THE JOURNAL OF COMPARATIVE NEUROLOGY 304:357–372 (1991)

Distribution of Motoneurons Supplying Cat Sartorius and Tensor Fasciae Latae, Demonstrated by Retrograde Multiple-Labelling Methods

D.C. GORDON, G.E. LOEB, AND F.J.R. RICHMOND
Department of Physiology and Biomedical Engineering Unit, Queen's University at Kingston, Kingston, Ontario, Canada K7L 3N6

ABSTRACT

Sartorius (SART) and tensor fasciae latae (TFL) in the cat hindlimb are functionally heterogeneous muscles with regions that differ in their skeletal actions and electromyographic recruitment during normal activity. The topographical organization of motoneurons supplying different regions of SART or TFL has been investigated by exposing cut nerve branches supplying different peripheral territories to a combination of retrograde tracers, including Fast Blue (FB), Fluorogold (FG), and horseradish peroxidase (HRP). Motoneurons supplying medial, central, and anterior regions of SART were intermixed extensively throughout a single columnar nucleus located in the ventrolateral part of segments L4 and L5. Within this column, motoneurons supplying medial SART tended to lie more rostrally than those supplying anterior regions, but the gradient was modest and showed some cat-to-cat variation. Two major branches entered anterior SART at different proximodistal levels. When these two branches were exposed to different tracers, most motoneurons contained a single tracer; only a few double-labelled cells were apparent. The labelling suggests that anterior SART may contain two separate, in-series divisions of motor units. In TFL, motoneurons supplying nerve branches to posterior, central, and anterior parts of the muscle were intermingled indiscriminately in a single ventrolateral cell column in L6 and rostral L7. These results suggest that topographical organization in lumbar motor nuclei does not always reflect the highly ordered biomechanical and functional specialization evident in the peripheral organization of the muscles themselves.

Key words: motor nuclei, horseradish peroxidase, fluorogold, fast blue, compartmentalization

In 1951, Romanes showed that motoneurons supplying single hindlimb muscles were clustered into anatomically distinct regions, which have come to be called motor nuclei. Since that time, much attention has been given to the motor nucleus as a functional as well as an anatomical entity, governed by rules of orderly recruitment. According to such rules, motoneurons are activated in a stereotyped progression starting with those that supply the weakest and most fatigue-resistant muscle units and followed progressively by the stronger and more fatigue-susceptible units as additional force becomes required (Henneman et al., '65a,b; Burke, '81). To achieve this orderly recruitment, motoneurons in all parts of the nucleus must receive qualitatively similar inputs from the descending and segmental systems that control the action of the muscle. Thus, in a muscle with a single pattern of orderly recruitment, the topography of motoneurons in a motor nucleus should neither reflect nor influence the order in which a motoneuron is recruited.

However, not all motor nuclei supply muscles in which motor units are recruited in a single pattern of orderly progression. A number of hindlimb muscles including sartorius, tensor fasciae latae, and biceps femoris have distributed origins or insertions arranged so that contractions in one part of the muscle will produce a different mechanical action on the limb than will contractions in another part (English and Weeks, '87; Chanaud et al., '91a,b; Pratt and Loeb, '91). These muscles have been shown to contain two or more separate subvolumes of motor units that are innervated by anatomically separate branches of the muscle nerve (English and Weeks, '87; Loeb et al., '87; Chanaud et al., '91a,b). EMG recordings have

Accepted October 9, 1990.

shown that individual regions in a single muscle can be activated at different times or at different relative intensities during various behavioral tasks, thus violating the rules of orderly recruitment that would be expected if the muscle were to behave as a single entity (Hoffer et al., '87; English and Weeks, '87; Chanaud and Macpherson, '91; Chanaud et al., '91b; Pratt and Loeb, '91; Pratt et al., '91). The fact that different parts of a single muscle can be activated independently is particularly significant because it suggests that functional subsets of motoneurons exist in a single motor nucleus and these can be distinguished from one another by descending or segmental projections. The factors responsible for this selectivity are not yet understood. Some have proposed that selective connections might be made on the basis of differences in the physiological activity or biochemical make-up of cells supplying different targets (called "species" recognition by Wyman, '73) (e.g., Sperry, '63; Eccles, '64; Lichtman et al., '84). However, others have argued that nonuniform connections might come about because of differences in the locations of motoneurons supplying specific muscle regions. Topographic differences would provide a "place-specific" substrate for organizing the heterogeneous synaptic input and would obviate the need for "species-specific" markers on the individual motoneurons (Wyman, '73; Lüscher and Vardar, '89).

