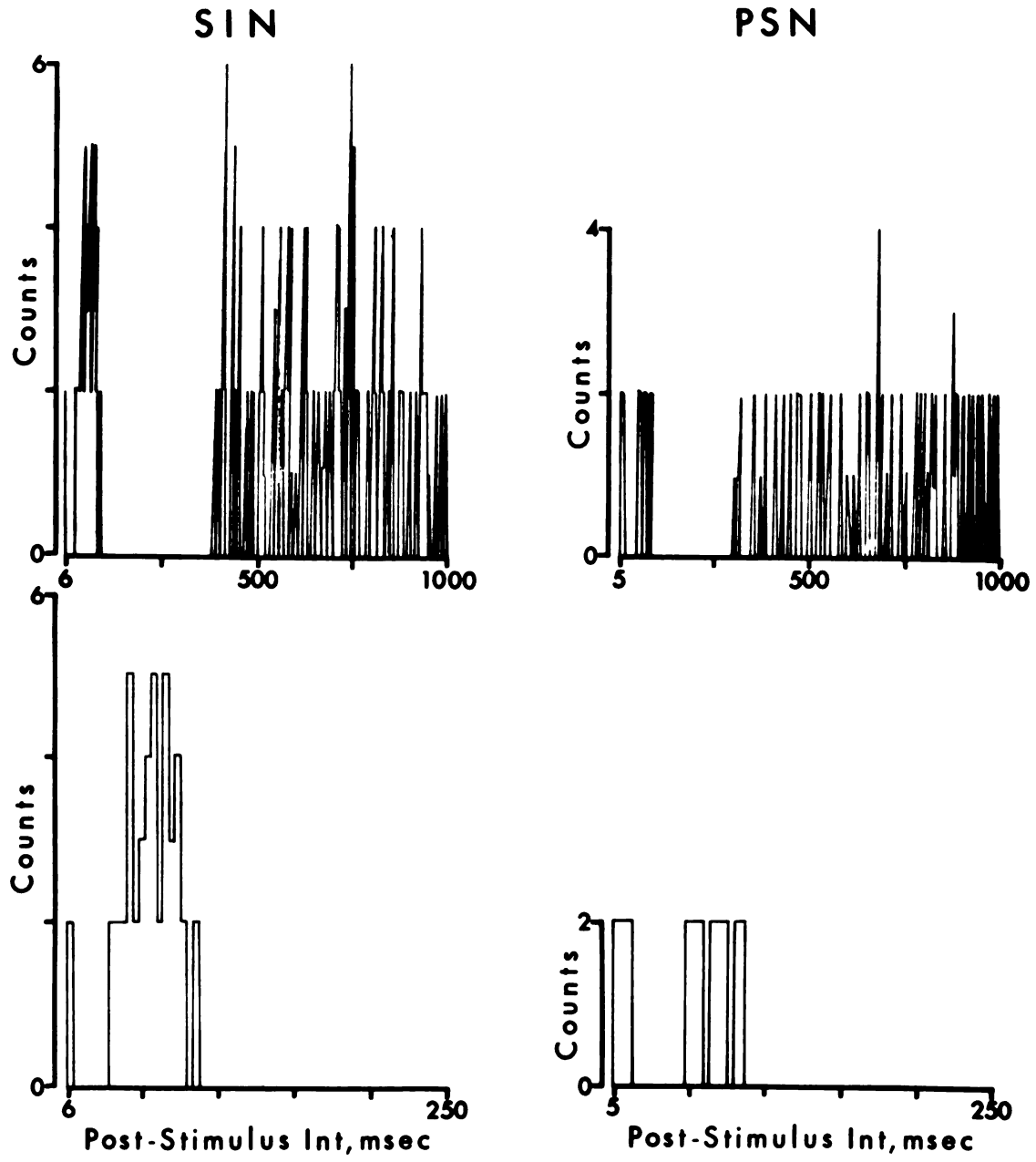

Figure 10

1967; Miura and Reis, 1969; Homma et al., 1970). Preganglionic (4 of 10 units) and non-antidromically activated neurons (5 of 6 units) whose spontaneous discharges were correlated in time with the R wave exhibited two periods (early and late) of inhibition. The remaining units exhibited only the late phase of inhibition. The temporal characteristics of the two periods of inhibition of spinal unitary discharge are summarized in Table 3. The onset latencies of both periods of unitary inhibition compare favorably with those reported by Taylor and Gebber (1975) for the early and late phases of whole splanchnic nerve inhibition evoked by stimulation of the carotid sinus nerve or paramedian nucleus (when minimum conduction time (10 msec) in the splanchnic nerve is taken into account). The early phase of splanchnic nerve inhibition was shown by Gootman and Cohen (1971) and Taylor and Gebber (1975) to be mediated at a spinal locus. In this regard, conduction velocity over the medullospinal inhibitory pathway was essentially the same whether calculated on the basis of the onset latency of the early period of depression of the discharges of either preganglionic or non-antidromically activated neurons (Table 3). This observation, as well, suggests that the two spinal neuronal types were closely adjacent and interconnected components of the same sympathetic pathway.

4. Presumed Preganglionic Neurons

Ten non-antidromically activated units exhibiting the properties of antidromically identified preganglionic neurons were encountered during the present study. The spontaneous discharges of these cells were correlated in time with the R wave. Interspike intervals of less than 50 msec were not observed. In addition, these

TABLE 3

Temporal Characteristics of Early and Late Periods of Depression of Spinal Unitary Discharge Produced by 5 msec Trains of 3 Pulses Applied to Depressor Sites in Medulla

Characteristic	SIN		PSN	
	Early Inhibition n=5	Late Inhibition n=6	Early Inhibition n=4	Late Inhibition n=10
Onset latency, msec	11±3 (6-23)	74±13 (50-130)	17±4 (5-28)	93±16 (46-179)
Duration, msec	36±6 (23-27)	198±41 (100-328)	45±5 (34-55)	186±33 (60-360)
Conduction velocity[a], M/sec	11.3±2.4 (4.4-16.7)	---	12.0±3.7 (4.4-20.0)	---

Values are mean ± standard error with ranges in parenthesis. n = number of cells.
[a]Conduction velocity in medullo-spinal pathway mediating the early period of inhibition; calculated on basis of onset latency of inhibition and distance between stimulating and recording electrodes.

units responded only once with a variable onset latency to single shock stimulation of the medullary pressor region. These neurons were presumed to be preganglionic units whose axons were distributed in nerves (perhaps cardiac) other than the cervical sympathetic trunk.

B. Spinal Interneurons Contained Within Sympathoinhibitory Pathways

The results presented above indicate that the non-antidromically activated units were interneurons interposed between the terminals of sympathoexcitatory reticulospinal fibers and preganglionic neurons. However, no evidence was uncovered to suggest the existence of sympathetic inhibitory interneurons in the IML cell column. In no instance did stimulation of the medullary depressor region or baroreceptor reflex activation excite IML units whose spontaneous discharges were correlated in time with the R wave. This point is important since it has been established that sympathoinhibition of baroreceptor origin is mediated at spinal levels (Fig. 10; also see Gebber et al., 1973; Snyder and Gebber, 1973; Coote and Macleod, 1974b; Lipski and Trzebski, 1975; Taylor and Gebber, 1975; Gebber, 1976), as well as supraspinal levels (Koizumi et al., 1971; Gebber et al., 1973; Taylor and Gebber, 1975; Gebber, 1976). In view of the data presented above, it is possible that spinal sympathoinhibition is mediated directly by the terminals of bulbospinal fibers. Alternatively, reticulospinal fibers of the baroreceptor reflex pathway might terminate on and excite inhibitory interneurons located in spinal sympathetic nuclei other than the intermediolateral (IML) cell column. The purpose of the present series of experiments was to continue the search for spinal interneurons that mediate sympathoinhibition of baroreceptor reflex origin. The search was centered in the medial portions of the

zona intermedia of the cat spinal cord. This region contains the intermediomedial (IMM) nucleus.

1. Effect of Bilateral Occlusion of Common Carotid Arteries (BLCO) on Spinal Unitary Discharge

The effect of BLCO was tested on the spontaneous discharge of 332 units located in the medial portions of the zona intermedia (T_1-T_3 spinal segments) in cats in which the vagus and aortic depressor nerves were sectioned. Occlusion was accomplished by sliding polyethylene tubing over ligatures which were passed around the carotid artery. In this manner, the artery could be clamped without stretching the carotid sinus (Miura and Reis, 1972). BLCO was limited to less than 5 sec so as to minimize reflex changes in blood pressure which might disrupt the position of the recording electrode. The discharges of 29 neurons were interrupted within one heart beat after BLCO was initiated (Figure 11). Spontaneous unitary discharge returned within a cardiac cycle after deocclusion. These observations suggest that certain spinal units are driven by carotid sinus baroreceptor nerve discharge. Units which consistently increased their firing rate during BLCO were not found in the medial regions of the zona intermedia.

The distribution in the zona intermedia of those units whose discharges abruptly ceased upon BLCO is shown in Figure 12. For the purpose of simplification, the positions of all 29 units are plotted at the level of the third thoracic spinal segment. The units were located 0.2 to 0.4 mm lateral to the dorsal median sulcus and from 1.6 to 2.1 mm beneath the dorsal surface of the spinal cord. The IMM is contained within this area (Rexed, 1964; Petras and Cummings, 1972) which, henceforth, will be referred to as the vicinity of IMM.

Figure 11. Interruption of discharges of a spinal unit during BLCO. Top trace is blood pressure (mmHg). Bottom trace shows 5-v pulses from window discriminator monitoring unitary discharge. Period of BLCO is shown by bar beneath time base (1 sec/division).

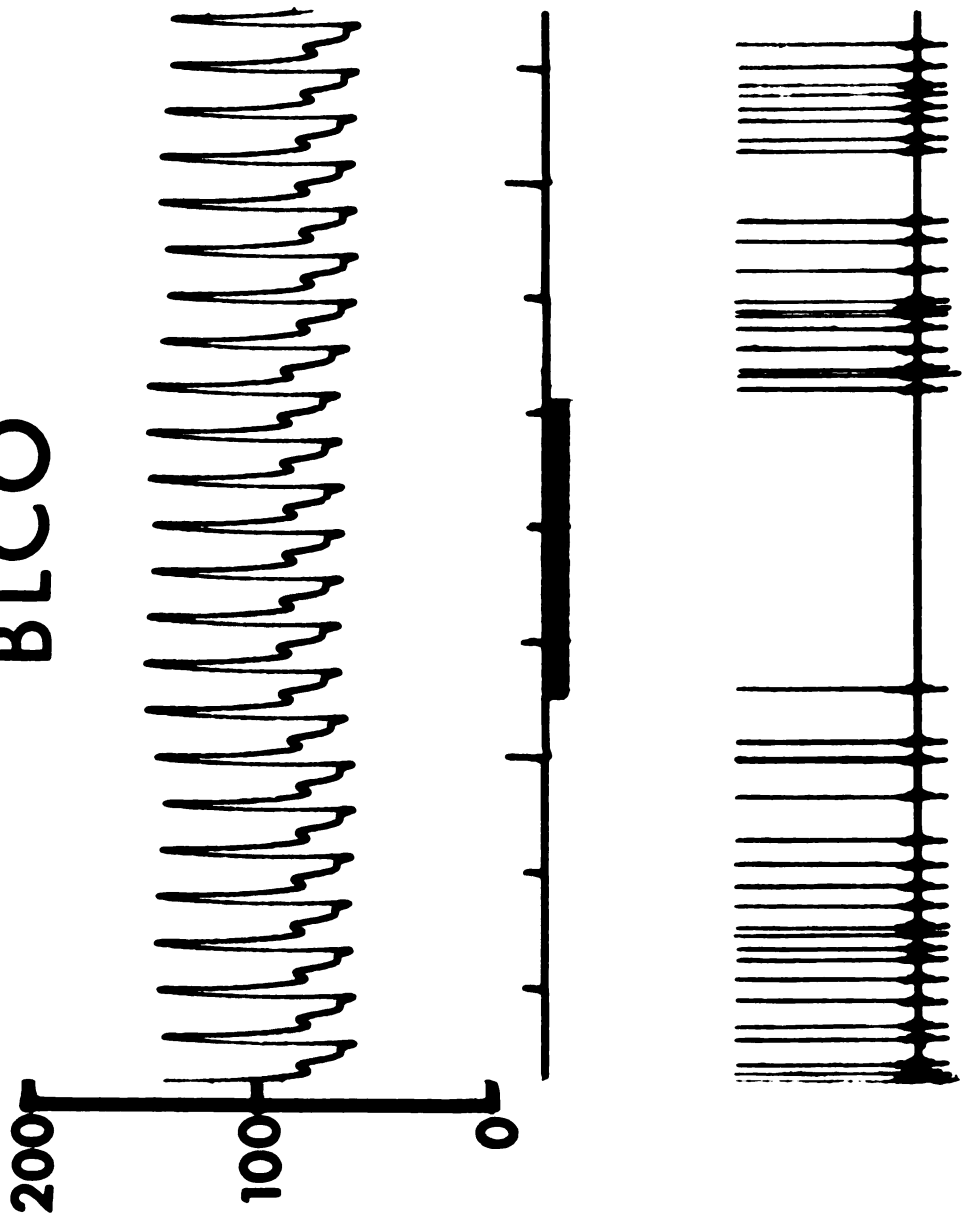

Figure 11

Figure 12. Distribution of recording sites in zona intermedia of 3rd thoracic spinal segment for units whose discharges were interrupted by BLCO. Unfilled circles show recording sites that were lesioned with anodal current (2 mA for 5 sec). Filled circles show recording sites within histologically identified electrode tracks. DH is dorsal horn. IML is intermediolateral horn. VH is ventral horn.

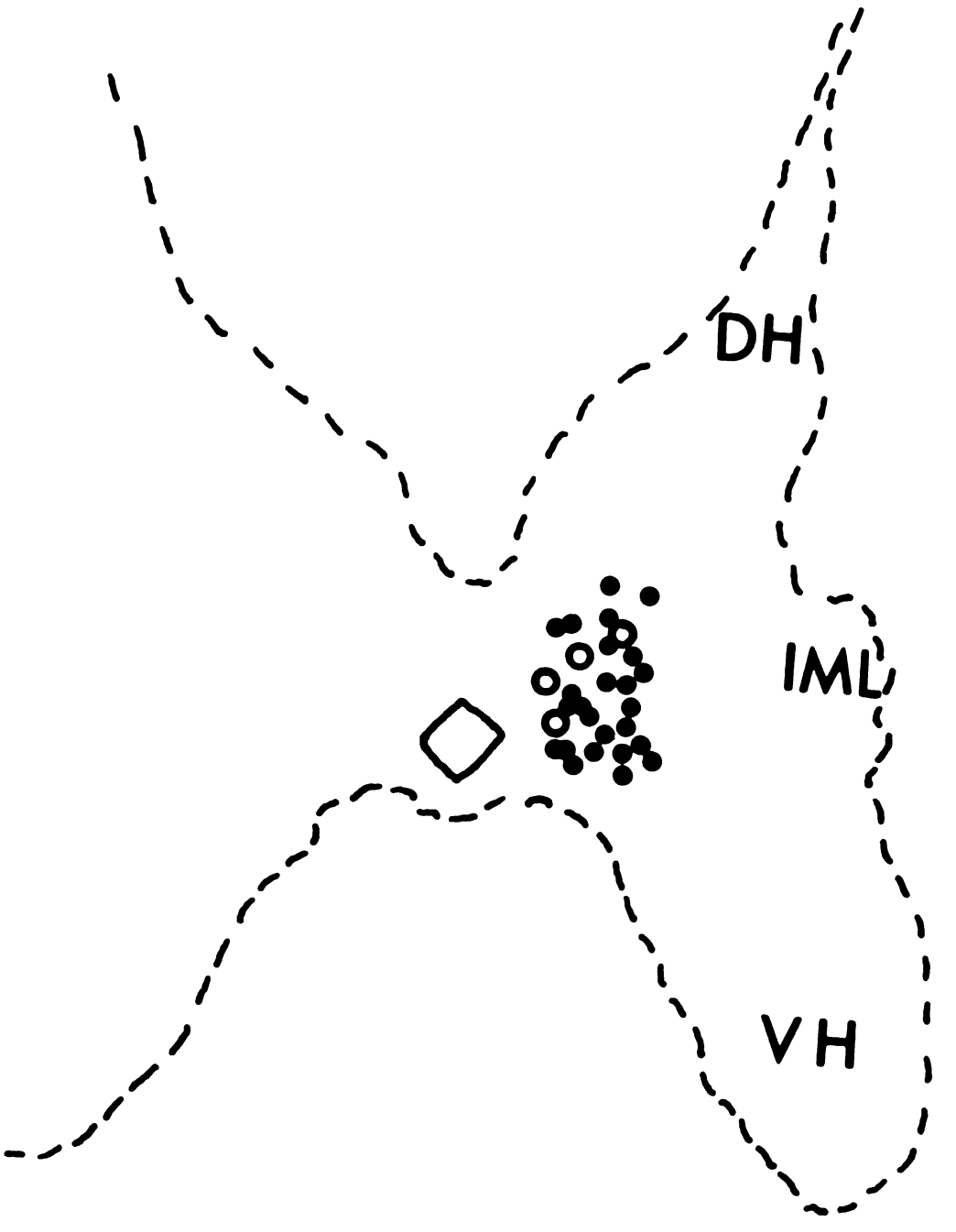

Figure 12

It should be stressed that units whose discharges were interrupted by BLCO never were found in clusters in any given experiment. Moreover, such units invariably were closely adjacent to cells whose discharges were unaffected by BLCO.

2. R-Wave Related Discharges of Spinal Units Affected by BLCO

It was reasoned that the pulse synchronous component of carotid sinus nerve activity should be reflected in the spontaneous discharges of interneurons in the baroreceptor reflex arc. This contention was tested by constructing post-R wave TIH for 19 of the 29 spinal neurons whose discharges were interrupted during BLCO. The probability of discharge of 10 of the 19 units was correlated in time with the R wave. Three patterns of R-wave locked unitary discharge were observed. Representative examples are shown in Figure 13-15. The abscissa in each histogram approximates the period of one cardiac cycle (R-R wave interval). Oscillographic traces of the spontaneous discharges of the unit appear above each histogram.

The post-R wave TIH of 3 units were multimodal containing 4 or more distinct peaks (Figure 13). The first 4 peaks in the histograms of the 3 neurons occurred 30±2 msec, 70±3 msec, 134±7 msec and 209± 2 msec after the R wave. Mean discharge rate of the 3 units was 10.5± 3.6 Hz.

