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PROPERTIES OF SINGLE CENTRAL Ia AFFERENT FIBRES PROJECTING TO MOTONEURONES

BY JOHN B. MUNSON AND GEORGE W. SYPERT

From the Departments of Neuroscience and Surgery, University of Florida College of Medicine and Veterans Administration Hospital, Gainesville, Florida 32610, U.S.A.

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SUMMARY

1. Electrical potentials in the cat lumbosacral spinal cord evoked by the action of single medial gastrocnemius Ia afferent fibres were recorded using low impedance, bevelled micropipette electrodes and the spike triggered averaging technique.

2. Axonal potentials from the Ia fibres recorded extracellularly appeared as brief triphasic predominantly negative potentials.

3. Terminal potentials recorded in regions of Ia afferent termination appeared as brief diphasic positive–negative waves, often with additional wavelets.

4. Focal synaptic potentials, recorded extracellularly in regions of the medial gastrocnemius Ia afferent termination, appeared as slow (about 10 msec duration) negative potentials following terminal potentials.

5. Excitatory post-synaptic potentials, recorded intracellularly in Ia target cells of the medial gastrocnemius, appeared as slow (about 10 msec duration) positive potentials following terminal potentials.

6. Analysis of the temporal progression of these potentials through the spinal cord allowed calculations of the Ia conduction velocity in the dorsal funiculus stem axon (50–60 m/sec), in major collateral branches (8–19 m/sec) and in terminal branches (0·2–1·0 m/sec).

7. The number of major collateral branches (nine or fewer) and their spacing along the spinal cord (1071 μm mean value) were determined by analysing the pattern of single fibre potential latency minima and maxima along the rostrocaudal extent of the triceps surae motoneurone column.

8. The structural and functional properties of medial gastrocnemius Ia afferent fibres are discussed in relation to recent single fibre anatomical data and the present single fibre electrophysiological data.

INTRODUCTION

The Ia motoneurone synapse and the associated afferent and efferent neuronal pathways have provided the basis for much of what is known about normal and abnormal neuronal physiology. Anatomical studies of the Ia afferent pathway have provided a detailed picture of the fine structure of that pathway (Szentagothai, 1967; Scheibel & Scheibel, 1969; Conradi, 1969; McLaughlin, 1972; Iles, 1976;

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Brown & Fyffe, 1978; Hongo, Ishizuka, Mannen & Sasaki, 1978; Burke, Walmsley & Hodgson, 1979). Physiological studies of population responses of group Ia afferents have described their conduction velocity in the dorsal funiculus (Lloyd & McIntyre, 1950) and their regions of synaptic activation (Eccles, Fatt, Landren & Winsbury, 1954).

The present work is a physiological investigation of single central medial gastrocnemius Ia afferents, investigating such properties as their rostrocaudal distribution, branching patterns, terminal fields and conduction velocity. We have sought answers to the following questions.

(1) Over what rostrocaudal extent of the spinal cord do major collateral branches of medial gastrocnemius Ia afferents innervate the triceps surae motoneurone pool?

(2) How many major collateral branches do medial gastrocnemius Ia afferents have and what is their pattern of distribution?

(3) What are the conduction velocities of central portions of medial gastrocnemius Ia afferents?

In addressing these questions, we have also documented the precision with which signal averaging techniques can be applied to functional anatomical investigations.

METHODS

This study is based on data from twenty-one cats of either sex, of unknown age, weighing 1·5–3 kg. Animals were anaesthetized with I.P. injections of sodium pentobarbitone (40 mg/kg) which was supplemented over the course of the experiment. The trachea was cannulated. The left cephalic vein was cannulated and a continuous slow drip of lactated Ringer solution provided. The fourth ventricle was opened at the atlanto-occipital membrane to drain cerebrospinal fluid, thus preventing its accumulation in the spinal cord pool. The animal was placed in a spinal frame with the head lowered.

The left hind limb and hip were denervated by cutting the femoral nerve and all accessible branches of the sciatic nerve except the medial gastrocnemius and the combined lateral gastrocnemius and soleus branches. These latter branches, in continuity, were placed on separate bipolar hook electrodes in the popliteal fossa for electrical stimulation. A suture was tied to the cut Achilles tendon to stretch the triceps surae muscles and thus excite the Ia spindle afferents. A pool was formed from the skin of the leg; the nerves and muscles were immersed in warm mineral oil.