In the present study, we have examined the distribution of motoneurons supplying different parts of two muscles, sartorius (SART) and tensor fasciae latae (TFL) in the cat. Both muscles have distributed insertions and regionally differentiated patterns of recruitment. The feline SART is a long biarticular muscle that can be divided into two biomechanically specialized parts that lie in parallel. Both parts share a flexor action at the hip. However, the anterior part of SART inserts onto the patella and acts to extend the knee, whereas the medial part inserts along the tibia and flexes the knee (Sherrington, '10). The differing mechanical actions of anterior and medial SART are reflected in their patterns of EMG activity during normal walking. The anterior part is recruited in two bursts per step cycle, one in mid-to-late stance and the other in the mid-to-late swing phase. The medial part is recruited only once, early in the swing phase when it helps to lift the foot by flexing both the hip and the knee (Hoffer et al., '87; Pratt and Loeb, '91).

Tensor fasciae latae has also been reported to contain two identifiable regions, a longer anterior region that inserts on the fascia close to sartorius and a more posterior part that inserts on a wide connective tissue sheet (the fascia latae) covering the lateral thigh. Anterior TFL flexes the hip and extends the knee (Chanaud et al., '91a,b). During walking, it is most active during flexion at the beginning of the swing phase. Posterior TFL has a more complicated role in "hip abduction, flexion and/or internal rotation" (Chanaud et al., '91b) and is activated during stance. In both SART and TFL, the boundary between the two functionally distinct regions is not marked by clear fascial planes or differences in fiber orientation. However, the regions are innervated by separate nerve branches whose stimulation leads to localized contractions within the target region rather than throughout the muscle as a whole (Chanaud et al., '91b).

We have taken advantage of the separated branching pattern of SART and TFL nerves to study topography within SART and TFL motor nuclei using a retrograde multiple-labelling method that combines the use of fluorescent tracers and horseradish peroxidase (HRP) (Gordon and Richmond, '89, '90). In the past, studies of topography in hindlimb motor nuclei have used only a single tracer, horseradish peroxidase (HRP), applied to an entire muscle nerve in one limb and to a single primary branch of the same muscle nerve in the contralateral limb (Pratt et al., '84; Weeks and English, '85, '87). Using that method in SART, Pratt and her colleagues ('84) described a topographical gradient in which the motoneurons supplying the medial part of the muscle tended to lie more rostrally in the motor nucleus than those supplying the anterior part. In the present study, multiple-labelling methods showed that this gradient was often quite subtle. Furthermore, they revealed an unexpected level of complexity in the organization of motoneurons supplying anterior SART and suggested that anterior SART may be divided into two in-series compartments of muscle units. In TFL, topographical gradients in the motor nucleus were difficult or impossible to detect, despite clear anatomical and functional heterogeneity within the muscle itself. These results are discussed in the context of the general "partitioning" hypothesis (Stuart et al., '88) and the possible roles played by topographical factors in the differential recruitment of muscles or muscle compartments.

METHODS

Surgery and nerve branch identification

Experiments were conducted on thirteen cats (2.9–3.7 kg) anesthetized with sodium pentobarbital (Nembutal, Abbott; initial dose 35 mg/kg ip, supplemental doses 5 mg/kg iv). An antibiotic (Penlong S, Rogan/STB) was injected im on the day prior to surgery and again 24 hours later. Under aseptic conditions nerves supplying the leg muscles sartorius (SART) or tensor fasciae latae (TFL) were exposed as shown in Table 1. Major nerve branches supplying different regions of the muscle were dissected as far distally as possible. The course and branching pattern of the nerves were recorded using line drawings or photographs (Fig. 1). Each nerve branch was stimulated electrically with single pulses (0.1 msec duration, 2–4 × threshold for just detectable contraction) to identify the extent of the contracting muscle territory. In one cat, the muscle nerve supplying gluteus maximus superficialis was also dissected.

Nerve branches serving muscle regions of interest were cut distally. The area around the base of each nerve was coated with molten paraffin wax (melting point 40°C) so that only a short length of its proximal end emerged above the wax. After the wax had begun to solidify, a depression was carved in the wax adjacent to the nerve branch. Each nerve was placed in its own depression, which served as a reservoir into which a specific tracer could be placed without contaminating adjacent nerves or tissues (Swett et al., '86; Gordon and Richmond, '90). A drop of distilled water was placed in each reservoir to test it for leakage and to cause osmotically induced swelling in the cut nerve end. After 10 minutes the distilled water was removed and replaced with approximately 30 μl of tracer solution. One reservoir was filled with horseradish peroxidase (HRP) (30%, Boehringer, Grade 1), the second with Fluorogold (FG) (5–7%, Fluorochrome, Inc.) (Schmued and Fallon, '86) and the third with Fast Blue (FB) (2%, Sigma Chemical Co., St. Louis, MO). All tracers were dissolved or suspended in 0.9% saline to which was added 2% dimethylsulphoxide (DMSO) (Huisman et al., '82). Each filled reservoir was

TABLE 1. Tracer Applications in SART and TFL[1]

	Sartorius				Tensor fasciae latae			
	Medial	Central	Anterior		Posterior	Central	Anterior	Gluteus
STFL 2	FG	FB						
3	FB	FG						
4					FG		FB	HRP
6					FB	FG	HRP	
7					FB	FG	HRP	
8	FG	FB	HRP					
9	FB	HRP	FG					
10*	FB	HRP	FG					
11*					FG	HRP	FB	
			Prox	Dist				
12		FB	HRP	FG				
13		FB	HRP	FG				

