THE MAKE-UP OF SPINAL CORD CIRCUITS WHICH PROCESS INPUTS FROM THE FEMORAL-SAPHENOUS VEIN

By

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Several characteristics of the afferents which conduct inputs from the femoral-saphenous vein to the spinal cord and the neurons within the cord which process these inputs were described. Experiments using the retrograde transport of horseradish peroxidase (HRP) from the vein to the dorsal root ganglia were performed to determine the number, distribution and sizes of the cell bodies from which the femoral-saphenous venous afferents originate. These experiments showed that afferents arising along the entire length of the vein project to very localized spinal levels and that these afferents are few in number. Most of the cell bodies labeled by the application of HRP to the femoral-saphenous vein were small in size

(diameter less than 35 micrometers). However, some large cell bodies (diameter greater than 50 micrometers) were also noted. It was estimated that over 70% of the femoral-saphenous venous afferents are C fibers; at least 13% were estimated to be A fibers. The largest venous afferents were predicted to conduct action potentials at approximately 60 m/sec.

The minimal spinal cord processing time for inputs elicited by stimulating large fibers arising in the femoral-saphenous vein was approximately the same as for inputs from skin; thus, di- or tri-synaptic pathways appeared to be the shortest circuits separating the primary afferents and motoneurons. Mappings of field potentials as well as the recording of single unit activity from reconstructed sites suggested that most of the first neurons activated by this afferent input are found in lamina V. Thus, it is likely that many of the large primary femoral-saphenous venous afferents terminate in this region.

Recordings were done from single spinal neurons driven by large venous afferents. The interneurons characterized were excited or both excited and inhibited for long durations following stimulation of the femoral-saphenous vein. These neurons were located predominantly in laminae V and VII. Most neurons could also be activated by stimulation of large muscle and cutaneous afferents. Some of the neurons were intracellularly stained; the reconstructed cells had large cell bodies and extensive dendritic fields.

CHAPTER I
GENERAL INTRODUCTION

The study of the neural substrate of relatively simple reflex pathways allows an investigator to gain insight into how the central nervous system organizes and coordinates motor behavior. A spinal reflex which appears to be produced by relatively simple central nervous system circuitry is elicited when receptors in the walls of the femoral-saphenous vein are stimulated (Thompson and Barnes, 1979; Thompson and Yates, 1983, 1984, 1986; Thompson et al., 1982, 1983). The femoral-saphenous venous afferents include fibers excited by intravenous perfusion pressures as low as 2-3 mm Hg (Thompson et al., 1983). Electromyographic recordings following low-threshold electrical stimulation of these afferents showed that they can elicit the simultaneous contraction of flexor and extensor hindlimb muscles (Thompson et al., 1982). It is interesting that both flexors and extensors are simultaneously excited by stimulation of afferents arising from the femoral-saphenous vein, as other known hindlimb reflexes involve the reciprocal facilitation and inhibition of motoneurons. Stimulation of cutaneous afferents or other exteroceptors generally results in flexor facilitation and extensor inhibition (Sherrington, 1910; Lloyd, 1943a; Hagbarth, 1952). Activation of muscle afferents typically produces the reciprocal facilitation and inhibition of agonists and antagonists (Lloyd,

1943a; Laporte and Lloyd, 1952; Matthews, 1969). The unique segmental reflex elicited by venous afferent stimulation has been suggested to provide a rapid means of controlling the counterpressure applied to the high-capacitance intramuscular veins; changes in this counterpressure are potentially important in compensating for orthostatic blood shifts (Thompson and Yates, 1983, 1984, 1986).

It is well established that other inputs from the internal environment, in addition to those from the femoral-saphenous vein, converge with somatic inputs in the spinal cord on interneurons that are part of somatic reflex pathways (Downman, 1955; Evans and McPherson, 1958, 1959, 1960; Evans, 1963). Spinal neurons which send their axons into ascending tracts, including the postsynaptic dorsal column pathway (Rigamonti and Hancock, 1974; Rigamonti et al., 1978), the spinocervical pathway (Rigamonti and Michelle, 1977) and the spinoreticular and spinothalamic pathways (Hancock et al., 1975; Foreman and Weber, 1980; Blair et al., 1981, 1984; Milne et al., 1981; Rucker and Holloway, 1982; Ammons et al., 1984; Rucker et al., 1984), also receive visceral inputs. These inputs arise in part from Pacinian corpuscles that sometimes discharge in phase with cardiovascular pulsations (Gammon and Bronk, 1935), thermoreceptors that are located at least in part along blood vessels (Thompson and Barnes, 1970; Rawson and Quick, 1972; Riedel, 1976), vascular receptors that respond to visceral movement or distention (Bessou and Perl, 1960; Floyd and Morrison, 1974; Floyd et al., 1976), proprioceptors found in the intercostal muscles and in the diaphragm

(Yamamoto et al., 1960; Remmers and Tsiaras, 1973; Davenport et al., 1985; Marlot et al., 1985), mechanoreceptors located in the vagina (Komisaruk and Wallman, 1977; Henry, 1983) and chemoreceptors innervating blood vessels (Moore and Moore, 1933; Lim et al., 1962; Potter et al., 1962; Besson et al., 1972). It would be of great interest to compare the spinal cord circuits which process inputs from the femoral-saphenous vein with the circuits that process other inputs from the internal environment.

The present study examined several of the characteristics of the afferents which conduct inputs from the femoral-saphenous vein to the spinal cord and the neurons within the cord which process these inputs. Experiments using the retrograde transport of horseradish peroxidase from the vein to the dorsal root ganglia were performed to determine the number, distribution and sizes of the cell bodies from which the femoral-saphenous venous afferents originate. The results of these experiments are described in Chapter II. In addition, field potential mappings were performed to determine the location of the first interneurons excited by the venous afferents. These mappings also demonstrated the shortest pathway between the primary afferents and motoneurons; this information is presented in Chapter III. The electrophysiological properties of the venous afferent-activated interneurons are discussed in Chapter IV. Interneurons with different locations in the cord were shown to be affected by venous afferent stimulation in different ways; the variability in how the excitabilities of neurons in the different laminae of Rexed were altered by vein stimulation is presented in Chapter V. Finally,

Chapter VI considers the hypothesis that the characteristics of the spinal cord circuitry which processes inputs from the femoral-saphenous vein are similar to the characteristics of the circuitry, described in previous studies, which processes other inputs from the internal environment.

CHAPTER II
TRACING OF AFFERENT PATHWAYS FROM THE FEMORAL-SAPHENOUS VEIN TO THE DORSAL ROOT GANGLIA USING TRANSPORT OF HORSERADISH PEROXIDASE

Introduction

In the mammal, intracranial (Davis and Dostrovsky, 1985; Keller et al., 1985; McMahon et al., 1985; Norregaard and Moskowitz, 1985), extracranial (McMahon et al., 1985; Norregaard and Moskowitz, 1985), and visceral (Bessou and Perl, 1960; Lim et al., 1962; Floyd and Morrison, 1974; Floyd et al., 1976; Guilbaud et al., 1977; Vance and Bowker, 1983; Barja and Mathison, 1984) blood vessels receive an extensive afferent innervation. Anatomical studies have also demonstrated the presence of large, myelinated fibers coursing along the walls of large veins of the hindlimb and into adjacent nerve trunks (Woollard, 1926; Hinsey, 1928; Truex, 1936; Polley, 1955). These observations are further supported by experiments in which electrical stimulation of carefully isolated segments of the femoral-saphenous vein elicited (i) compound action potentials recordable from the femoral nerve and dorsal root fibers entering the lower lumbar spinal cord (Thompson and Barnes, 1979; Thompson and Yates, 1986), (ii) evoked field potentials recordable from the lumbar cord (Thompson and Barnes, 1979; Thompson and Yates, 1986), (iii) changes in firing patterns recordable from single spinal motoneurons (Thompson et al., 1982) and (iv) electromyographic activity recordable from hindlimb muscles (Thompson et al., 1982). The

injection of fluid pulses into the femoral-saphenous vein or longitudinal stretch of the vein wall similarly elicited field potentials in the lumbar spinal cord and activity recordable from ventral roots (Thompson et al., 1983).

The study presented in this chapter was performed in order to gain insight concerning the projection pattern of these femoral-saphenous venous afferents to the spinal cord. The retrograde transport of horseradish peroxidase (HRP) from the femoral-saphenous vein to the dorsal root ganglia was used to determine the range of afferent sizes, the relative numbers of these afferents, and the spinal cord segments to which they project.

Methods and Materials

HRP Application Procedures

Experiments were conducted on kittens (5-6 weeks of age) and adult cats (weighing 2.5-3.8 kg). Animals were anesthetized using an intraperitoneal (ip) injection of sodium pentobarbital (Butler, 38 mg/kg) or an intramuscular (im) injection of Ketaset (ketamine hydrochloride, Bristol, 10 mg/kg) combined with Rompun (xylazine, Haver-Lockhart, 0.5 mg/kg). Maintenance doses of sodium pentobarbital (12 mg/kg) or Ketaset (5 mg/kg) were delivered as necessary to maintain areflexia during the time that tissues were in contact with HRP.

The skin of either of the hindlimbs was opened ventrally to expose the femoral-saphenous vein and underlying muscle. In 3 kittens and 9 adult cats, one to three 10-15 mm segments of the vein

were separated from surrounding tissues and placed on rubber dams. A segment of the proximal femoral vein (overlying the vastus medialis and adductor femoris muscles) was always prepared for HRP application; other HRP application sites were located distally and were spaced 3-6 cm apart. The tissues adjacent to the isolated vein segments were covered with a thick layer of petroleum jelly to prevent spread of the tracer. Uptake of HRP was promoted by making small tears in the outer vein wall with sharp forceps so that fibers coursing there would be damaged. A 30% solution of Sigma Type VI HRP in 2% dimethylsulfoxide was applied to the prepared vein segments; after 3-4 hrs the enzyme was removed from the vein and the application sites were irrigated with saline. Incisions were closed using wound clips and the animals were returned to their cages and allowed to recover.

As a control for these experiments, different HRP application sites were used in 2 adult cats. In these animals segments of the femoral-saphenous vein were separated from the adjacent muscle as described above. However, the HRP solution was applied to the muscle which lay directly underneath the 10-15 mm segment of vein that had been dissected away; these tissues were the most likely to be accidentally exposed to HRP during those experiments in which the tracer was applied to segments of the femoral-saphenous vein. In one experiment 2 application sites located 31 mm apart were used; in the other 3 application sites separated by 20 and 22 mm were used. The distribution of cells in the dorsal root ganglia labeled by this procedure was compared with that of cells labeled after the enzyme was applied to the femoral-saphenous vein.

Histological Procedures

After a recovery period of 50-60 hrs in kittens and 90-96 hrs in adult cats, the animals were reanesthetized; kittens were transcardially perfused with 250-500 ml of chilled saline containing 0.1% sodium nitrite and 1.5 units of heparin per ml followed by 2 l of chilled 1.25% glutaraldehyde/0.6% paraformaldehyde fixative. After an additional 30 min the fixative was replaced with chilled 0.1 M phosphate buffer (pH 7.4). Adult cats were similarly transcardially perfused; however, twice the volume of solutions were used than in the smaller animals.

The spinal cord and lumbosacral dorsal root ganglia were exposed by dorsal laminectomy. The L3-S1 dorsal root ganglia (on the side exposed to HRP) and the L6-S1 spinal cord segments were removed and placed in 0.1 M phosphate buffer (pH 7.4). The tissues were cut into 50 micron sagittal sections using a vibratome (Oxford). Histochemistry was performed using the chromagen tetramethylbenzidine according to the procedures of Mesulam (1982). Tissues were counterstained using neutral red, rapidly dehydrated and cleared with xylenes. Sections were observed using both bright- and dark-field illumination. Neuronal cell bodies in the dorsal root ganglia were noted to be oval in shape (see Figure 2-1). Both the long (major) and short (minor) axes of labeled cells were measured using an eyepiece graticule. The mean of these two measurements was taken to give the average cell diameter. In addition, the dimensions of unlabeled cells in representative sections were also measured to indicate the range of sizes of cell bodies present in the ganglia.

Controls for the Diffusion of HRP

In all experiments the ventral horn of the spinal cord was scrutinized for the presence of labeled motoneuron cell bodies. In those experiments in which HRP was applied to the muscle underlying the femoral-saphenous vein numerous densely labeled motoneuron cell bodies were noted. Thus, the presence or absence of labeled motoneurons appeared to be an adequate indicator as to whether HRP had diffused into the muscle underlying the femoral-saphenous vein. If any labeled cell bodies were noted in the ventral horn of a preparation in which HRP was applied to isolated segments of the femoral-saphenous vein, the experimental results were discarded and not analyzed further.

Results

Five of the 12 experiments (42%) in which HRP was applied to the femoral-saphenous vein were successful; in these experiments labeled neuronal cell bodies were present in the dorsal root ganglia and no labeled motoneuron cell bodies could be detected in the spinal cord. Examples of cell bodies in the dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein are shown in Figure 2-1.

Following the application of HRP to the femoral-saphenous vein, an average of 289 +/- 402 (mean +/- S.D.)[1] labeled cell bodies in the dorsal root ganglia was counted per experiment; an average of 182

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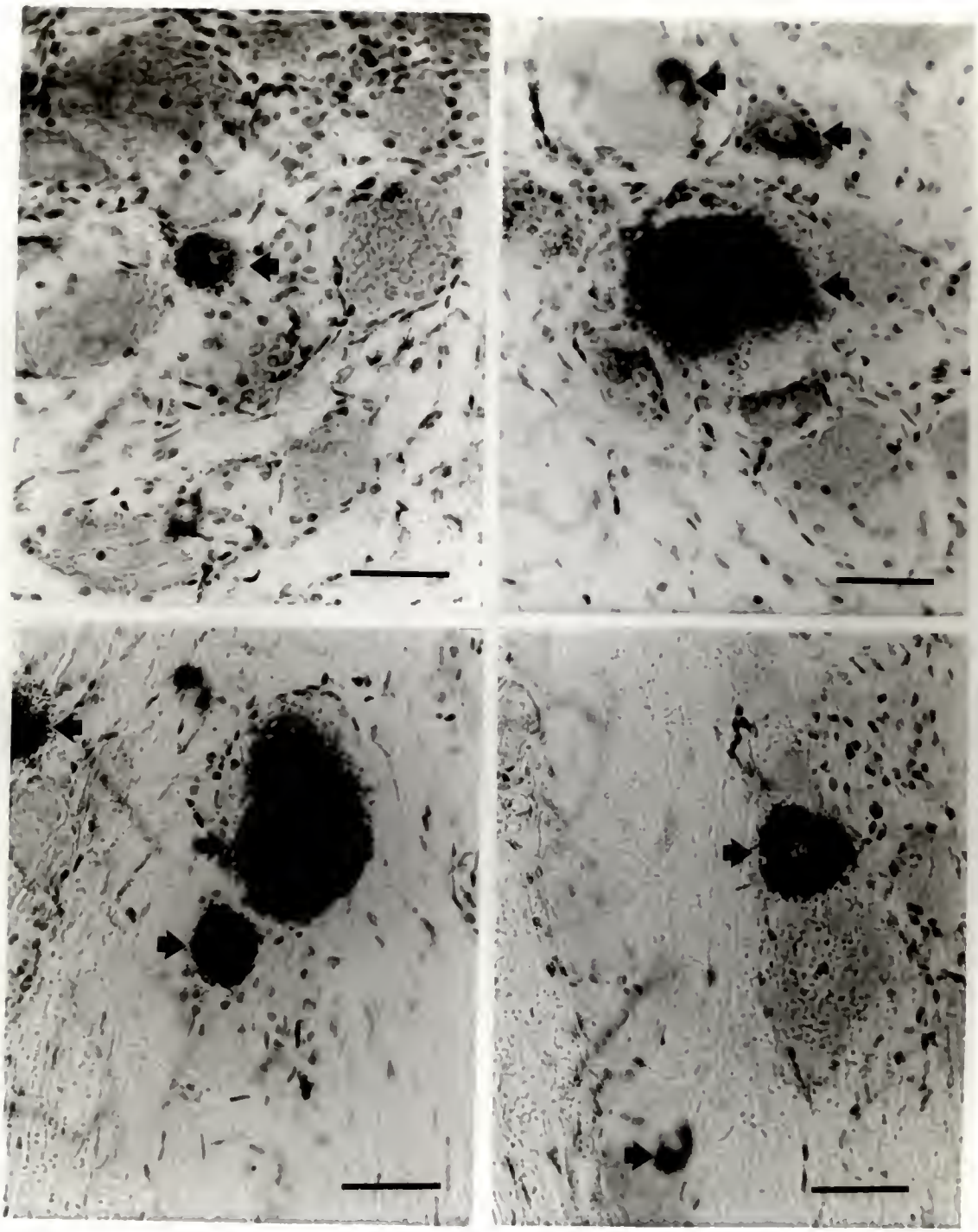
[1]Other confidence intervals presented here also represent mean +/- standard deviation.

Figure 2-1. Examples of cell bodies in the L5 and L6 dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein. Calibration bars represent 50 micrometers. Labeled neuronal cell bodies are denoted by arrows.



+/- 232 cells was counted per vein segment exposed to HRP. The top portion of Table 2-1 shows the locations of cell bodies in the dorsal root ganglia labeled by the application of HRP to the vein. In 3 preparations more than 95% of the labeled cell bodies counted were confined to the L6 dorsal root ganglion; in 1 preparation all of the labeled cell bodies were confined to the L5 dorsal root ganglion; in the other preparation 77% of the labeled cell bodies counted were located in the L5 dorsal root ganglion, and the other 23% were located in the L6 ganglion. In total 78% of the labeled cells counted were located in the L6 dorsal root ganglion, 21% were located in the L5 ganglion and less than 1% were located at other levels.

The numbers and locations of cell bodies in the dorsal root ganglia labeled by the application of HRP to muscle underlying the femoral-saphenous vein (bottom portion of Table 2-1) were quite different from those described above. In one experiment 6285 labeled cells were counted; in the other 6251 were counted. The cell bodies labeled by the application of HRP to the muscle underlying the femoral-saphenous vein had a more widespread distribution than did the cell bodies labeled by HRP application to the vein itself. In total 3.9% of the labeled cells counted were located in the L3 dorsal root ganglion, 7.3% were located in the L4 ganglion, 8.6% were located in the L5 ganglion, 21.2% were located in the L6 ganglion, 30.5% were located in the L7 ganglion and 28.5% were located in the S1 ganglion. It is reassuring that most of the cell bodies labeled by the application of HRP to muscle underlying the femoral-saphenous vein were located at levels caudal to those containing the cell bodies labeled by HRP application to the vein itself.

Table 2-1. Percentage of cell bodies in different dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein (top) or to the tissues underlying the vein (bottom).

HRP Applied to the Femoral-Saphenous Vein

Experiment #	# of Sites to which HRP Was Applied	# of Cell Bodies Labeled	Percentage of Labeled Cell Bodies Counted in Each Ganglion					
			L3	L4	L5	L6	L7	S1
1	1	391	0	0	77	23	0	0
2	2	953	0	0	3	96	1	0
3	2	45	0	0	0	100	0	0
4	3	51	0	0	0	100	0	0
5	3	7	0	0	100	0	0	0
SUMMATION	---	1447	0	0	21	78	<<1	0

HRP Applied to the Muscle Underlying the Femoral-Saphenous Vein

1	2	6251	0	4	10	25	36	25
2	3	6285	8	11	7	18	25	32
SUMMATION	---	12536	4	7	9	21	31	29

Figure 2-2 shows the diameters of cell bodies in the dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein of the kitten; the figure also shows the diameters of unlabeled cells measured in representative sections to indicate the range of sizes of cell bodies present in the ganglia. Figure 2-3 shows similar data collected from adult cats. Data derived from kittens and adult cats are shown separately because the cell bodies in young animals were noted to be smaller than those in the adult. Cell bodies in the kitten labeled by the application of HRP to the femoral-saphenous vein had an average diameter of 25.4 +/- 8.6 micrometers. The majority (87.5%) of the labeled cells were small in size (diameter < 35 micrometers); only 1.6% were large (diameter > 50 micrometers). A total of 694 unlabeled cells were measured in the kitten for comparison with the labeled cells. Unlabeled cells had an average diameter of 30.5 +/- 11.3 micrometers; this mean was significantly larger than that for labeled cells by Student's t-test ($p < 0.0001$). Seventy-three percent of the unlabeled cells were small in size (diameter < 35 micrometers); 6.5% were large (diameter > 50 micrometers).