The spinal cord was exposed by a dorsal laminectomy of the L1 or L4 through L7 vertebrae. A pool was formed from the skin and the spinal cord was covered with warm mineral oil. Body and oil pool temperatures were maintained at 38 °C by a heating blanket and infra-red lamp. Dorsal roots L6 and rostral L7 were cut to provide access to the medial and lateral gastrocnemius and soleus branches of the motoneurone pools. Small filaments of rostral S1 or caudal L7 dorsal roots in continuity were placed on fine (0·1 mm) bipolar platinum hook electrodes for recording. Single Ia afferents were identified by conventional criteria (constant amplitude and discharge rate of action potentials, phasic response to muscle stretch, 'pause' in discharge following muscle contraction, latency from peripheral nerve stimulation of 1·3–2·0 msec (conduction distance usually about 160 mm), low threshold to muscle stretch). To verify that the afferent conducted into the spinal cord, a silver ball electrode was placed on the ipsilateral dorsal funiculus at vertebral level L4 and the ascending stem branch of the Ia afferent was stimulated antidromically. If the Ia afferent were intact, this antidromic action potential would collide with and block the orthodromic action potential initiated 2–3 msec later by electrical stimulation of the peripheral nerve.

Conduction velocity of the ascending stem branch of the Ia afferent in the dorsal funiculus was also calculated with this collision technique. The ipsilateral dorsal funiculus was stimulated to initiate an antidromic action potential in the Ia afferent. Following the arrival of the antidromic

action potential in the periphery, an orthodromic action potential could be initiated by electrical stimulation in the popliteal fossa and recorded in the dorsal roots. The more rostral the dorsal funiculus stimulating electrode the longer the time required for the antidromic action potential to reach the periphery and thus fail to collide with a subsequently initiated orthodromically conducted action potential. Conduction velocity in the dorsal funiculi was thus calculated as the change in maximum effective conditioning stimulus-test stimulus interval divided into the change in rostrocaudal location of the dorsal funiculus stimulating electrode.

Recordings from the spinal cord were made with low impedance (3–5 MΩ) bevelled micropipettes filled with 3 M-KCl or 3 M-NaCl. The amplified signal was led into an average response computer which was triggered by action potentials recorded from the Ia afferent. A 50 μV, 1 msec calibration pulse was averaged with the intracellular signals. Sweep durations were usually 10·24 or 15·36 msec, with bin widths of 10 or 15 μsec, respectively. Averaged data were digitized and stored on magnetic tape for subsequent analysis. By use of a cursor, time and amplitude values could be determined for each bin.

RESULTS

1. *Electrical potentials generated by single central medial gastrocnemius Ia afferent fibres in the spinal cord*

Four types of electrical potentials evoked by single Ia fibres were recorded in the spinal cord using micropipette electrodes and signal averaging techniques. These potentials are illustrated in Fig. 1.

(*a*) *Single fibre axonal potential* (*a.p.*). A.p.s (Fig. 1*E*) were negative or triphasic positive–negative–positive potentials recorded in extracellular space in spinal grey outside of pools of target cells. They were of small amplitude ($< 10\ \mu$V) and brief duration ($< 300\ \mu$sec).

(*b*) *Single fibre terminal potential* (*t.p.*). T.p.s were recorded in regions of synaptic contact between the Ia afferent and its target cells (e.g. spinal cord laminae VI, VII and IX). These field potentials were readily recorded with the micro-electrode tip in extracellular space in the target neuronal pool (Fig. 1*B*) or inside a motoneurone (Fig. 1*A*). Amplitude of t.p.s was as large as 50 μV, but generally was from 5 to 15 μV. T.p. amplitudes did not differ whether recorded intra- or just extracellularly ($12{\cdot}0 \pm 0{\cdot}8\ \mu$V *vs.* $12{\cdot}3 \pm 1{\cdot}1\ \mu$V; $n = 120$; $P < 0{\cdot}8$; two-tailed t test). T.p.s produced by medial gastrocnemius Ia afferents in medial motoneurones or lateral motoneurones did not differ in amplitude ($11{\cdot}83 \pm 0{\cdot}57\ \mu$V *vs.* $11{\cdot}70 \pm 0{\cdot}92\ \mu$V; $n = 165$; $P < 0{\cdot}9$; two-tailed t test).

T.p.s were classified as *simple* (Fig. 1*C*) or *compound* (Fig. 1*D*). Simple ones consisted of a single positive–negative wave (Fig. 1*A*, *B*, *C*) but were occasionally purely positive or purely negative in polarity. Compound t.p.s typically exhibited two or more positive peaks (Fig. 1*D*) and appeared to be composed of two or more simple superimposed t.p.s, each with a different latency from the trigger point. Rise times of simple t.p.s were 60–80 μsec; time from positive peak to negative peak was 80–100 μsec; total duration of simple t.p.s was about 400 μsec; Temporal qualities of compound t.p.s were more variable.