The post-R wave TIH of 5 units contained one peak occurring 30±2 msec after the R wave. As shown in Figure 14, these neurons exhibited a high level of background discharge (relative to the number of spike occurrences in the peak of the histogram) throughout the cardiac cycle. Thus, a significant component of the spontaneous

Figure 13. Multimodal post-R wave TIH of spinal unit located in vicinity of IMM. Number of spike occurrences (counts) is plotted against post-R wave interval in milliseconds. Abscissa approaches period of one cardiac cycle in this and in Figures 14-16, 18-19. Number of computer sweeps was 200. Address bin was 2.5 msec. Oscillographic traces of blood pressure (180/125 mmHg) and unitary discharge are shown above histogram. Horizontal calibration is 200 msec. Vertical calibration is 100 μV.

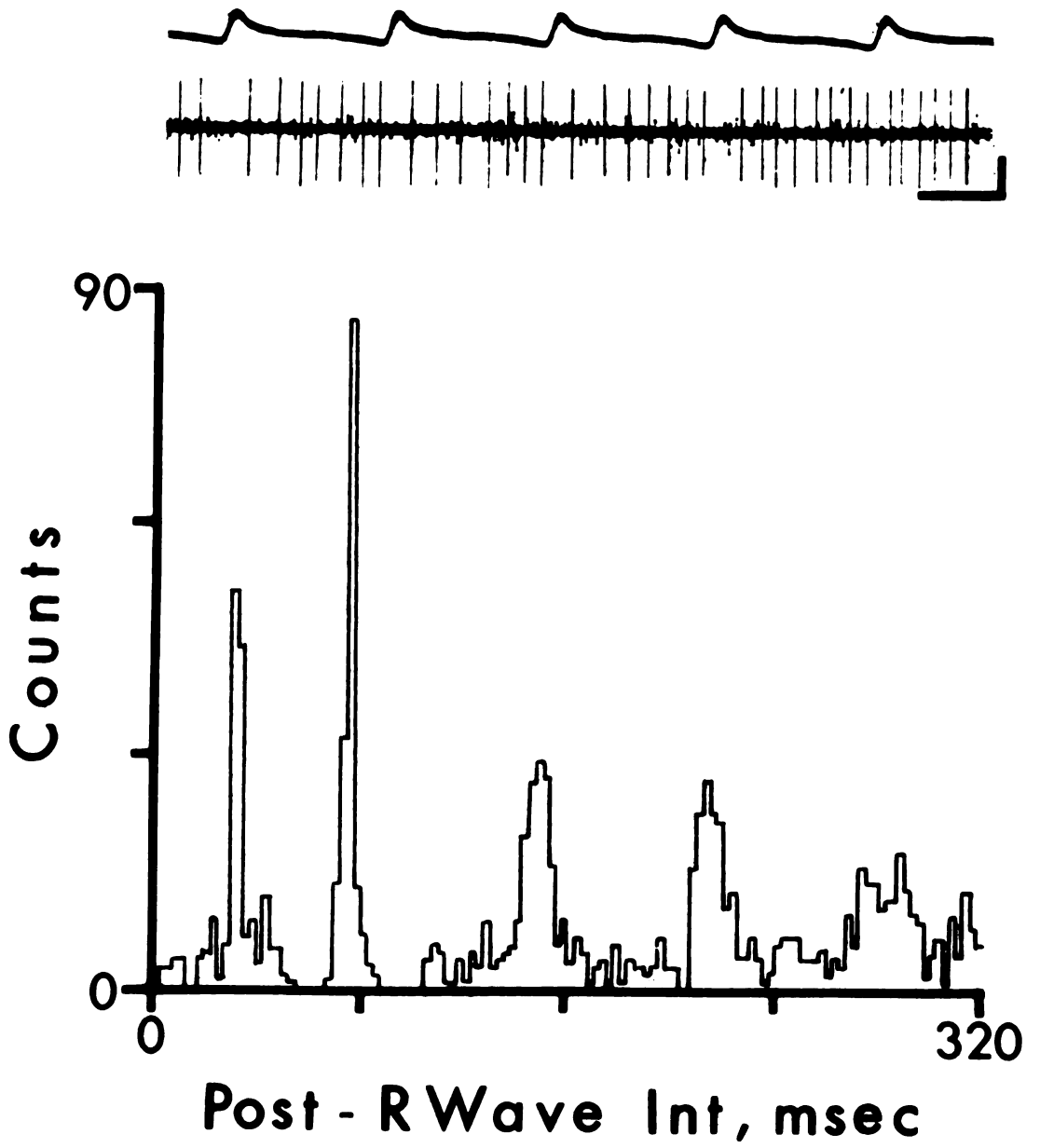

Figure 13

Figure 14. Unimodal post-R wave TIH of spinal unit. Number of computer sweeps was 250. Address bin was 9 msec. Oscillographic traces of blood pressure (145/95 mmHg) and unitary discharge are shown above histogram. Horizontal calibration is 200 msec. Vertical calibration is 100 μV.

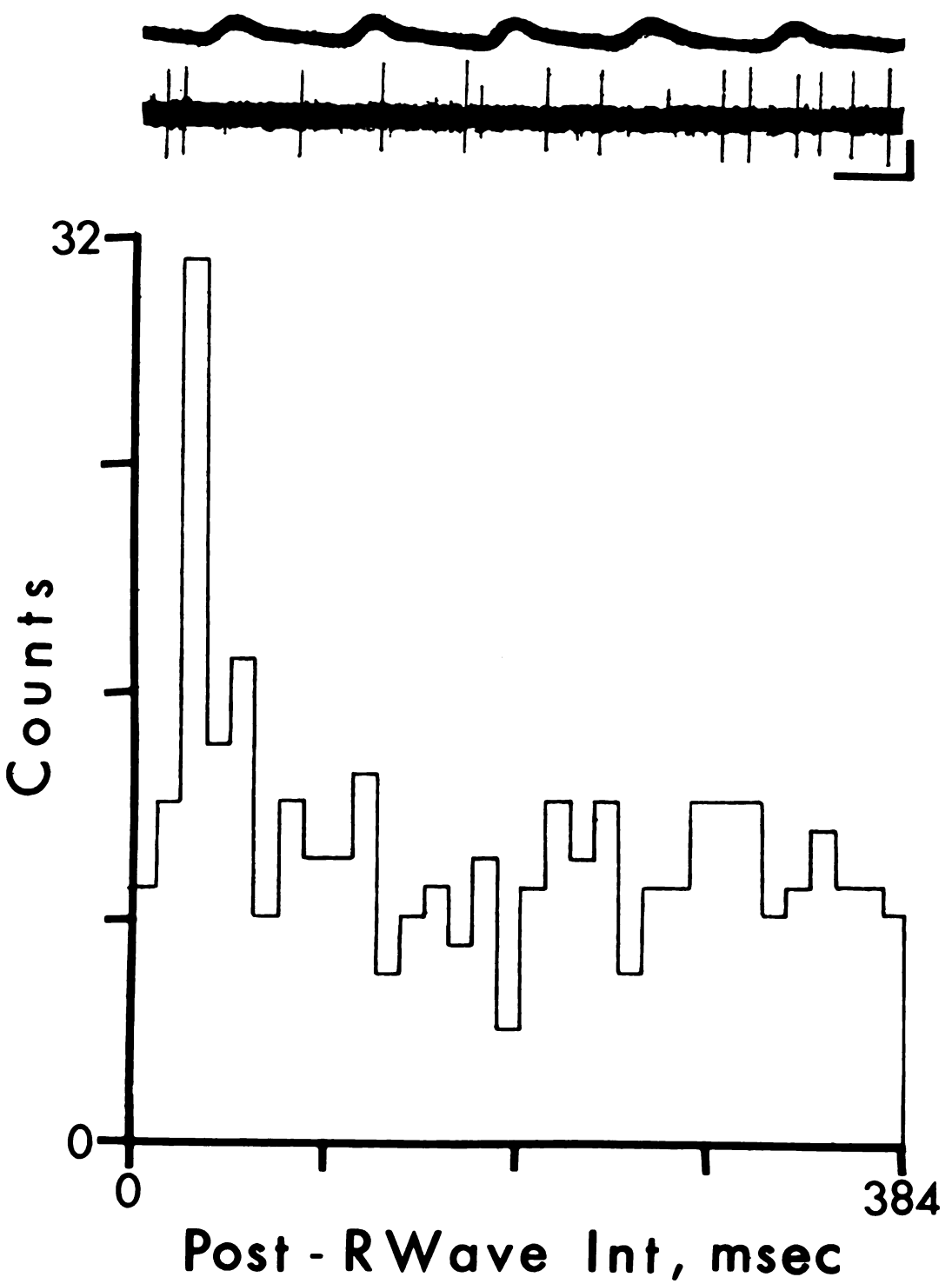

Figure 14

Figure 14. Unimodal post-R wave TIH of spinal unit. Number of computer sweeps was 250. Address bin was 9 msec. Oscillographic traces of blood pressure (145/95 mmHg) and unitary discharge are shown above histogram. Horizontal calibration is 200 msec. Vertical calibration is 100 μV.

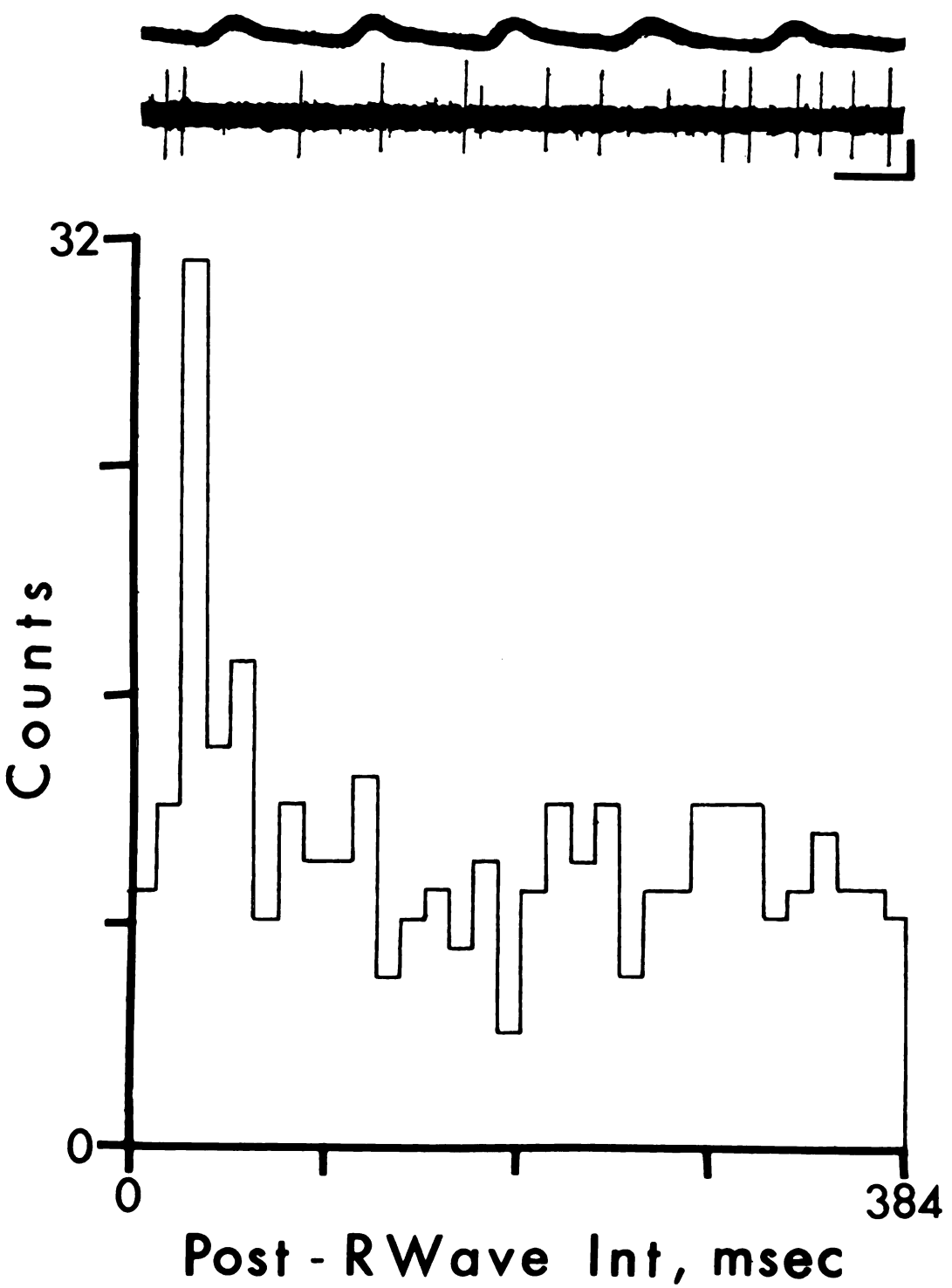

Figure 14

discharges of units with the unimodal histogram was not related in time to the R wave. Mean firing rate of the 5 units was 6.2±1.3 Hz. Heart rate remained essentially constant in each of the 4 cats in which units with the unimodal histogram were located. The period of the cardiac cycle (R-R wave interval), however, ranged from 270 to 400 msec in these 4 animals. This spread was much greater than the range (25-35 msec) for the interval between the R wave and the peak in the unimodal histograms of unitary discharge. Thus, it seems most likely that the peak in the histogram shown in Figure 14 was associated with an event occurring early in the cardiac cycle whose R wave was used to trigger the computer sweep, rather than with an event occurring in the preceding cardiac cycle.

The post-R wave TIH of two units contained two peaks. The histogram for one of these neurons whose mean firing rate was 5.3 Hz is shown in Figure 15. Peak probability of discharge occurred 46 msec and 225 msec after the R wave.

The probability of discharge of the remaining 9 cells, whose activity was interrupted during BLCO, was not correlated in time with the R wave (Figure 16). That is, the post-R wave TIH of these cells failed to exhibit distinct peaks. This observation indicates that pulse synchronous carotid sinus nerve discharge was not always transmitted to spinal elements of the baroreceptor reflex arc. The mean firing rate (2.4±0.3 Hz) of the 9 units was low in comparison with neurons whose discharges were correlated in time with the R wave.

Figure 15. Bimodal post-R wave TIH of spinal unit. Number of computer sweeps was 290. Address bin was 5 msec. Oscillographic traces of blood pressure (165/100 mmHg) and unitary discharge are shown above histogram. Horizontal calibration is 200 msec. Vertical calibration is 100 μV.

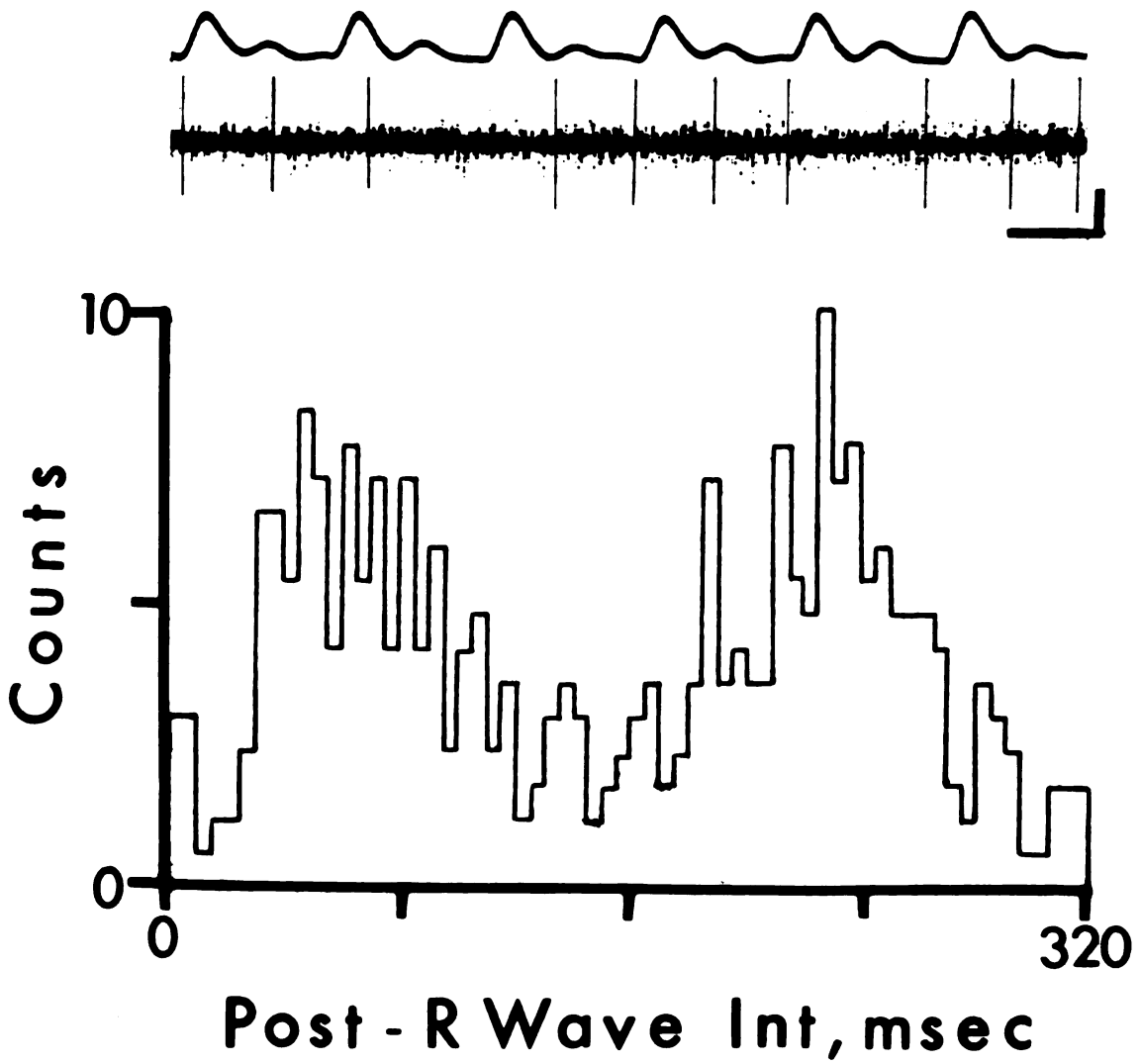

Figure 15

Figure 16. Negative post-R wave TIH of spinal unit whose discharges were interrupted by BLCO. Number of computer sweeps was 250. Address bin was 2.5 msec. Oscillographic traces of blood pressure (160/100 mmHg) and unitary discharge are shown above histogram. Horizontal calibration is 200 msec. Vertical calibration is 100 μV.