[1]Results are reported for 11 cats (STFL series) in which all tracers produced strong labelling. Asterisks denote those cases in which spinal cords were sectioned transversely; the other cords were cut horizontally. Columns labelled medial, central, anterior, and posterior show the particular nerve branches exposed to the tracers Fast Blue (FB), Fluorogold (FG), and horseradish peroxidase (HRP). Prox, proximally directed branches of anterior SART; Dist, distally directed branches of anterior SART.

sealed with a new layer of wax, and wax was spread over any exposed tissues to prevent dehydration. The reservoirs were left undisturbed for 3–4 hours. The wax overlying each reservoir was then removed. The integrity of the pools was checked to ensure that tracer had not leaked out during the period of exposure. The tracer solutions were blotted and the nerve ends were rinsed with saline. The remaining wax was removed and the incisions were closed.

Problems were seldom encountered with the dipping procedure for SART nerves. However, the central nerve branches serving TFL (Fig. 1) usually ran with a blood vessel that was difficult to microdissect from the nerve bundle prior to nerve section. If the nerves and the blood vessel were cut together, bleeding was difficult to stop, presumably because the DMSO in the tracer solutions hampered normal coagulation. Thus, the tracer solution became diluted over time and uptake of the tracer was poor. In the present report, experimental results concerning the TFL motor nucleus are described only for the four cats in which all three subpopulations of motoneurons were labelled strongly by their respective tracers. Results from two other cats (STFL 1,5) were not used for data analysis.

Perfusion and histology

Following an 8-day survival period (Gordon and Richmond, '90), cats were reanesthetized with sodium pentobarbital (35 mg/kg i.p.) and perfused by delivering fluid under a controlled driving pressure of 25 kPa through a cannula introduced into the descending aorta. Two liters of normal saline were perfused followed by 1.5 liters of fixative A, containing 4% paraformaldehyde in 0.1 M acetate buffer (pH 6.5) and 1.5 liters of fixative B containing 4% paraformaldehyde in 0.1 M borate buffer (pH 9.5) (modified from Gerfen and Sawchenko, '84). The spinal cord from L4 to L7 was removed and the distances between segmental boundaries were measured with respect to the ends of the tissue block. All tissues were transferred through a graded series of sucrose solutions (10%, 20%, and 30%) in 0.1 M borate buffer (pH 9.5) over the subsequent 48-hour period. The fixed tissue was frozen and cut serially into 50 μm horizontal or transverse sections. Sections were processed histologically for HRP activity using tetramethylbenzidine (TMB) as the chromogen (Mesulam, '78). Slides were coverslipped with synthetic mounting medium (DPX, Luka) and were examined using dark field and epi-fluorescence illumination (wavelengths 340–380 nm). The positions of all motoneurons were mapped onto large drawings made using a camera lucida attachment. As a rule, individual cells were counted only if their nuclei could be identified within the cell soma. However, some cells were stained so darkly that their nuclei were obscured. These cells were counted if they were comparable in size to other well-filled cells in the vicinity and at least three primary dendrites could be seen to emerge from the cell body. Where cell sizes are reported, they are based on measurements of "mean equivalent diameter," determined by averaging the maximum and minimum diameters of the cell in cross-section (Burke et al., '77).

The positions of labelled motoneurons were also mapped as a series of X,Y coordinates using a digitizing pad (Scriptel, RDT 1218) whose output was fed to a Macintosh II computer. In each case, the X axis was defined arbitrarily as a line drawn parallel to the long axis of the labelled column so that it intersected the most rostral of the labelled motoneurons. The coordinates of this motoneuron were designated as (0,0). The relative distributions of three labelled subpopulations were compared statistically using the Kruskal-Wallis one-way analysis of variance by ranks and the Mann-Whitney U-test (Siegel, '56). These methods tested whether statistically significant differences existed between the distributions of motoneurons supplying different primary branches of SART or TFL.

RESULTS

The nerve bundle supplying sartorius (SART) characteristically divides into a series of major branches of different thicknesses that enter the muscle at intervals from its medial to its anterior edge (Fig. 1). These branches have been reported to innervate long, narrow strips of muscle fibers running from the origin to the insertion of the muscle (Loeb et al., '87). In medial SART, most branches run distally from the parent nerve bundle before entering the muscle but one or two branches are also proximally directed. As the nerve bundle approaches the anterior edge of SART, it bifurcates into distinct proximally and distally directed branches that run for short distances along the surface parallel to muscle fascicles before entering the substance of the muscle (Fig. 1). In the earliest five experiments different tracers were applied to 1) the most medial major branch, 2) one or more central branches, and 3) one or both of the anterior branches of SART as shown in Table