(*c*) *Single fibre focal synaptic potential* (*f.s.p.*). F.s.p.s (Fig. 1*B*) were slow potentials of negative polarity recorded when the micro-electrode tip was located in a pool of target neurones of the Ia afferent (i.e. laminae VI, VII and IX). They often appeared as the attenuated mirror image of an e.p.s.p. recorded in the immediate vicinity,

i.e. their time course is the same as that of the e.p.s.p. and not that of the underlying current. While f.s.p.s were usually recorded with the electrode tip in extracellular space, we have occasionally recorded negative slow potentials from within motoneurones. These were distinguished from inhibitory postsynaptic potentials by the fact that they did not reverse polarity following Cl injection. In these cases, we

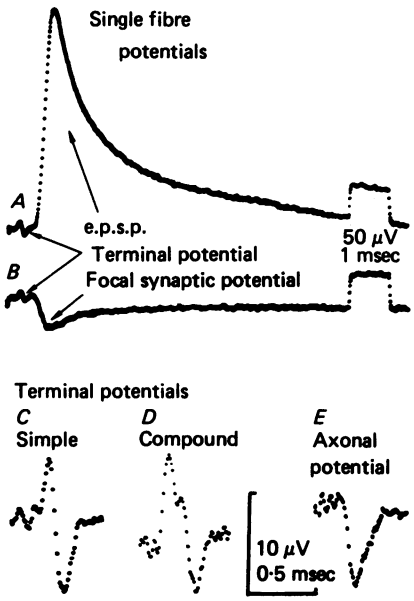

Fig. 1. Single fibre potentials generated by single medial gastrocnemius Ia afferent in the cat spinal cord. *A*: intracellular recording from motoneurone showing t.p. and e.p.s.p. *B*: recording immediately extracellular to motoneurone of *A*, showing t.p. and f.s.p. Calibration square wave for *A* and *B*: 1 msec, 50 μV. *C*: simple t.p. from medial gastrocnemius motoneuronal pool. *D*: compound t.p. from motoneuronal pool. *E*: a.p. recorded from lamina VI dorsomedial to Ia terminal region. Calibration shown for *C–E*. Averages accumulated from 1024 sweeps. Details presented in text.

assume that the triggering Ia afferent made either no or only very weak excitatory synaptic contact with the impaled motoneurone, and that the negativity recorded was an f.s.p. generated by an adjacent motoneurone (Watt, Stauffer, Taylor, Reinking & Stuart, 1976).

(*d*) *Single-fibre excitatory post-synaptic potential* (*e.p.s.p.*). Intracellular recording in triceps surae motoneurones revealed that e.p.s.p.s (Fig. 1*A*) were generated in 85% of homonymous (i.e. medial gastrocnemius) and in 60% of heteronymous (i.e. lateral gastrocnemius and soleus) motoneurones. Their characteristics are discussed in detail in the companion paper (Munson & Sypert, 1979).

2. *Cross-sectional analysis of single fibre potentials*

A cross-sectional analysis of single fibre potentials was achieved by making successive penetrations across a transverse plane of the spinal cord (Fig. 2*A*). Histological controls using small electrolytic lesions were used to verify the electrode tracts and depths of the penetrations. Potentials at successive depths are shown for five penetrations at 500 μm intervals (Fig. 2*B*, *a–e*). Penetration *a* was located dorsal

of the most dorsal group of target cells of medial gastrocnemius Ia afferents. Accordingly, the only single fibre potentials recorded were a.p.s. Penetration *b* traversed lamina VI, composed in part of interneurones activated by medial gastrocnemius Ia afferents (Szentagothai, 1967). Consistently, single fibre t.p.s and f.s.p.s were seen at depths of 1000–1500 μm. Penetration *c* traverses lamina VII, the pool of inhibitory interneurones activated by medial gastrocnemius Ia afferents (Szentagothai, 1967).

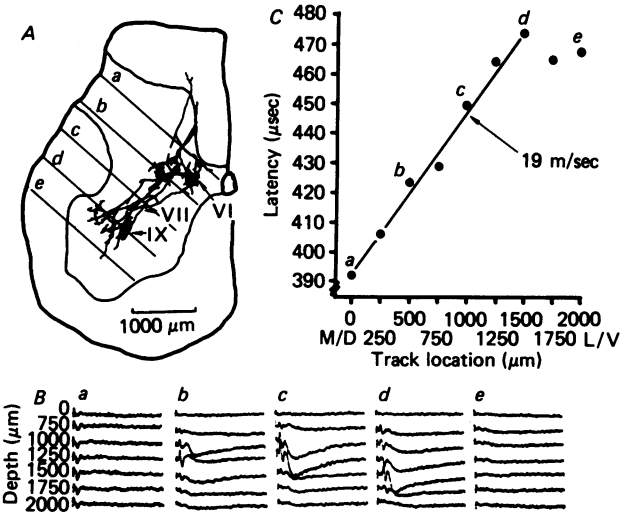

Fig. 2. Cross-sectional analysis of single fibre potentials in cat spinal cord. *A*: schematic presentation of spinal cord in cross-section showing electrode tracts (*a–e*) with superimposition of terminal distributions of three medial gastrocnemius Ia afferents redrawn from Brown & Fyffe (1978). *B*: single fibre potentials from cat spinal cord at indicated depths determined from micrometer reading; *a–e* correspond to *a–e* in *A*. Note that the progression from *a* to *e* is accompanied by a progression from a.p.s to t.p.s, f.s.p.s and e.p.s.p.s and then to potentials indistinguishable at this amplification. Note also the progressively longer latency of single fibre potentials recorded more ventrolaterally. *C*: calculation of conduction velocity in major collateral branch based on progression of mean latencies with progressively more ventrolateral electrode tracts. Analysis includes also penetrations made between each of *a–e* depicted in *A* and *B*. Slope of line connecting points predicts conduction velocity of 19 m/sec for this major collateral branch. M/D: medial dorsal. L/V: lateral ventral. Full details in text.