The relative sizes of cell bodies in the dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein and unlabeled cell bodies were similar in the adult cat to the relative sizes in the kitten. Cell bodies in adults labeled by the application of HRP to the femoral-saphenous vein had an average diameter of 32.9 +/- 14.0 micrometers. Most (71.8%) of the labeled cells were small in size (diameter < 35 micrometers); only 13.2% were large

Figure 2-2. Diameters of cell bodies in the dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein of the kitten. The diameters of unlabeled cells measured in representative sections to indicate the range of sizes of cell bodies present in the ganglia are also shown.

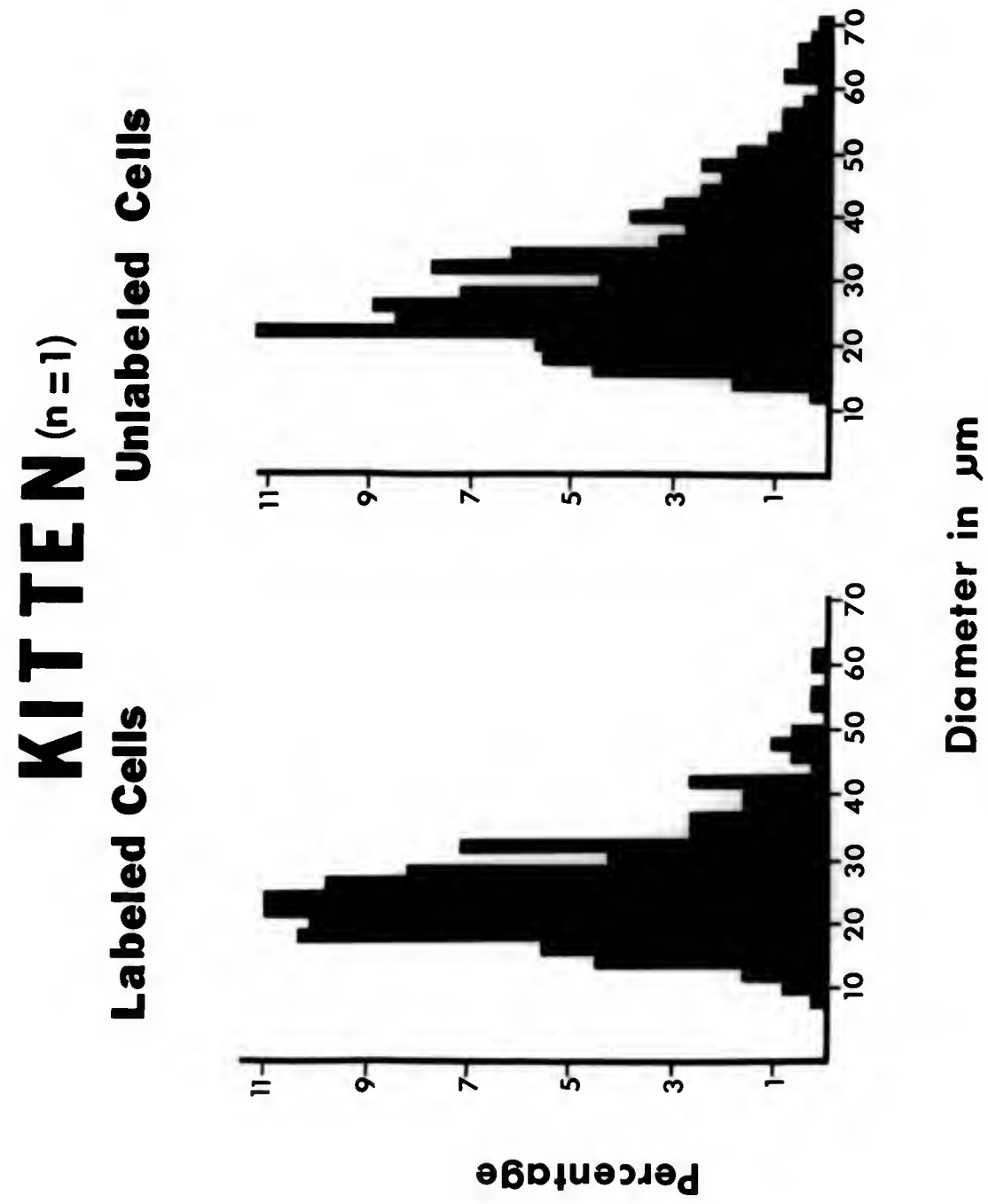
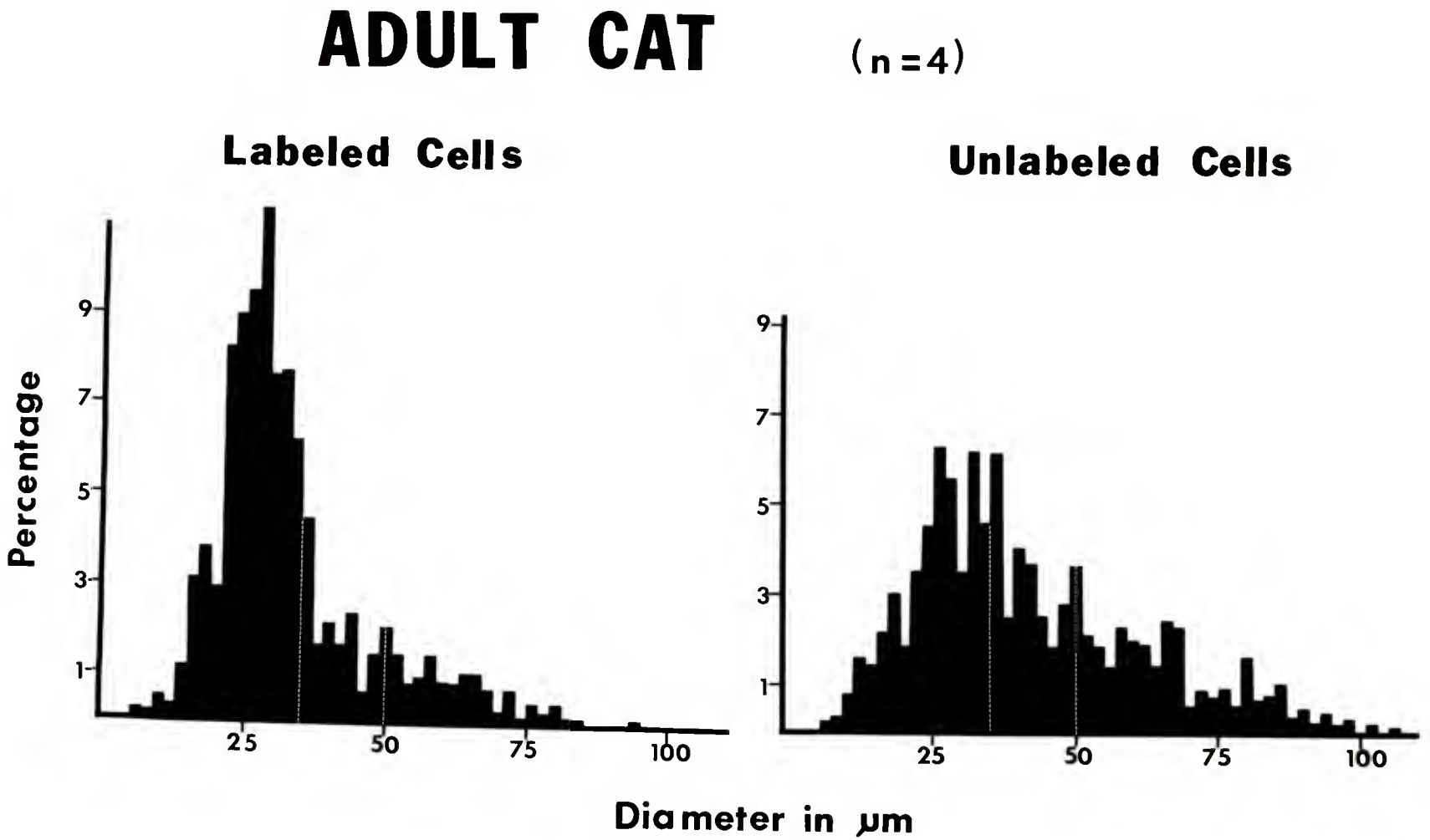

KITTEN (n=1)
Labeled Cells
Unlabeled Cells
Percentage
11
9
7
5
3
1
10 20 30 40 50 60 70
Diameter in µm

Figure 2-3. Diameters of cell bodies in the dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein of the adult cat. The diameters of unlabeled cells measured in representative sections to indicate the range of sizes of cell bodies present in the ganglia are also shown. Vertical dashed lines indicate divisions into small, intermediate and large-sized cells according to the scheme proposed by Lee et al. (1986).


ADULT CAT
(n=4)
Labeled Cells
Unlabeled Cells
Percentage
9
7
5
3
1
25
50
75
100
Diameter in µm

(diameter > 50 micrometers). A total of 1255 unlabeled cells were measured in the adults for comparison with the labeled cells. Unlabeled cells had an average diameter of 41.7 +/- 19.5 micrometers; this mean was significantly larger than that for labeled cells by Student's t-test ($p < 0.0001$). Forty-five percent of the unlabeled cells were small in size (diameter < 35 micrometers); 24.1% were large (diameter > 50 micrometers).

Both large and small cell bodies in the dorsal root ganglia of cats were labeled by the application of HRP to the femoral-saphenous vein; however, most of the labeled cells were small. Labeled cell bodies were smaller, on the average, than other cell bodies located in the ganglia; in addition, the percentage of large labeled cell bodies counted in the ganglia was smaller than the percentage of large unlabeled cell bodies. However, the anterograde transport of HRP from the dorsal root ganglia to afferent terminals in the spinal cord was far less extensive than the retrograde transport from the vein to the ganglia, if it occurred at all. The transganglionic labeling of intraspinal portions of afferents was never detected using either bright- or dark-field illumination following the application of HRP to the femoral-saphenous vein.

Discussion

Previous electrophysiological studies in the cat have shown that electrical or mechanical stimulation of isolated segments of the femoral-saphenous vein can elicit potentials recordable from the lumbar spinal cord (Thompson and Barnes, 1979; Thompson and Yates, 1983, 1984, 1986; Thompson et al., 1982, 1983). This study extends

these findings by providing an anatomical demonstration of the number, distribution and sizes of the cell bodies from which these femoral-saphenous venous afferents originate. The selective labeling of afferents arising in the walls of the femoral-saphenous vein in these preparations was supported by several lines of evidence. No labeling of spinal motoneurons could be detected in these studies, suggesting that HRP had not diffused from the vein to adjacent muscle. Further evidence suggesting that no spread of tracer occurred is that the application of HRP to the tissues adjacent to the femoral-saphenous vein produced labeling of cell bodies found predominantly in dorsal root ganglia caudal to those containing labeled cell bodies following application of HRP to the vein itself. These data also suggest that the afferents which were labeled terminated in the walls of the femoral-saphenous vein and were not fibers of passage coursing along the vein but arising in muscle. It would be very unlikely for all fibers of passage from muscle to be sensory afferents; if fibers arising in muscle had picked-up HRP in these experiments, the labeling of motoneuron cell bodies should have occurred. In addition, if fibers of passage from muscle had transported HRP, the distribution of labeled cell bodies in the dorsal root ganglia should have been similar to that following the application of tracer directly to the muscle. Since the cells labeled by the application of HRP to the femoral-saphenous vein had a different distribution, it is likely that they also had a different origin. Similarly, it is unlikely that fibers of passage arising in skin were labeled in these studies. The skin overlying the

femoral-saphenous vein in the intact animal is innervated by the saphenous nerve; this nerve projects to several spinal levels (Bernhard, 1953; Crouch, 1969). If fibers of passage from skin had picked-up HRP in these experiments, a more widespread distribution of labeled cell bodies in the dorsal root ganglia would have been expected. It is also noteworthy that the cell bodies in the dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein were significantly smaller, on the average, than other cell bodies in the ganglia. If a general class of hindlimb afferents, and not a specific population, had taken up HRP in these studies, the diameters of the labeled cells should have been identical to those of other cells in the ganglia.

A recent study by Lee et al. (1986) has made it possible to correlate the diameter of a cell body in a dorsal root ganglion with the conduction velocity along the peripheral process arising from it. These investigators made intracellular recordings from single cell bodies in the dorsal root ganglia of adult cats using microelectrodes filled with an HRP solution, determined the conduction velocity along the peripheral process to the cell body, and then injected the tracer into the neuron so it could later be visualized. They reported that cell bodies less than 35 micrometers in diameter gave rise to peripheral processes that carried impulses at less than 2.5 m/sec (C fibers). Cell bodies greater than 50 micrometers in diameter were reported to give rise to peripheral processes that carried impulses at greater than 2.5 m/sec (A fibers). For these neurons there was a linear correlation between the diameter of the cell body and the

impulse conduction velocity; this relationship was defined by the following equation:

Conduction Velocity = (0.84 * Diameter of Cell Body) - 20.3.

However, there was no predictable relationship between the diameters of intermediate-sized cell bodies (35-50 micrometers) and the conduction velocities along their peripheral processes.

Most (71.8%) of the cell bodies labeled by the application of HRP to the femoral-saphenous vein of the adult cat were smaller than 35 micrometers in diameter; these cell bodies would be expected to give rise to slowly conducting peripheral processes. However, 13.2% of the cell bodies labeled by the application of HRP to the vein were larger than 50 micrometers in diameter; these cell bodies should give rise to rapidly conducting peripheral processes. The estimated conduction velocities along these processes are shown in Table 2-2; the table also shows the predicted conduction velocities of other afferents based upon the diameters of cell bodies that were not labeled following the application of HRP to the vein. The largest femoral-saphenous venous afferents were estimated to have conduction velocities of approximately 60 m/sec. Fifteen percent of the cell bodies labeled by the application of HRP to the femoral-saphenous vein were intermediate in size (35-50 micrometers); no estimate could be made of the conduction velocities along these afferents. Blood vessels other than the femoral-saphenous vein have previously been shown to be innervated by thermoreceptors (Fruhstorfer and Lindblom, 1983), chemoreceptors (Moore and Moore, 1933; Lim et al., 1962; Potter et al., 1962; Besson et al., 1972) and slowly conducting

Table 2-2. Estimated conduction velocities along the peripheral processes of large primary femoral-saphenous venous afferents and other large primary sensory neurons determined from the diameters of the cell bodies.

Conduction Velocity Range	Percentage of Cells Falling into the Range	
	Femoral-Saphenous Venous Afferents	Other Afferents
20-30 m/sec	47	37
30-40 m/sec	36	32
40-50 m/sec	15	19
50-60 m/sec	2	10
> 60 m/sec	0	2

mechanoreceptors (Bessou and Perl, 1960; Floyd and Morrison, 1974; Floyd et al., 1976). It is likely that the femoral-saphenous vein is also innervated by one or more of these receptor types as well as by rapidly conducting afferents that have not been reported to be associated with other vessels.

Only a small number of afferents were labeled by the application of HRP to the femoral-saphenous vein (an average of 182 per vein segment exposed to the enzyme), and only a small fraction of the afferents labeled were large. However, electrical activation of A-alpha/beta femoral-saphenous venous afferents has been reported to produce large field potentials recordable from the cord (Thompson and Barnes, 1979; Thompson and Yates, 1986). For the anatomy and electrophysiology to be in register, it would seem that the divergence of the inputs from the femoral-saphenous vein to the spinal cord is substantial. Such divergence could be accomplished by either the extensive branching of the intraspinal portions of the afferents or the activation of second-order spinal interneurons that, in turn, make contacts with a large number of higher-order cells. Visceral afferent inputs into the lower thoracic spinal cord of the cat have previously been shown to exhibit a similar widespread divergence (Cervero et al., 1984).

CHAPTER III
PROPERTIES OF SPINAL CORD PROCESSING OF FEMORAL-SAPHENOUS VENOUS AFFERENT INPUT REVEALED BY ANALYSIS OF EVOKED POTENTIALS

Introduction

The study presented in Chapter II suggested that afferents with both large and small diameters project from the femoral-saphenous vein to the lumbosacral spinal cord of the cat. In most preparations the L6 segment was the input cord segment for these primary afferents. The study discussed in this chapter examined two characteristics of the interneurons which link inputs along these afferents with spinal motoneurons. The minimum time required for activation of spinal motoneurons by the femoral-saphenous venous afferents was measured to estimate the least number of interneurons interposed between the primary afferents and motoneurons. In addition, focal synaptic field potentials were mapped to determine the location of the interneurons monosynaptically excited by stimulation of the femoral-saphenous vein.

The field potential mapping techniques utilized in this study were first described by Gasser and Graham (1933); the intracord mapping of potential fields has since been performed in a number of studies (Campbell, 1945; Austin and McCouch, 1955; Howland et al., 1955; Coombs et al., 1956; Fernandez de Molina and Gray, 1957; Willis et al., 1973; Fu et al., 1974; Beall et al., 1977; Foreman et al., 1979) and has proven to be an effective preliminary method of

describing the spatial location of the interneuronal pools, activated by a particular stimulus. The most prominent field potential recordable from the dorsal horn is the result of ionic movements at excitatory axo-dendritic and axo-somatic synapses (Willis, 1980). When the synapses are activated, positive ionic current leaves the extracellular space at the synapses (sinks) and reappears along the axons of the interneurons (sources). Because the majority of dorsal horn interneurons ventral to the substantia gelatinosa have ventrally projecting axons (Matsushita, 1969, 1970), the dorsal horn takes on a net negative charge and the ventral horn takes on a net positive charge. The negativity of shortest latency is focussed in a region which contains a high density of interneurons monosynaptically excited by primary afferents (Yates et al., 1982).

Methods and Materials

Surgical Preparation of Animals

Data were recorded from 25 adult cats of either sex. Intraspinal potential fields were recorded from 7 of these animals; the minimum times between the arrival of the primary afferent volleys along muscle, cutaneous and venous afferents and detectable discharges in the ventral roots were determined in the other 18. Animals were initially anesthetized with 3% Fluothane (halothane, Ayerst laboratories) vaporized in a mixture of nitrous oxide and oxygen. A tracheostomy was performed and either of the common carotid arteries was cannulated; the other common carotid artery was ligated. The tracheostomy allowed for artificial ventilation with a

Harvard respiration pump; cannulation of the carotid allowed for measurement of arterial blood pressure. The left cephalic vein was also cannulated to allow the intravenous administration of drugs and fluids. Animals were rendered decerebrate at the midcollicular level by transection of the brainstem and aspiration of the portions of the brain rostral to this level. Following decerebration, anesthesia was discontinued; this occurred at least 4 hours prior to the beginning of the recording session. The spinal cord was also transected at T10. Animals were immobilized in a spinal unit (David Kopf); paralysis was induced through intravenous injection of Flaxedil (gallamine triethiodide, Davis-Geck, 2 mg/kg). Flaxedil was readministered when any muscle contractions were noted. Spinal cord segments L3-S1 were exposed by laminectomy, the dura mater was opened and pinned away from the cord dorsum, and the exposed tissues were covered with warmed mineral oil. Body and oil pool temperatures were maintained at 37 degrees C using a heating pad and a heat lamp.