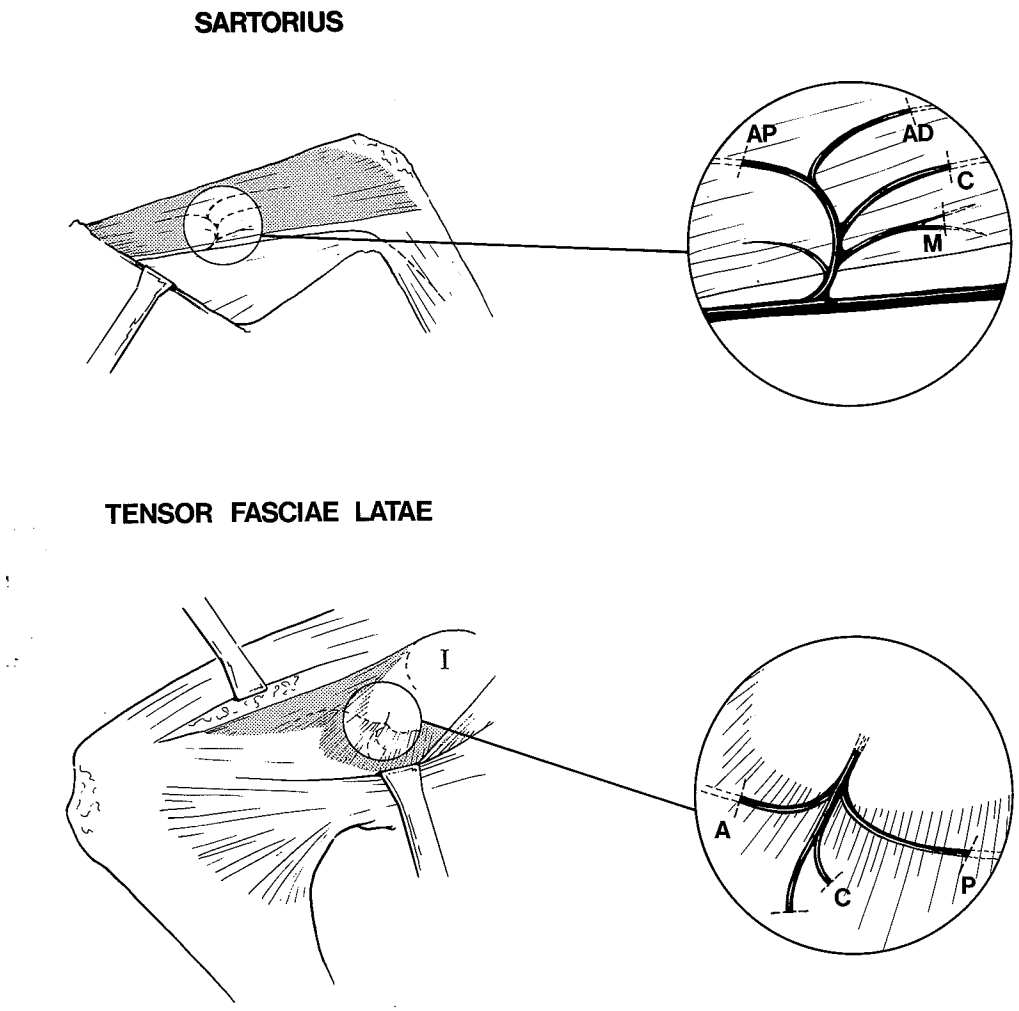


Fig. 1. Innervation of TFL and SART. Line drawings of SART, on the medial aspect of the hindlimb, (top left) and TFL, on the lateral aspect, (bottom left) show the anatomical features of each muscle in relation to its underlying nerve supply. The typical branching pattern of the nerves is shown in the circle on the right. A, anterior branch; C, central branches; P, posterior branch; M, medialmost branch in SART; AP, anterior, proximally directed branch; AD, anterior; distally directed branch. I, Ischium.

1. In an additional two experiments, all of the medial and central branches were exposed to one tracer and the two different tracers were placed respectively on the proximally directed and distally directed branches that entered anterior SART.

The nerve axons supplying tensor fasciae latae (TFL) courses in 3–5 branches that enter the muscle on its ventral surface (Fig. 1). One long branch consistently courses anteriorly for several mm. Electrical stimulation of this branch produced a contraction that was localized to the long anterior part of the muscle. The wide posterior part of TFL is innervated by a grouping of 2–4 nerve branches. One to three relatively fine nerve branches enter the central portion of the muscle directly from the main nerve bundle. A single, longer branch enters TFL more caudally. Stimulation of the centrally directed branches usually produced muscle contractions in the middle part of the muscle, whereas stimulation of the caudally directed branch generally produced contractions in the caudal one third of the muscle. In this study, the three tracers were placed on 1) the most anterior nerve branch, 2) the most posterior nerve branch, and 3) the central branches taken together (Table 1).