Clear evidence of single fibre t.p.s and f.s.p.s is found through much of this penetration. Penetration *d* passes through lamina IX, the triceps surae motoneuronal pool (Szentagothai, 1967). Again single fibre t.p.s and f.s.p.s are evident. Penetration *e* is largely lateral and ventral of medial gastrocnemius Ia target cells where little evidence of single fibre activity appears. The low amplitude and somewhat shorter latency of these latter signals presumably represents a volume conducted signal originating from greater distances.

These data permit a calculation of conduction time and, thus, an estimate of conduction velocity in this portion of the major collateral branch. To achieve this, mean latency was determined for the t.p.s or a.p.s of each penetration. These latencies were then plotted as a function of location of the electrode track (Fig. 2*C*). In the example shown, latency of single fibre potentials increased linearly in successive

penetrations from medial–dorsal to lateral–ventral. This progression of time over distance corresponds to a conduction velocity in the major collateral branch of 19 m/sec. The accuracy of this estimation depends on the fact that the major collateral branch being analysed is anatomically parallel with the series of loci analysed electrophysiologically. Similar calculations from five other experiments gave values of 8–17 m/sec for major collateral branches.

3. *Rostrocaudal Analysis of t.p.s*

Micro-electrode penetrations were made at 200 μm intervals over the approximately 10 mm rostrocaudal extent of the triceps surae motoneurone pool. At each rostrocaudal location explorations were made to maximize antidromic field potentials from electrical stimulation of the medial gastrocnemius and lateral gastrocnemius and soleus nerves, thus assuring that the micro-electrode was in the triceps surae motoneuronal pool. Electrical activity time-locked to the Ia afferent action potential was then recorded at each site. This activity consisted of t.p.s, f.s.p.s and e.p.s.p.s.

Results of one such experiment are presented in Fig. 3. In Fig. 3*B*, minimum latency of the t.p. recorded at each rostrocaudal level is shown. This latency is calculated as the time from the beginning of the averaged record to the first positive peak. It represents conduction time for the Ia afferent action potential from the dorsal root. There appear to be nine levels at which these latencies achieve minima (i.e. 1·6 mm, 2·8 mm, etc.). On either side of these minima (i.e. rostral and caudal) t.p. latencies increased. We interpret that each of these minima corresponds to the location of a ventrally directed major collateral branch of the Ia afferent. The longer t.p. latencies observed between the minima are interpreted to represent rostrally and caudally directed terminal branches of the major collateral branch. At other rostrocaudal locations (e.g. 7·4–7·8), no single fibre potentials were detectable even after extensive exploration of the motoneuronal pool, suggesting that no afferent terminals were directed into this region. The point at 9·4 may represent a slowly conducting major collateral branch with a very limited terminal field. The location and terminal field territory for each of the nine major collateral branches are shown in Fig. 3*A* (I–IX). Distances between the major collateral branches may be estimated by the distances between the latency minima. For Fig. 3 these intervals range from 600 to 2000 μm, the mean being 1050 μm.

A progressive increase in latency of the successive minima is seen as one proceeds rostrally from major collateral branch II. A line connecting these minima (Fig. 3*B*) predicts a conduction velocity of 41 m/sec for the ascending Ia stem axone in the dorsal funiculus, assuming that conduction time in each of the ventrally directed major collateral branches is equal (see below). The conduction velocity of this Ia afferent from the popliteal fossa to the dorsal root recording site was 102 m/sec.

Data pertinent to this type of analysis were obtained from four other experiments; these are summarized in Table 1. Animals 21 and 24 are in excellent agreement in all respects. The entire rostrocaudal extent of the triceps surae motoneuronal pool was explored in both animals. Based upon these analyses, medial gastrocnemius Ia afferents are characterized by up to nine major collateral branches descending into the triceps surae motoneuronal pool, at intervals from 400 to 2400 μm (mean = 1071 μm). The rostrally directed stem axone in the dorsal column conducts