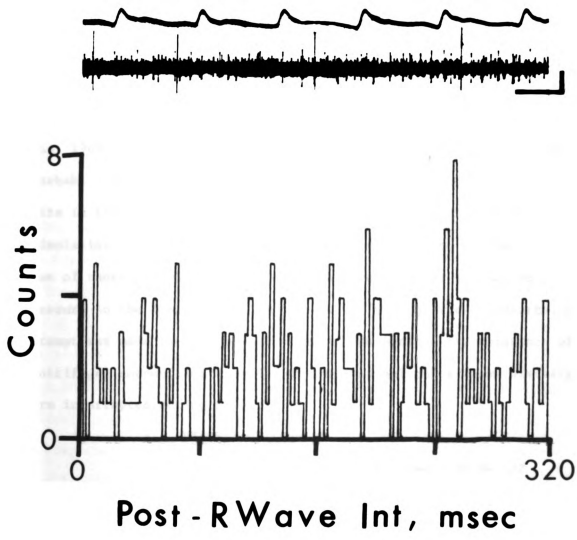

Figure 16

On occasion, units commonly affected by BLCO, but whose discharges were related in different ways or not at all to the R wave, were located under comparable conditions in the same cat. Thus, it seems that the pattern of R-wave locking of unitary discharge was not solely dependent upon the level of baroreceptor nerve activity or blood pressure.

3. Evidence for a Connection Between NTS and Spinal Units in Vicinity of IMM

NTS is the primary site of central termination of arterial baroreceptor fibers (Cottle, 1964; Humphrey, 1967; Miura and Reis, 1969, 1970; Seller and Illert, 1969). Furthermore, Lipski and Trzebski (1975), and Trzebski et al. (1975) reported that certain units in the vicinity of NTS can be antidromically activated by stimulation of their axons in the cervical spinal cord of the cat. Some of these neurons were excited orthodromically by increasing pressure in the isolated carotid sinus. In view of these resports, an attempt was made in the present study to demonstrate the existence of centrifugal connections from NTS to those spinal units whose discharge were interrupted during BLCO.

a. NTS Stimulation

Stimulation (2-7 v; 0.1-0.2 msec; 20 Hz) of histologically verified sites in NTS (within 1 mm anterior or posterior to the obex) for 15 sec lowered blood pressure by 10-40 mmHg in 7 experiments. Single shocks applied once every 2 sec to the same sites elicited short latency (8±1 msec) discharges in 9 spinal units whose activity was interrupted by BLCO. Conduction velocity from NTS to the recording microelectrode was 15±2 m/sec. The PSH and

oscillographic traces in Figure 17 are typical of the spinal unitary response pattern to NTS stimulation. The unit responded once to each shock applied to NTS and faithfully followed frequencies of stimulation up to 5 Hz. The evoked response was followed by a short period of depressed spontaneous activity.

Two additional spinal units, whose activity was interrupted by BLCO, were excited by single shocks (10 v; 0.5 msec) applied to depressor sites in the paramedian reticular nucleus. The latencies of activation of spinal units from NTS and from the paramedian nucleus were similar.

b. Unitary Discharge Patterns in NTS

NTS was surveyed in an attempt to locate unitary discharge patterns similar to those observed in the vicinity of IMM. The vagus and aortic depressor nerves were cut in these experiments. As reported by others (Humphrey, 1967; Miura and Reis, 1972; Middleton et al., 1973; Werz et al., 1974; Schwaber and Schneiderman, 1975), units whose spontaneous discharges were related in time to the R wave were located within the confines of the solitary complex (medial and lateral nuclei (Berman, 1968) near the level of the obex. BLCO partly or completely interrupted the activity of these neurons. The post-R wave TIH of 8 cells were similar in form to the multimodal histograms exhibited by spinal units. A typical example is shown in Figure 18. Multimodal histograms for units in NTS contained 3 to 5 peaks. The first 3 peaks occurred 39±2 msec, 83±6 msec and 161±8 msec after the R wave. The oscillographic trace in Figure 18 shows that these units discharged in bursts several times during each cardiac cycle. Mean firing rate of the 8 units was 7.6±1.7 Hz.

Figure 17. Post-stimulus histogram (PSH) of spinal unitary discharge evoked by single shocks (3 v, 0.1 msec) applied once every 2 sec to a depressor site in left NTS. Spontaneous discharges of this unit were interrupted by BLCO. Number of spike occurrences (counts) is plotted against interval (msec) after stimulus (90 trials). Address bin was 1 msec. Insert shows 5 superimposed traces of evoked unitary discharge. Horizontal calibration is 2 msec. Vertical calibration is 200 μV.

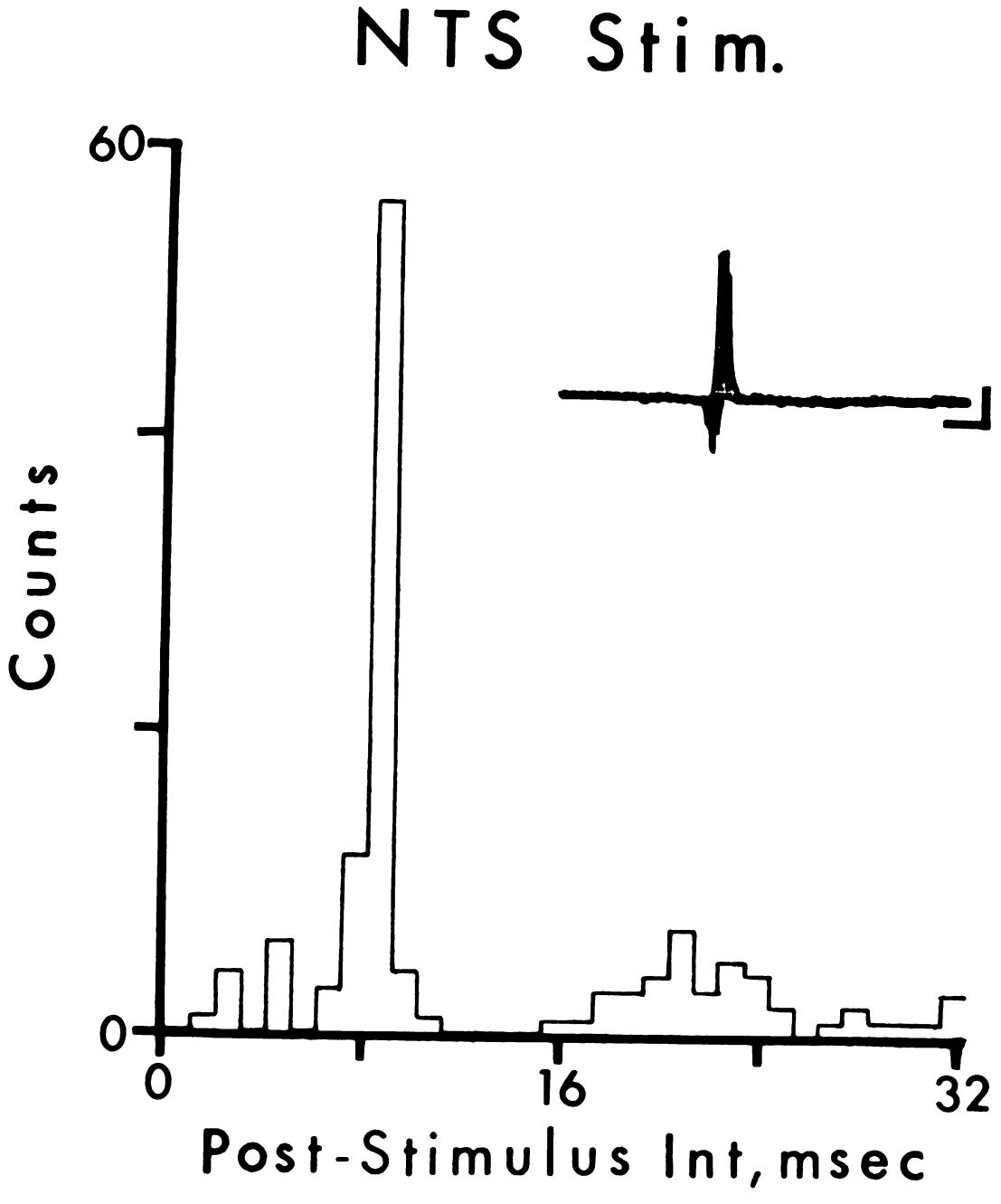

Figure 17

Figure 18. Multimodal post-R wave TIH of unit located in NTS. Number of computer sweeps was 500. Address bin was 5 msec. Oscillographic traces of blood pressure (175/110 mmHg) and unitary discharge are shown above histogram. Horizontal calibration is 200 msec. Vertical calibration is 100 μV.

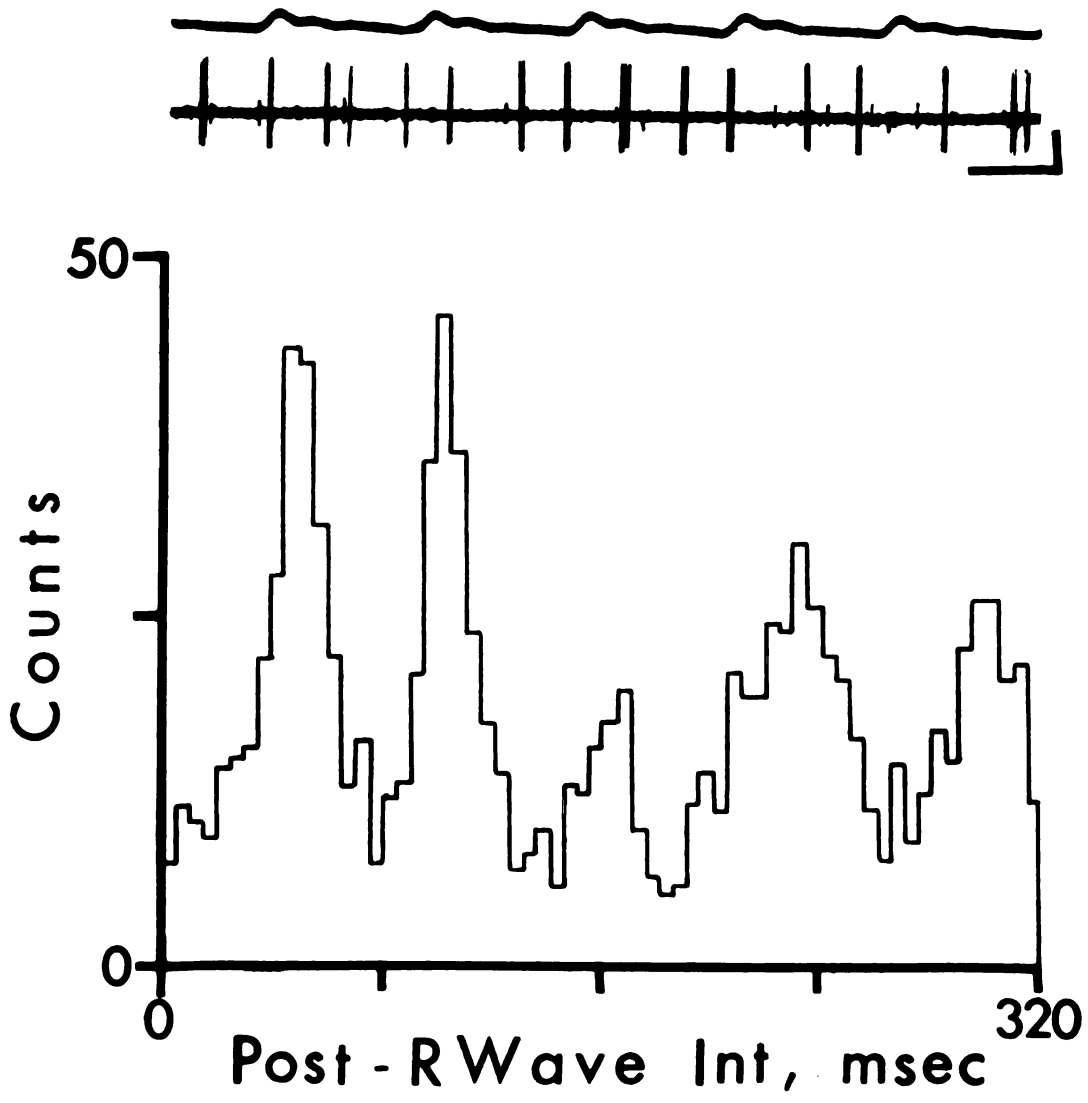

Figure 18

Post-R wave TIH of 6 additional units in NTS were unimodal (Figure 19). Mean firing rate of these units was 7.4±1.9 Hz. Peak discharge occurred 85±5 msec after the R wave. The peak in the unimodal histograms corresponded most closely in time with the second peak of the multimodal post-R wave TIH of spinal (Figure 13) or NTS (Figure 18) units. As shown in the oscillographic trace in Figure 19, units in NTS with a unimodal histogram discharged in bursts near the beginning of the femoral arterial pulse wave. Unitary discharges in NTS with the prominent cardiac periodicity shown in Figure 19 have been previously reported (Humphrey, 1967; Miura and Reis, 1969; Werz et al., 1974; Schwaber and Schneiderman, 1975).

Units in NTS exhibiting unimodal histograms with an early peak (<50 msec) similar to those of spinal units (Figure 14) were not found in the present study. However, such neurons have been located in NTS of the cat by Werz et al. (1974).

4. Orthodromic Activation of Neurons in Spinal Cord and in NTS by Stimulation of Inferior Cardiac Nerve

The pulse synchronous component of carotid sinus nerve discharge in the cat occurs approximately 70 msec after the R wave (Gebber, 1976). Thus, the timing of the peak in the unimodal post-R wave TIH shown in Figure 14 and perhaps all but the second peak in the histograms shown in Figures 13 and 18, is inconsistent with that of pulse-synchronous carotid sinus baroreceptor discharge. Furthermore, the vagus and aortic depressor nerves were cut in these experiments. Therefore, it would appear that certain cells in the spinal cord and in NTS receive input from sources in addition to the IX and X cranial nerves. The experiments described below indicate that one such source may be derived from afferents passing through the stellate ganglion.

Figure 19. Unimodal post-R wave TIH of unit located in NTS. Number of computer sweeps was 427. Address bin was 2 msec. Oscillographic traces of blood prssure (145/90 mmHg) and unitary discharges are shown above histogram. Horizontal calibration is 200 msec. Vertical calibration is 100 μV.

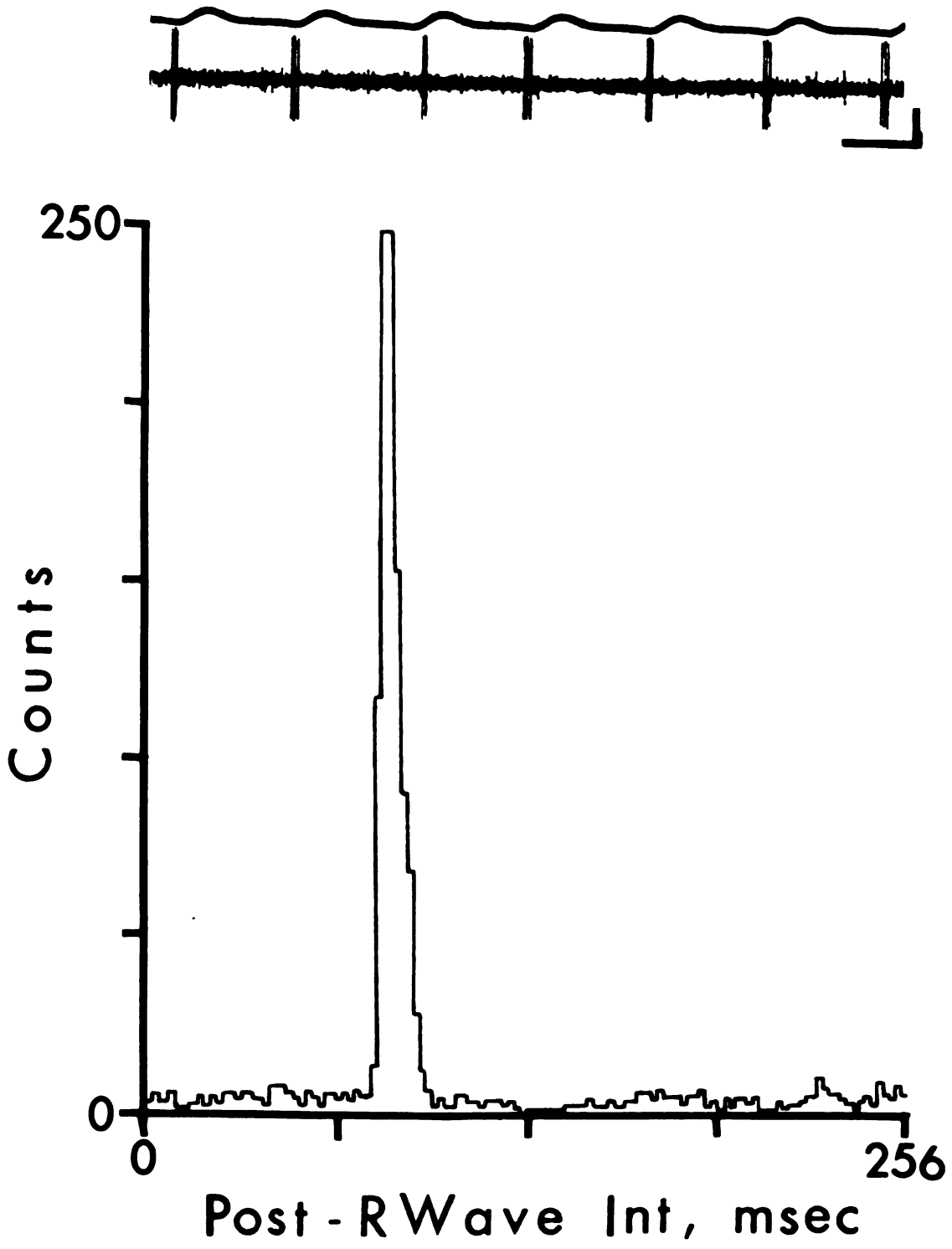

Figure 19

Stimulation (2-7 v; 0.05-0.2 msec; 5-10 Hz) of the left intact inferior cardiac nerve lowered blood pressure 10-35 mmHg in 10 cats. This observation supports the view that sympathoinhibitory afferents pass through the left stellate ganglion (Malliani et al., 1971; Pagani et al., 1974; Koizumi et al., 1975; Weaver, 1976). Single shocks of the same intensity and duration applied once every 2 sec to the inferior cardiac nerve elicited discharges in units located in the vicinity of IMM (n=7) and in NTS (n=11). The spontaneous activity of these neurons was related in time to the R wave. However, not all of the post-R wave TIHs of these units contained an early (<50 msec) peak.

The spinal units responded with an early (4.8±0.1 msec) and a late (12.1±0.7 msec) discharge to each shock applied to the inferior cardiac nerve (Figure 20A). The insert in Figure 20A shows that the evoked unitary discharges were preceded by a positive field potential of unknown origin. The early discharge faithfully followed frequencies of stimulation up to 7 Hz. The late discharge failed at lower frequencies of stimulation.

The units in NTS usually discharged in a short burst of 2 or 3 spikes to each shock applied to the inferior cardiac nerve (Figure 20B). Modal discharge onset latency in the spike train was 6.1±0.2 msec for 11 cells. The first spike in the train faithfully followed frequencies of stimulation up to 5 Hz. The later occurring spikes failed at lower frequencies of stimulation.