The skin of either of the hindlimbs was opened dorsally to expose a segment of the femoral-saphenous vein. A 10-15 mm segment of the vein was separated from surrounding connective tissue and gently placed upon a silver bipolar stimulating electrode. A plastic membrane was placed beneath the stimulated vein segment to prevent accidental contact of the electrode with surrounding tissues. In those experiments in which the minimum time between the arrival of the primary afferent volley and a detectable discharge in the ventral roots was determined, a segment of one or more of the following nerves was also prepared, in a manner similar to that used to prepare

the vein, for bipolar electrical stimulation: the sural nerve, posterior tibial nerve, gastrocnemius nerve, deep peroneal nerve, plantaris nerve or deep posterior nerve (branch of the tibial innervating foot musculature). Bipolar stimuli were sometimes also applied to the skin overlying the femoral-saphenous vein. In addition, the L6 and L7 ventral roots were cut at dural entry and were placed across bipolar hook recording electrodes.

Stimulation Procedures

Bipolar electrical stimulation was used in these experiments to excite the femoral-saphenous venous afferents. A number of previous studies have also utilized electrical stimulation to activate the venous afferents (Thompson and Barnes, 1979; Thompson and Yates, 1983, 1984, 1986; Thompson et al., 1982); these studies provided considerable evidence that this stimulation only excited afferent fibers arising in the vein wall. Application of ligatures to portions of the vein immediately proximal and distal to the stimulating electrode abolished the evoked potentials elicited by passing current along that vein segment (Thompson and Yates, 1986). This result was apparently due to the blockade of impulse conduction in afferents coursing along the vein wall (Gelfan and Tarlov, 1956). Stimulation of isolated segments of the femoral-saphenous vein elicited a unique spinal reflex; the stimulation produced the simultaneous excitation of hindlimb flexor and extensor muscles acting on the thigh, crus and foot (Thompson et al., 1982). Activation of cutaneous afferents produces the reciprocal excitation and inhibition of flexors and extensors; activation of large muscle

afferents also elicits the reciprocal excitation and inhibition of hindlimb muscles (see Chapter I). It is thus likely that the afferents which were excited by the stimulation of isolated segments of the femoral-saphenous vein belonged to a unique class arising in the vein wall and were not fibers of passage coursing along the vein but arising in muscle or skin. The studies presented in the last chapter involved the application of HRP to isolated vein segments; the procedures used to isolate portions of the femoral-saphenous vein for exposure to this tracer and for electrical stimulation are similar. The results presented in Chapter II suggested that only afferents arising in the vein were labeled by the application of HRP to the isolated vein segments; thus, muscle and cutaneous afferents apparently were not coursing along these segments. It is clear that electrical stimulation is a technique which allows for the selective activation of femoral-saphenous venous afferent fibers.

During experiments in which intracord field potentials were mapped, the prepared segment of the femoral-saphenous vein was stimulated using single-shock square-wave pulses of current, 0.2 msec in duration, repeated at a frequency of 1 Hz. Stimuli were delivered by a digital stimulator (Frederick Haer Pulsar 6i). Stimuli 3 times threshold for eliciting field potentials recordable from the cord dorsum were used to excite the afferent fibers. This stimulus intensity was chosen because previous studies had shown it sufficient to elicit compound action potentials recordable from the dorsal roots with A-beta components of maximal amplitude. The A-delta and C fibers shown in Chapter II to also arise from the femoral-saphenous

vein were not excited by this stimulus intensity (Thompson and Yates, 1986); thus, a very synchronous spinal input was elicited. During the experiments in which the minimal times required for activation of spinal motoneurons were measured, the femoral-saphenous vein was stimulated at an intensity 2-3 times that necessary to evoke field potentials recordable from the cord dorsum. The hindlimb skin and the sural nerve were stimulated at twice cord dorsum threshold. Stimuli applied to muscle nerves were graded to evoke only monosynaptic reflexes recordable from the ventral roots or to elicit both monosynaptic and polysynaptic reflexes.

Recording Procedures

Intraspinal potential fields. Intraspinal field potentials were recorded using tungsten microelectrodes insulated with epoxy or glass except for a 10-20 micrometer tip which had initial impedances of 0.5-4.0 megohms. Potentials were recorded at 0.2 mm intervals from the cord dorsum to 4.4 mm in depth; electrodes were positioned using a hydraulic microdrive (Narishige). Five tracks were conducted across the L6 cord segment. Penetrations were optimally made at midline and 0.5, 1.0, 1.5 and 2.0 mm lateral to midline; slight alterations in the choice of penetration site were often necessary to avoid puncturing large blood vessels. Potentials were referenced to animal ground; the reference electrode was positioned in the wound margin adjacent to the cord. Potentials were led into A.C. preamplifiers (Grass P511) with a time constant of 250 msec and subsequently into a tape recorder (Crown-Vetter model A) which contained preamplifiers with a bandwidth of D.C. to 15 kHz. The

potentials were displayed on an oscilloscope and were averaged by an Ortec computer. Records of averaged potentials were made with an X-Y plotter (Hewlett-Packard).

A radiofrequency lesion was placed at the bottom of each track to facilitate the histological localization of the track. The depths of lesions were also analyzed as a control for tissue shrinkage by fixative and other factors (such as cord dimpling) which might cause depths measured from histological material to deviate from those measurements read from the vernier of the hydraulic microdrive.

Cord dorsum potentials. Cord dorsum potentials were recorded from L6 using silver ball electrodes and were referenced to animal ground. The potentials were led into A.C. preamplifiers, etc, as for potentials recorded from microelectrodes. Cord dorsum potentials were recorded in these experiments for two reasons. The measurement of cord dorsum potentials provided a means to determine the latency at which input along the fastest-conducting afferents arrived at the cord; several studies have shown that the initial triphasic spike of the cord dorsum potential is a compound action potential propagating through large primary afferent fibers (Austin and McCouch, 1955; Howland et al., 1955). In addition, the amplitude of cord dorsum potentials is known to vary in proportion to spinal cord excitability (Bernhard and Koll, 1953; Gelfan and Tarlov, 1956; Molt et al., 1978). Thus, the measurement of cord dorsum potentials provided a control to assure that the level of spinal cord excitability remained constant throughout the recording session and that little cord damage was produced by electrode penetrations in

those experiments in which intraspinal field potentials were mapped. Data recorded from animals in which the cord dorsum potentials varied in amplitude during the recording session were not analyzed.

Compound action potentials. Compound action potentials were recorded from the L6 and L7 ventral roots during those experiments in which the minimal time required for an afferent input to activate motoneurons was determined. The bipolar recording electrodes were positioned in close proximity to the spinal cord in these experiments. Compound action potentials were led into A.C. preamplifiers, etc, as described above.

Histological Procedures

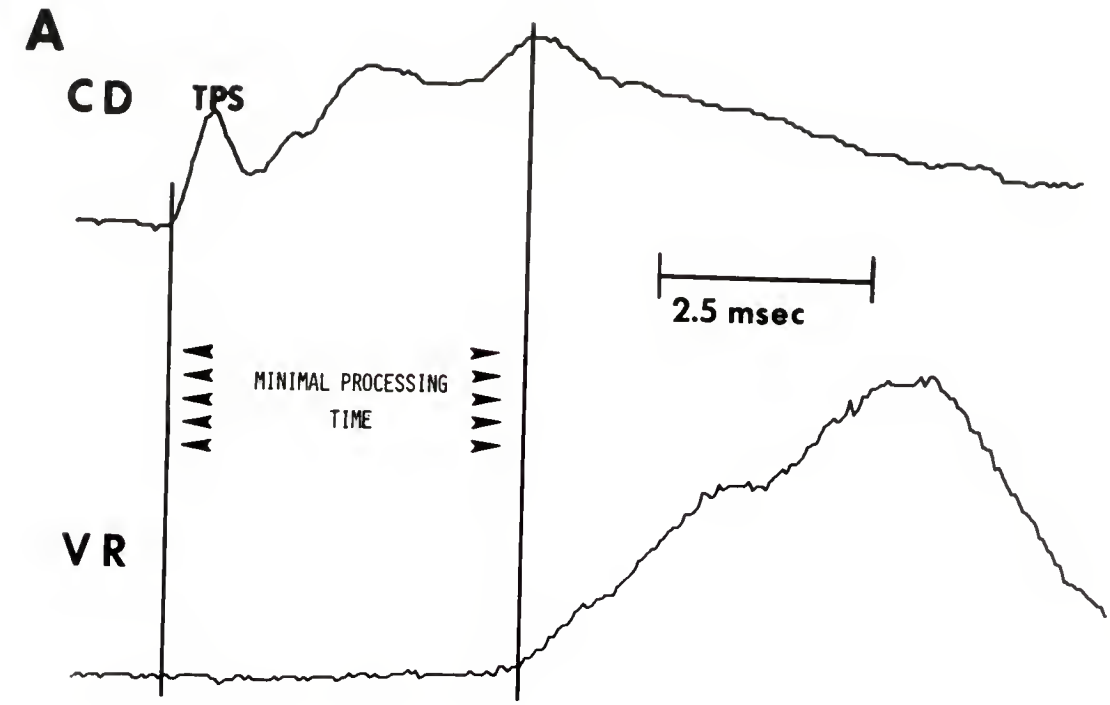
At the conclusion of intraspinal field potential mappings, animals were perfused through the heart with 1 l of heparinized saline followed by 1.5 l of 1.25% glutaraldehyde/1% paraformaldehyde fixative. The L6 segment was removed, quick-embedded in gelatin-albumin, cut into 40 micrometer sections with a freezing microtome and stained with cresyl violet. Most electrode tracks could easily be reconstructed from histological material; only data recorded along reconstructed tracks were considered for analysis. If fewer than 4 tracks were reconstructed in a particular animal, data recorded from that animal were not analyzed. Identification of the laminae in which the recording sites were located was done through microscopic examination of histological material and by comparison with published accounts of laminar organization (Rexed, 1952, 1954).

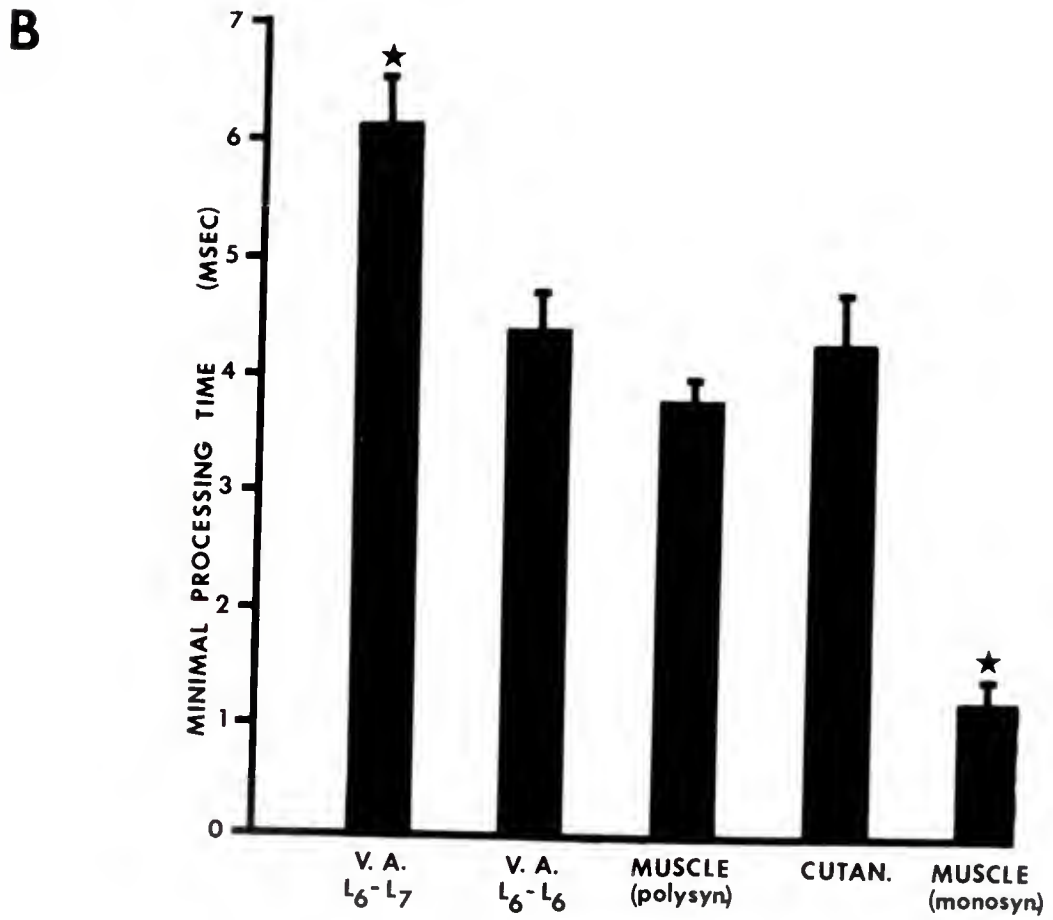
Results

Figure 3-1 shows the results of experiments in which minimal times required for activation of spinal motoneurons by inputs from muscle, skin, and the femoral-saphenous vein were examined. A determination of this minimal time for femoral-saphenous venous afferent input is illustrated in part "A" of the figure. This latency represents a combination of conduction time along the intraspinal portions of primary afferents, synaptic delay times, and conduction time along the processes of spinal neurons. However, a comparison of the central delay following venous afferent stimulation with the delay following stimulation of cutaneous and muscle afferents, the synaptology of which is well established (Brown, 1981), provides an estimate of the minimal number of synapses interposed between the primary afferents and alpha-motoneurons.

The minimal times between the arrival of the primary afferent volley and a detectable discharge in the ventral roots are indicated in part "B" of Figure 3-1. The abscissa shows the afferent type stimulated. Data regarding muscle afferents represent the pooling of delays measured following stimulation of the following nerves: posterior tibial, gastrocnemius, deep peroneal, deep posterior or plantaris. The latencies from triphasic spike to onset of both monosynaptic and polysynaptic reflexes recorded from the ventral root are shown. Data regarding cutaneous afferents represent the pooling of delays measured following stimulation of the sural nerve or the skin overlying the femoral-saphenous vein. Latencies reported for muscle and cutaneous input reflect the processing time by the input

Figure 3-1. Minimal spinal cord processing time for inputs from muscle, skin and the femoral-saphenous vein. (A) The method used to determine minimal spinal cord processing time. (B) A comparison of these latencies. Abbreviations: CD, cord dorsum potential; VR, potential recorded from the ventral roots; TPS, triphasic spike.


A
CD
TPS
2.5 msec
MINIMAL PROCESSING
TIME
VR


B
MINIMAL PROCESSING TIME (MSEC)
0
1
2
3
4
5
6
7
V. A.
L6-L7
V. A.
L6-L6
MUSCLE
(polysyn)
CUTAN.
MUSCLE
(monosyn)

cord segment for the stimulated afferents (L7 for the stimulated muscle nerves and sural nerve and L6 for the skin overlying the femoral-saphenous vein). Two central delay times following stimulation of the femoral-saphenous vein are reported: the latency separating the L6 triphasic spike and activity recorded from L6 ventral roots as well as the latency separating the L6 triphasic spike and activity recorded from the L7 ventral roots. Thick bars indicate mean minimal spinal cord processing time; thin bars indicate the standard error. Stars indicate values shown to differ significantly from all others by an analysis of variance procedure in combination with either a Waller-Duncan K-ratio T test or Duncan's multiple range test (alpha=0.05). The delay separating the triphasic spike elicited by muscle afferent stimulation and the monosynaptic reflex recorded from the ventral root was significantly shorter than all others; the delay separating the L6 triphasic spike elicited by femoral-saphenous venous afferent stimulation and ventral root activity recorded from L7 was significantly longer than all others.

Figure 3-2 shows a series of waveforms recorded following femoral-saphenous venous afferent stimulation at various depths along a typical microelectrode track through L6. The potentials were recorded as a series of negativities near the cord dorsum or in the dorsal gray matter (waveforms A-D). As the microelectrode tip advanced into the ventral horn, the negative waves decreased in amplitude and eventually reversed in polarity (waveforms E and F).

Figure 3-3 shows the focus of the short latency negative waves elicited by stimulation of the femoral-saphenous vein. The dots

Figure 3-2. Potentials recorded along a typical electrode track through L6 following stimulation of the femoral-saphenous vein.