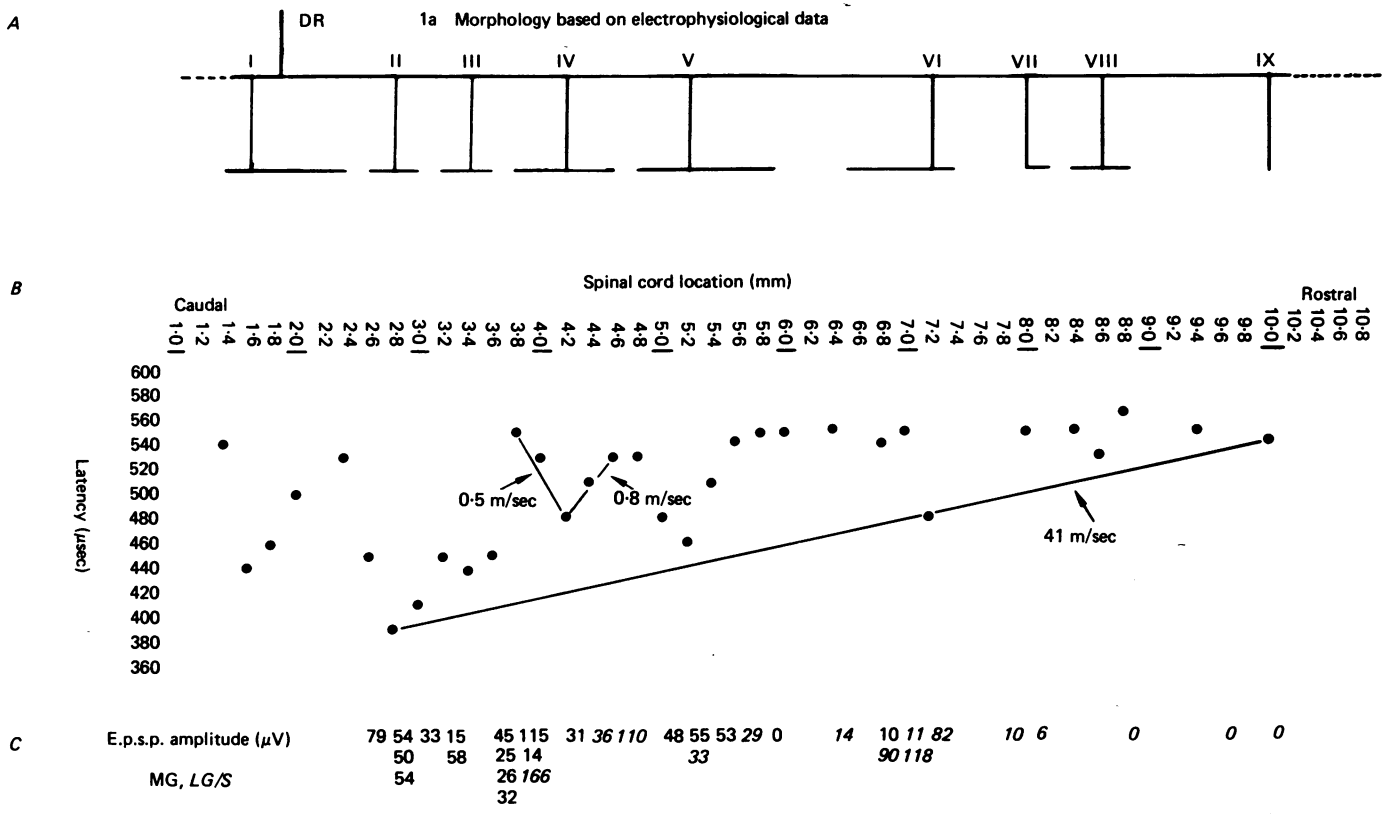

Fig. 3. Rostrocaudal analysis of single fibre potentials in cat spinal cord. *A*: reconstruction of single medial gastrocnemius (MG) Ia afferent based upon electrophysiological data presented in *B* and *C*. I–IX are nine major collateral branches descending into terminal fields. Single fibre potentials were recorded over an 8·6 mm rostrocaudal extent; interrupted lines indicate uncertainty concerning limits of stem axon. DR: dorsal root. *B*: minimum latency measured from dorsal root recording site for single fibre t.p. at 200 μm intervals along spinal cord. Potentials were recorded with micro-electrode tip in triceps surae motoneurone pool, determined by antidromic stimulation of MG and lateral gastrocnemius/soleus (LG/S) muscle nerves in popliteal fossa. Line connecting progressive latency minima predicts conduction velocity in stem axon of 41 m/sec. Line connecting latency minima for branch IV predicts conduction velocity in terminal branch of 0·5 and 0·8 m/sec. Further details in text. *C*: amplitude of e.p.s.p.s recorded in MG and LG/S (italicized) motoneurones in course of rostrocaudal exploration. Projection frequency was 18/19 homonymous and 12/15 heteronymous. Full details in text.

with a mean conduction velocity of about 48 m/sec or about 52 % of the peripheral conduction velocity of the Ia afferent.