Figure 20. PSH of spinal and medullary unitary discharge evoked by single shocks (5 v; 0.1 msec in A; 7 V; 0.1 msec in B) applied once every 2 sec to left inferior cardiac nerve. Units in A and B are from 2 different experiments. A: Spinal unit in vicinity of IMM (100 trials). Address bin was 1 msec. B: NTS unit (70 trials). Address bin was 1 msec. Inserts in A and B show 5 superimposed traces of evoked unitary discharge. Horizontal calibrations are 4 msec. Vertical calibrations are 200 μV in A and 100 μV in B.

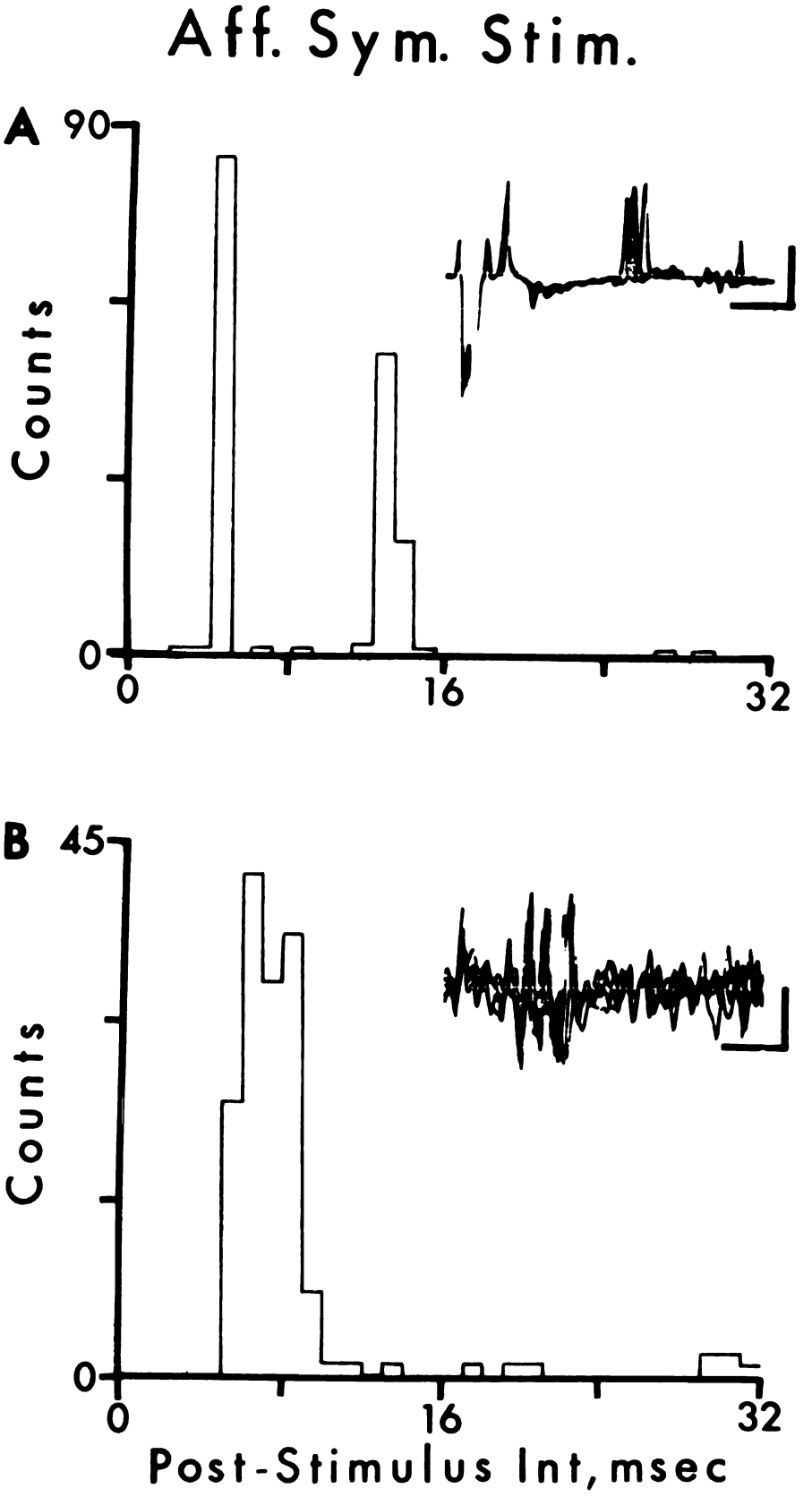
Aff. Sym. Stim.
A
90
Counts
0
0
16
32
B
45
Counts
0
0
16
32
Post-Stimulus Int, msec

Figure 20

The spinal units orthodromically activated by stimulation of the inferior cardiac nerve also were excited by single shocks (2-15 v; 0.1-0.5 msec) applied to the intact thoracic sympathetic chain just central to the stellate ganglion. The response pattern elicited by thoracic sympathetic chain stimulation was essentially the same as that produced by electrical activation of the inferior cardiac nerve. Importantly, the responses of spinal units to thoracic sympathetic chain stimulation failed to follow frequencies above 7 Hz. Thoracic preganglionic neurons in IML faithfully followed frequencies of antidromic stimulation of the cervical sympathetic nerve in excess of 50 Hz (see above, also see Taylor and Gebber, 1973). Thus, units in the vicinity of IMM whose discharges were related in time to the R wave were orthodromically but not antidromically activated by stimulation of the thoracic sympathetic chain. This observation supports the contention that these units were spinal interneurons rather than preganglionic neurons.

II. Convergence of Rhythmically-Active Inputs to Single PSNs

The spontaneous discharges of sympathetic nerve bundles often reveal mixtures of the three periodicities of SND (Cohen and Gootman, 1970; Taylor and Gebber, 1975; Barman and Gebber, 1976). Data presented in the following section indicate that a common pool of PSNs exhibit the cardiac-related, respiratory-related, and 10 c/sec periodic components in SND. In this series of experiments the discharges of single fibers teased from the cervical sympathetic preganglionic nerve were analyzed using time interval and autocorrelation analysis. The latter method enabled me to determine the periodic components in the discharge of single PSNs. Experiments were performed on spontaneously breathing, vagotomized cats.

A. Co-existence of the 3 c/sec and Respiratory-related Periodicities in the Discharges of Single PSNs

The oscillographic traces in (1) of Figure 21 (panels A-C) illustrate the relationship between the spontaneous discharges of 3 PSNs and the femoral arterial pulse wave. As described above, PSNs discharged at low frequencies (1.1±0.7 impulses/sec; n=140) and did not exhibit an obvious cardiac rhythm. That is, preganglionic units failed to discharge in a significant number of cardiac cycles. An insidious cardiac-related discharge of PSNs could be demonstrated by determining the probability of PSN discharge during the phases of the cardiac cycle. The discharge of 83 of 140 PSNs were to one degree or another probabilistically related in time to the R wave of the ECG. Such relationships presumably were established via baroreceptor phasing mechanisms (Taylor and Gebber, 1975). The post-R wave TIHs of two PSNs (units A and B) whose discharges were related in time to the R wave are shown in Figure 22 Peak probability of discharge occurred approximately 190 msec after the R wave of the ECG. The discharges of unit C in Figure 21 were not related temporally to the R wave (Figure 22C).

Comparison of the post-R wave TIHs and post-expiratory TIHs for units A-C in Figure 22 illustrates that only units whose discharges were related in time to the R wave exhibited a respiratory-related periodicity. Polygraphic traces of tracheal pressure and PSN discharges from which the post-expiratory TIHs were derived are shown in (2) of panels A-C in Figure 21. With respect to changes in tracheal pressure, the probability of discharge of units A and B (Figure 22) increased during late expiration and reached a maximum during

Figure 21. Spontaneous discharges of 3 cervical PSNs (A-C) from different vagotomized cats. (1) in A-C shows oscillographic traces of femoral arterial pulse wave (top) and unit discharge (bottom). (a) in A-C shows polygraphic traces of tracheal pressure (top) and standardized 5v pulses derived from unit discharge. The downward deflection in the records of tracheal pressure denotes the beginning of expiration. Inspiration begins at the start of the upward deflection. Blood pressure was in mmHg: 190/130 in A; 185/120 in B; and 200/125 in C. Vertical calibrations are 200 μV. Horizontal calibration is 1.3 sec for records in (1) and 2 sec for records in (2).

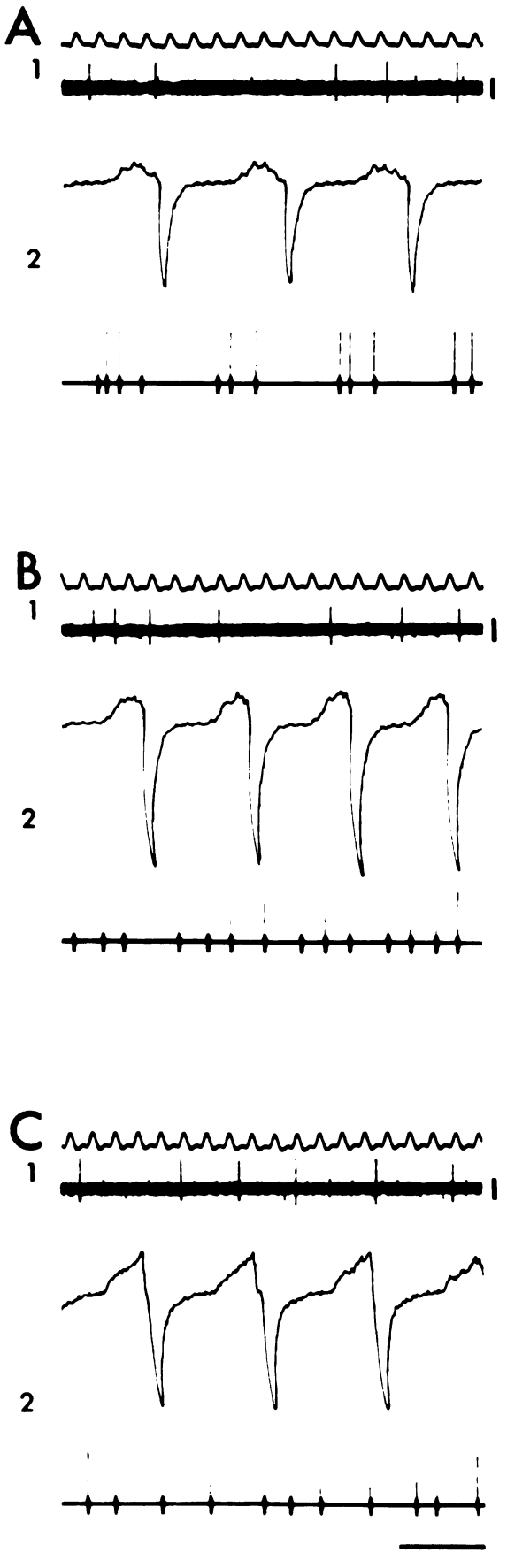

Figure 21

Figure 22. Post-R wave and post-expiratory TIHs for 3 PSNs (A-C) whose discharges are shown in Figure 21. Number of spike occurrences (counts) is plotted against the interval following the R wave (left) and after the start of expiration (right). The abscissa approximates the period of one cardiac cycle in the post-R wave TIHs and that of one respiratory cycle in the post-expiratory TIHs. Post-R wave TIHs: Address bin was 2.5 msec in A, and 3.0 msec in B and C. Number of computer sweeps was 500. Post-expiratory TIHs: Address bin was 40 msec. Number of computer sweeps was 100. I indicates start of inspiration.

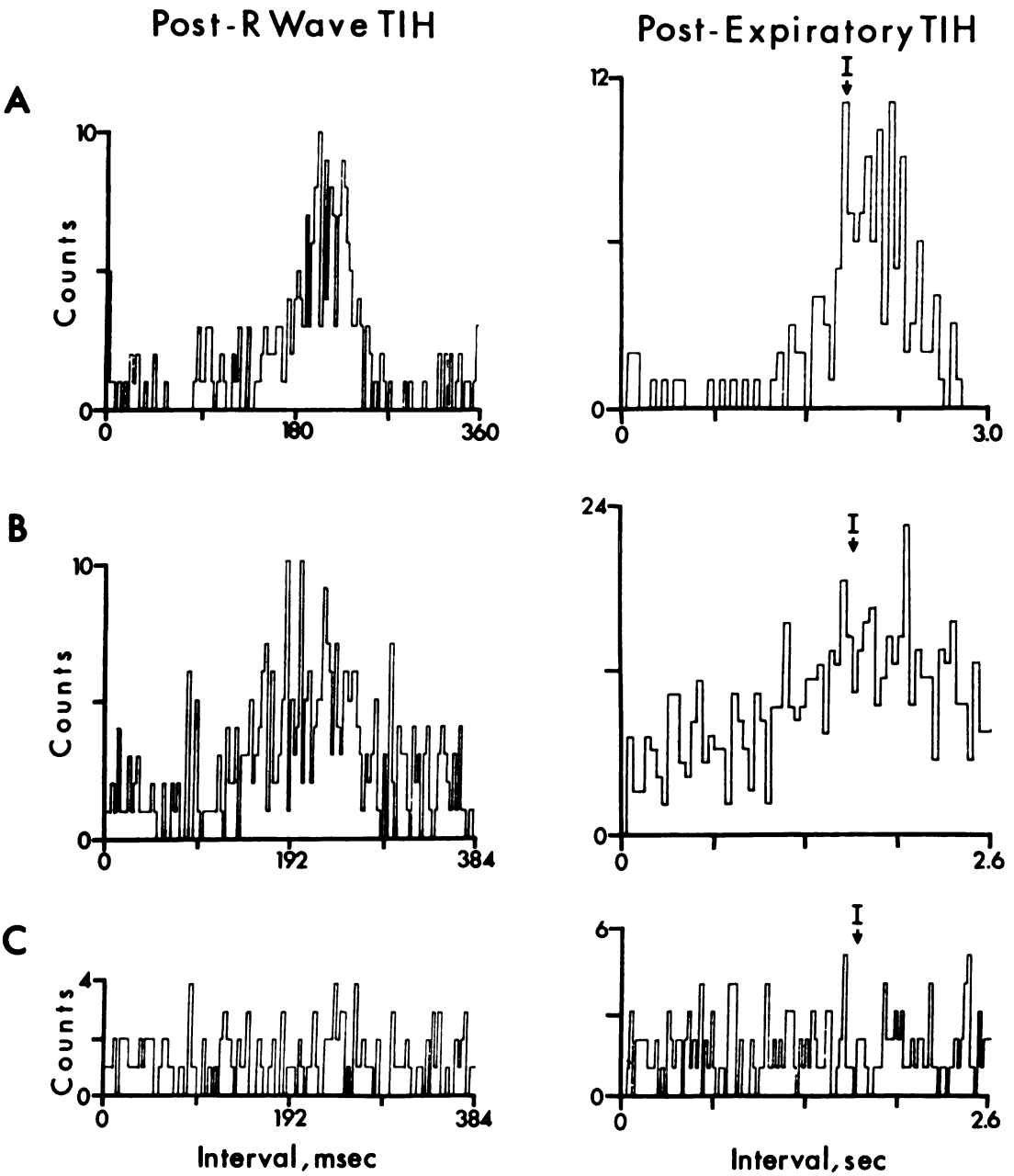
Post-R Wave TIH
Post-Expiratory TIH
A
B
C
Counts
10
0
180
360
12
3.0
I
24
2.6
4
192
6
Interval, msec
Interval, sec

Figure 22

early or mid-inspiration. This pattern of respiratory-related activity resembles one of several reported in recordings of sympathetic nerve bundles (Cohen and Gootman, 1970; Koizumi et al., 1971; Barman and Gebber, 1976) and individual PSNs (Preiss et al., 1975). The discharges of units which exhibited a negative post-R wave relationships also were unrelated in time to the central respiratory cycle (Figure 22C).

The data in Figures 21 and 22 suggest that a relationship exists between the degree of cardiac- and respiratory-related discharges of individual preganglionic cervical sympathetic fibers. To further test this possibility it was necessary to quantitate and compare the degree of cardiac- and respiratory-related unitary discharge. Therefore, post-R wave and post-expiratory TIHs were integrated in order to transform these probability distributions into cumulative distributions. Figure 23 illustrates the cumulative distributions derived from the integration of the time interval histograms shown in Figure 22A. The slopes during periods of maximum and minimum activity within one cardiac or one respiratory cycle were drawn for each histogram. A modulation index was calculated by determining the ratio between the slopes of maximum and minimum unit activity. The "cardiac related modulation index" (CRMI) for the units illustrated in Fig. 6 A, B and C was 10.3, 3.5, and 1.0, respectively. The "respiratory related modulation index" (RRMI) for these units was 10.9, 2.1, and 1.0, respectively.

Ratios depicting the probability of preganglionic unitary discharge with respect to the phases of the cardiac and respiratory cycle were determined in three experiments. The plot in Figure 24

Figure 23. Integrated post-R wave and post-expiratory time interval histograms. Cumulative distributions were derived from time interval histograms shown in Figure 22A.