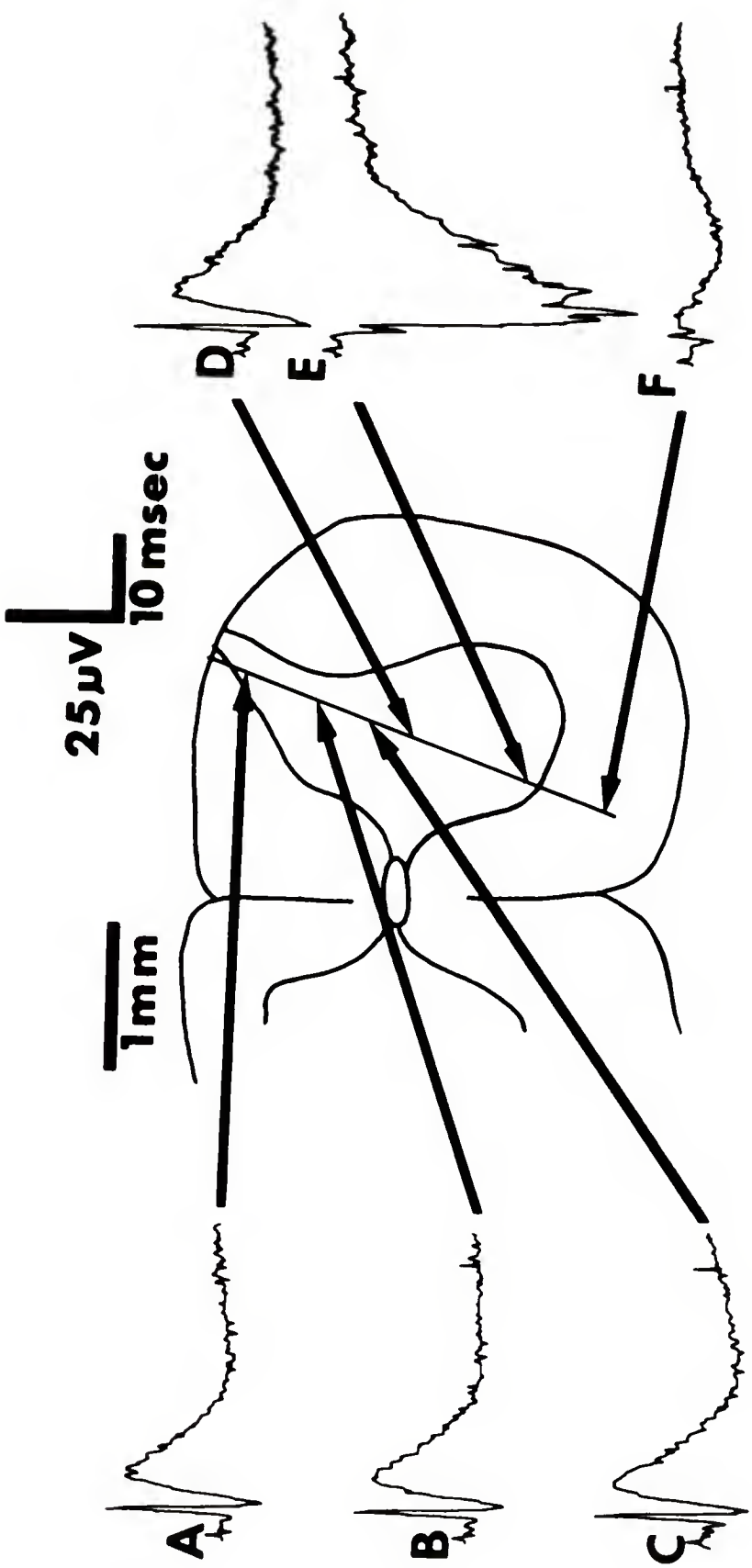
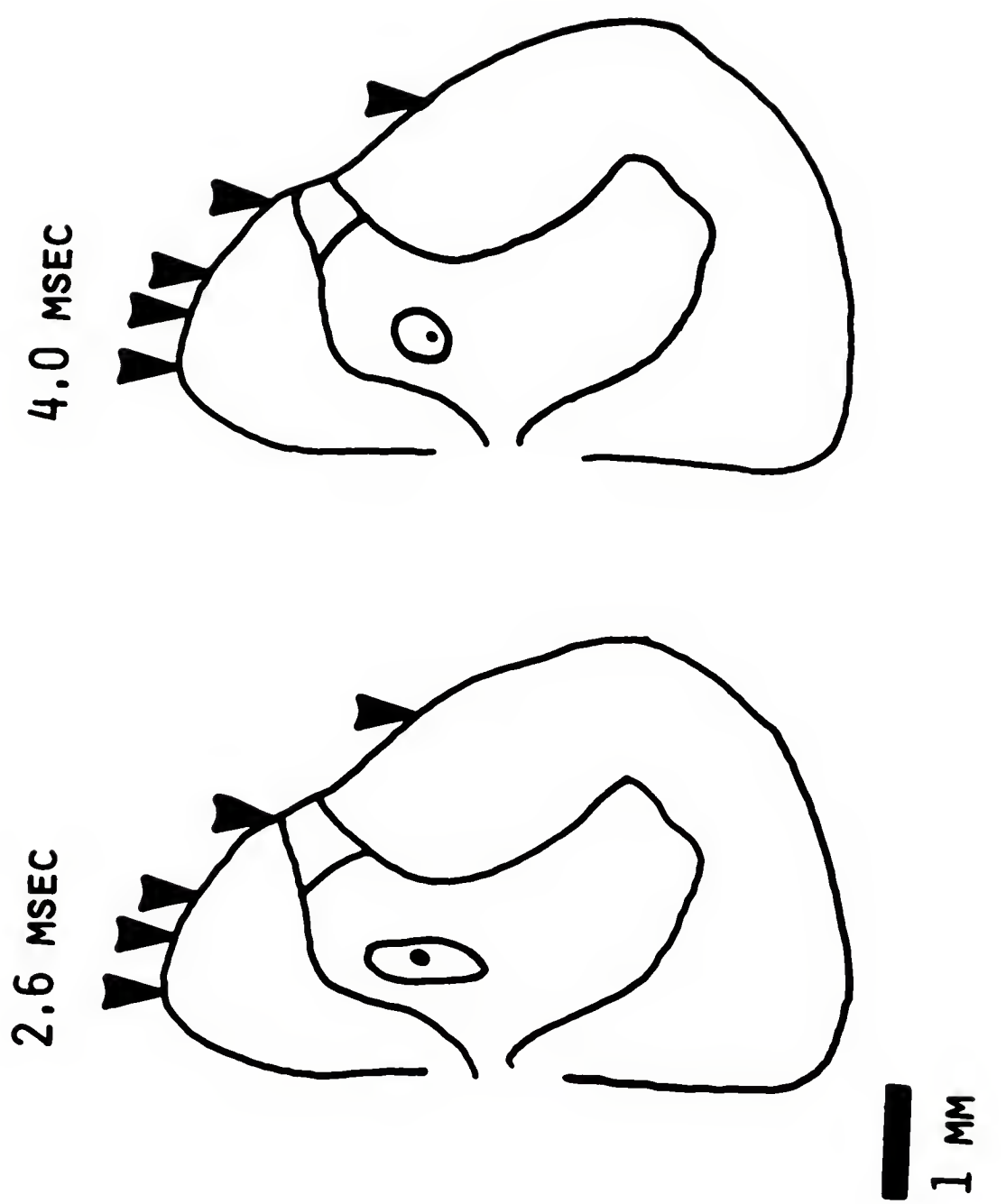

A
B
C
D
E
F
25μV
10 msec
1mm

Figure 3-3. The location of the focus of the short latency negative waves elicited by stimulation of the femoral-saphenous vein. Symbols are explained in the text.


4.0 MSEC
2.6 MSEC
1 MM

indicate points where maximal-amplitude potentials were recordable; the lines surrounding the dots indicate the area in which potentials 80% or more of maximal could be recorded; the arrows at the top of both diagrams indicate the location and orientation of electrode tracks used to collect data; the time over each diagram indicates latency from the onset of the triphasic spike of the cord dorsum potential. In most experiments (5 of 7), the initial negativities were focussed in the neck of the dorsal horn; this region corresponds to Rexed's lamina V (Rexed, 1952, 1954). In 4 of 7 experiments, the initial negativities were also focussed, along the medial-to-lateral axis, roughly in the center of the gray matter; however, foci both medial and lateral to this point were also noted. It is likely that the inconsistencies in results observed in a few experiments were due more to limitations in the field potential mapping technique than to physiological variability between animals. If a microelectrode failed to pass directly through a potential generator during a recording session, the spatial location of that potential generator could appear displaced from its actual location.

Discussion

The latency separating evoked activity recordable from the dorsal and ventral roots of L6, the input segment for femoral-saphenous venous afferents, following stimulation of the femoral-saphenous vein (4.4 msec) was approximately the same as the latency noted following stimulation of cutaneous afferents (4.3 msec). Minimal spinal cord processing time, estimated in the same way for inputs from muscle which reach alpha-motoneurons through

polysynaptic circuits, was only slightly shorter (3.8 msec). It is known that the fastest circuits which link cutaneous inputs and muscle inputs with interneurons and then motoneurons are di- and tri-synaptic (Lloyd, 1943b, 1943c). These data are consistent with a di- or tri- synaptic circuit being the shortest pathway between primary femoral-saphenous venous afferents and alpha-motoneurons.

Following electrical stimulation of the femoral-saphenous vein, negative waves of the cord dorsum potential can be recorded in spinal animals for 20 msec or more (Thompson and Yates, 1986). These waves reflect the activation of dorsal horn interneurons (Coombs et al., 1956; Fernandez de Molina and Gray, 1957; Willis et al., 1973; Fu et al., 1974; Beall et al., 1977; Foreman et al., 1979; Willis, 1980; Yates et al., 1982). In addition, maximal ventral root action potentials elicited in spinal animals by femoral-saphenous venous afferent stimulation have a duration of 20-25 msec (Thompson et al., 1982). Thus, although relatively short di- and tri- synaptic circuits interconnect venous afferents and motoneurons, much higher order circuits also appear to exist.

The minimal latency separating L6 dorsal root activity and L7 ventral root activity elicited by FVA stimulation was significantly longer (by at least 1.7 msec) than other processing times estimated. Increased intraspinal conduction distances can account for only a portion of this longer latency; the conduction time along primary femoral-saphenous venous afferents from L6 to L7 is less than 1 msec (Thompson and Yates, 1986). It is likely that at least one additional synapse is interposed between femoral-saphenous venous

afferents and L7 motoneurons than is interposed between the afferents and L6 motoneurons.

A distribution of intracord potential fields similar to that produced by stimulation of the femoral-saphenous vein (negativities in the dorsal horn and positivities in the ventral horn) is also elicited by stimulation of other afferent types. Previous studies have shown that the negative waves of shortest latency in the dorsal horn can be attributed to monosynaptic excitation of interneurons by primary afferents (Coombs et al., 1956; Fernandez de Molina and Gray, 1957; Willis et al., 1973; Fu et al., 1974; Beall et al., 1977; Foreman et al., 1979; Willis, 1980; Yates et al., 1982). Since the largest negative waves of shortest latency were recorded from Rexed's lamina V, it appears that most of the first interneurons excited by the large venous afferents are found in this region.

CHAPTER IV
PHYSIOLOGICAL PROPERTIES OF SINGLE SPINAL CORD NEURONS ACTIVATED BY STIMULATION OF THE FEMORAL-SAPHENOUS VEIN

Introduction

The data presented in Chapter III suggested that at least one or two interneurons are interposed between large (A-beta) primary femoral-saphenous venous afferents and motoneurons. It was also concluded that the first interneurons activated by these large afferents are located in Rexed's lamina V. The study discussed in this chapter examined the responses of single spinal cord neurons following stimulation of the femoral-saphenous vein. In addition, the patterns of convergence of inputs from muscle and skin on the venous afferent-activated interneurons were examined. This study was conducted to show whether venous afferent inputs were highly processed by spinal interneurons and, if so, whether these neurons were modality-specific.

Methods and Materials

Surgical Preparation of Animals

Experiments were performed on 19 unanesthetized, decerebrate cats with spinal cords transected at T12 and 5 intact cats anesthetized using alpha-chloralose. The two preparations were used because a comparison of the data obtained from each type would suggest whether an elicited input had strong or weak effects on the excitabilities of the neurons being studied. It is known that the

excitabilities of spinal interneurons in intact animals anesthetized using alpha-chloralose are lower than in decerebrate-spinal preparations. The lower excitabilities in these anesthetized preparations are due to the influences of descending supraspinal inputs to the spinal interneurons as well as to direct effects of the anesthetic on the neurons (Alvord and Fuortes, 1954; Balis and Monroe, 1964; Brown, 1981). If spinal neurons are driven in both decerebrate and anesthetized preparations by a particular input, it is likely that the input has powerful effects on these neurons. However, if spinal neurons are activated by the input in decerebrate-spinal preparations, but not anesthetized preparations, it is likely that the input has only weak influences on the excitabilities of these neurons.

Anesthesia was induced in the group of animals which would not be decerebrated using the intravenous injection of alpha-chloralose (Merck, 75 mg/kg). Maintenance doses of the drug (25 mg/kg) were routinely delivered every four hours. However, the electrocardiogram and arterial blood pressure were continuously monitored during the experiment; if either the heart rate or blood pressure increased, indicating that the animal was recovering from anesthesia, additional alpha-chloralose was administered until both of these measurements stabilized at their original level. The other animals were rendered decerebrate using the procedures described in Chapter III.

Most of the surgical procedures used to prepare cats for single unit recordings were similar to those described in Chapter III for

preparing animals for the recording of field potentials. Several additional procedures were also used to provide better mechanical stability for recording. A dural hammock was formed by placing stitches through the dura and then by suspending small weights from the threads. In this way the cord was lifted away from underlying vertebral bodies and pulsating blood vessels. In many animals the bladder was catheterized so that it would remain empty; this helped to stabilize the blood pressure and prevented blood pressure surges due to a full bladder. A bilateral pneumothorax was also used to minimize movements of the spinal cord during respiration. In addition to these procedures for increasing mechanical stability, holes were made in the arachnoid and pia overlying the L6 spinal cord using sharp forceps. This procedure permitted the insertion of microelectrodes into the cord without breaking the tips.

Stimulation Procedures

Bipolar electrical stimulation was used to activate femoral-saphenous venous afferents, cutaneous afferents coursing in the sural nerve and muscle afferents running in the posterior tibial, deep posterior, lateral gastrocnemius and hamstring nerves. Square-wave pulses of current, 0.2 msec in duration, were used to excite afferent fibers. While data were being collected, stimulus frequencies of 1 Hz were used. However, stimulation frequencies up to 100 Hz were used to differentiate afferent responses from responses generated by interneurons (see below). Throughout the time microelectrode penetrations were being done, the femoral-saphenous vein was continuously stimulated at an intensity 3 times that

necessary to evoke a field potential recordable from the cord dorsum. As discussed in Chapter III, this stimulus intensity provides a maximal A-beta volley to the spinal cord but does not excite the A-delta and C afferents. High current intensities were not used in an attempt to recruit the small-diameter venous afferents due to concerns regarding stimulus spread. Nerves were stimulated at intensities up to 10 times the threshold for eliciting a cord dorsum potential; the minimal current intensity for affecting the excitability of a unit was also determined.

Recording Procedures

Intracellular and extracellular single unit recordings were done using glass micropipette electrodes filled with 3 M KCl or 8% HRP in tris-buffered 0.15 M KCl (pH 7.3). Electrodes filled with 3 M KCl had initial impedances of 15-30 megohms; electrodes filled with the HRP solution had initial impedances of 30-90 megohms. Microelectrodes were positioned using a hydraulic microdrive (Narishige). The L6 dorsal columns ipsilateral to the stimulated vein and nerve segments were usually impaled by the electrodes; however, a few penetrations were also made through the L6 dorsal root entry zone. The microdrive was angled sharply towards the midline during these latter penetrations. When electrodes filled with 3 M KCl were used to record responses, only units located at 700-1500 micrometers depth were studied. At these depths, the electrode tips should have remained in the dorsal horn or in the most superficial portion of lamina VII (Rexed, 1952, 1954); thus, no motoneurons should have been encountered. In addition, the units were tested for

their responses to high frequency stimulation; it is known that action potentials recorded from primary afferents show constant latency with high stimulation frequencies, whereas the latencies of responses recorded from interneurons are altered by high frequency stimulation (Yates et al., 1982). If responses recorded from a unit were invariant in latency with 100 Hz stimulation, these responses were not analyzed. The use of electrodes filled with the HRP solution allowed recording sites to be marked either by the intracellular staining of the characterized neuron or by the extracellular iontophoresis of the tracer; the procedures used to reconstruct recording sites are discussed in Chapter V. Only data recorded from units located outside of lamina IX were analyzed.

All potentials were referenced to animal ground; the reference electrode was located in the wound margin adjacent to L6. Potentials were led into a microelectrode pre-amplifier (Winston model 1090A) and subsequently into an A.C. amplifier (Grass P511) with a time constant of 250 msec, tape recorder (Vetter model D) with a 10 db bandwidth of 0-2500 Hz and oscilloscope. The direct output of the microelectrode pre-amplifier was also led into an oscilloscope and into the tape recorder. Analysis of data was facilitated by the time histogram unit of an Ortec computer (model 4621); the latter unit generated poststimulus time histograms from the data. Hardcopy records of histograms were generated using an X-Y plotter (Hewlett-Packard).

At the conclusion of single unit recordings from 11 animals, small bundles of the L7 dorsal rootlets were cut proximally and

placed across a silver bipolar recording electrode. The sural and posterior tibial nerves were then stimulated using the same current intensities applied to activate interneurons during the single unit recordings. Potentials were led into an A.C. amplifier, tape recorder and oscilloscope. Potentials were also averaged using the Ortec computer. In addition, the conduction distances from the nerve stimulation sites to the dorsal root recording site were measured. These data were used to determine the conduction velocities of the afferents activated by the minimal current intensities necessary to alter the excitabilities of interneurons.

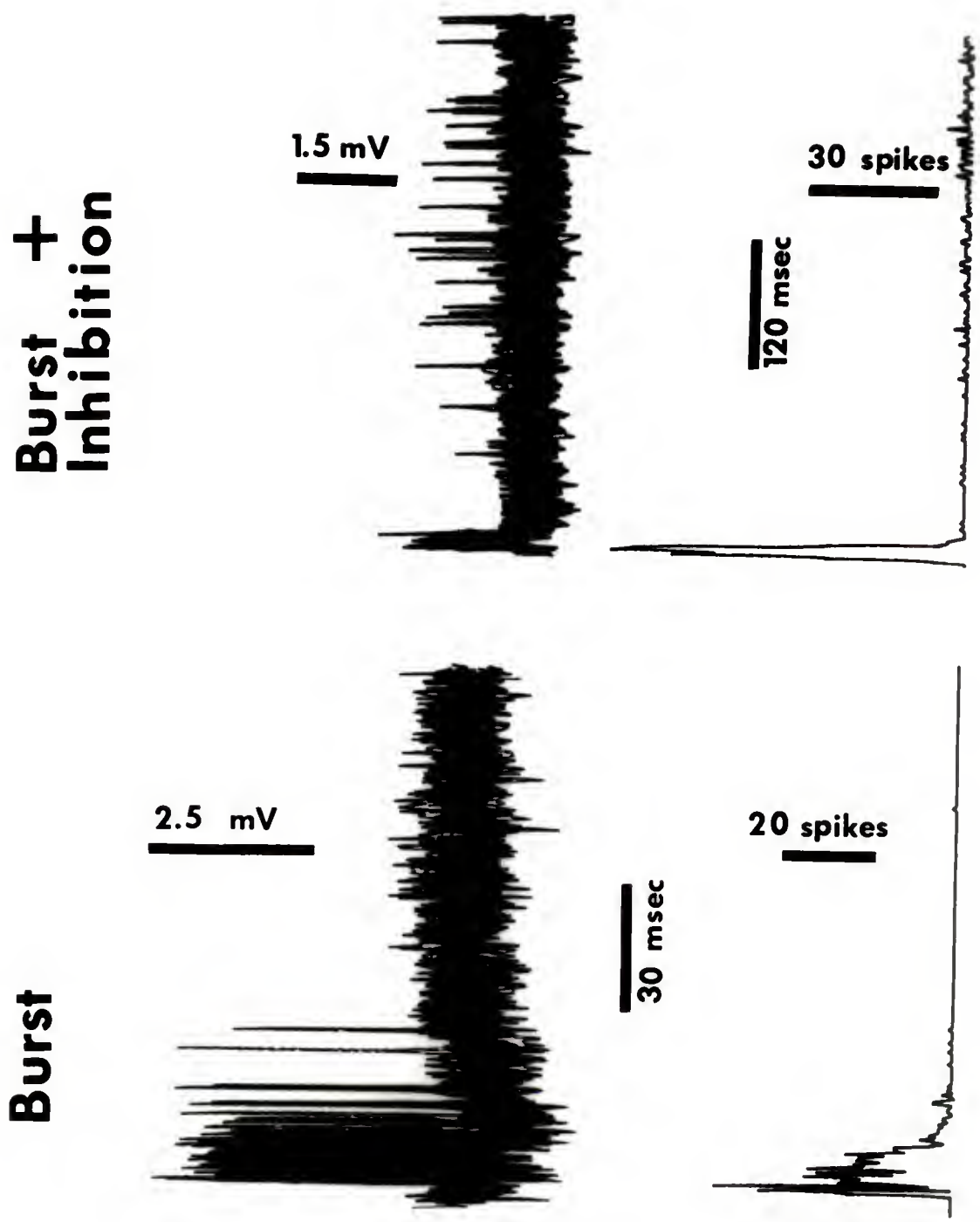
Results

Responses of Interneurons Following Stimulation of the Femoral-Saphenous Venous Afferents

Data were recorded from 81 neurons activated by stimulation of the femoral-saphenous vein in decerebrate cats; detailed analyses were made of the data recorded from 66 units. While only the latter data were useful in quantitative determinations of such parameters as the onset latency of evoked activity in neurons, etc., all of the data were useful in determining the patterns of convergence of femoral-saphenous venous afferent input with inputs from muscle and skin. In anesthetized animals, data were recorded from 14 units; the responses of all of these neurons following femoral venous afferent stimulation were characterized in detail.

Figure 4-1 illustrates the types of responses recorded from interneurons following femoral-saphenous venous afferent stimulation at an intensity 3 times that necessary to evoke field potentials

Figure 4-1. The effects of stimulation of the femoral-saphenous vein on the excitabilities of interneurons. The top traces show 16 superimposed oscilloscope sweeps. The bottom traces show poststimulus time histograms generated from 64 consecutive sweeps. The stimulus was delivered at the onset of the traces.


Burst + Inhibition
1.5 mV
30 spikes
120 msec
Burst
2.5 mV
30 msec
20 spikes

recordable from the cord dorsum. A burst of action potentials was recorded from 42 out of 66 (64%) of the venous afferent-driven neurons following stimulation of the femoral-saphenous vein in decerebrate animals. The other 24 neurons (36%) responded to vein stimulation with a combination of one or more bursts of action potentials and a period during which the firing rate of the unit was depressed below the spontaneous rate. The neurons studied in cats anesthetized using alpha-chloralose responded in similar ways to femoral-saphenous venous afferent stimulation; half of the 14 neurons characterized were excited by vein stimulation, and the other half were both excited and inhibited.