A similar analysis was made of conduction velocities in the rostrally and caudally directed terminal branches of the major collateral branches. An example of such an analysis is shown for major collateral branch IV of Fig. 3*A*. These rostral and caudal

TABLE 1. Anatomical and physiological characteristics of single central medial gastrocnemius Ia afferent fibres, based on analysis of single fibre potentials

Animal	Rostro–caudal distance explored (μm)	No. of major collateral branches	Intercollateral/Intracellular distance (μm)		Conduction velocity (m/sec)	
			Mean	Range	Dorsal funiculus	Peripheral nerve
7	4800	3	870	700–1000	40	94
18	5400	4	1250	600–2400	67	90
21	9000	7	1057	600–2000	35	97
23	4600	3	600	400–800	55	73
24	8600	9	1050	600–2000	41	102
Mean	6480	5·2	1071	580–1640	47·6	91·2

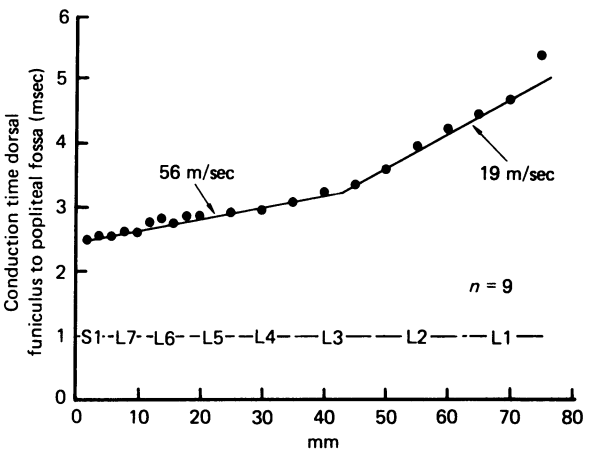

Fig. 4. Mean conduction velocity of nine group Ia afferents in spinal cord segments S1 through L1 measured with impulse collision technique. Details in text.

terminal branches each appear to extend over a 400 μm rostrocaudal extent of the spinal cord (other terminal branches in this animal ranged from 200 to 800 μm in rostrocaudal extent). Mean conduction velocity for the terminal branches in the experiment of Fig. 3 was 0·7 m/sec, with a range of 0·2–1 m/sec. These are minimum values based on the assumption that the terminal branches are oriented in parallel with the rostrocaudal loci of points analysed (see Discussion).

The triceps surae motoneuronal pool may be divided into three zones with regard to the species of motoneurones encountered, i.e. a caudal region where only motoneurones of the medial gastrocnemius were encountered, a transition region where both medial and lateral branch motoneurones were encountered, and a rostral region where only lateral branch motoneurones were encountered (Fig. 3*C*). The triggering Ia afferent elicited e.p.s.p.s in 95 % of homonymous and 79 % of heterony-

mous motoneurones in this sample of thirty-four motoneurones. Mean homonymous e.p.s.p. amplitude was 44 μV; mean heteronymous e.p.s.p. amplitude was 59 μV.

4. *Collision testing of Ia fibres in the dorsal column*

An impulse collision technique was also used to calculate the conduction velocity of individual afferent stem axones in the dorsal funiculus (see Methods). Data on dorsal funiculus for nine group Ia afferents from four cats are summarized in Fig. 4. These fibres conducted at a mean velocity of 56 m/sec at lower lumbar levels and at 1/3 that rate at upper lumbar levels. The mean peripheral velocity of these group Ia afferents was 87 m/sec; that of the lower lumbar of 56 m/sec was thus 64 % of the peripheral.

DISCUSSION

Using spike-triggered averaging and low impedance micropipette electrodes, we have distinguished and analysed four types of single fibre potentials in the cat spinal cord temporally related to action potentials in Ia muscle spindle afferents. These have been interpreted to provide evidence concerning the anatomy of single central Ia afferents, and conduction velocities in dorsal funiculus stem axons, major collateral branches and terminal branches. Based upon this analysis, a second paper (Munson & Sypert, 1979) will describe in detail the nature of Ia motoneurone synaptic transmission.

1. *Interpretation of single fibre potentials*

(*a*) *Axonal potentials.* Distinguishing features of a.p.s are their small amplitude ($< 10\,\mu$V), limited locus, and primarily negative polarity. We attribute their diminutive amplitude and limited locus to the fact that they are generated by single major collateral branches of Ia afferents, spaced at mean intervals of about 1000 μm (Brown & Fyffe, 1978); Hongo *et al.* 1978; this research). The predominantly negative polarity is in accord with theoretical discussions and experimental findings of Renshaw, Forbes & Morison (1940), Brooks & Eccles (1947), Lorente de No (1947) and Katz & Miledi (1965). Inward current flow at the active axonal site is responsible for focal negativity; to the extent that the recording site is a current source for the approaching and departing action potential, it will also demonstrate positivity before and after the negativity, in proportion to the current density at the site. The fact that there is an orderly increase in latency of a.p.s progressing ventrolaterally across the spinal cord is consistent with this interpretation.