Morphology and distribution of motoneurons

Figure 2 shows the typical appearance of SART motoneurons labelled with FB, FG, and HRP. Cells stained with FB were bright blue in color under fluorescence illumination, whereas cells filled with FG contained densely distributed, fine gold particulate substance. Cells labelled with HRP were opaque under fluorescence illumination but were

Fig. 2. Staining appearance of motoneurons labelled with FG, FB and HRP. **A:** Low-power photomicrograph of the TFL motor nucleus in cross-section. Cells labelled with different tracers are intermingled. The section was illuminated concurrently with fluorescence and dark-field methods, so that the extensive dendritic labelling of HRP-filled motoneurons (copper-coloured) could be seen. **B–D:** Labelled cells in the SART motor nucleus at higher magnification. B shows the intimate relationships between cells and dendrites labelled from the medial (FB-blue cells), anterior-distal (FG-yellow cells) and anterior-proximal (HRP-copper-coloured cells) parts of the muscle. In C and D, three SART motoneurons are photographed before and after dark-field illumination. Note the apparent absence of double-labelling in the labelled motoneurons. bar = 100 μm.

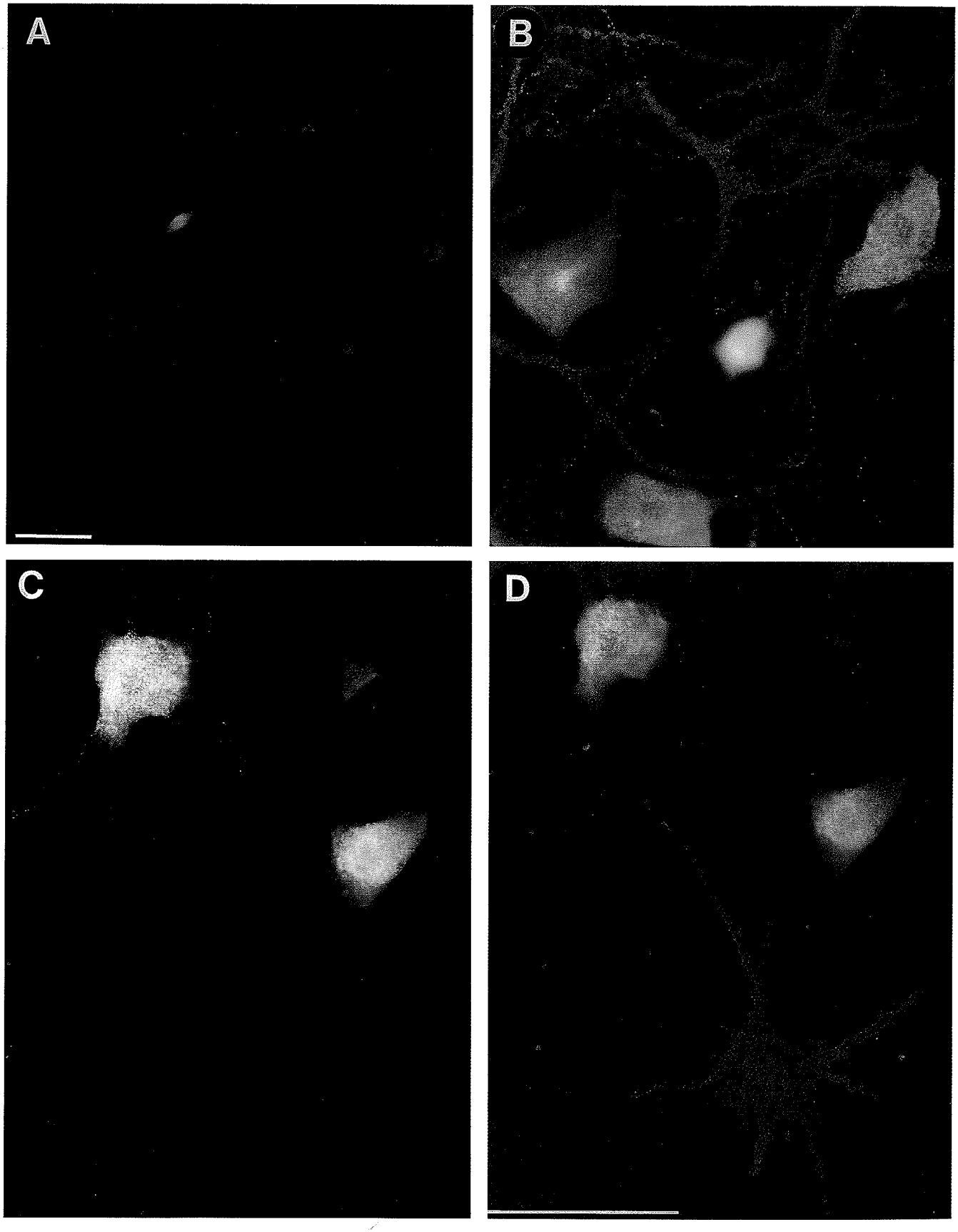
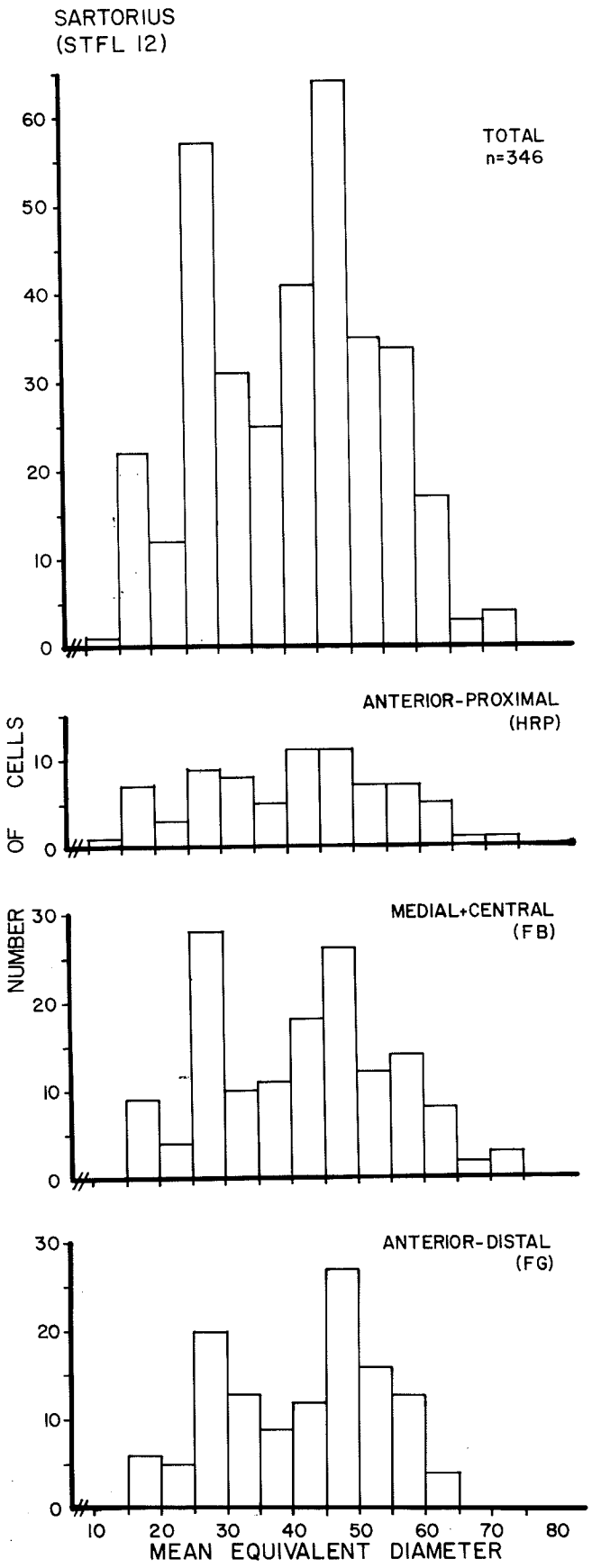