The quantitative aspects of the response properties of these neurons activated by stimulation of the femoral-saphenous vein are summarized in Table 4-1. The mean spontaneous firing rates of the neurons were similar in decerebrate and anesthetized animals (15.5 +/- 16.7 spikes per second in the former and 14.3 +/- 20.3 spikes per second in the latter). Clearly, most of the units examined had substantial ongoing activity in the absence of stimulation; however, a number of units showed no recordable spontaneous firing. Twelve of the units (18%) characterized in decerebrate cats were silent in the absence of stimulation; 5 of the units (36%) in anesthetized animals exhibited no spontaneous firing. The occurrence of these silent units is important, since a unit which does not fire spontaneously will be classified as only being excited by elicited inputs, whether or not it receives inhibitory inputs. It is only possible to show that the firing rate of a unit decreases below the spontaneous level

Table 4-1. Responses of spinal interneurons to electrical stimulation of the femoral-saphenous venous afferents, posterior tibial nerve and sural nerve. All confidence intervals represent mean +/- standard deviation. Onset latencies were measured from the onset of the triphasic spike of the cord dorsum potential. All means except those for spontaneous firing rate are in msec; mean spontaneous firing rates are in spikes per sec.

	Decerebrate Animals		
	'Burst' Units	'Burst + Inhibition' Units	Both Types Combined
Spontaneous firing rate	13.5 +/- 17.2 (n = 42)	19.1 +/- 15.6 (n = 24)	15.5 +/- 16.7 (n = 66)
Onset latency of activity from vein stimulation	13.5 +/- 19.6[a] (n = 42)	5.6 +/- 4.9[a] (n = 24)	10.6 +/- 16.3 (n = 66)
Duration unit was excited by vein stimulation	34.0 +/- 65.0 (n = 42)	59.7 +/- 82.7 (n = 24)	43.3 +/- 72.4 (n = 66)
Duration unit was inhibited by vein stimulation	----------	41.3 +/- 26.4 (n = 24)	----------
Total duration excitability was altered by vein stimulation	34.0 +/- 65.0[a] (n = 42)	101 +/- 88.6[a] (n = 24)	58.4 +/- 80.6 (n = 66)
Total duration excitability was altered by tibial nerve stimulation	102 +/- 186 (n = 11)	89.8 +/- 73.7 (n = 11)	95.8 +/- 138 (n = 22)
Total duration excitability was altered by sural nerve stimulation	42.6 +/- 48.0 (n = 10)	45.0 +/- 30.4 (n = 8)	43.7 +/- 40.0 (n = 18)

[a]Values for 'burst' and 'burst + inhibition' units were significantly different by Student's t-test ($p < 0.05$).

Table 4-1--continued.

Anesthetized Animals		
'Burst' Units	'Burst + Inhibition' Units	Both Types Combined
4.0 +/- 9.6 (n = 7)	24.6 +/- 23.4 (n = 7)	14.3 +/- 20.3 (n = 14)
6.8 +/- 6.2 (n = 7)	4.0 +/- 4.2 (n = 7)	5.4 +/- 5.3 (n = 14)
17.4 +/- 19.8 (n = 7)	18.5 +/- 20.8 (n = 7)	17.9 +/- 19.4 (n = 14)
----------	43.6 +/- 63.3 (n = 7)	----------
17.4 +/- 19.8 (n = 7)	62.1 +/- 66.4 (n = 7)	38.5 +/- 51.9 (n = 14)
18.6 (n = 1)	75.1 +/- 81.1 (n = 4)	63.8 +/- 64.6 (n = 5)
5.1 +/- 5.9 (n = 3)	81.5 +/- 68.6 (n = 5)	52.9 +/- 65.3 (n = 8)

following stimulation if the unit fires spontaneously. Thus, some of the units with a low spontaneous rate, classified in this study as being only excited by venous afferent stimulation, may indeed also have been inhibited by this input.

Table 4-1 also shows the onset latencies of the responses elicited by venous afferent stimulation and the durations that the excitabilities of the units were altered by this stimulation. The variances in these values were enormous. The changes in the excitabilities of neurons elicited by stimulation of the femoral-saphenous vein had an average onset latency of 10.6 +/- 16.3 msec following the onset of the triphasic spike of the cord dorsum potential in decerebrate animals and 5.4 +/- 5.3 msec in anesthetized animals. These means were shown to be significantly different by Student's t-test ($p < 0.039$). The triphasic spike is a compound action potential propagating through the largest active primary afferent fibers (Austin and McCouch, 1955; Howland et al., 1955; Yates et al., 1982); thus, the onset latency measured from the triphasic spike represented the onset latency of activity following the arrival of femoral-saphenous venous afferent input at the cord. In decerebrate cats, there was also a significant difference ($p < 0.016$) in the onset latency of elicited activity in the units excited by venous afferent stimulation and the units both excited and inhibited by this input; the mean latency was 13.5 +/- 19.6 msec in the former and 5.6 +/- 4.9 msec in the latter. A similar significant difference in the onset latency of activity elicited by venous afferent stimulation was not shown between units of the two types in anesthetized cats.

The excitabilities of the units receiving inputs from the femoral-saphenous vein were modulated for long durations following vein stimulation. The mean duration the excitabilities of the neurons were altered by vein stimulation was 58.4 +/- 80.6 msec in decerebrate animals and 38.5 +/- 51.9 msec in anesthetized animals. In decerebrate animals there was also a significant difference in the duration that units excited by venous afferent stimulation and neurons both excited and inhibited by this stimulation were affected by inputs from the femoral-saphenous vein ($p < 0.0008$).

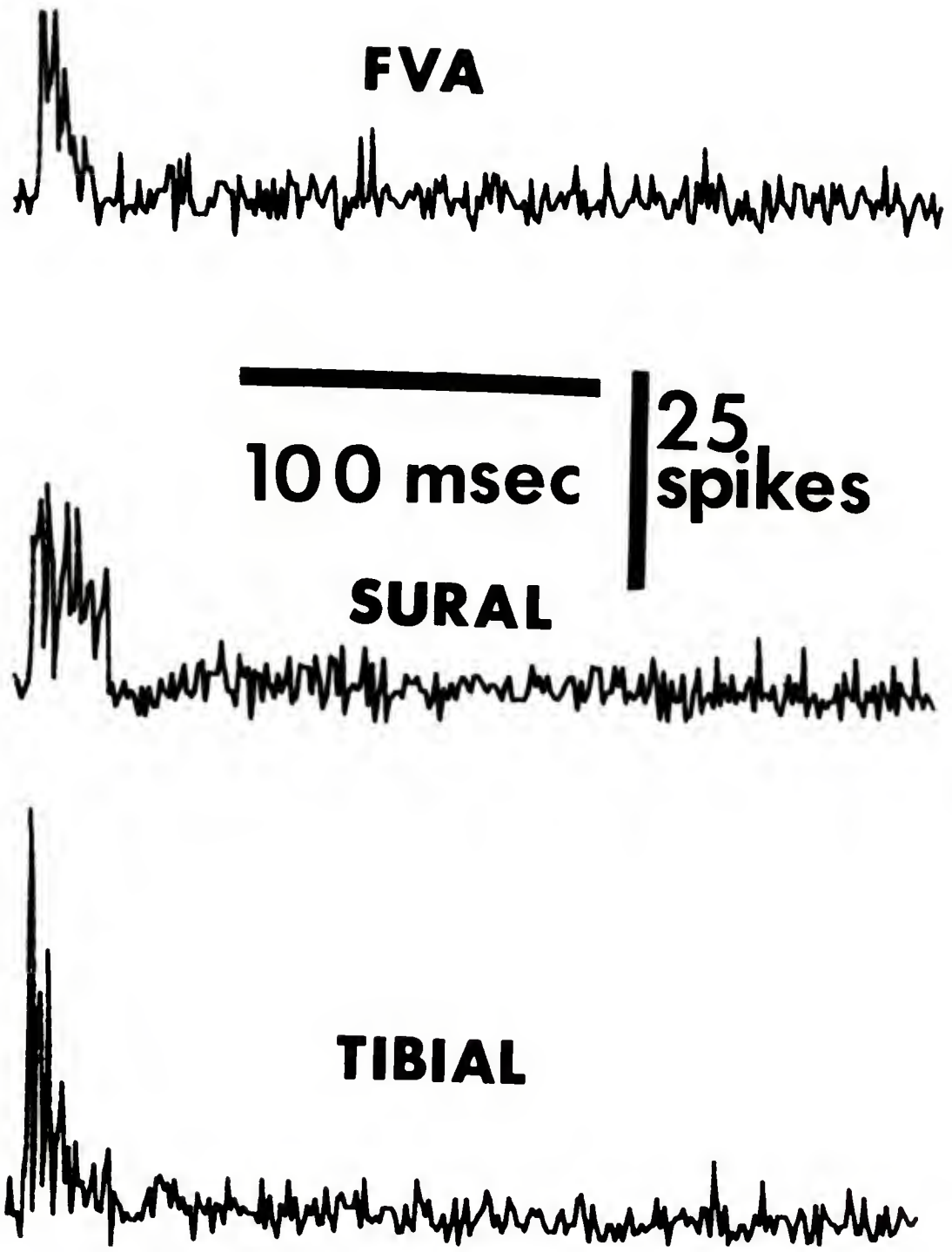
Convergence of Inputs from the Femoral-Saphenous Vein with Inputs from Muscle and Skin on Single Spinal Neurons

The effects of stimulation of the posterior tibial nerve, the largest muscle nerve of the hindlimb, and the sural nerve, the largest cutaneous nerve of the hindlimb, on the excitabilities of units receiving input from the femoral-saphenous vein were also studied. The posterior tibial nerve innervates a number of muscles of the crus and foot, including the triceps surae, tibialis posterior, flexor hallucis longus, lumbrical muscles and interosseus muscles. The sural nerve innervates skin overlying the foot and ankle (Jefferson, 1954; Crouch, 1969). In decerebrate animals, 78 out of 80 (98%) of the venous afferent-activated units tested could also be activated by stimulation of the tibial nerve at an intensity 10 times threshold for eliciting a cord dorsum potential; 58 out of 78 (74%) of the tested units were also driven by sural nerve stimulation at an intensity 10 times that necessary to evoke a cord dorsum potential; 56 out of 77 (73%) of the neurons were activated by

stimulation of both nerves. In anesthetized animals, 9 out of 13 (69%) of the venous afferent-activated units examined could also be activated by stimulation of the tibial nerve; 10 out of 13 (77%) of the tested units were also driven by sural nerve stimulation; 7 out of 13 (54%) could be activated by stimulation of both nerves.

Figure 4-2 shows poststimulus time histograms generated from data recorded from a single unit following stimulation of the femoral-saphenous vein, the sural nerve and the tibial nerve. The activity elicited by tibial nerve stimulation had an average onset latency of 6.6 +/- 10.3 msec following the triphasic spike produced by stimulation of this nerve in decerebrate animals and 3.9 +/- 2.8 msec in anesthetized animals. The activity elicited by sural nerve stimulation had an average onset latency of 8.2 +/- 5.4 msec following the triphasic spike produced by stimulation of this nerve in decerebrate animals and 4.4 +/- 4.9 msec in anesthetized animals. The venous afferent-activated neurons were excited, inhibited or both excited and inhibited by stimulation of the sural and tibial nerves. Most of these units (37 out of 54 or 69%) studied in decerebrate animals were both excited and inhibited by stimulation of the tibial nerve; 16 units (30%) were excited by tibial nerve stimulation and 1 neuron (2%) was inhibited by stimulation of this nerve. In contrast, most of these units (22 out of 37 or 59%) were only excited by sural nerve stimulation in decerebrate cats. Only 13 units (35%) were both excited and inhibited by sural nerve stimulation; 2 cells (5%) were inhibited by the stimulation of this nerve. In anesthetized animals, 40% of the tested units were excited by tibial

Figure 4-2. Poststimulus time histograms showing the effects of stimulation of the femoral-saphenous vein, posterior tibial nerve and sural nerve on the excitability of a single spinal neuron. The histograms were each generated from 64 consecutive sweeps. The stimulus was delivered at the onset of the traces.


FVA
100 msec
25 spikes
SURAL
TIBIAL

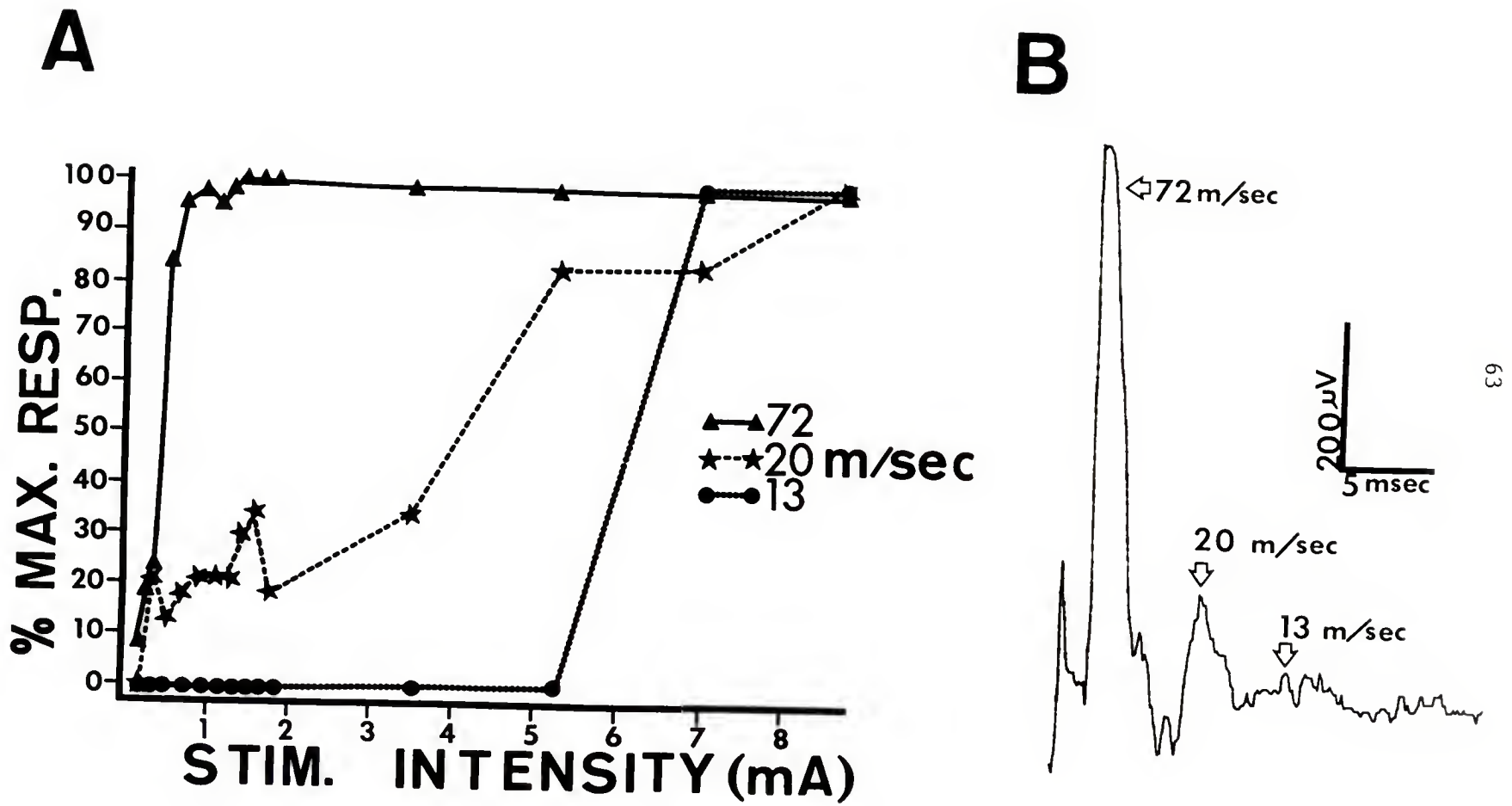
nerve stimulation, 40% were both excited and inhibited, and 20% were inhibited. In the same animals, half of the units examined were excited by sural nerve stimulation, and the other half were both excited and inhibited by the stimulation of this nerve. There were no obvious simple relationships between how a neuron responded to stimulation of the femoral-saphenous vein and how it responded to stimulation of the tibial and sural nerves. The excitabilities of the neurons were modulated for long durations following stimulation of the sural and tibial nerves; these durations are shown in Table 4-1.

To better determine the patterns of convergence of muscle afferent input on femoral-saphenous venous afferent-activated spinal interneurons, the effects of stimulation of 3 muscle nerves--the hamstring nerve, the lateral gastrocnemius nerve and the deep posterior nerve--were tested on 17 units in decerebrate cats. The nerves were stimulated using current intensities 10 times that necessary to evoke a field potential recordable from the cord dorsum. The hamstring nerve innervates 3 flexors of the thigh, the semimembranosus, the semitendinosus and the biceps femoris; the lateral gastrocnemius nerve innervates 2 extensors located in the crus, the lateral gastrocnemius and the soleus; the deep posterior nerve innervates a number of muscles located in the foot (Jefferson, 1954; Crouch, 1969). All of the 17 venous afferent-activated units examined could also be driven by stimulation of the deep posterior or the lateral gastrocnemius nerve; 12 of the units could also be driven by stimulation of the hamstring nerve. It was clear that muscle

inputs from many parts of the hindlimb and from both flexors and extensors converged on single interneurons also receiving inputs from the femoral-saphenous vein.

Following recordings from many interneurons, small bundles of the L7 dorsal rootlets were cut proximally and placed across a silver bipolar recording electrode. The tibial and sural nerves were then exposed to the same current intensities which were necessary to alter the excitabilities of interneurons. Compound action potentials, recorded from the dorsal rootlets, elicited by these stimulus intensities were analyzed to determine the conduction velocities of the excited fibers. In most cases, low stimulus intensities elicited A-alpha and A-beta volleys, whereas higher stimulus intensities also elicited A-delta volleys; the results of a typical study are shown in Figure 4-3. This figure shows the effects of different stimulus intensities applied to the sural nerve on the amplitudes of 3 components of the evoked dorsal root action potential. In decerebrate cats, 18 out of 29 units (62%) for which an analysis was done were driven by stimulus intensities applied to the tibial nerve which only elicited A-alpha and A-beta components in the compound action potentials recorded from the dorsal roots. The other 11 units (38%) were activated by current intensities that produced A-delta volleys as well as A-alpha and A-beta volleys. Sixteen out of the 17 units (94%) for which an analysis was done were driven by stimulus intensities applied to the sural nerve which only elicited A-alpha and A-beta components in the compound action potentials recorded from the dorsal roots. Only 1 unit was driven by current intensities that

Figure 4-3. The response amplitude versus stimulus intensity relationship for cutaneous afferents. (A) The relationship between stimulus intensity and the peak amplitudes of 3 components of the dorsal root action potential evoked by sural nerve stimulation. (B) A maximal-amplitude compound action potential; the waveform represents the average of 64 consecutive sweeps.


A
100
90
80
70
60
50
40
30
20
10
0
% MAX. RESP.
1
2
3
4
5
6
7
8
STIM. INTENSITY (mA)
72
20 m/sec
13
B
72 m/sec
200 μV
5 msec
20 m/sec
13 m/sec

elicited A-delta as well as A-alpha and A-beta volleys. The results in anesthetized animals were quite similar. Six out of 7 units (86%) for which an analysis was done were driven by stimulus intensities applied to the tibial nerve which only elicited A-alpha and A-beta components in the compound action potentials recorded from the dorsal roots. The other unit was activated by current intensities that produced A-delta volleys as well as A-alpha and A-beta volleys. Two out of the 3 units for which an analysis was done were driven by stimulus intensities applied to the sural nerve which only elicited A-alpha and A-beta components in the compound action potentials recorded from the dorsal roots. The other unit was driven by current intensities that elicited A-delta as well as A-alpha and A-beta volleys. However, at minimal stimulus intensities necessary to evoke A-delta dorsal root volleys, the A-alpha and A-beta volleys were often still increasing in amplitude. This was especially true for dorsal root action potentials elicited by stimulation of the tibial nerve. Thus, in most cases in which both A-delta volleys and volleys along larger afferents were elicited by stimulus intensities minimally necessary to activate a neuron, it was impossible to conclude which fiber group carried the input which activated the unit. Nonetheless, it was clear that large muscle and cutaneous afferents were included in the population which carried inputs to the spinal cord that altered the excitabilities of neurons also driven by inputs from the femoral-saphenous vein.