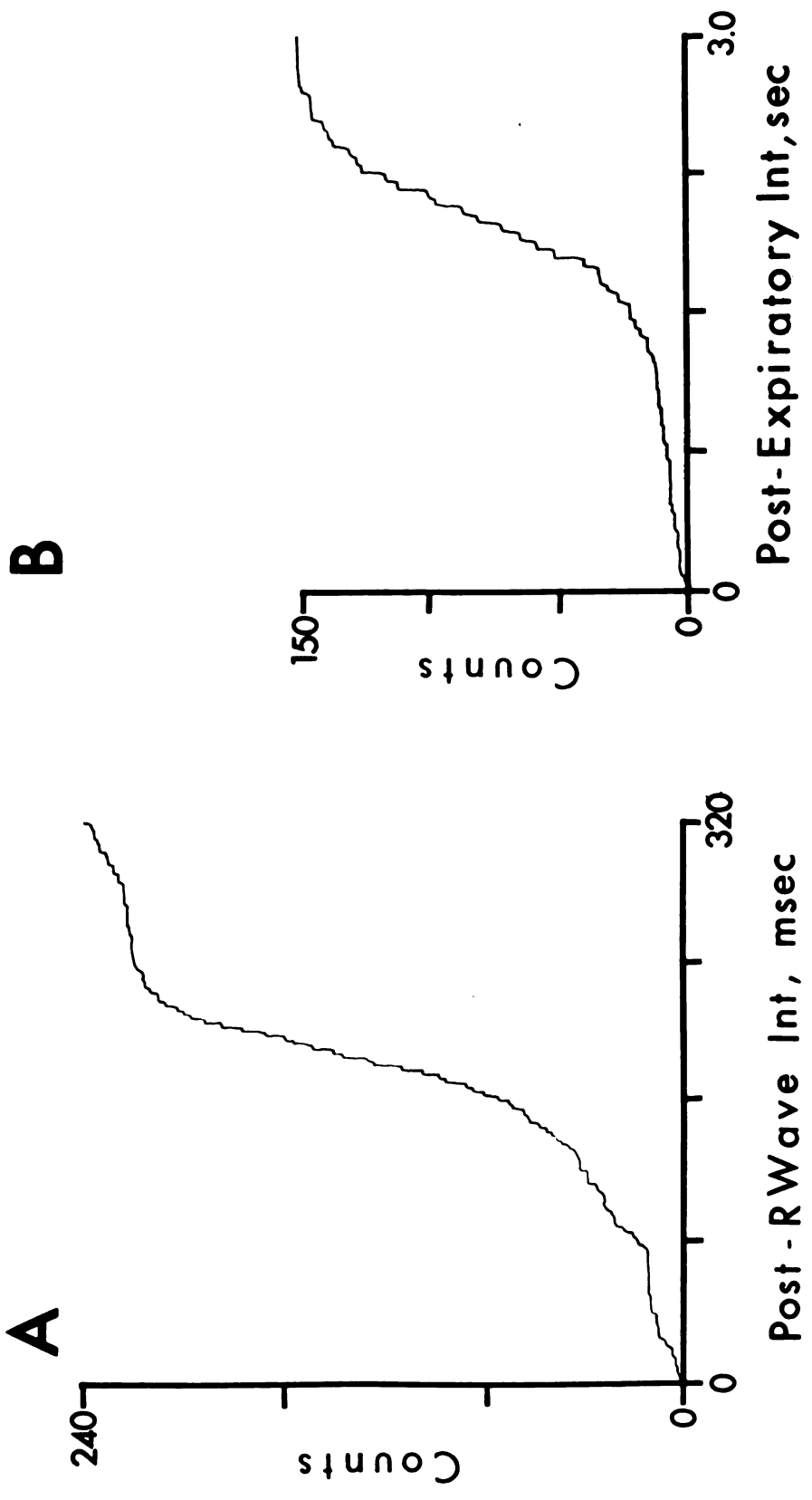
A
240
Counts
0
0
320
Post-RWave Int, msec
B
150
Counts
0
0
3.0
Post-Expiratory Int,sec

Figure 23

Figure 24. Relationship between modulation indices of cardiac-related (CRMI) and respiratory-related (RRMI) discharges of 22 PSNs in same vagotomized cat. Method for calculation of modulation indices is described in text.

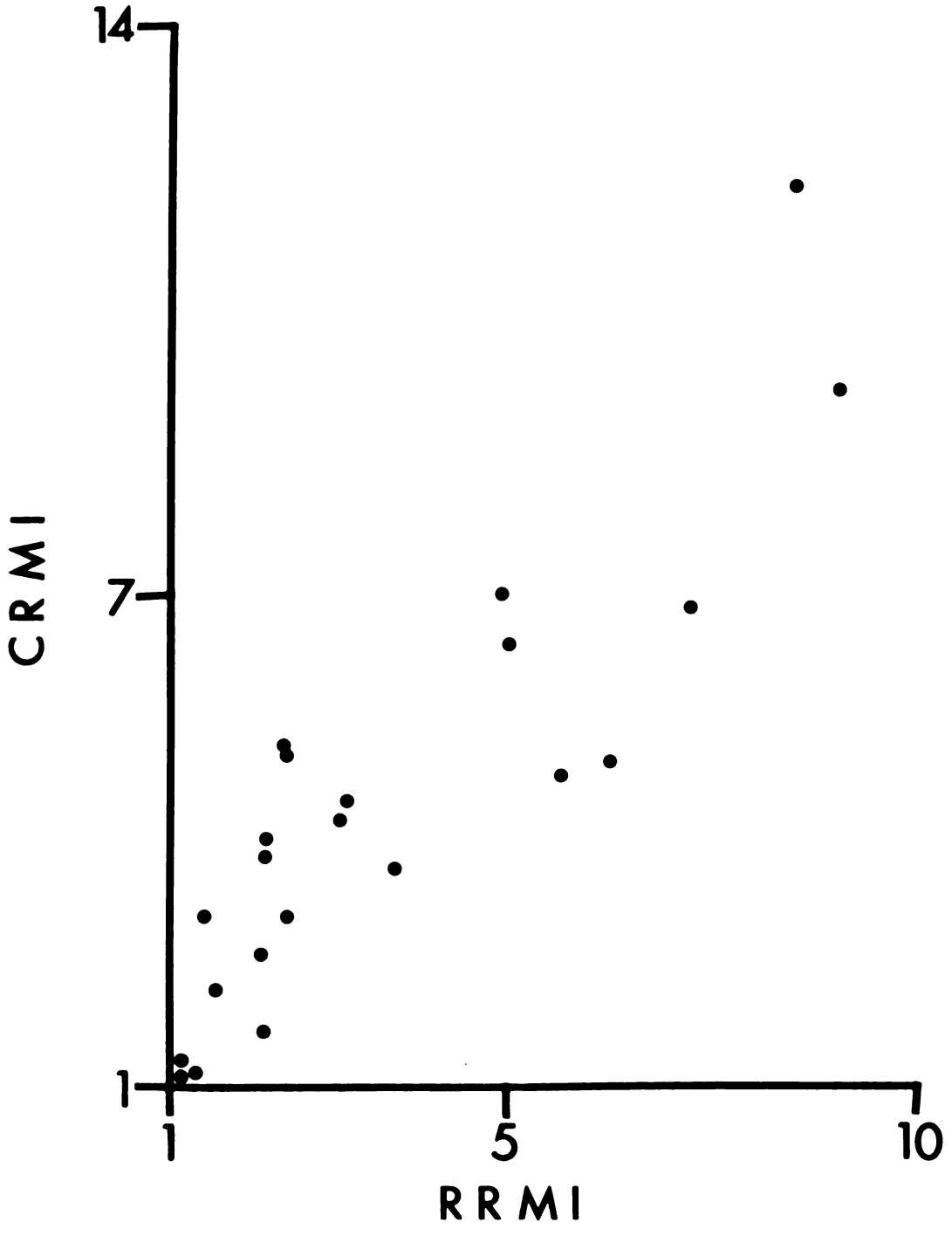

Figure 24

indicates the existence of a direct relationship between the degree of cardiac-related and respiratory-related discharges of individual PSNs. Data was collected from 22 PSNs in the same cat during a time span in which blood pressure and respiratory rate remained essentially constant. The plot shows a significant correlation (r=0.91) between the degree of cardiac- and respiratory-related discharges of PSNs. Plots similar to the one shown in Figure 24 were obtained in the other two experiments. The co-existence of the cardiac- and respiratory-related periodicities in the discharges of PSNs indicate that these neurons serve as the final common pathway for both the cardiac and the respiratory central rhythm generating mechanisms.

B. Co-existence of the 3 c/sec and 10 c/sec Periodicities in Discharges of Single PSNs

The 3 c/sec and 10 c/sec periodicities can be viewed in combination or independently in recordings made from whole sympathetic nerves (McCall and Gebber, 1976). The following experiment was designed to determine if these periodicities of SND are mediated by the same or different populations of PSNs. Autocorrelation analysis was used to determine the periodic components within the discharges of individual PSNs. By determining the average firing probabilities at various times after a spike, this method brought out periodic groupings otherwise masked by firing variability (see Mannard and Polosa, 1973). The autocorrelograms shown in Figure 25 indicate that the discharges of individual PSNs may contain one or a combination of the 3 and 10 c/sec periodic components of SND. The autocorrelogram of unit A exhibited a marked 3 c/sec periodicity. The period of the correlogram fluctuation was equivalent to the duration of the cardiac cycle (i.e.,

Figure 25. Autocorrelation of the discharges of 3 preganglionic neurons (PSNs). Histograms were constructed by triggering the sweep of the computer with a spontaneous spike of the PSN. Histograms were based on 371 trials in A, 314 trials in B, and 160 trials in C. Address bin was 8 msec for each histogram.

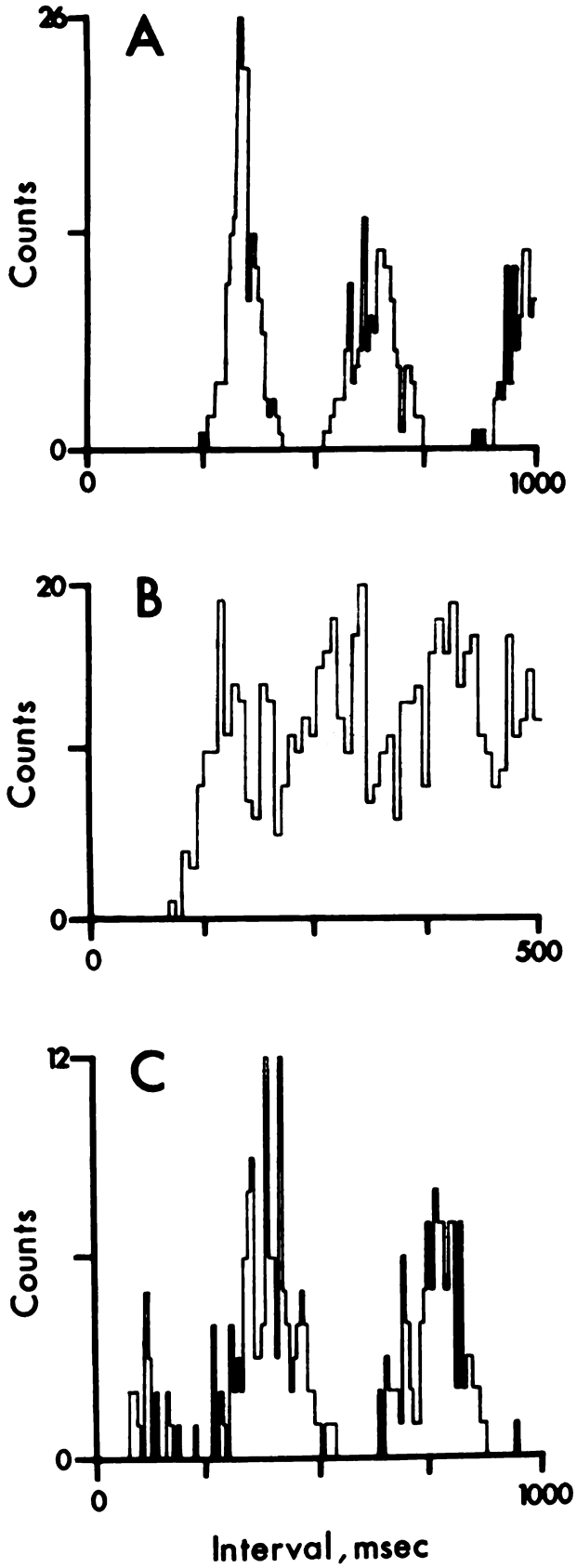
A
26
Counts
0
0
1000
B
20
Counts
0
0
500
C
12
Counts
0
0
1000
Interval, msec

Figure 25

320 msec). Indeed the discharges of this unit were strongly related in time to the R wave (not shown). The autocorrelogram in Fig. 25B is representative of one of two units which exhibited a 10 c/sec periodicity. This unit had a negative post-R wave TIH relationship. Six neurons exhibited both the 3 c/sec and 10 c/sec periodic components in their discharges (Figure 25C). Note that the probability of discharge of PSN C increased markedly at 100 msec and 350 msec (the duration of the cardiac cycle) after the spike that initiated the analysis. The discharges of unit C were related in time to the R wave of the ECG (CRMI: ≈3.5). These results indicate that mixtures of 3 c/sec and 10 c/sec periodicities recorded from whole preganglionic sympathetic nerve bundles can arise, at least in part, from the same population of PSNs.

C. Relationship Between the CRMI of Cervical Sympathetic Fibers and Their Peripheral Conduction Velocities

The data presented in Figure 24 indicate that preganglionic units whose discharges showed markedly different degrees of correlation in time with the R wave often were located in the same cat during a time span when blood pressure remained essentially unchanged. This observation raises the possibility that PSNs subserving vasoconstrictor functions are more sensitive to phasic baroreceptor input than those which subserve noncardiovascular functions. Indeed cardiac periodicity is most prominent in the discharges of sympathetic nerve bundles that contain a high percentage of vasoconstrictor fibers (Koizumi et al., 1971; Gootman and Cohen, 1973; Gebber et al., 1975; Taylor and Gebber, 1975). Therefore, attempts were made to differentiate between the CRMI of preganglionic fibers on the basis of their peripheral conduction velocities. In this regard a functional

differentiation of the four groups of fibers (S_1-S_4) in the preganglionic cervical sympathetic nerve of the cat has been described on the basis of their conduction velocities (Eccles, 1935). Fibers of the S_2 group (conduction velocity between 2.0 and 7.0 m/sec) are thought to mediate vasoconstriction. Figure 26 shows that the cardiac modulation index of unit discharge is not related to the conduction velocity of the axon of the PSN. Fiber conduction velocity was calculated on the basis of the onset latency of unit discharge produced by single shock stimulation (2-10 v; 0.1 msec) of the whole sympathetic nerve at a site approximately 5 cm from the recording electrode. The conduction velocities approximated the total range of the four components of the compound action potential reported previously for preganglionic neurons in the cat cervical sympathetic nerve (Eccles, 1935). If the view relating conduction velocity to function is accepted, then it is clear that the CRMI cannot be used to predict the function of a PSN.

D. Relationship Between the CRMI of PSN Discharge and the Percent Inhibition of Neuronal Activity During Baroreceptor Reflex Activation

Figure 27 depicts the relationship between the cardiac modulation index of PSN discharge and the percent inhibition of neuronal activity produced when mean blood pressure was raised to 200 mmHg or higher by the intravenous injection of 1-2 μg/kg of norepinephrine. Percent inhibition was calculated on the basis of the number of spikes elicited by the PSN during 20 sec periods at control blood pressure and during the pressor action of norepinephrine. Gebber et al. (1973) have reported that inhibition of sympathetic nervous discharge during the pressor action of norepinephrine is eliminated by baroreceptor denervation in cats. A significant, albeit weak,

Figure 26. Relationship between CRMI and axonal conduction velocity for 32 PSNs. Method for determining axonal conduction velocity is described in text.

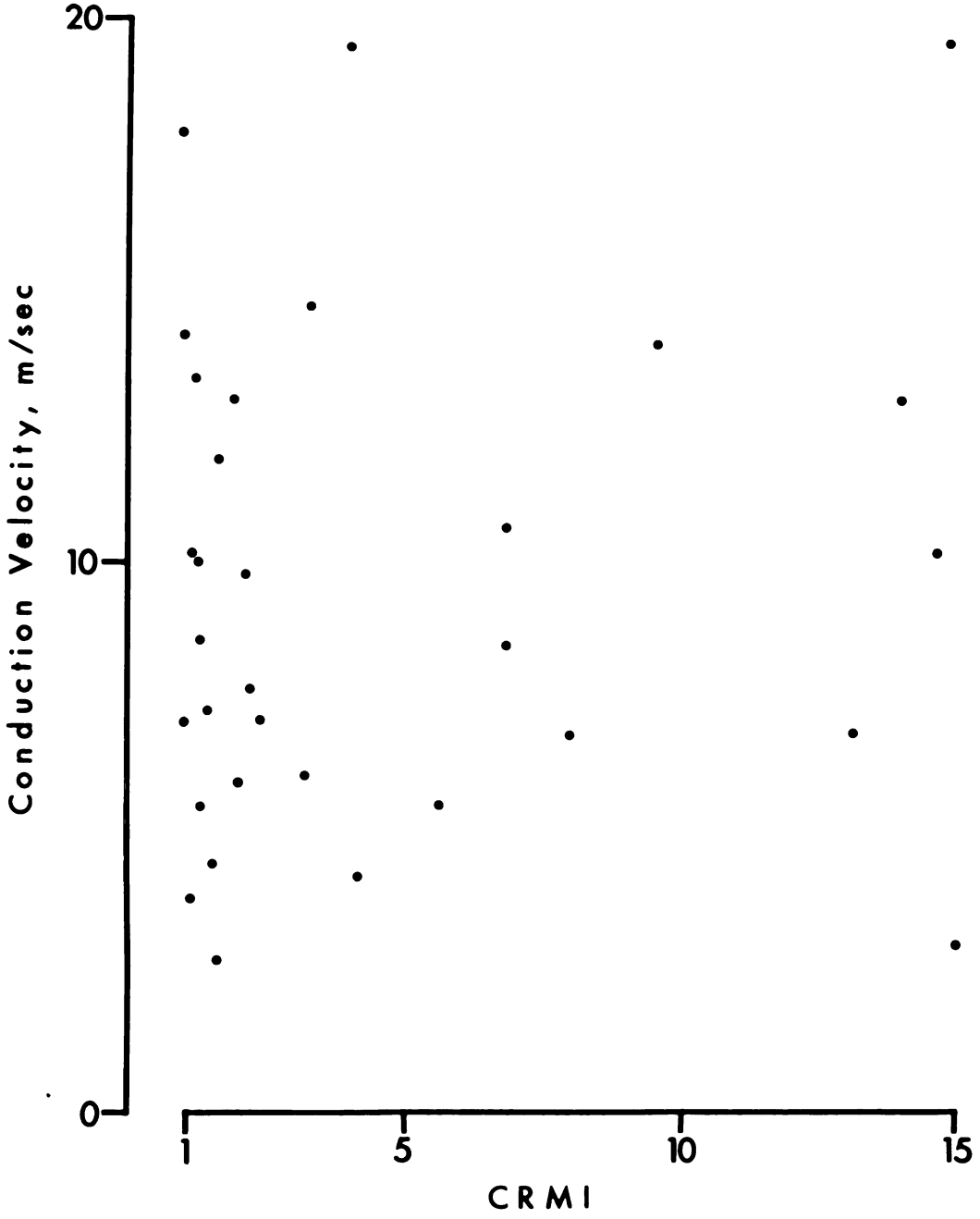

Figure 26

Figure 27. Relationship between CRMI and percent inhibition of spontaneous discharges during pressor action of norepinephrine for 52 PSNs. CRMI was determined at control blood pressure. Method for calculation of percent inhibition during elevation of blood pressure is described in text.