Figure 2



clearly visualized by their content of dense, copper-colored reaction product when trans-illuminated in darkfield.

The morphology of SART and TFL motoneurons was consistent with previous descriptions of hindlimb motoneurons (e.g., Zwaagstra and Kernell, '81). Because each tracer filled the proximal portion of the primary dendrites, most motoneurons had a stellate appearance. In cells stained with HRP, a considerably larger portion of the dendritic tree could be usually seen (Fig. 2). All three tracers labelled cells throughout the full range of motoneuronal sizes, as shown in Figure 3.

The SART motor nucleus was located in a column oriented longitudinally in the ventrolateral portion of the L4 and L5 ventral horn as previously described by Romanes ('51) (Fig. 4). The motor nucleus extended approximately 1.5 segments but the exact rostrocaudal position of the column with respect to segmental landmarks showed some variation from one cat to another. In some cats, the rostral tip of the nucleus was located close to the L3/L4 border. At the other extreme the rostral tip of the nucleus in one cat lay in the caudal part of L4 and cells were found as far caudally as the L5/L6 segmental border.

The TFL motor nucleus also occupied a column in the ventrolateral portion of the ventral horn (Fig. 5). It had a shorter rostrocaudal extent than SART, approximately one spinal segment. The nucleus had its rostral tip in rostral or middle L6 and straddled the L6/L7 segmental border. In the four cats studied here, more labelled TFL motoneurons were located in L6 than in L7.

In one cat (STFL 4) the anterior and posterior branches supplying TFL and a rostral branch supplying the adjacent gluteus maximus were exposed to different tracers. Motoneurons supplying the gluteus muscle were found in a more caudal column coextensive with that of TFL in the ventrolateral part of the ventral horn. As shown in the histograms of Figure 6, a small proportion of gluteus motoneurons (<30%) were intermingled with TFL motoneurons in the caudal part of L6, but the majority were found in the rostral part of L7.