Discussion

Venous Afferent Effects on the Excitability of Spinal Neurons

The response properties of the neurons driven by stimulation of the femoral-saphenous vein were similar to those reported by others for units activated by electrical stimulation of cutaneous and muscle afferents. These previous studies have shown that most spinal cord neurons, especially those in decerebrate preparations, have a substantial spontaneous firing rate. However, units which do not fire in the absence of stimulation have also been reported (LeBars et al., 1975; Light and Durkovic, 1984). In addition, the onset latency and duration of activity elicited in spinal neurons by venous afferent stimulation are included in the range of values reported following electrical stimulation of large fibers in the hindlimb nerves (Feldman, 1975).

Neurons were either excited or both excited and inhibited by stimulation of the femoral-saphenous vein. In this study, as well as in previous studies, spinal interneurons were also shown to be excited or both excited and inhibited by electrical stimulation of muscle and cutaneous afferents (Hongo et al., 1966; Hillman and Wall, 1969; Wagman and Price, 1969; Feldman, 1975). Units both excited and inhibited by venous afferent stimulation are likely to receive inputs through several separate pathways, some responsible for the excitation, others for the inhibition. The excitation could either be due to direct inputs from primary afferents to the unit or to inputs from higher-order spinal neurons driven by venous afferent stimulation. The inhibition is also likely due to inputs from

venous afferent-driven neurons to the unit; however, the neurons responsible for the inhibition are likely to be different from the neurons that are responsible for the excitation. An alternate possibility is that the inhibition is due to the venous afferent-induced presynaptic inhibition of the tonically active afferents responsible for the high spontaneous firing rate observed in these neurons. However, the presynaptic inhibition of primary spinal afferents has a time course of 200 msec or more (Eccles et al., 1954, 1962, 1963a, 1963b; Eccles and Krnjevic, 1959; Wall, 1962). The period of inhibition produced by venous afferent stimulation in most units had a time course of 50 msec or less; in many units the duration was less than 20 msec. Thus, it is likely that the inhibition recorded from most of the units following venous afferent stimulation is due to postsynaptic inhibition, and not primary afferent depolarization. However, in the few units that were inhibited for long durations following venous afferent stimulation, it is possible that primary afferent depolarization also contributed to the reduced firing rate. Data recorded from one such unit are shown in Figure 4-1.

Since units that were both excited and inhibited by venous afferent inputs were likely to receive these inputs through a number of pathways, the venous afferent influences on the excitabilities of these units appear to be powerful. Units that responded in this manner following venous afferent stimulation were common, suggesting that venous afferent inputs, in general, have a powerful impact on the excitabilities of spinal cord neurons. Units both excited and

inhibited by venous afferent inputs also probably are involved with the integration and processing of these inputs, although the purpose of this integration cannot be determined in studies using electrical stimulation. In contrast, units that are only excited by an afferent input are more likely to have a role of relaying the input without significantly processing it. These latter units may fire for a long duration after receiving the afferent input, however, thereby prolonging its effects in the nervous system.

Stimulation of the femoral-saphenous vein had prolonged effects on the excitabilities of the units examined in both decerebrate and anesthetized animals. This is further evidence that the inputs carried by the large femoral-saphenous venous afferents have a potent influence on the excitabilities of spinal neurons.

Convergence of Inputs on Single Neurons

Stimulation of A-beta femoral-saphenous venous afferents activated many spinal interneurons that could also be driven by electrical stimulation of muscle and cutaneous afferents. Previous studies of the properties of single spinal interneurons that received noxious inputs from the internal environment showed that widespread convergence of inputs from muscle and skin occurred on these units as well (Pomeranz et al., 1968; Hancock et al., 1973, 1975; Gokin et al., 1977; Guilbaud et al., 1977; Foreman and Weber, 1980; Milne et al., 1981; Blair et al., 1981, 1984; Cervero, 1982; McMahon and Morrison, 1982; Rucker and Holloway, 1982; Takahashi and Yokota, 1983; Rucker et al., 1984). Pomeranz et al. (1968, p. 528), in fact, "failed to detect cells which responded only to visceral afferents"

in the thoracic spinal cord. Thus, spinal neurons which processed venous afferent inputs were similar to those that process noxious inputs from the viscera, in that both types of neurons received widespread convergent inputs from muscle and skin.

Comparison of Responses in Decerebrate and Anesthetized Animals

The responses of spinal neurons following venous afferent stimulation differed somewhat in decerebrate and in anesthetized preparations. These differences could be explained by a generalized lower neuronal excitability in the latter animals. The onset latency of the activity elicited by venous afferent stimulation was significantly shorter in anesthetized cats than in decerebrate cats. Inputs to neurons mediated through multi-synaptic connections are more likely to be affected by a lowered state of excitability than inputs mediated through connections comprised of only a few synapses. Thus, neurons activated at long latency would be expected to be more affected by anesthesia than neurons activated at short latency. The mean onset latency of elicited activity in the neurons in anesthetized animals would accordingly be expected to be shorter than in decerebrate animals.

However, many of the properties of the venous afferent-activated units were similar in both anesthetized and decerebrate preparations. In both groups of animals the convergence of venous, muscle and cutaneous inputs on single neurons was common. This suggests that muscle and cutaneous inputs have powerful influences on the excitabilities of these units. It was also common for units to be both excited and inhibited following venous afferent stimulation in

the two types of preparations. As discussed above, it is likely that these units which responded to inputs from the femoral-saphenous vein in a complex manner are involved with the integration and processing of the inputs. Thus, it appears that venous afferent inputs are highly processed in the spinal cord by interneurons.

CHAPTER V
THE VARIABILITY IN THE RESPONSES OF NEURONS LOCATED IN DIFFERENT LAMINAE FOLLOWING STIMULATION OF THE FEMORAL-SAPHENOUS VEIN

Introduction

The data presented in Chapter IV suggested that spinal neurons respond in a wide variety of ways following the electrical activation of A-beta femoral-saphenous venous afferent fibers. Some neurons were excited by stimulation of the femoral-saphenous vein; others were both excited and inhibited. Some units were driven within a few milliseconds following stimulation of the vein; others were driven only at long latency. The excitabilities of some neurons were affected only a few milliseconds by vein stimulation; the excitabilities of others were modulated for over 100 msec. In addition, most neurons driven by stimulation of the femoral-saphenous vein could also be activated by stimulation of muscle and cutaneous afferents; however, a few units were not activated by the stimulation of hindlimb nerves. It would be of interest to determine whether the neurons that responded in similar ways following stimulation of the femoral-saphenous vein had similar locations in the spinal cord. The study discussed in this chapter examined this possibility.

Methods and Materials

Experiments were performed on 12 unanesthetized, decerebrate cats with spinal cords transected at T12 and 5 intact cats anesthetized using alpha-chloralose. The responses recorded from

these animals were described in Chapter IV; this chapter will describe the locations of the units with different response patterns following stimulation of the femoral-saphenous venous afferents, tibial nerve and sural nerve. Surgical procedures used to prepare animals for recordings and the techniques used to record single unit responses were described in detail in Chapter IV. The data described in this chapter were recorded using microelectrodes filled with an 8% solution of HRP in tris-buffered 0.15 M KCl (pH 7.3).

After a neuron had been characterized extracellularly, an attempt was made to penetrate the element studied. If the attempt was successful, HRP was iontophoretically injected with 1-3 nA positive-going rectangular pulses, 500 msec in duration, at 1 Hz. Current was injected through the electrode for 7.5-15 min and was delivered through the companion bridge unit (Winston BR-1) to the microelectrode pre-amplifier. This bridge unit had been custom-modified by the manufacturer to make it capable of presenting high voltages. If the characterized neuron could not be impaled, HRP was iontophoresed extracellularly using 5 nA pulses for 20-30 min. A blank electrode was inserted into the opposite side of the cord parallel to each track which yielded data. Once positioned, the blank electrode was broken using scissors so that only 1-2 mm protruded above the surface of the cord. These electrode tips were left in place through the fixation process; they produced easily observed tracks in the tissue which were used to help identify sections from which data were recorded. A rostrocaudal distance of at least 1 mm separated sites of HRP iontophoresis.

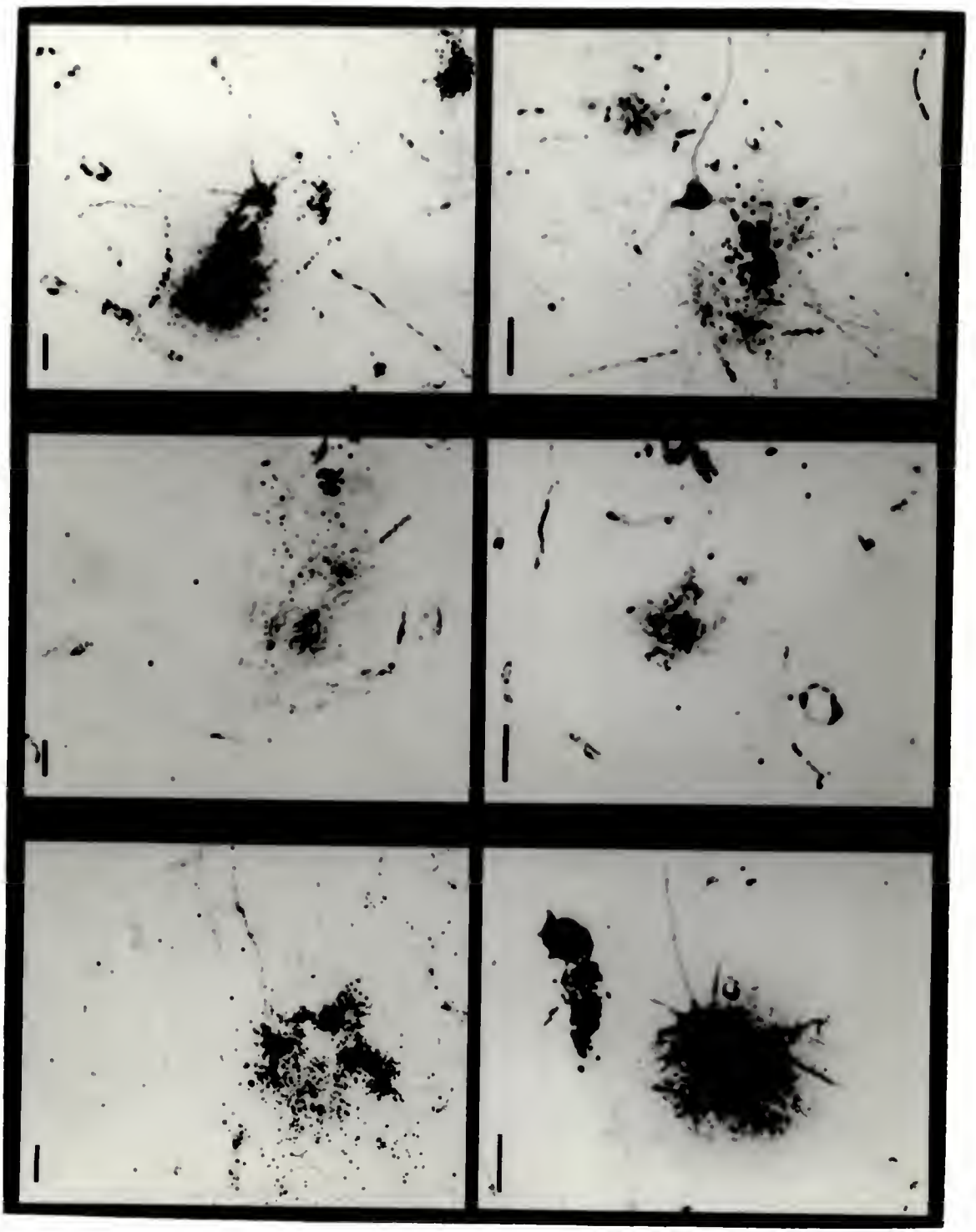
After at least an hour following the last marking attempt, the animal was perfused through the carotid artery with 1 l of heparinized saline containing 0.1% sodium nitrite followed by 2 l of 2.5% glutaraldehyde/1.2% paraformaldehyde fixative. The L6 cord was removed and stored overnight in the fixative. The tissue was cut into sections 50 micrometers thick using a vibratome (Oxford). The sections were then incubated for 30 min at room temperature in a solution comprised of 0.05% 3, 3' diaminobenzidine tetrahydrochloride and 0.1% hydrogen peroxide in 0.1 M tris buffer at pH 7.6. The sections were then rinsed in 0.1 M phosphate buffer (pH 7.4), mounted onto slides, dried, counterstained using 1% neutral red, dehydrated, cleared in xylene and coverslipped. Two-dimensional camera lucida reconstructions were made of stained neurons and extracellular recording sites.

Pugh and Stern (1984) have shown that HRP can be very reliably used to label extracellular single unit recording sites. Examples of histologically reconstructed recording sites are shown in Figure 5-1. Injection site diameters ranged from 50 to 250 micrometers. In addition to the visible brown reaction product in the extracellular space, internalization of HRP by neurons provided positive identification of the center of the injection site which was readily distinguishable from damaged vascular or neural elements mechanically produced by the electrode.

Results

Fifty sites at which responses were recorded from venous afferent-activated neurons were reconstructed; 36 of these

Figure 5-1. Examples of recording sites marked through the extracellular iontophoresis of HRP. The injection sites were easily identified by the presence of brown reaction product in the extracellular space. Most of the injection sites were light in color towards the borders, but were much darker towards the center. Neuronal elements in the center of the injection site typically also contained HRP reaction product. The region of the injection site which was darkest in color, and which contained neurons that had internalized HRP, was assumed to be the location from which extracellular responses had been recorded. Calibration bars represent 50 micrometers.



reconstructed recording sites were in decerebrate animals and the other 14 were in anesthetized animals. The locations of the recording sites are shown in Figure 5-2. In decerebrate cats, neuronal activity elicited by stimulation of the femoral-saphenous vein was recorded from Rexed's laminae IV-VIII and X; in anesthetized cats, venous afferent-elicited neuronal activity was recorded from laminae V and VI and from the dorsal portion of lamina VII. Most of the recording sites were located either in lamina V (11 out of 50) or in the most superficial portion of lamina VII, dorsal to the dorsalmost border of lamina VIII (15 out of 50).

Units located in the different laminae had similar spontaneous firing rates; these values are shown in Table 5-1. However, the units which were excited by venous afferent stimulation and the units both excited and inhibited by vein stimulation were highest in density in different regions of spinal gray matter. The locations of the units of the two types are shown in Figure 5-3. Most of the units in the dorsal horn (12 out of 20 or 60%) were both excited and inhibited by stimulation of the vein. In contrast, most of the units in the ventral horn (22 out of 30 or 73%) responded to venous afferent stimulation with only a burst of action potentials. Only 2 units located deeper than the dorsalmost border of lamina VIII were both excited and inhibited by venous afferent stimulation; clearly, units of this latter type were rare deep in the ventral horn.

An investigation was also done to determine the locations of the neurons driven at monosynaptic latencies by femoral-saphenous venous afferents. The cord dorsum potential elicited by venous afferent

Figure 5-2. Locations of sites at which responses were recorded from venous afferent-activated interneurons.

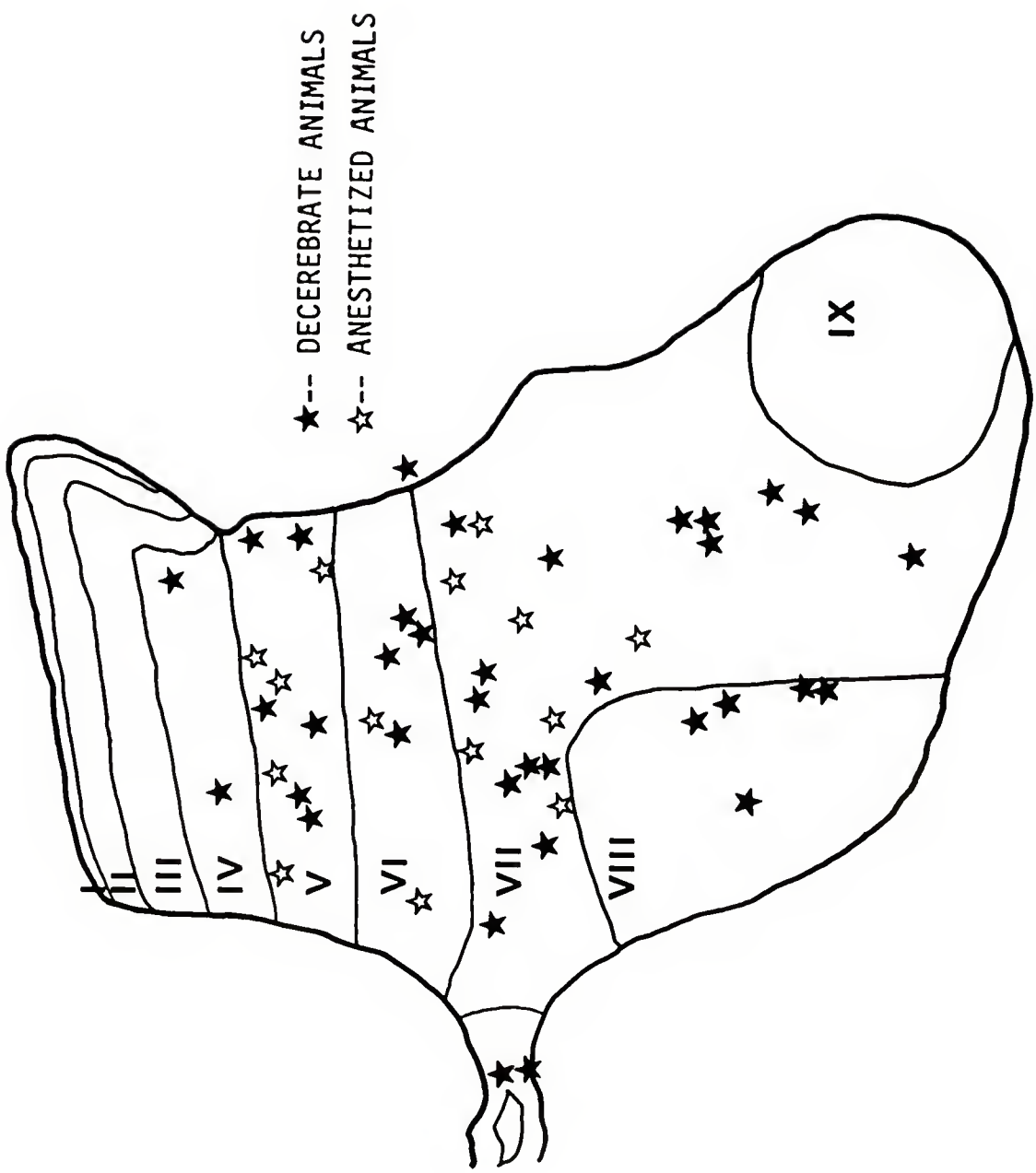
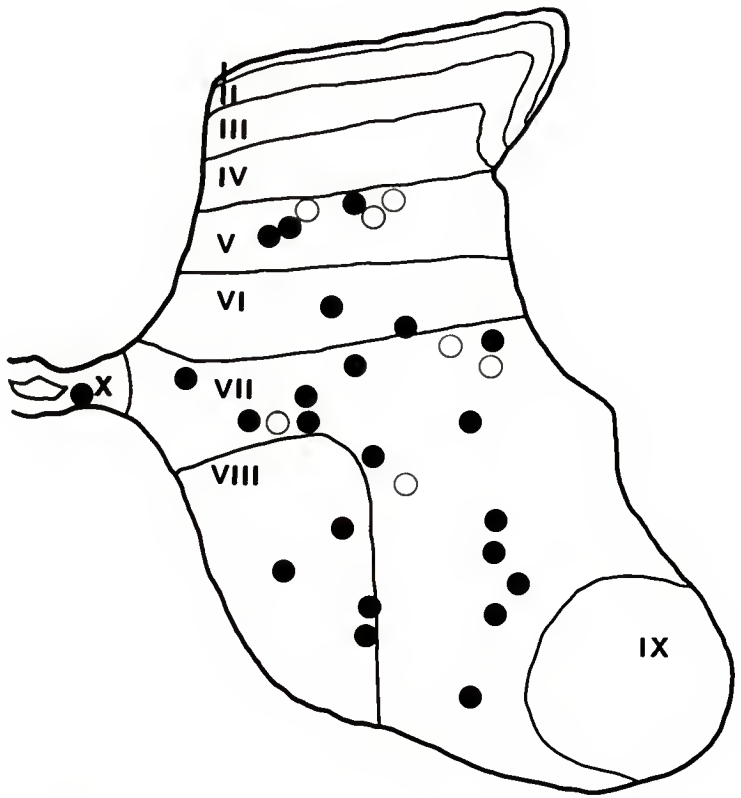

★-- DECEREBRATE ANIMALS
☆-- ANESTHETIZED ANIMALS
II
III
IV
V
VI
VII
VIII
IX

Table 5-1. Spontaneous firing rates of neurons located in different laminae. Confidence intervals represent mean +/- standard deviation. All spontaneous firing rates are in spikes per second.