(*b*) *Terminal potentials.* Distinguishing features of t.p.s are their relatively larger (than a.p.) amplitude (up to 50 μV), their restriction to target neuronal pools and their predominantly positive–negative polarity. The large amplitude is attributed to a number of factors. Firstly, the large number of afferent terminals increases both the amount of excitable membrane depolarized and the probability of recording from the vicinity of one or several terminals. Secondly, the relatively large size of the terminal boutons compared with the smaller axons results in a greater current density during depolarization. The positive–negative polarity is consistent with theoretical arguments and laboratory findings on sealed end recordings (Lorente de No, 1947; Brooks & Eccles, 1947; Takeuchi & Takeuchi, 1962; Katz & Miledi,

1965). The positive peak most probably signals the maximum rate of rise of the action potential invading the presynaptic terminal, reversing to negativity during the decaying phase. Our interpretation of these positive–negative potentials is further supported by the progression of latency with distance rostral and caudal from each major collateral branch (Fig. 2*B*) as well as additional data presented in the companion paper (Munson & Sypert, 1979).

(*c*) *Focal synaptic potentials.* Distinguishing features of f.s.p.s are their negative polarity, their restriction to regions of Ia target neurones, their tendency to occur in conjunction with t.p.s and their appearance (i.e. time course and configuration) as inverted e.p.s.p.s. F.s.p.s have been identified as extracellular concomitants of e.p.s.p.s by Brooks & Eccles (1947); this concept was further developed by Eccles, Fatt, Landgren & Winsbury (1954) and by Fu, Santini & Schomburg (1974), who showed their distribution (following whole nerve stimulation) in spinal cord laminae VI, VII and IX, i.e. the loci of Ia target neurones (Szentagothai, 1967).

(*d*) *Excitatory post-synaptic potentials.* Distinguishing features of e.p.s.p.s are their positive polarity when recorded intracellularly, their tendency to occur in conjunction with t.p.s, and their time course (rise time $< 3{\cdot}0$ msec, mean half-width about 4 msec). They have been described in detail (e.g. Mendell & Henneman, 1971) and will be further analysed in the succeeding paper (Munson & Sypert, 1979).

2. *Anatomy of Central Ia Afferents*

Electrophysiological evidence shows that medial gastrocnemius Ia afferents branch upon entering the spinal cord. The stem axon (including the descending branch) in the dorsal funiculus gives up to nine major collateral branches into the triceps surae motoneurone pool. The range of distances between adjacent major collateral branches is 400–2400 μm; the mean distance is 1071 μm. T.p.s, f.s.p.s and e.p.s.p.s appear in three regions, corresponding to laminae VI, VII and IX, thus confirming the existence of Ia synaptic terminals in these regions. Within the motoneuronal pool, t.p. latencies increase both rostrally and caudally up to 800 μm from each latency minimum. This was interpreted to show the existence of terminal branches extending rostrally and caudally from the major collateral branch in the motoneuronal pool.

This scheme of the morphology of the central branches of medial gastrocnemius Ia afferents which has emerged from our physiological data clarifies and complements earlier anatomical investigations (Szentagothai, 1967; Scheibel & Scheibel, 1969; Iles, 1976). The most detailed anatomical descriptions of single central Ia afferents have been presented by Brown & Fyffe (1978), Hongo, Ishizuka, Mannen & Sasaki (1978) and Burke, Walmsley & Hodgson (1979). Whereas the previous studies stained multiple afferents, these latter studies were accomplished by injecting single identified Ia stem axons in the dorsal funiculus with horseradish peroxidase (HRP) permitting the detailed morphological analysis of their terminal distribution. The results are in essential agreement and will be summarized together. Medial gastrocnemius Ia afferents were observed to branch rostrally and caudally upon entering the spinal cord and to give off about six major collateral branches (range 3–11) over their rostral–caudal extent of 4–13 mm. Collateral branch spacing was 100–3000 μm (mean 1000–1100 μm). Collateral branches descended directly to lamina IV or V, where they branched medially to innervate lamina VI and ventrolaterally at about 45 degrees towards the region of the triceps surae motor nucleus. Terminal fields

of adjacent collateral branches in laminae VI, VII and IX were found to ramify into virtually continuous rostral–caudal columns. Terminal branches were found to ramify 300–500 μm in the saggital plane. These anatomical studies are particularly valuable in detailing the fine structure of afferent terminations. Interpretation of the morphology of an individual afferent is not possible when multiple afferents have been stained (e.g. Szentagothai, 1967; Scheibel & Scheibel, 1969; Iles, 1976); even when only a single afferent is stained the filling of all branches has been assumed to be incomplete (Brown & Fyffe, 1978). Our functional data relate directly to this question. In experiments in which the entire rostral–caudal extent of the triceps surae motoneurone pool was explored, single fibre potentials were recorded over a maximum distance of 8600 μm. Up to nine major afferent collateral branches at intervals of 400–2400 μm (mean = 1071 μm) were identified. These values are in excellent agreement with the HRP studies and suggest the completeness of staining in those studies.