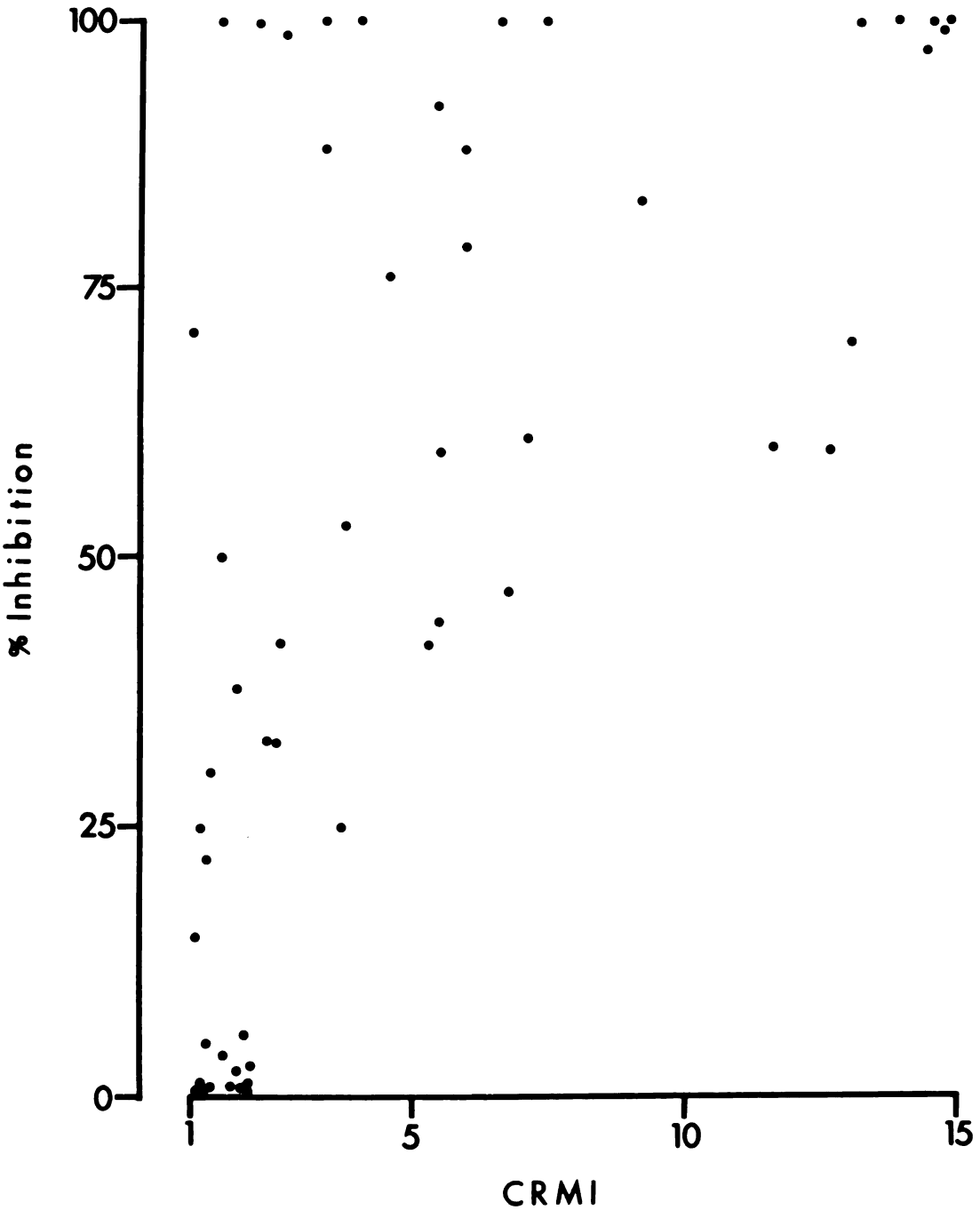

Figure 27

correlation (r=0.55) existed between the degree of cardiac-related unit discharge and inhibition of activity produced during the elevation of blood pressure (i.e., baroreceptor reflex activation). This observation indicates that, as might be expected, PSN whose discharges were related to the R wave (via baroreceptor phasing mechanisms) were the units most sensitive to baroreceptor-induced inhibition.

III. Characteristics of Sympathetic Nervous Discharge Recorded from Nerve Bundles

A. Spinal Origin of 10 c/sec Periodicity of SND

The objective of this series of experiments was to determine the origin of the 10 c/sec periodicity of SND. A high pass filtering circuit of 1 Hz was employed in order to transform bursts of SND into slow waves. The patterns of SND recorded from the renal nerve of cats are shown in Figures 28-30. Both the 3 and 10 c/sec periodicities were observed in cats with an intact neuraxis (Figure 28). Oscillations of SND locked in a 1:1 relation to the cardiac cycle (≈3 c/sec) are illustrated in Figure 28A. Faster activity was superimposed on the slow wave. This pattern was noted in 14 of 23 experiments. SND was synchronized into slow waves with a period approximating 100 msec (10 c/sec periodicity) in 3 cats in which mean arterial blood pressure was below 100 mmHg (Figure 28B). A mixture of the 3 and 10 c/sec periodicities was observed in the remaining experiments performed on intact cats (Figure 28C).

Figure 29 illustrates the respiratory related periodicity in the spontaneous discharges of the renal nerve of vagotomized cats with an intact neuraxis. As described above SND usually was synchronized into bursts which were locked in a 1:1 relation to the cardiac cycle.

Figure 28. Patterns of renal SND in 3 intact cats (A-C). Top traces: Arterial blood pressure (mmHg). Bottom traces: Renal SND. Horizontal calibration is 0.5 sec. Vertical calibration is 40 μV.

Figure 29. Respiratory periodicity in SND of vagotomized cat. Top trace is blood pressure (mmHg). Middle trace shows spontaneous discharges of external carotid postganglionic sympathetic nerve (negativity recorded as an upward deflection). Bottom trace shows RC-integrated phrenic nerve discharge (inspiration recorded as an upward deflection). Time base (below blood pressure) is 1 sec/division and applies to all traces. Vertical calibration is 40 μV and applies to SND.

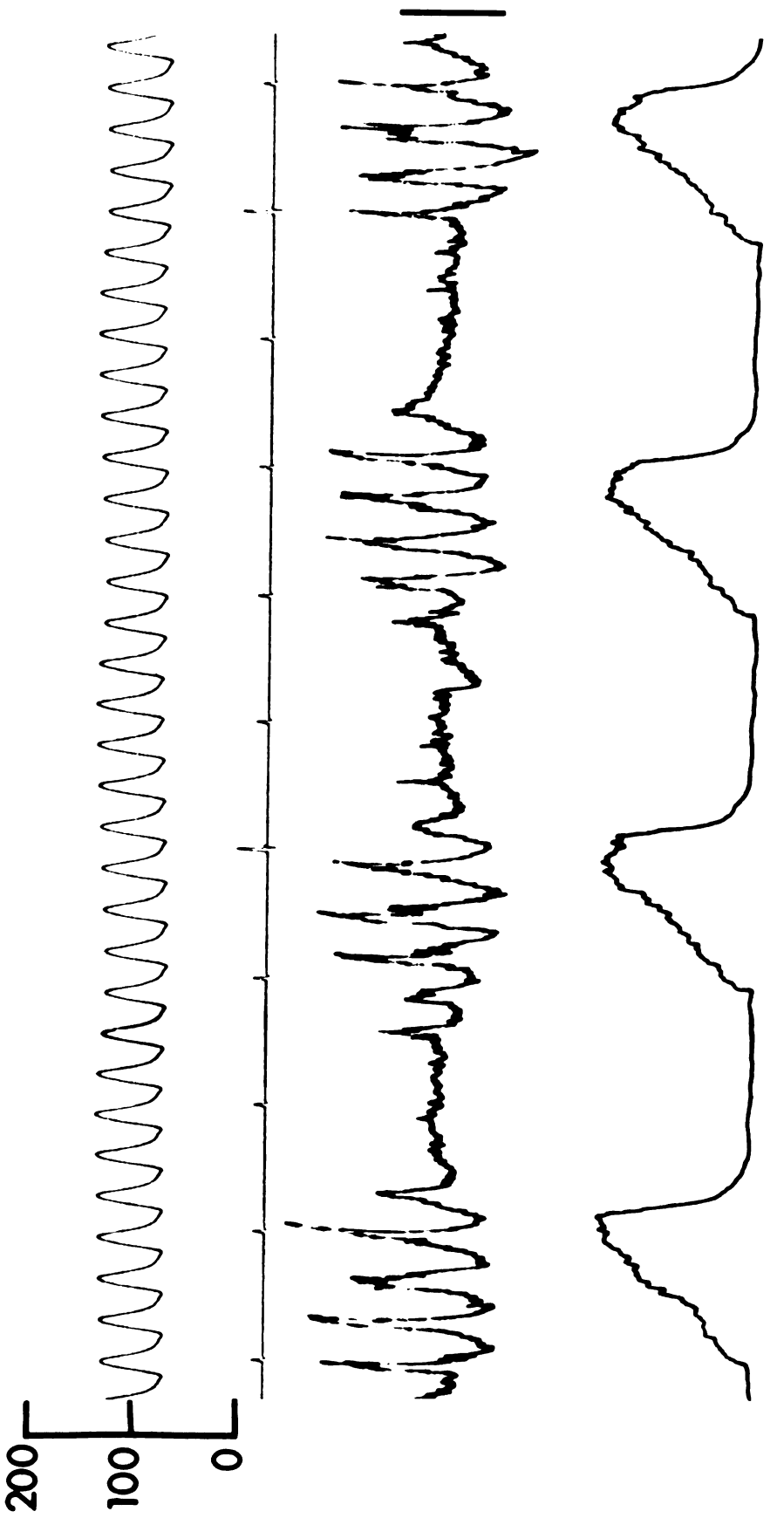

Figure 29

The cardiac related bursts of SND occurred almost exclusively during the inspiratory phase of the central respiratory cycle (monitored by RC integrated phrenic nerve activity) in the example shown in Figure 29. In other experiments SND began to increase from a minimum in early expiration and reached a maximum during inspiration.

The periodicities of renal SND observed in intact cats were compared to that seen in animals in which the spinal cord was sectioned at the level of the first cervical vertebra. Although SND in spinal cats (10 experiments) was minimal and irregular in form under resting conditions (Figure 30A), renal nerve discharge was synchronized into slow waves with a period approximating 100 msec during 1) asphyxia (20-40 sec after the artificial respirator was turned off) or 2) high frequency (20-50 Hz) stimulation (6-12 v square wave pulses) of descending pressor tracts located in the dorsolateral white columns of the mid-cervical spinal cord (Kerr and Alexander, 1964; Illert and Gariel, 1970; Gebber et al., 1973; Taylor and Brody, 1976). Faster activity superimposed on the 10 c/sec slow waves also was observed. The effects of asphyxia and spinal stimulation are illustrated in Figures 30B and 30C, respectively. However, the 3 c/sec and respiratory-related periodic components of SND never appeared in the spinal cat. Ganglionic blocking doses of hexamethonium (5 mg/kg, i.v.) abolished activity in the renal nerve of spinal cats (Figure 30D).

Autocorrelation analysis was performed to provide a quantitative measure of the periodicities of renal nerve discharge in intact and spinal cats. Periodicities approaching 3 or 10 c/sec were evident in the autocorrelation functions of SND from intact cats (Figure 31A and B). The autocorrelation function in spinal cats showed a minimal

Figure 30. Renal SND in a spinal cat. A: Traces of arterial blood pressure in mmHg (top) and renal SND (bottom) recorded 3 h after C_1 transection of spinal cord. B: During asphyxia (30-36 sec after artificial respirator was turned off). C: During stimulation (8 v; 0.5 msec; 40 Hz) of a midcervical spinal pressor site. D: Renal nerve recording 2 min after i.v. injection of hexamethonium (5 mg/kg). Horizontal calibration is 0.5 sec. Vertical calibration is 20 μV.

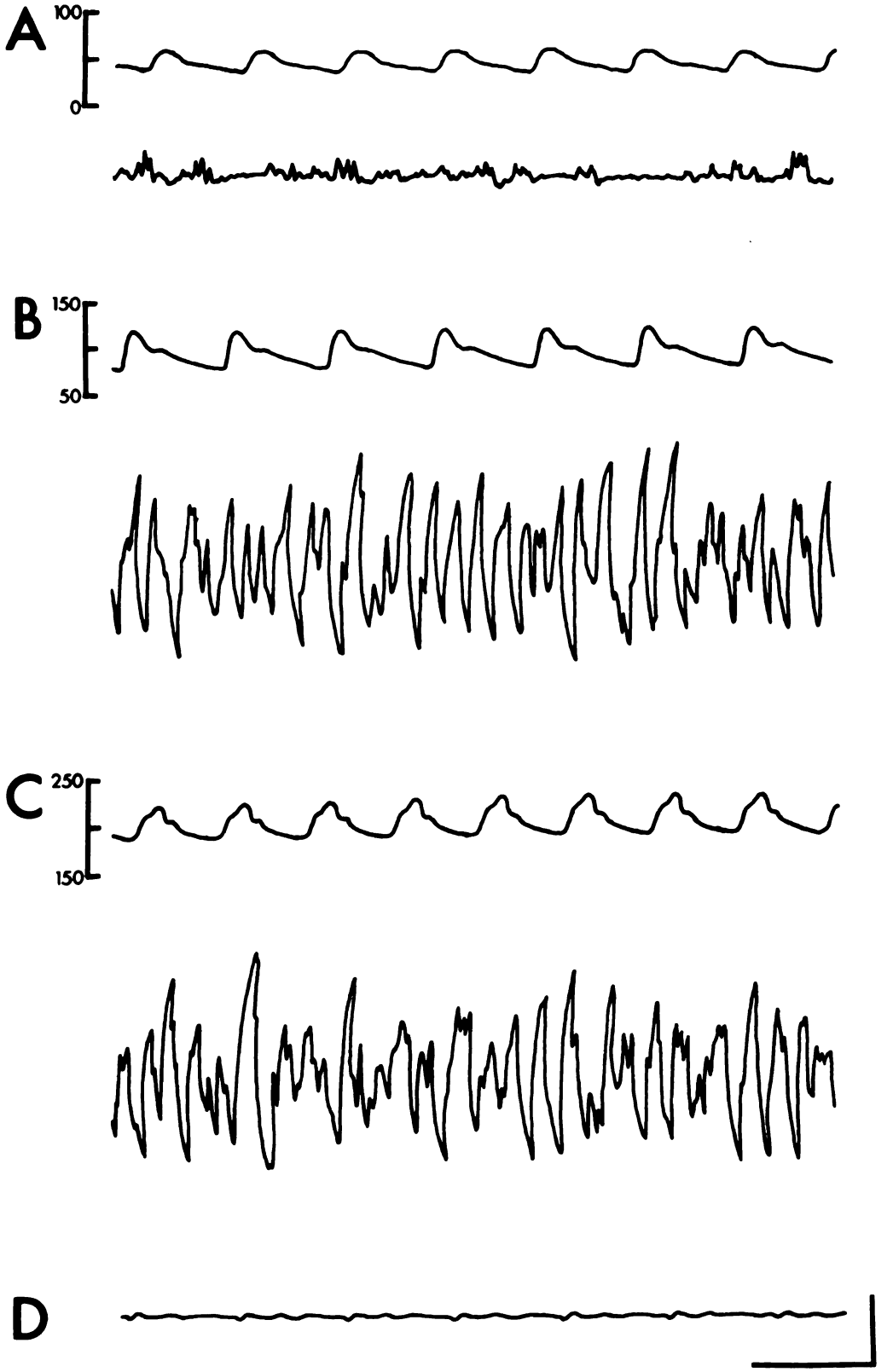

Figure 30

Figure 31. Autocorrelation functions of renal SND in intact and spinal cats. Autocorrelograms from 2 intact cats show ≈3 c/sec (A) and ≈10 c/sec (B) periodic components. Autocorrelograms from a spinal cat under resting conditions (C) and during asphyxia (D). Address bin was 2 msec in A-D. Sample run was 4 min in A-C and 3 min in D. Autocorrelogram D was constructed from data obtained during six 30 sec periods of asphyxia. Horizontal calibration is 500 msec for A and 250 msec for B-D.

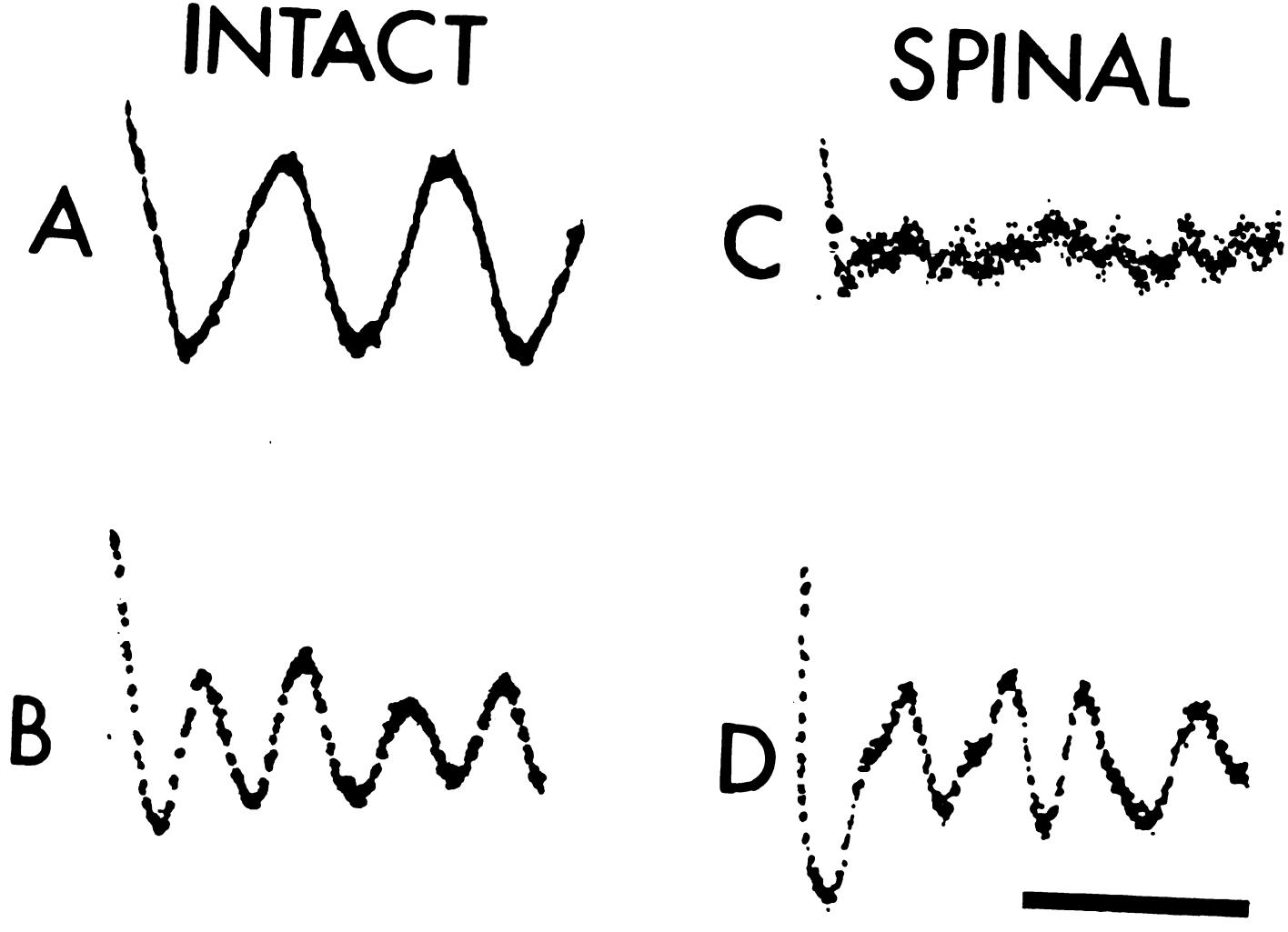

Figure 31

degree of periodicity under resting conditions (Figure 31C). However, a pronounced periodic component (≈100 msec) was present in the auto-correlograms derived from spinal cats during asphyxia (Figure 31D) or stimulation of mid-cervical spinal pressor sites. These observations demonstrate that SND is synchronized into 10 c/sec slow waves at the level of the spinal cord.