Topographical organization within motor nuclei

Motoneurons supplying the different branches of SART were intermingled extensively. When the distribution of motoneurons supplying medial, central, and anterior muscle regions was compared, all three subpopulations of motoneurons were distributed over almost the entire length of the motor nucleus to within 1–2 mm of the rostral or caudal ends (Figs. 4 and 7). However, modest differences could be detected in the relative density of motoneurons at different rostrocaudal levels. Motoneurons supplying medial SART tended to concentrate more rostrally, whereas motoneurons supplying anterior SART were more common caudally. Thus, the median distance of motoneurons from the rostral pole of the nucleus was greater for anterior than medial motoneurons in all cats (Fig. 7). In some cats (e.g., STFL 9, Fig. 4), the differences in distribution were subtle; in others (e.g., STFL 12, Fig. 8), the shift was more marked. However, in all cats, nonparametric statistical testing

Fig. 3. Size distributions of SART motoneurons labelled using different tracers. The top histogram combines data from all labelled motoneurons; the motoneuronal populations labelled by single tracers are shown below.

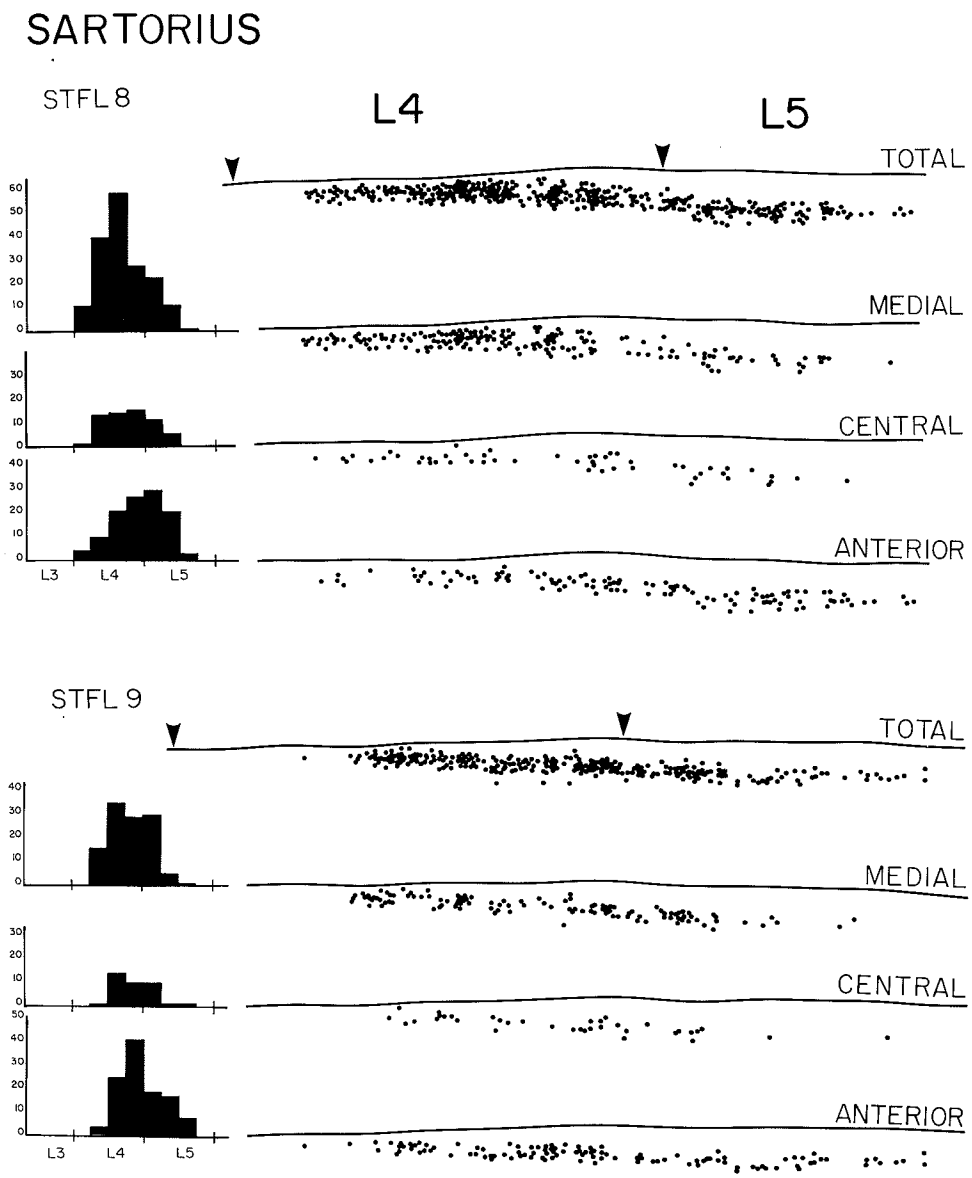


Fig. 4. Rostrocaudal distribution of motoneurons supplying anterior, central, and medial primary branches of SART in two different cats (STFL 8,9). Labelled motoneurons are shown as dots in relation to the lateral border of the ventral horn shown by the horizontal line. The arrows mark the locations of L4 segmental borders. In each cat, the composite distribution of all labelled cells (top) is followed by maps showing cells labelled from individual nerve branches. The histograms (left) indicate the numbers of cells at different rostrocaudal levels, determined by dividing each segment into fourths.

suggested that motoneurons supplying the most medial and the anterior branches differed significantly in their rostrocaudal distributions ($P < .01$). In one cat in which topographical segregation was most marked, the distribution of cells with mean diameters greater than 35 μm was compared with that of the whole cell population to determine whether topographical differences might be greater within large- than small-motoneuron populations. However, no difference was seen in the rostrocaudal distributions of large and small cells. No obvious double-labelling was observed in SART motoneurons when medial, central, and anterior branches were exposed to different tracers.