Lamina of Rexed	n	Mean Spontaneous Firing Rate	Range of Spontaneous Firing Rates
IV	2	17.5 +/- 21.1	2.5 - 32.4
V	11	8.8 +/- 11.7	0.0 - 33.3
VI	7	15.6 +/- 12.9	1.1 - 37.2
VII	22	20.8 +/- 21.0	0.0 - 60.0
VIII	5	9.9 +/- 9.6	0.0 - 20.8
X	2	5.0 +/- 7.1	0.0 - 10.0

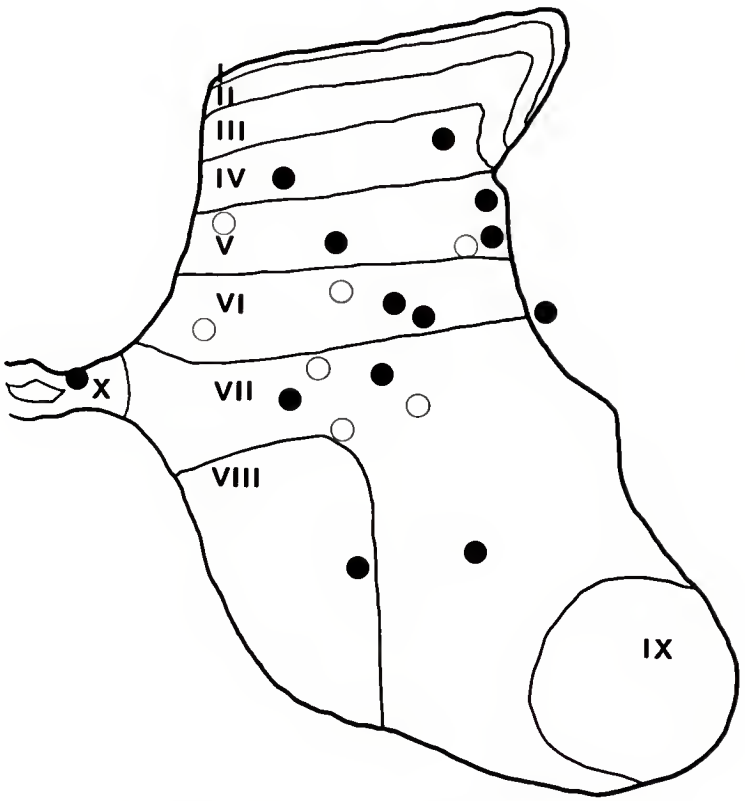
Figure 5-3. Locations of units excited or both excited and inhibited by stimulation of femoral-saphenous venous afferents.

Burst



●–DECEREBRATE

Burst +
Inhibition



○–ANESTHETIZED

stimulation was used to estimate this latency at which units were first activated by inputs from the vein. The cord dorsum potential is comprised of an initial triphasic spike, a series of negative waves and a slow positive wave. The triphasic spike is a compound action potential reflecting activity in the largest afferent fibers excited by the stimulus, the negative waves are generated by dorsal horn interneurons, and the positive wave is produced by the presynaptic inhibition of afferent fibers (Austin and McCouch, 1955; Howland et al., 1955; Eccles et al., 1963a, 1963b; Willis, 1980; Yates et al., 1982). Thus, the latency of the first negative wave of the cord dorsum potential corresponds to the latency at which interneurons are being directly excited by large primary afferent fibers. Neurons excited at the same latency as that of the peak of the first negative wave of the cord dorsum potential, or at shorter latencies, were assumed to receive monosynaptic, or at least relatively direct, inputs from from primary femoral-saphenous venous afferents. Figure 5-4 illustrates these procedures used to determine which units received direct inputs from the venous afferents. The average latency of the peak of the first negative wave of the cord dorsum potential was 2.67 +/- 0.71 msec from the onset of the triphasic spike in decerebrate animals and 2.06 +/- 0.85 msec in anesthetized animals.

Based on these criteria, 8 of the 50 units with recording sites reconstructed were classified as being directly excited by the primary venous afferents; 4 of these units were in anesthetized cats, and the other 4 were in decerebrate cats. The locations of these

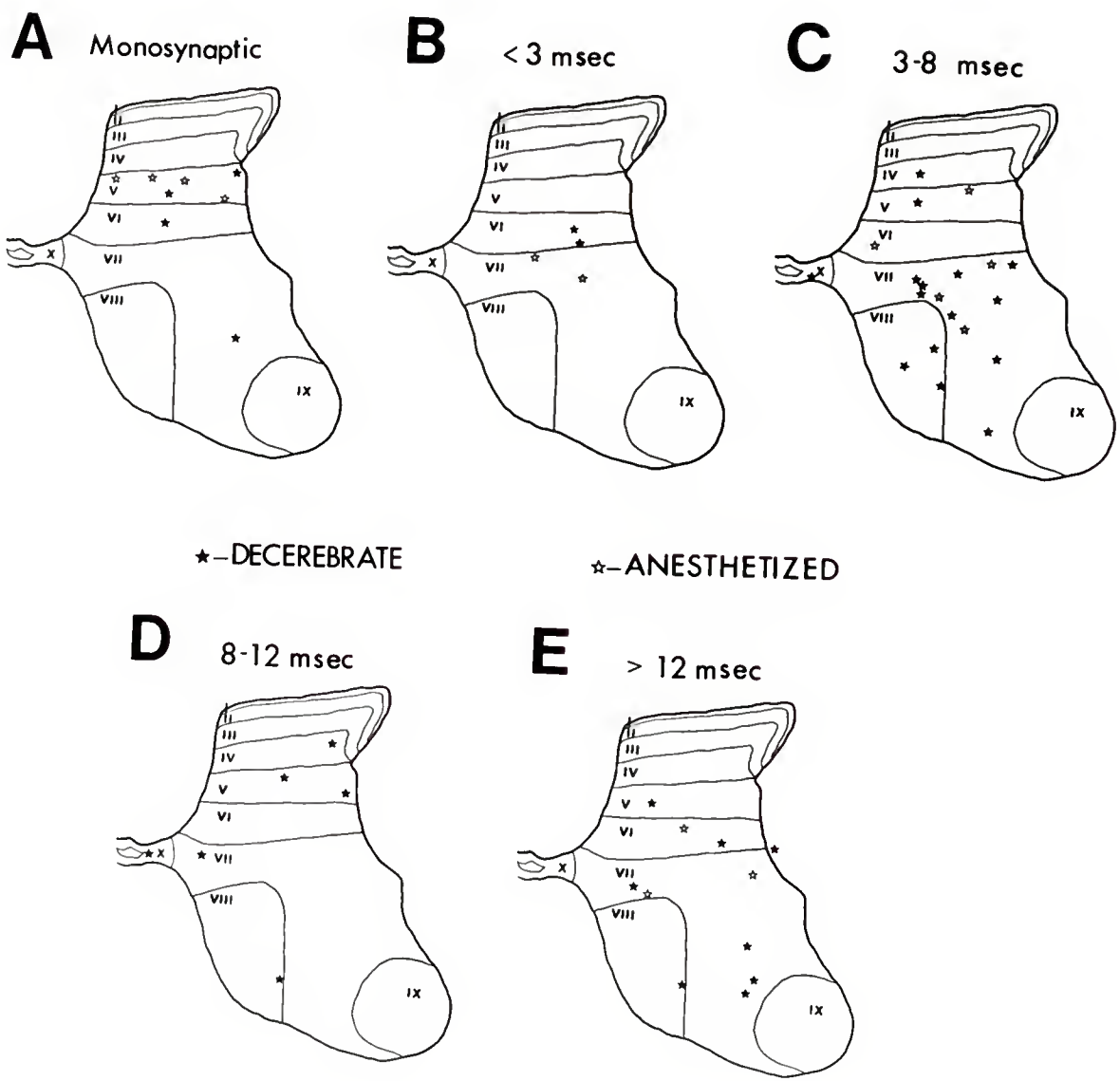
Figure 5-4. Procedures used to determine whether a unit is activated at monosynaptic latencies by primary femoral-saphenous venous afferents. The lefthand portion of the figure compares the latency of the peak of the first negative wave of the venous afferent-elicited cord dorsum potential with the onset latency of activity evoked in a unit. The cord dorsum potential is the average of 32 consecutive sweeps; unit activity is shown by a poststimulus time histogram generated from 64 consecutive sweeps. Further responses recorded from the same unit are shown in the lefthand portion of Figure 4-1. The first negative wave of the cord dorsum potential is preceded by an intramedullary spike, which is produced by action potentials propagating into the afferent termini (Austin and McCouch, 1955). The righthand portion of the figure shows the location of the unit from which activity was recorded.

Cord Dorsum Potential
Stimulus Artifact
Triphasic Spike
Spike
N1
40 μV
2 msec
Unit Activity
10 spikes
II
III
IV
V
VI
VII
VIII
IX
X
XI

units are shown in part "A" of Figure 5-5; most (6 out of 8 or 75%) were located in lamina V, 1 was located in lamina VI and the other was located much more ventrally in lamina VII. These units driven at short latency by venous afferent stimulation in decerebrate animals had a mean spontaneous firing rate of 8.2 +/- 7.2 spikes per second and were activated at a mean latency of 1.8 +/- 0.72 msec following the triphasic spike of the cord dorsum potential. Two of the units studied in decerebrate animals were excited by venous afferent stimulation, and the other two were both excited and inhibited. The excitabilities of these units were modulated for 32.6 +/- 44.2 msec. The spontaneous firing rates of the units driven by venous afferent stimulation at short latency in anesthetized animals were much lower than those in decerebrate cats; the mean rate was only 0.24 +/- 0.28 spikes per second. Two of the units in anesthetized animals were silent in the absence of stimulation; it was impossible to determine whether these units were both excited and inhibited by venous afferent stimulation or only excited. It is only possible to show that the firing rate of a unit decreases below the spontaneous level following stimulation if the unit fires spontaneously. The other two units in anesthetized cats were both excited and inhibited by vein stimulation. These four units were activated at a mean latency of 1.06 +/- 0.55 msec following the triphasic spike of the cord dorsum potential; the excitabilities of the units were modulated by venous afferents for a mean duration of 20.4 +/- 12.8 msec.

The locations of the units driven at longer latencies by the stimulation of the femoral-saphenous vein are shown in parts "B" to

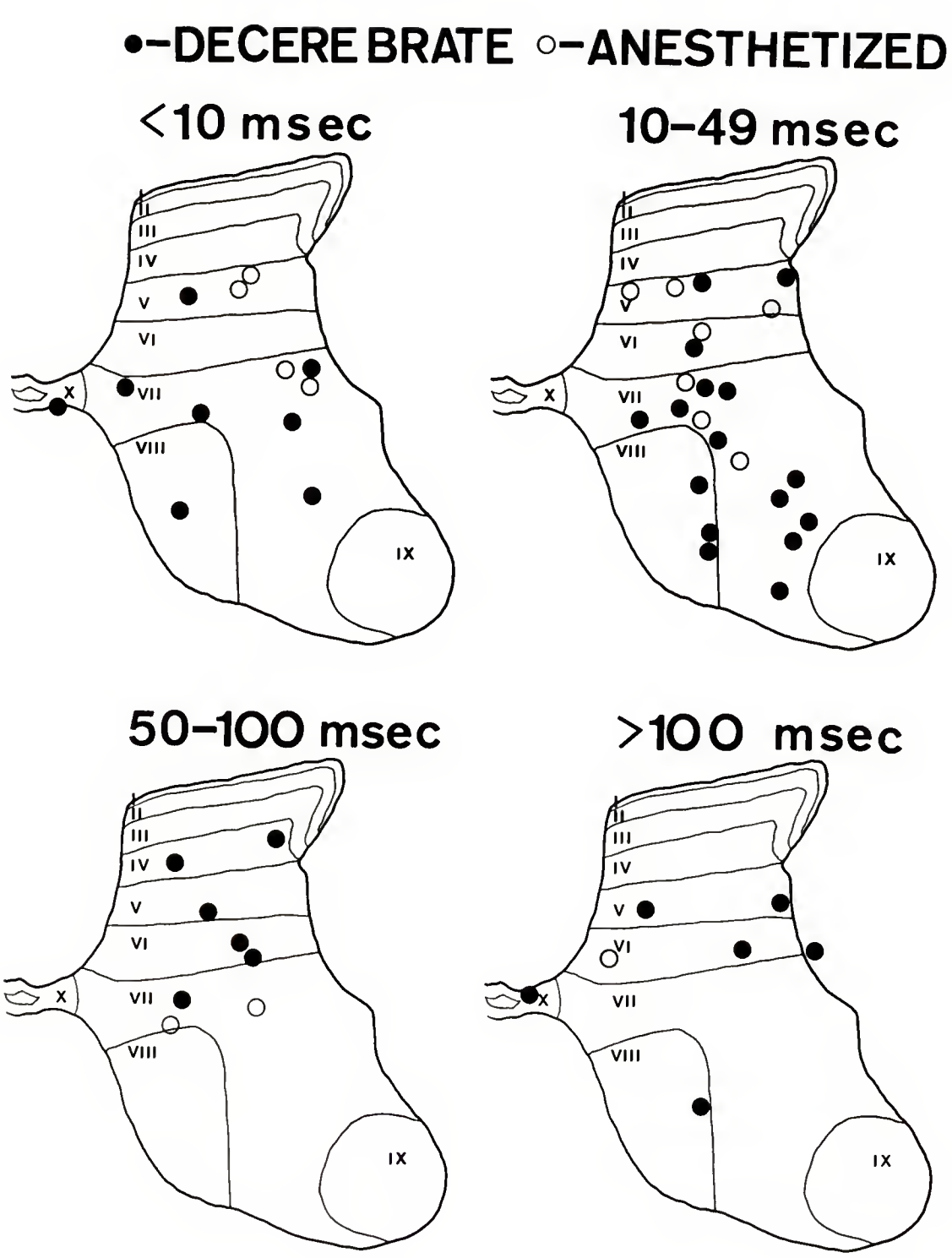
Figure 5-5. Locations of units which were activated at different minimal latencies following stimulation of the femoral-saphenous vein. (A) Locations of units driven at monosynaptic latencies. (B-E) Locations of units activated at longer latencies. The time over each diagram indicates latency following the onset of the triphasic spike of the cord dorsum potential.


A
Monosynaptic
B
< 3 msec
C
3-8 msec
★–DECEREBRATE
☆–ANESTHETIZED
D
8-12 msec
E
> 12 msec
II
III
IV
V
VI
VII
VIII
IX
X

"E" of Figure 5-5. Within 3 msec following the triphasic spike of the cord dorsum potential, but at latencies longer than that of the peak of the first negative wave of the cord dorsum potential, a few units located in laminae VI and VII were activated by vein stimulation. At slightly longer latencies, many units located in the ventral horn were activated. However, units in the dorsal horn were also activated at these longer latencies. The onset latency of neuronal activity produced by venous afferent stimulation ranged to over 12 msec following the arrival of the afferent volley at the cord; neurons activated at latencies longer than 12 msec following the triphasic spike were located both in the dorsal and ventral horn.

Figure 5-6 shows the durations that the excitabilities of units were modulated following stimulation of the femoral-saphenous vein; neurons were activated from less than 10 msec to over 100 msec. The excitabilities of units located in the dorsal horn and in the superficial portion of lamina VII were modulated for both long and short durations. However, most of the units located deeper than the dorsalmost border of lamina VIII were only activated for short durations (less than 50 msec). The excitabilities of units located in the dorsal horn and superficial portions of lamina VII of decerebrate cats were modulated for a mean duration of 74.8 +/- 86.1 msec following vein stimulation, whereas units located deeper were activated for only an average duration of 26.2 +/- 44.5 msec. These means were shown to be significantly different by Student's t-test ($p < 0.035$).

Figure 5-6. Locations of units activated for different durations following the stimulation of the femoral-saphenous vein.


●-DECEREBRATE ○-ANESTHETIZED
<10 msec
10-49 msec
50-100 msec
>100 msec
II
III
IV
V
VI
VII
VIII
IX
X

As discussed in the last chapter, most of the venous afferent-driven units could also be activated by stimulation of the tibial and sural nerves; however, a few units were not driven by the stimulation of hindlimb nerves. Table 5-2 shows the locations of the venous afferent-driven neurons that also received convergent inputs from muscle or skin. In anesthetized animals most of the units which did not receive convergent inputs from muscle or skin were located in lamina V, the region which contains most of the neurons driven at shortest latency by stimulation of the femoral-saphenous vein. Accordingly, 2 of the 3 units in anesthetized animals that were not driven by stimulation of the sural nerve were activated at monosynaptic latencies by stimulation of the femoral-saphenous vein. The other unit was driven by vein stimulation at a fairly short latency (3.6 msec following the onset of the triphasic spike of the cord dorsum potential). Two of the 4 units in anesthetized animals that were not driven by stimulation of the tibial nerve were activated at monosynaptic latencies by stimulation of the femoral-saphenous vein. The other two units were activated at 3.6 and 3.8 msec following the triphasic spike of the cord dorsum potential. Only one unit studied in decerebrate animals, located in lamina VII, could neither be activated by stimulation of the sural nerve or the tibial nerve; this unit was driven at monosynaptic latencies by stimulation of the femoral-saphenous vein. However, the other four units characterized in decerebrate cats that were not driven by sural nerve stimulation were activated by venous afferent stimulation only at long latency (11.5 +/- 1.9 msec following the

Table 5-2. Locations of neurons activated by electrical stimulation of the femoral-saphenous vein that also receive convergent inputs from muscle or skin.