DISCUSSION

The purpose of the present investigation was to build a model of the organization of spinal sympathetic pathways based on single-unit data. The results indicate that the spinal cord contains complex sympathetic networks which are capable of integrating neuronal activity.

A. Characterization of Spinal Sympathetic Interneurons

Great difficulty has been encountered in identifying central neurons involved in regulating the discharges of preganglionic sympathetic nerves. As reviewed by Calaresu et al. (1975), Gootman et al. (1975) and Koepchen et al. (1975), a common approach has been to search for single units with the rhythmic firing pattern exhibited by populations of baroreceptor or preganglionic sympathetic fibers. With the exception of some neurons in the nucleus of the solitary tract, attempts to locate central units which discharge during each cardiac cycle have yielded little success. This suggests that an obvious cardiac-related rhythm in the discharges of central sympathetic units may be masked by firing variability. In this case, an insidious cardiac rhythm might be superimposed on the random basal discharge of a central sympathetic neuron (Gootman et al., 1975; Koepchen et al., 1976). On the other hand, the lack of a cardiac rhythm in the discharges of central sympathetic units may reflect the fact that these units fail to discharge during every cardiac cycle. In this regard,

the mean firing rate of individual pre- and postganglionic sympathetic neurons is less than the average heart rate under basal conditions (Green and Heffron, 1968a; Jänig and Schmidt, 1970; Mannard and Polosa, 1973; Seller, 1973; Taylor and Gebber, 1973). Therefore, the identification of sympathetic neurons whose processes lie within the central nervous system, and thus cannot be excited by antidromic stimulation of peripheral nerves, requires methods which present a picture of the probability of unitary discharge during the phases of the cardiac cycle or of the discharge pattern of whole sympathetic nerves (Gebber, 1975; Gootman et al., 1975). One of these methods, the post-R wave TIH was employed in the present study to identify spinal interneurons within pathways responsible for basal sympathetic nervous discharge. The results indicate that spinal interneurons contained within sympathoexcitatory and sympathoinhibitory pathways are located in the IML and IMM nuclei, respectively.

1. Sympathoexcitatory Interneurons Located in the IML Nucleus

A number of observations lead to the conclusion that the non-antidromically activated units in the IML cell column whose spontaneous discharges were correlated in time with the R wave were interneurons interposed between the terminals of reticulospinal fibers and preganglionic neurons. First, the positive relationship between the probability of unitary discharge and the phases of the cardiac cycle indicated that these neurons were contained within a sympathetic pathway. Second, the discharge patterns of these cells were distinctly different from those of antidromically identified preganglionic neurons. Non-antidromically activated units discharged spontaneously in bursts, sometimes with interspike intervals as short

as 2 msec. Preganglionic neurons, on the other hand, rarely discharged more than once during a cardiac cycle. Single shocks applied to medullary pressor sites evoked trains of spikes in non-antidromically activated units. Preganglionic neurons usually discharged only once to medullary pressor stimulation.

The question may be raised whether the non-antidromically activated neurons were preganglionic units whose axons were distributed in nerves other than the cervical sympathetic trunk. This possibility seems unlikely for two reasons. First, as described above, the discharge patterns of these neurons were distinctly different than those of antidromically identified preganglionic units. Second, other non-antidromically activated units whose discharge patterns were essentially identical to those of antidromically excited preganglionic neurons also were encountered in the vicinity of the intermediolateral cell column. These neurons are more likely candidates for preganglionic units whose axons did not run in the cervical sympathetic nerve.

The possibility that the high frequency discharges were recorded from descending reticulospinal fibers is remote. First, the recording microelectrode had a tip diameter of only 1 μm. Second, multiple rather than single discharges were elicited by each shock applied to pressor sites in nucleus reticularis ventralis, a medullary structure known to give rise to reticulospinal fibers (Brodal, 1957). It is also improbable that the multiple discharges of non-antidromically activated units in the IML nucleus were recorded from dendrites of PSNs. First, these high frequency discharging neurons could not be antidromically activated. Second, the dendrites of PSNs are short

(≈50 μm; Fernandez De Molina, 1965) and oriented in a longitudinal direction (Réthelyi, 1972). In this regard, the maximum spike height of the non-antidromically excited cells usually was observed about 75-100 μm ventral to the point where spike amplitude of preganglionic units in the same field was greatest. In some cases, the preganglionic unitary spike(s) disappeared from the recording field when the electrode was positioned to record maximum spike amplitude of the non-antidromically excited cell. Third, intracellular recordings of PSNs provided no evidence of repetitive dendritic firing in these cells (Fernandez De Molina, 1965).

The spinal sympathetic interneurons located in the IML nucleus were contained within pathways mediating sympathoexcitation. First, these neurons were excited by shocks applied to the medullary pressor region. Second, they were inhibited by stimulation of the medullary depressor region. Third, the spontaneous discharges of these neurons were inhibited during the pressor action produced by the i.v. injection of norepinephrine (i.e., baroreceptor reflex activation (Gebber et al., 1973)). In addition, it appears that sympathetic interneurons and PSNs in the IML cell column were closely adjacent and interconnected components of the same sympathoexcitatory pathway. First, conduction velocity from the medullary pressor region to the recording sites was not significantly different for the two neuronal types (Table 2). Second, conduction velocity over the medullospinal inhibitory pathway was the same whether calculated on the basis of the onset latency of the early period of depression of the discharges of either PSNs or sympathoexcitatory interneurons (Table 3). Third, the onset latencies of the late period of inhibition of the discharges of these two sympathetic elements evoked by stimulation of medullary

depressor sites was equivalent. Finally, post-R wave TIH analysis indicated that both sympathetic elements were contained within the pathway transmitting activity synchronized (i.e., 3 c/sec) in brain stem sympathetic networks.

As previously reported by Seller (1973), it was noted in the present study that the spontaneous discharges of some preganglionic units were not at all correlated in time with the R wave even though their discharge rate was markedly or completely suppressed during the pressor action of norepinephrine. Other preganglionic units or interneurons in the IML cell column whose spontaneous discharges exhibited a strongly positive R wave relationship often were found in the same cat. This observation raises the possibility that sympathetic neurons subserving vasoconstrictor function are more sensitive to phasic baroreceptor input than those which subserve noncardiovascular function. Indeed, cardiac periodicity is most prominent in the discharges of sympathetic nerve bundles which contain a high percentage of vasoconstrictor fibers (Koizumi et al., 1971; Taylor and Gebber, 1975). However, the data presented in Figure 27 indicate that the cardiac-related modulation index of preganglionic unitary discharge is not related to their axonal conduction velocities. If the view relating conduction velocity to function (Eccles, 1935) is accepted, then it is clear that the cardiac-related modulation index cannot be used to predict the function of PSNs or sympathoexcitatory interneurons. The function (sympathetic or non-sympathetic) of those non-antidromically activated units in the vicinity of the intermediolateral cell column which exhibited a negative post-R wave relationship remains to be determined (see below).

The results of the present study must be viewed in relation to those reported by Henry and Calaresu (1974a,b,c). These investigators observed short (3 msec) and long latency (20 msec) unitary discharges in the intermediolateral cell column of the thoracic spinal cord of cats upon stimulation of cardioacceleratory sites in the medulla. It was suggested that the long latency responses were recorded from preganglionic units and that the short latency responses were elicited in interneurons interposed between rapidly conducting medullospinal fibers (conduction velocity = 63 m/sec) and preganglionic units. The mean difference between the discharge onset latencies of the two spinal neuronal types (17 msec) was purported to account for the above mentioned discrepancies in the values reported for conduction velocity along descending sympathoexcitatory pathways. In contrast, the conduction velocity from pressor and depressor medullary sites to spinal sympathoexcitatory interneurons characterized in the present study, was considerably lower than 63 m/sec. Values reported in the current study for conduction velocity along medullospinal sympathoexcitatory pathways (2-3 m/sec) were within the range of those published by others (Gootman and Cohen, 1971; Gebber et al., 1973; Foreman and Wurster, 1973; Taylor and Gebber, 1973). In addition, values of conduction velocity from cardiovascular reactive medullary sites to spinal interneurons and to preganglionic cells in the IML nucleus were equivalent (Table 2). This observation indicates that, as might be expected, the delay between activation of spinal interneurons and preganglionic units was quite short, rather than the 17 msec value reported by Henry and Calaresu (1974c). With regards to this point, Henry and Calaresu neglected to determine whether either

of the two spinal neuronal types studied could be antidromically activated by stimulation of peripheral sympathetic nerves. This omission is important since they observed that the spinal units eliciting the long latency response discharged repetitively with short interspike intervals to single shocks applied to medullary cardioacceleratory sites. Thus, units classified as preganglionic cells by Henry and Calaresu (1974c) had at least one of the characteristics of those defined as spinal sympathetic interneurons in the present study. These neurons never responded with short latency (3 msec) discharges to stimulation of the medullary pressor region. Consequently, we are forced to conclude that neither medullary units with axonal conduction velocities exceeding 60 m/sec nor spinal neurons which elicit short latency responses upon medullary stimulation are contained within circuits responsible for basal sympathetic nerve discharge. The function of these two neuronal types remains to be determined.

Taylor and Gebber (1975) have described two distinct components of baroreceptor-induced sympathoinhibition. Inhibition of spontaneously occurring splanchnic or renal nerve discharge produced by 5 msec trains of 3 pulses applied to the carotid sinus nerve or the medullary depressor region was viewed as a computer-summed positive potential. Positivity produced by stimulation of the baroreceptor reflex arc contained an early and a late component. Importantly, discharges elicited in the splanchnic nerve by stimulation of descending spinal pressor tracts were inhibited during the time course of the early but not the late positive potential. This observation led to the conclusion that the early positive potential monitored sympathoinhibition exerted at a spinal level, while the late positive potential monitored inhibition in the brain stem. Gebber (1976) subsequently

demonstrated that both periods of inhibition were time-locked to the pulse-synchronous component of naturally occurring carotid sinus nerve discharge.

The results obtained in the present study shed further light on the nature of the interaction between sympathoexcitatory and baroreceptor reflex pathways. First, individual spinal units in the IML cell column exhibited both the early and late periods of inhibition upon electrical activation of intramedullary components of the baroreceptor reflex arc. This observation demonstrates that both spinal and brain stem inhibition were mediated on the same sympathoexcitatory pathway. Second, the observation that the onset of early inhibition recorded at the unit level was shorter than the earliest discharge evoked by stimulation of the medullary pressor region (see Tables 2 and 3) reinforces the view (Taylor and Gebber, 1975) that early inhibition was mediated at a spinal locus. Third, the observation that nonantidromically activated units in the IML nucleus exhibited an early phase of inhibition indicates that spinal inhibition was mediated at the level of the interneuron rather than directly on the preganglionic cell. This conclusion is at odds with that made by Wyszogrodski and Polosa (1973) and Kirchner et al. (1975). They reported that glutamate-evoked discharges of preganglionic units could be suppressed by stimulation of the medullary depressor region, cervical spinal depressor tracts and somatic afferent nerves in cats. The observation that chemically-induced excitation (iontophoretic application of glutamate) could be suppressed led these authors to conclude that spinal inhibition was mediated directly on the preganglionic

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cell. This conclusion must be viewed cautiously with respect to the data presented in the current study. First, it is possible that suppression of glutamate-induced preganglionic unitary discharge was related to disfacilitation (via inhibition of interneuronal discharge) rather than to direct inhibition of the preganglionic cell. Second, a component of the discharge of preganglionic units produced by glutamate might have been induced by the drug's exictatory effects on closely adjacent sympathoexcitatory interneurons. Although untested, the possibility also exists that spinal inhibition is mediated both at the level of the preganglionic cell and interneurons.

2. Sympathoinhibitory Interneurons Located in the IMM Nucleus

A number of observations made in the present study indicate that bulbospinal projections of the baroreceptor reflex arc terminate on and excite interneurons located in the medial portions of the zona intermedia (i.e., vicinity of IMM). First, the spontaneous discharges of 29 spinal units were interrupted during BLCO in cats in which the aortic depressor and vagus nerves were sectioned. This observation suggests that a primary source of driving input to these cells was of carotid sinus baroreceptor origin. Second, the same neurons were activated by single shocks applied to depressor sites in the medullary nucleus of baroreceptor fiber termination (i.e., NTS). Third, components of the spontaneous discharges of some of these units were correlated in time with the R wave of the ECG. Furthermore, the discharges of certain neurons in NTS and in the vicinity of IMM showed similar patterns of R wave locking. This observation also suggests the existence of connections between the nucleus of baroreceptor fiber termination and interneurons in the spinal cord.

Several possibilities exist concerning the function of the spinal neurons whose discharges were interrupted by BLCO. First, were the interneurons contained within bulbospinal pathways to expiratory motoneurons of the intercostal nerves? In this regard, medullary expiratory neurons are excited (via disinhibition) by baroreceptor activation (Richter and Seller, 1975), and some of these neurons send their axons to the spinal cord (Bianchi, 1971). This possibility seems unlikely, however, since as shown in Figures 24-27, spinal units whose activity was interrupted during BLCO did not exhibit the periodic high frequency discharge pattern characteristic of medullary and spinal respiratory neurons. Second, were the interneurons contained within the loop of the baroreceptor pathway responsible for inhibition of spinal somatic reflexes? This possibility seems remote since Coote and MacLeod (1974b) demonstrated that the latency of inhibition of thoracic spinal somatic reflexes produced by carotid sinus nerve stimulation was greater than 100 msec. The spinal units located in the present study were excited 8 msec after single shock stimulation of NTS. Third, were the spinal units whose discharges were interrupted by BLCO contained within the sympathetic cholinergic vasodilator pathway? This possibility also seems unlikely since the preganglionic neurons of the cholinergic dilator pathway are quiescent under basal conditions (Horeyseck et al., 1976). In addition, sympathetic cholinergic dilation is mediated over a ventrolateral medullary pathway (Schramm and Bignall, 1971) far removed from those nuclei (NTS; PRN) whose electrical activation led to short latency responses of units in the vicinity of IMM.

The data presented in this study indicate that IMM interneurons serve as the final link in the spinal pathway responsible for baroreceptor-induced inhibition of the spontaneous discharges of sympathoexcitatory elements located in the IML cell column. First, no evidence was obtained to suggest that sympathoinhibitory interneurons exist in the IML nucleus. Second, the value (11± 3 msec) for the onset of inhibition of sympathoexcitatory elements in the IML nucleus by PRN stimulation was close to that (8±1 msec) for the latency of activation of units in the IMM by NTS or PRN stimulation. The difference (3 msec) is not inconsistent with the possibility that spinal sympathoinhibition was mediated directly by the interneurons in the vicinity of the IMM nucleus. In this regard, a tract connecting the IMM and IML nuclei has been described (Bok, 1928).

Comparison of the data presented in this and previous studies also indicate that IMM interneurons are contained within the spinal pathway responsible for baroreceptor-induced inhibition of SND. Taylor and Gebber (1975) and Gebber (1976) found that the conduction velocity in the bulbospinal pathway mediating baroreceptor-induced inhibition of splanchnic nerve discharge was ≈12 m/sec. Similarly, conduction velocity from the intramedullary components (NTS, PRN) of the baroreceptor reflex arc to interneurons in the IMM nucleus was 15±2 m/sec. Further comparisons can be drawn between the results of the present investigation and those reported by Gebber (1976), Gootman and Cohen (1971) and Taylor and Gebber (1975). When peripheral conduction time in the splanchnic nerve (≈10 msec) is taken into account, the onset of spinal inhibition of whole sympathetic nerve activity produced by a) the pulse synchronous component of carotid sinus nerve

discharge (Gebber, 1976), b) stimulation of the carotid sinus or aortic depressor nerves (Taylor and Gebber, 1975), and c) stimulation of PRN (Gootman and Gohen, 1971; Taylor and Gebber, 1975) is close to the latency of excitation of IMM spinal units (8±1 msec) elicited by NTS or PRN stimulation. These comparisons indicate that spinal units in the IMM nucleus, whose discharges were interrupted during BLCO, mediate baroreceptor-induced inhibition of spontaneously occurring sympathetic nerve activity.

It has been established that vagal and glossopharyngeal afferents arising from different cardiovascular and respiratory chemo- and mechanoreceptors converge onto a common pool of neurons in NTS (Biscoe and Sampson, 1970; Gabriel and Seller, 1970; Middleton et al., 1973; Werz et al., 1974). The results of the present study indicate that interneurons within the baroreceptor reflex arc receive input from additional sources as well. In cats in which the aortic depressor and vagus nerves were sectioned, the peak (30±2 msec) in the unimodal post-R wave TIH of spinal cells (Figure 15) occurred before the onset (69 msec after the R wave (Gebber, 1976)) of the pulse synchronous component of carotid sinus nerve discharge. Indeed, the timing of all but the second peak in the multimodal histograms of cells in the spinal cord (Figure 14) and in NTS (Figure 19) was inconsistent with that of the pulse synchronous component of carotid sinus nerve discharge. Input to these neurons from the carotid sinus nerves, however, was significant since their discharges were interrupted within one heart beat of occluding the common carotid arteries. This observation suggests that auxiliary afferent input responsible

for the early and late peaks in the post-R wave TIH was insufficient to discharge these units in the absence of carotid sinus baroreceptor activity.

A logical source of auxiliary input to interneurons of the baroreceptor reflex arc might be from rhythmically active cardiac and/or thoracic vascular afferents. Regarding this point, inhibition of pre-and postganglionic sympathetic nerve discharge has been reported in spinal and in intact preparations upon mechanical or electrical activation of afferent fibers which pass through the stellate ganglion (Malliani et al., 1971; Pagani et al., 1974; Koizumi et al., 1975; Weaver, 1976). Although no attempt was made in the present study to determine the effect of removal of the stellate ganglia on peaks within unitary post-R wave TIH, it was established that units in the spinal cord and in NTS, whose discharges were related in time to the R wave, could be orthodromically activated by single shocks applied to the inferior cardiac nerve. Spinal units responded twice to each shock. The latency of the early discharge (5 msec) was shorter than that (8 msec) for activation of spinal units by NTS stimulation (Figure 21). This observation suggests that the early discharge of units in the vicinity of IMM produced by stimulation of the inferior cardiac nerve was mediated over a spinal reflex pathway. The latency of the late spinal unitary discharge (12 msec) evoked by inferior cardiac nerve stimulation is consistent with activation over a supraspinal reflex pathway which perhaps includes NTS. Regarding this point, the sum of the latencies of activation of NTS units by inferior cardiac nerve stimulation, and of spinal units by NTS stimulation in different experiments was 14 msec.