3. *Conduction velocity of central branches of the medial gastrocnemius Ia afferents*

(*a*) *Dorsal funiculus.* We have used two methods to calculate conduction velocity of the Ia afferent stem axon in the dorsal funiculus. The impulse collision method gave a mean velocity of 56 m/sec at lower lumbar levels. Calculations based on the progression of latencies of t.p.s at successively higher levels of the spinal cord gave a mean value of 47·6 m/sec. Sampling errors may contribute to this difference, since mean peripheral conduction velocity for the impulse collision group was higher than that for the single fibre potential group (91·2 *vs.* 86·6 m/sec). Furthermore, our estimate of dorsal funiculus conduction velocity was based on the assumption that conduction times in each of the major collateral branches was equal. In fact, this is probably not the case, since the spinal cord increases in size in rostral L7 compared with caudal L7 and S1. To the extent that a greater proportion of conduction time is thus expended in the more rostral major collateral branches of the medial gastrocnemius Ia afferent, a smaller proportion is therefore spent in the dorsal funiculus stem axon segment. The mean estimate of 47·6 m/sec is thus probably an underestimate of true conduction velocity of the dorsal funiculus. Similar estimates of Ia stem axon conduction velocity in the dorsal funiculus are obtainable from previous studies (Lloyd & McIntyre, 1950; Wall & Werman, 1976).

(*b*) *Major collateral branches.* Plotting of the progressive increase in a.p. and t.p. latency with increasing cross-sectional depth gave an estimate of conduction velocity for major collateral branches at 8–19 m/sec. Iles (1976) and Hongo *et al.* (1978) have found major collateral branches reaching the ventral horn with axon cylinder diameters of 2·5–3·0 μm. At a rate of 6 m/sec.μm (external diameter of myelinated axons, Hursh, 1939) a conduction velocity of 15–18 m/sec is predicted, but must be tempered since these measurements on branching collaterals were not of external diameters. Fu & Schomburg (1974), Wall & Werman (1976) and Brooks and Eccles (1947) suggest an 'intraspinal conduction velocity' of about 20 m/sec, derived from conduction partly in the major collateral branch and partly in the dorsal funiculus stem axon. Since the stem axon conducts at greater than 20 m/sec, the major collateral branch must conduct at less than 20 m/sec. Our data are in agreement with those derived from more traditional approaches.

(*c*) *Terminal branches.* Iles (1976) found that terminal arborizations of Ia afferent

collaterals are oriented primarily across the cord grey matter, in contrast with Brown & Fyffe (1978) who find them oriented in a rostral–caudal direction. Our functional data support the existence of both. Penetrations into the motoneuronal pool from 45 degrees lateral showed t.p.s of decreasing latency as the electrode approached the pool, suggestive of laterally conducting terminals. Increasing t.p. latencies rostrally and caudally of the minima (Fig. 3) suggest the existence of rostrally and caudally directed terminal branches, extending up to 800 μm but usually less than half of that. Conduction velocity in the rostral–caudal terminal branches ranged from 0·2 to 1·0 m/sec, similar to those of Wall & Werman (1976). Our calculations may underestimate true velocity to the extent that the orientation of the terminal branch is not precisely parallel to the locus of points analysed electrophysiologically.

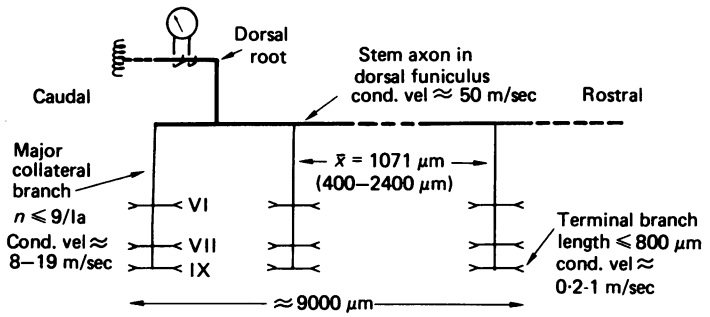

Fig. 5. Morphology of single central medial gastrocnemius Ia afferent fibres projecting to motoneurones, based on electrophysiological data. Details in text.

Electroanatomy of single central medial gastrocnemius Ia afferents

Based upon interpretation of our electrophysiological data, a composite picture of single central Ia afferents emerges (Fig. 5). Upon entering the spinal cord, the afferent fibre bifurcates rostrally and caudally. The rostrally directed stem axon in the dorsal funiculus conducts at about 50–60 m/sec as far as the L3 segment, where it slows to about one third that rate through the L1 segment. Major collateral branches (nine or fewer) descend into the spinal cord at about 1 mm intervals over the approximately 1 cm rostrocaudal extent of the triceps surae motoneuronal pool at spinal cord levels L7 and S1. Conduction velocity in these major collateral branches is in the range of 8–19 m/sec. Three distinct regions of synaptic activation exist: laminae VI, VII and IX. Terminal branches in the motoneuronal pool extend for several hundreds of μm laterally, rostrally and caudally from the major collateral branch. Conduction velocity in terminal branches is 0·2–1 m/sec. These data, derived from the use of signal averaging techniques, are in excellent agreement with previous data based upon anatomical and classical electrophysiological methods.

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