Lamina Containing Recording Site	Decerebrate Animals				Anesthetized Animals			
	Tested Units also Driven by Tibial N. Stim.		Tested Units also Driven by Sural N. Stim.		Tested Units also Driven by Tibial N. Stim.		Tested Units also Driven by Sural N. Stim.	
	Number Tested	Percent Driven	Number Tested	Percent Driven	Number Tested	Percent Driven	Number Tested	Percent Driven
IV	2	100	2	50	-	--	-	--
V	6	100	6	67	5	40	5	40
VI	5	100	5	80	2	100	2	100
VII	11	91	10	90	6	83	6	100
VIII	4	100	4	100	-	--	-	--
TOTAL	28	96	27	81	13	69	13	77

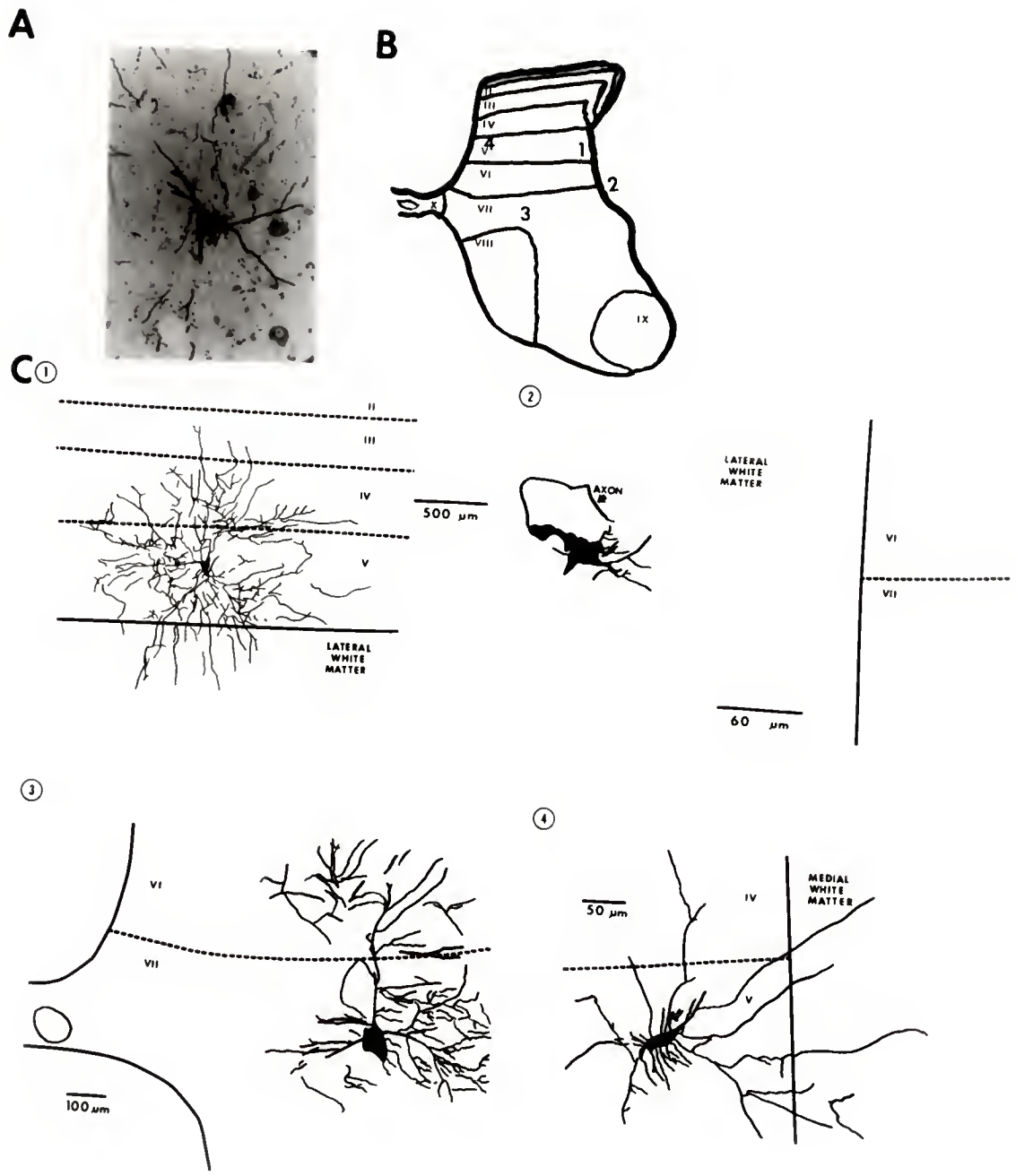
onset of the triphasic spike of the cord dorsum potential). Five out of the 8 units classified as receiving monosynaptic input from primary femoral-saphenous venous afferents could not be driven by stimulation of the tibial nerve, the sural nerve or both.

Four of the venous afferent-activated neurons were stained through the intracellular iontophoresis of HRP. Figure 5-7 shows the locations of the cell bodies of these neurons as well as the two-dimensional reconstructions of the neuronal elements which were stained. The cell bodies of 2 neurons were located in lamina V, 1 cell body was located in the dorsal portion of lamina VII and the other cell body was located in the lateral white matter beside the border between laminae VI and VII. These cell bodies were large; they had an average diameter of 37.1 +/- 18.2 micrometers. Only the cell body located in the lateral white matter had a diameter of less than 25 micrometers. The dendritic trees of three of the neurons were reconstructed; these dendritic trees were large and highly branched. They had a mean rostrocaudal spread of 963 +/- 809 micrometers and a mean mediolateral spread of 492 +/- 83 micrometers. The dendrites also spanned several laminae.

Discussion

This study made use of the marking of extracellular single unit recording sites to determine the locations of neurons with different response properties following the stimulation of the femoral-saphenous vein. The sites of such extracellular recordings are generally assumed to correspond to the locations of the cell bodies of the units characterized (Willis and Coggeshall, 1978).

Figure 5-7. Reconstructions of venous afferent-activated interneurons that were intracellularly stained using HRP. (A) A photomicrograph of the cell body of a stained neuron. (B) The locations of the cell bodies of the stained neurons. (C) Reconstructions of the cell bodies and dendritic trees of the neurons. The numbers in (B) and (C) correspond. Unless otherwise marked, all neuronal processes shown are dendrites. Neuron "1" was reconstructed from somewhat oblique sagittal sections, explaining the unconventional transitions in lamination. The other neurons were reconstructed from cross sections.


A
B
II
III
IV
V
VI
VII
VIII
IX
1
2
3
C ①
②
II
III
IV
V
500 µm
LATERAL WHITE MATTER
AXON
LATERAL WHITE MATTER
VI
VII
60 µm
③
VI
VII
100 µm
④
50 µm
IV
MEDIAL WHITE MATTER
V

Since cell bodies are by far the largest neuronal elements from which action potentials can be recorded, it is probable that most stable extracellular recordings will be done in the vicinity of these structures. However, stable recordings from axons at distances far from the cell body are sometimes also achieved. It is important to keep this fact in mind when drawing conclusions about the locations of neurons based on extracellular marking of recording sites. The region which contains most of the extracellularly characterized units is likely to also contain most of the cell bodies of these neurons. If a recording site is reconstructed outside of this area containing most of the other recording sites, it is not prudent to immediately conclude that the cell body of a neuron of interest is also found at this location. It is possible that such recording sites which do not fit the pattern are locations from which responses were recorded from axons.

Sites at which responses were recorded from venous afferent-activated units were reconstructed predominantly in Rexed's lamina V and in deeper laminae in both decerebrate and anesthetized cats. It is likely that these regions also contain many cell bodies of neurons receiving inputs from the femoral-saphenous vein. However, no driven units were found in anesthetized cats in lamina VIII or in the deep portions of lamina VII. As discussed in Chapter IV, the excitabilities of spinal neurons in intact animals anesthetized using alpha-chloralose are lower than in decerebrate-spinal preparations. Thus, if neurons are activated by an input in decerebrate-spinal preparations, but not anesthetized

preparations, it is likely that the input has only weak influences on the excitabilities of these neurons. This evidence suggests that neurons located deep in the ventral horn receive only weak inputs from femoral-saphenous venous afferents. It is also noteworthy that very few neurons that were both excited and inhibited by stimulation of the vein were found deeper than the dorsalmost border of lamina VIII. Evidence presented in Chapter IV suggested that these neurons which respond in a complex manner receive powerful inputs from the stimulated afferents. The observation that very few units which were both excited and inhibited were found deep in the ventral horn provided further support for the conclusion that neurons in this region receive only weak inputs from the femoral-saphenous vein. The validity of this conclusion is also bolstered by the fact that units located deep in the ventral horn were driven by venous afferent stimulation for significantly shorter durations than units located more superficially. The evidence presented in this chapter suggests that neurons located in laminae V, VI and the dorsal portion of lamina VII receive powerful inputs from the femoral-saphenous vein. In addition, neurons located in deeper regions appear to receive weaker inputs from these afferents.

A fairly large percentage of the neurons studied (8 out of 50 or 16%) were activated by venous afferent stimulation at latencies shorter than that of the first negative wave of the cord dorsum potential. These neurons were suggested to receive monosynaptic inputs from the venous afferents. The data presented in Chapter II suggested that only a very few afferents project from the

femoral-saphenous vein to the spinal cord. For the anatomical studies presented in Chapter II and the studies presented in this chapter to be in register, it would seem that the intraspinal portions of the primary femoral-saphenous venous afferents must branch extensively and make synaptic contacts with a large number of second order neurons. In contrast, anatomical studies have suggested that visceral afferents which enter the thoracic spinal cord do not branch to this great extent (Cervero and Connell, 1984). In this respect, venous afferents appear different from other afferents from the internal environment.

Most of the units activated by venous afferent stimulation at very short latencies were located in the neck of the dorsal horn, predominantly in lamina V. Responses from one unit driven at short latency, however, were recorded from deep within lamina VII. It is possible that these latter responses were recorded from the axon of an interneuron arising from a cell body located in the dorsal horn. In any case, these data suggest that most of the interneurons excited monosynaptically by large femoral-saphenous venous afferents are located in lamina V. The field potential mapping experiments discussed in Chapter III also suggested that most of the neurons activated at shortest latency by the large venous afferents are located in this region.

Units located in the dorsal portion of lamina VII were activated by venous afferent stimulation at latencies slightly longer than that of the first negative wave of the cord dorsum potential. Many of the lamina VII neurons which were driven only slightly later than the

neurons concluded to receive direct inputs from the venous afferents are likely to receive direct or relatively direct inputs from these monosynaptically driven neurons. It is also possible that some of these lamina VII neurons are premotor interneurons for the femoral-saphenous venous afferent-elicited spinal reflex. Data presented in Chapter III suggested that the shortest connection between primary venous afferents and alpha-motoneurons is di- or tri-synaptic. Studies using both the Golgi technique and the intracellular staining of neurons with HRP have suggested that few, if any, lamina V neurons send their axons into lamina IX. However, many of these neurons do project to superficial lamina VII. In turn, many neurons in the dorsal portion of lamina VII project to lamina IX (Matsushita, 1969, 1970; Czarkowska et al., 1976). These data suggest that the shortest pathway from primary venous afferents to motoneurons may be comprised of a lamina V neuron which projects to a lamina VII neuron that, in turn, projects to motoneurons.

An alternate possibility is that the lamina V neurons which are excited monosynaptically by primary venous afferents make contacts with the distal dendrites of motoneurons in laminae V or VII. However, EPSPs elicited in motoneurons by venous afferent stimulation are very large, typically 1 to 2 mV in amplitude, and have a fast rise time, typically 4 to 5 msec (Thompson and Yates, 1984). EPSPs elicited by contacts on distal dendrites have much slower rise times and much smaller amplitudes (Rall, 1970). Thus, it is likely that the venous afferent-elicited reflex is produced through synaptic interactions of lamina VII interneurons with proximal dendrites or cell bodies of motoneurons.

It is also likely that many of the venous afferent-activated units were tract cells, in addition to neurons that form parts of local circuits. It has previously been shown that stimulation of the femoral-saphenous vein elicits field potentials recordable from the sensorimotor cerebral cortex (Thompson et al., 1980). Most of the tract cells that relay information from the femoral-saphenous venous afferents probably send their axons into the spinoreticular and spinothalamic pathways. The neurons giving rise to these tracts are found in the ventral horn, which also contains many venous afferent-driven neurons (Trevino and Carstens, 1975; Trevino et al., 1973; Corvaja et al., 1977; Fields et al., 1977). The other major tracts projecting to the brain from the lumbosacral gray matter, the postsynaptic dorsal column and the spinocervical pathways, arise from cell bodies mainly in laminae III and IV (Petit, 1972; Rustioni, 1973; Craig, 1976; Brown et al., 1976). These regions contain few venous afferent-activated units.

Most of the neurons (5 out of 8 or 63%) that were classified as receiving monosynaptic inputs from primary femoral-saphenous venous afferents could not also be driven by stimulation of the tibial nerve, the sural nerve or both. In contrast, only 6 out of 36 (17%) of the units activated at longer latencies by vein stimulation could not also be driven by stimulation of one of the hindlimb nerves. It thus appears that, in general, neurons receiving the shortest latency inputs from the femoral-saphenous vein are less likely to receive convergent inputs from muscle or skin than are neurons activated by venous afferents at longer latency.

The intracellularly stained venous afferent-activated interneurons had large cell bodies and extensive dendritic spreads. A previous study using intracellular staining showed that neurons with these characteristics in laminae V and VII are wide dynamic range (receive inputs from both large and fine afferents). Neurons that received only noxious inputs (along fine afferents) had small cell bodies but extensive dendritic spreads; neurons that received only innocuous inputs (along large afferents) had large cell bodies but only small dendritic spreads (Ritz and Greenspan, 1985). The neurons characterized in this study were shown to receive inputs along large afferents arising in the femoral-saphenous vein, muscle and skin. It is likely that many of these neurons receive inputs along fine afferents as well.

CHAPTER VI
GENERAL DISCUSSION

The previous chapters have described several characteristics of the afferents which conduct inputs from the femoral-saphenous vein to the spinal cord and the neurons within the cord which process these inputs. Many of these characteristics are similar to those for visceral afferents. However, in some respects femoral-saphenous venous afferents appear different from other afferents from the internal environment.

Several previous studies have suggested that visceral afferents have smaller mean diameters than somatic afferents. Cervero et al. (1984) found that the application of HRP to visceral nerves entering the thoracic cord labeled a significantly smaller percentage of large cell bodies in the dorsal root ganglia (diameter > 40 micrometers) than did the application of HRP to somatic nerves entering the same segments. Similarly, Li et al. (1977) reported that the relative sizes of the A-alpha/beta and A-delta components of the compound action potentials recorded from the splanchnic nerve (a visceral nerve) were smaller than the relative sizes of these components recorded from the saphenous nerve (a cutaneous nerve). The data presented in Chapter II suggested that cell bodies in the dorsal root ganglia labeled by the application of HRP to the femoral-saphenous vein had significantly smaller mean diameters than other cell bodies

located in the ganglia. Thus, femoral-saphenous venous afferents, like visceral afferents entering the thoracic spinal cord, are comprised of fibers that are finer, on the average, than somatic afferents.

The number of visceral afferents entering the thoracic spinal cord has been reported to be small. In fact, wide field electron microscopic analysis has shown that the major visceral nerve entering the thoracic cord of the cat, the splanchnic nerve, contains only 3500 afferents. This nerve enters the spinal cord over 12 or more segments; thus, the average number of afferents entering any one segment is 300 or less (Kuo et al., 1982). Similarly, only an average of 182 cell bodies were labeled in the dorsal root ganglia per segment of the femoral-saphenous vein exposed to HRP. Clearly, both femoral-saphenous venous afferents and visceral afferents entering the thoracic spinal cord are few in number.

Although visceral afferents are few in number, stimulation of these afferents produces widespread effects in the spinal cord. For example, stimulation of the splanchnic nerve has been reported to drive over half of dorsal horn neurons in the thoracic spinal cord (Pomeranz et al., 1968; Cervero, 1982, 1983a, 1983b). Stimulation of only the A-beta population of the femoral-saphenous venous afferents was suggested in Chapter IV to activate many interneurons in the L6 spinal cord and in Chapter III to produce large field potentials recordable from this segment. The central gain for both visceral afferents and femoral-saphenous venous afferents is very high.

A high central gain for an afferent input can be accomplished through two means: the extensive branching of the intraspinal portions of the afferents or the activation of a few second order interneurons that, in turn, make contacts with a large number of higher-order cells. Several studies have suggested that the high central gain for visceral afferent inputs is due to the latter possibility. In one of these studies, the application of HRP to the cut end of the splanchnic nerve or to a somatic nerve of similar size was used to transganglionically visualize the central projections. The labeling in the cord was much fainter following the application of HRP to the visceral nerve; the authors proposed that this suggests that visceral afferents have a limited intracord branching (Cervero and Connell, 1984). Selzer and Spencer (1969) also showed that the largest-amplitude negative wave of the cord dorsum potential elicited by sympathetic chain stimulation occurred at long latency (20 msec or more following the stimulation). The short latency negative waves were small in amplitude. Since the negative waves are generated by the activity of dorsal horn neurons, these data suggest that few neurons are driven by the primary sympathetic afferents at short latency. In contrast, the data discussed in Chapter V suggested that many neurons are driven at short latency by stimulation of the femoral-saphenous vein; 16% of the neurons studied were classified as receiving monosynaptic inputs from large femoral-saphenous venous afferents. Although inputs carried along both visceral afferents and afferents arising in the femoral-saphenous vein have a high central gain, this gain appears to be accomplished through different means in the two cases.

A number of studies utilizing the transganglionic transport of HRP have suggested that visceral afferents entering both the thoracic and sacral spinal cord terminate in laminae I and V (Kuo et al., 1983, 1984; Cervero and Connell, 1984; Kuo and deGroat, 1985). The field potential mappings presented in Chapter III as well as the recordings of single unit activity from reconstructed sites discussed in Chapter V suggested that most of the first neurons activated by the large femoral-saphenous venous afferents are located in lamina V. Thus, it is likely that many primary venous afferents terminate in this region; the laminae containing terminals of visceral afferents and femoral-saphenous venous afferents appear to at least partially overlap. It is not known, however, which laminae contain the terminals of the fine venous afferents. It is possible that they project to lamina I. Until this information is obtained, it will not be possible to conclude whether the laminae containing terminals of afferents from the viscera and the femoral-saphenous vein are identical.

It is well established that inputs from viscera, muscle and skin converge on single spinal neurons (Pomeranz et al., 1968; Hancock et al., 1973, 1975; Gokin et al., 1977; Guilbaud et al., 1977; Foreman and Weber, 1980; Milne et al., 1981; Blair et al., 1981, 1984; Cervero, 1982; McMahon and Morrison, 1982; Rucker and Holloway, 1982; Takahashi and Yokota, 1983; Rucker et al., 1984). As discussed in Chapter IV, it is also common for inputs carried along muscle, cutaneous and venous afferents to converge on single neurons. Clearly, femoral-saphenous venous afferents, and the spinal

interneurons that process inputs carried along these afferents, have much in common with visceral afferents, and the neurons driven by stimulation of these latter afferents. There appear to be marked similarities in how the central nervous system processes many inputs from the internal environment. Yet, the mechanisms are not identical.

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BIOGRAPHICAL SKETCH

Billy J. Yates was born October 23, 1960, in Plant City, Florida. He grew up and attended primary and secondary schools in nearby Lakeland. He graduated from Lakeland High School in 1978 with an interest in medicine, and began premedical studies at the University of Florida in the fall of that year. During his early undergraduate studies, he came to work, quite by accident, in the laboratory of Dr. John Mathias. His experiences in this laboratory convinced him that a career in science would be much more challenging and rewarding than a career in medicine. He also developed an interest in the autonomic nervous system while working with Dr. Mathias. In order to gain further insight in this field, he began working with Dr. Floyd Thompson in January of 1981. In December of the same year he earned a B.S. degree. He immediately entered the doctoral program in the Department of Neuroscience and chose Dr. Thompson to supervise his dissertation research. While performing this research, he developed a keen interest in the organization of the mammalian spinal cord. He will pursue this interest during his postdoctoral career with Dr. Victor Wilson at the Rockefeller University in New York, New York.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Floyd J. Thompson, Chairman
Associate Professor of Neuroscience

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