3. Summary of the Organization and Interaction of Spinal Sympathetic Interneurons

A model of the organization and interaction of spinal sympathetic interneurons is presented in Figure 32. Interneurons contained within the sympathoexcitatory pathway are located in the IML cell column. Similarities in the conduction velocity from stimulation sites in the medulla to sympathoexcitatory interneurons (ESIN) and preganglionic neurons (P) indicate that these two sympathetic elements are closely adjacent and interconnected components of the same sympathoexcitatory pathway. ESIN may play an important role in the amplification of neuronal activity transmitted in sympathoexcitatory reticulospinal fibers (ERS). In this regard, the burst discharge of these interneurons may be necessary to bring preganglionic neurons to discharge threshold. Although untested, the possibility also exists that convergence of large numbers of ESIN onto PSNs may be crucial in the transmission of activity from brain stem synchronizing networks to PSNs. Observations in the present study also reveal that individual ESIN receive excitatory input from many ERS fibers. In this regard, the first order LH (Figure 10) showed that the variability of onset latency of the first interneuronal discharge in a spike train elicited by stimulation of a medullary pressor site was similar to that of PSNs (Table 2). As proposed by Gebber (1975), the redundancy of brain stem input, in combination with a continually changing population of brain stem neurons responsive to excitation, would lead to a degree of unpredictability regarding the onset latency of spinal unitary discharge to successive stimuli applied to the same medullary pressor site. In addition, random summation of electrically evoked and

Figure 32. Diagram of the organization and interaction of spinal sympathetic interneurons. Unfilled circles are excitatory terminals. Filled circle is an inhibitory terminal. B, baroreceptor afferent; CSA, cardiac sympathetic afferent; ERS, reticulospinal fiber of sympathoexcitatory pathway; ESIN, spinal interneuron in sympathoexcitatory pathway; IMIN, medullary interneuron in sympathoinhibitory pathway; IML, intermediolateral sympathetic nucleus; IRS, reticulospinal fiber in sympathoinhibitory pathway; ISIN, spinal interneuron in sympathoinhibitory pathway; NTS, nucleus of tractus solitarius; P, preganglionic sympathetic neuron; ZI, medial portion of zona intermedia including IMM. See text for description of neuronal connections.

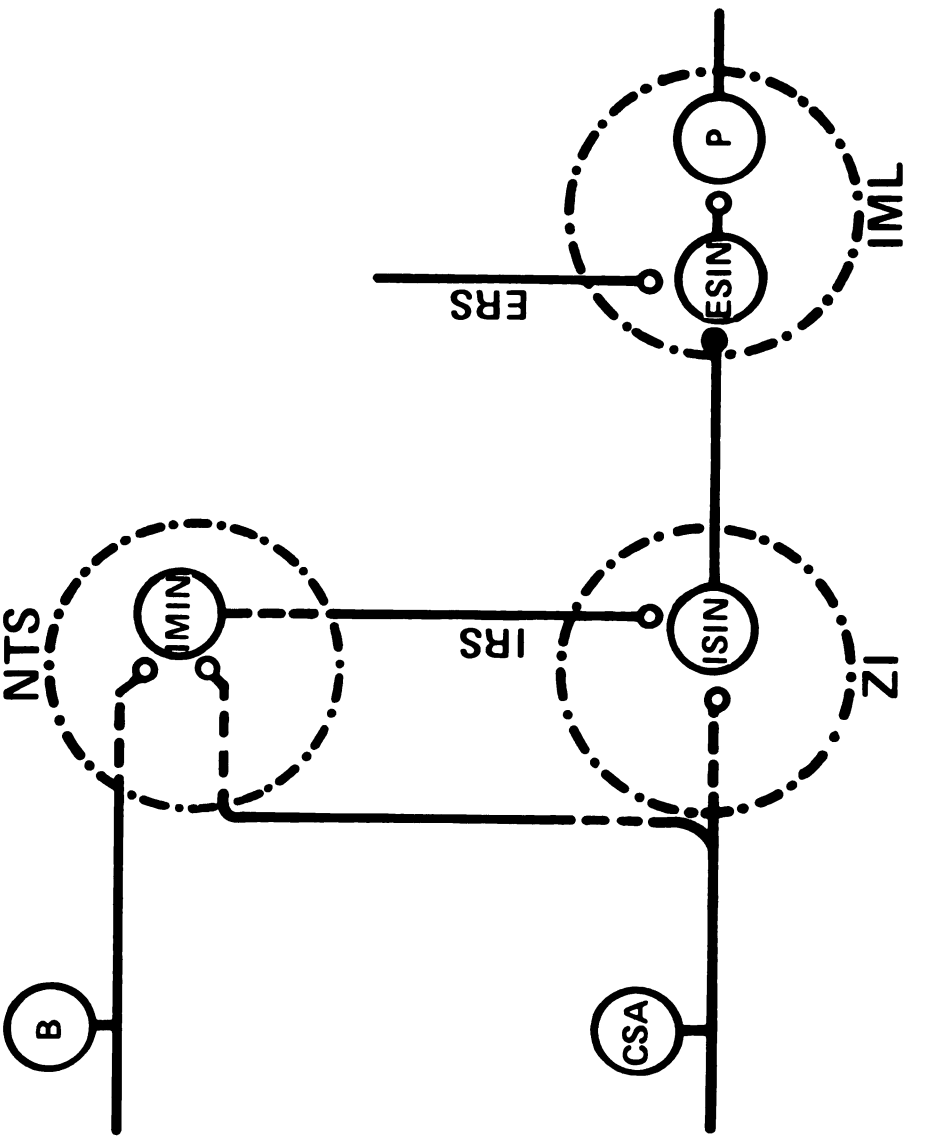

Figure 32

spontaneously occurring activity transmitted to ESIN over different pathway might contribute to the variability of discharge onset latency.

As illustrated in Figure 32, the interneuron contained within the baroreceptor reflex arc (ISIN) is located in the medial portion of the zona intermedia (ZI). The latency of excitation of ISIN by stimulation of intramedullary components of the baroreceptor reflex arc (NTS) was similar to that for the onset latency of inhibition of sympathoexcitatory elements in the IML cell column. This observation suggests that spinal inhibition is mediated directly by the terminals (filled circle) of ISIN. The present study also established that spinal inhibition of baroreceptor reflex origin occurs on sympathoexcitatory interneurons. Thus, the terminal of ISIN is directed to ESIN rather than to P. Neurons in the NTS (IMIN) project directly (Lipski and Trzebski, 1975; Trzebski et al., 1975) or indirectly (Humphrey, 1967; Miura and Reis, 1969,1972; Kirchner et al., 1975) through the reticular formation (IRS) to the inhibitory spinal interneuron. IMIN is driven by arterial baroreceptors (B) and by projections of afferents (CSA) which pass through the stellate ganglion. These afferents presumably arise from receptors in the heart and/or thoracic vessels. A direct spinal pathway for the activation of ISIN by CSA also appears to exist. This indicates that spinal sympathetic interneurons are important in the mediation of spinal reflexes. Further studies are clearly needed to determine the role of spinal sympathetic interneurons in the integration of spontaneous and reflex-evoked nerve activity.

B. Convergence of Rhythmically-Active Inputs to PSNs

The spontaneous discharges of sympathetic preganglionic nerve bundles often reveal mixtures of the 3 c/sec, 10 c/sec, and respiratory-related periodicities. The extent to which PSNs serve as the final common path for the three central rhythm generating networks is unknown. Therefore it was of interest to determine if a common pool of PSNs exhibit the cardiac-related, respiratory-related, and 10 c/sec periodic components of SND.

The direct relationship between the cardiac and respiratory modulation indices (Figure 25) leads to a number of conclusions concerning the convergence of inputs on PSNs. First, and most obvious, the co-existence of the cardiac- and respiratory-related periodicities in the discharges of PSNs demonstrate that these units serve as the final common path for both central rhtyhm generating networks. Second, the proportional nature of the plot in Figure 25 suggests that a long term depolarization of PSNs, produced by respiratory-related inputs, may allow synchronous activity from the 3 c/sec generating mechanism to bring PSNs to discharge threshold in a large percentage of cardiac cycles. That is, PSNs may be more receptive to cardiac-related inputs in the presence of a powerful respiratory-related depolarization. Convergence of cardiac- and respiratory-related activity may occur directly on PSNs or at a synaptic level central to the PSN. No attempt was made to determine if the discharges of sympathoexcitatory interneurons exhibited a prominent respiratory periodicity. However, Gootman et al. (1975) recorded from brain stem neurons whose discharge contained both cardiac- and repiratory-related periodicities. Third, the data in Figure 25 also indicate

that under comparable conditions in the same cat, PSNs exhibit different degrees of cardiac-and respiratory-related activity. This observation suggests that cardiac-and respiratory-related discharges are superimposed on a variable background of randomly occurring activity. The possibility may also be raised that PSNs which failed to exhibit cardiac- and respiratory-related activity were not anatomically connected to the rhythm generating networks. However, it is just as conceivable that rhythmic inputs to those neurons were weak and thus insufficient to bring them to discharge threshold.

The cardiac-related (≈3 c/sec) and 10 c/sec periodicities can be viewed in combination or independently in recordings made from whole sympathetic nerves (Figure 28). The data described in section B of the Results indicate that such is also the case for individual PSNs. Since the 10 c/sec periodicity of SND is not often temporally related to the cardiac cycle (Taylor and Gebber, 1975), autocorrelation analysis was used to determine the periodic components in the discharges of individual PSNs. Figure 26 shows that inputs from both the 3 and 10 c/sec generating mechanisms converge onto the same PSN. These data indicate that mixtures of these two periodicities recorded from whole sympathetic nerve bundles can arise, at least in part, from the same population of PSNs. Whether the networks responsible for the generation of the two rapid periodicities are connected in series at a level central to the PSN remains to be determined. These data also indicate that the 3 most common periodicities of SND are not associated with different functional pathways since they are mediated by the same PSNs.

C. Origin of the 10 c/sec Periodicity of SND

Cohen and Gootman (1969,1970) and Gootman and Cohen (1970,1971) noted a well defined 10 c/sec periodicity of SND in the cat splanchnic nerve which often was locked in 3:1 relation to the cardiac cycle. More recently Gootman and Cohen (1973) demonstrated that the spontaneous discharges of sympathetic nerves at different segmental levels have closely coupled 10 c/sec periodic components. On the basis of these observations they suggested that the 10 c/sec periodicity of SND reflects the fundamental organization of brain stem sympathetic centers. However, it is interesting to note that Cohen and Gootman (1969) and Gootman and Cohen (1971) observed that a computer summed early positive potential (i.e., spinal inhibition) evoked by stimulation of the medullary depressor region was followed by damped oscillations (10 c/sec) of SND. It is difficult to envision locking of the 10 c/sec periodicity to the electrical shock applied to a depressor site in the medulla unless the resultant spinal inhibition entrained a spinal rhythm. Therefore, it was of interest to determine the level of the neuraxis responsible for the synchronization of SND into 10 c/sec slow waves. Data presented in section C of the Results indicate that renal nerve discharge was synchronized into slow waves with periods approaching 100 msec during asphyxia or high frequency stimulation of descending pressor tracts in the high spinal animal (Figure 30). A pronounced 10 c/sec periodic component of SND was present in the autocorrelograms derived from spinal cats (Figure 31). These data demonstrate that the discharges of sympathetic nerve bundles are synchronized into 10 c/sec slow waves at the level of the spinal cord.

Previous experimentation (Taylor and Gebber, 1975) revealed that the 3 c/sec periodic component of SND is of central rather than baroreceptor reflex origin. Wave forms with periods approaching 300 msec were not observed in the spinal cat. Thus, the absence of this periodic component of SND in the spinal preparation supports the contention (Taylor and Gebber, 1975) that the 3 c/sec periodicity reflects the fundamental organization of brain stem sympathetic networks.

These data attest to the complexity of connections between spinal sympathetic elements. The occurrence of the 10 c/sec periodicity of SND during asphyxia or stimulation of descending pressor tracts in C_1 transected animals implies the existence of excitatory and/or inhibitory feedback networks at the spinal level. These networks are probably interneuronal in composition since Réthelyi (1972) demonstrated that the axons of preganglionic sympathetic neurons of the cat exit through the ventral roots without giving rise to recurrent collaterals. The observation that the spontaneous discharges of sympathetic nerves at different segemental levels have closely coupled 10 c/sec periodic components (Gootman and Cohen, 1973) is indicative of the fact that these spinal networks are normally dependent on supraspinal driving inputs. The existence of a second pathway between the terminals of excitatory reticulospinal fibers and preganglionic neurons is indicated since the 3 c/sec periodicity of SND was the most common pattern observed in intact cats. The present study indicates that an interneuron is contained within this pathway. Thus, it appears that a spinal pathway also exists which more or less faithfully follows the wave of activity synchronized in brain stem

sympathetic networks. Whether the two proposed spinal pathways are activated by the same or different reticulospinal systems remains to be elucidated.

The function of those non-antidromically activated units in the vicinity of the intermediolateral cell column which exhibited a negative post-R wave relationship remains to be determined. The possibility exists that these units were components of the spinal 10 c/sec generating mechanism. In this regard the 10 c/sec periodicity of SND is rarely locked to the R wave of the ECG (Taylor and Gebber, 1975). In addition, no evidence was obtained to suggest that sympathoexcitatory interneurons characterized in the present study transmitted rhythmic activity generated by a 10 c/sec synchronizing mechanism. The ISIH of these interneurons never exhibited a peak around 100 msec (Figure 16). In contrast interspike interval and autocorrelation analysis revealed that the discharges of some PSNs exhibited a 10 c/sec periodic component (Figures 17 and 26). Therefore, the sympathoexcitatory interneuron characterized in the present study was not the site of convergence of inputs from the 3 c/sec and 10 c/sec generating networks. Thus, it appears that two distinct populations of interneurons mediate the 3 and 10 c/sec periodicity of SND. The two populations of interneurons must converge on a single population of PSNs (Figure 26). Clearly, additional studies are needed to determine the organization of the 10 c/sec generating mechanism.

SUMMARY

The present investigation characterized the organization of spinal sympathetic networks. Computer-aided techniques were used to identify three distinct sympathetic elements in the zona intermedia of the cat thoracic spinal cord. Two of these cell types were located in the intermediolateral (IML) cell column. The first could be antidromically activated by stimulation of the cervical sympathetic nerve and thus were classified as preganglionic neurons. Post-R wave time interval analysis revealed that the discharges of many preganglionic neurons were correlated in time with the cardiac cycle. The discharges of the second cell type in the IML nucleus were also temporally related to the R wave of the ECG. However, these units were not antidromically activated by stimulation of the cervical sympathetic nerve.

A number of observations lead to the conclusion that the non-antidromically activated units in the IML cell column whose spontaneous discharges were correlated in time with the R wave were interneurons interposed between the terminals of reticulospinal fibers and preganglionic neurons. First, the positive relationship between the probability of unitary discharge and the phases of the cardiac cycle indicated that these neurons were contained within a sympathetic pathway. Second, the discharge patterns of these cells were distinctly different from those of preganglionic neurons. Sympathetic interneurons

discharged spontaneously in bursts with interspike intervals as short as 2 msec. In contrast, the interspike interval of preganglionic discharges was always greater than 75 msec. Single shocks applied to medullary pressor sites evoked trains of spikes in the non-antidromically activated units. Preganglionic neurons usually discharged only once to medullary pressor region stimulation. Finally, similarities in the conduction velocity from medullary pressor sites to sympathetic interneurons in the IML nucleus and preganglionic units indicated that these two spinal elements were closely adjacent and interconnected components of the same sympathoexcitatory pathway.

Sympathetic units in the IML cell column exhibited both an early and a late period of inhibition upon electrical stimulation of intramedullary components of the baroreceptor reflex arc. The onset of early inhibition recorded at the unit level was shorter than the earliest discharge evoked by stimulation of the medullary pressor region. This observation indicates that the early inhibition was mediated at a spinal locus. Finally the fact that sympathoexcitatory interneurons in the IML nucleus exhibited an early phase of inhibition indicates that spinal inhibition was mediated at the level of the interneuron rather than directly on the preganglionic cell.

The third distinct spinal sympathetic element characterized in the present study was located in the intermediomedial (IMM) nucleus of the zona intermedia. A number of observations indicate that these units were interneurons contained within the spinal component of the baroreceptor reflex pathway. First, the spontaneous discharges of 29 IMM units were interrupted during BLCO in cats in which the aortic depressor and vagus nerves were sectioned. This observation

suggests that a primary source of driving input to these cells was of carotid sinus baroreceptor origin. Second, the same neurons were activated by single shocks applied to the nucleus tractus solitarius (NTS) and the paramedian nucleus (PRN). Third, components of the spontaneous discharges of some of these units were correlated in time with the R wave of the ECG. Furthermore, the discharges of certain neurons in the NTS and in the vicinity of IMM showed similar patterns of R wave locking. This observation also suggests the existence of connections between the nucleus of baroreceptor fiber termination and interneurons in the spinal cord. The data presented in this study indicate that IMM interneurons serve as the final link in the spinal pathway responsible for baroreceptor-induced inhibition of the spontaneous discharges of sympathoexcitatory elements located in the IML cell column. First, no evidence was obtained to suggest that sympathoinhibitory interneurons exist in the IML nucleus. Second, the value (11±3 msec) for the onset of inhibition of sympathoexcitatory elements in the IML nucleus evoked by PRN stimulation was close to that (8±1 msec) for the latency of activation of units in the IMM by NTS or PRN stimulation. The difference (3 msec) is consistent with the possibility that spinal sympathoinhibition was mediated directly by the interneurons in the vicinity of the IMM nucleus. Finally the data indicate that IMM interneurons terminate directly on sympathoexcitatory interneurons rather than on preganglionic neurons.

The convergence of rhythmically-active inputs to preganglionic neurons was also studied in the present investigation. A direct relationship existed between the degree of cardiac- and respiratory-related discharges of preganglionic units. In addition, the discharges

of some preganglionic units exhibited both a 3 c/sec and a 10 c/sec periodicity. These observations indicate that individual preganglionic neurons serve as the final common path for the 3 c/sec, 10 c/sec, and respiratory-related sympathetic rhythm generating networks.

It was also of interest to determine the level of the neuraxis responsible for the synchronization of SND into 10 c/sec slow waves. Therefore, the pattern of renal SND was compared in intact and spinal cats. Renal nerve discharge was synchronized into slow waves with periods approaching 100 msec during asphyxia or high frequency stimulation of descending pressor tracts in spinal animals. These data demonstrate that the discharges of sympathetic nerve bundles are synchronized into 10 c/sec slow waves at the level of the spinal cord.

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