When nerve branches supplying different regions within medial SART were compared, differences in distribution were more difficult to demonstrate. As a rule, the median distance of motoneurons from the rostral pole of the nucleus was slightly shorter for the most medial nerve branch (Fig. 7) than for a central branch. However, the differences in distribution of medial vs. central motoneurons were not found to be significant statistically in any single animal.

In two cats, the proximally directed and distally directed branches supplying anterior SART were labelled using the tracers FG and HRP. Large numbers of motoneurons could

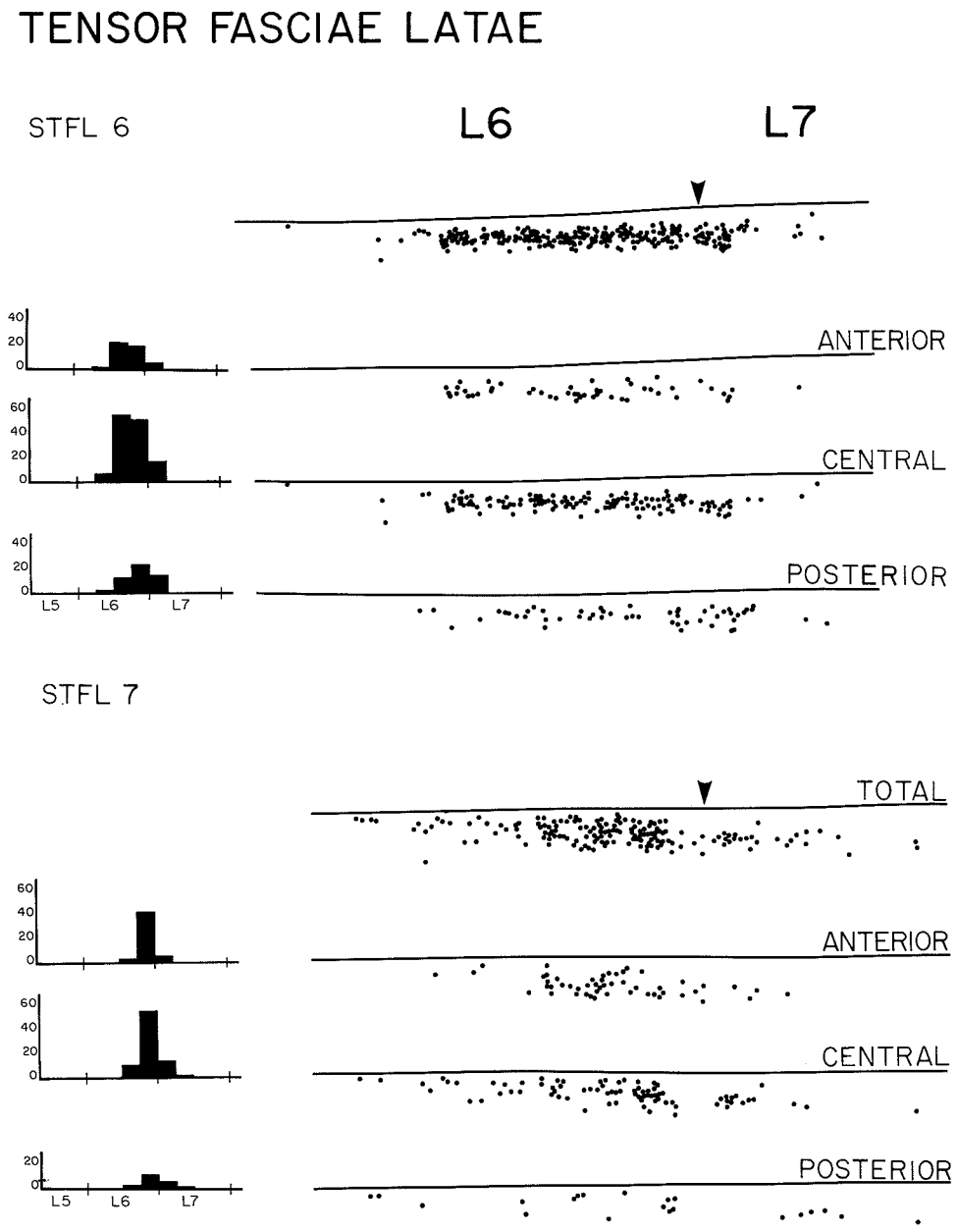
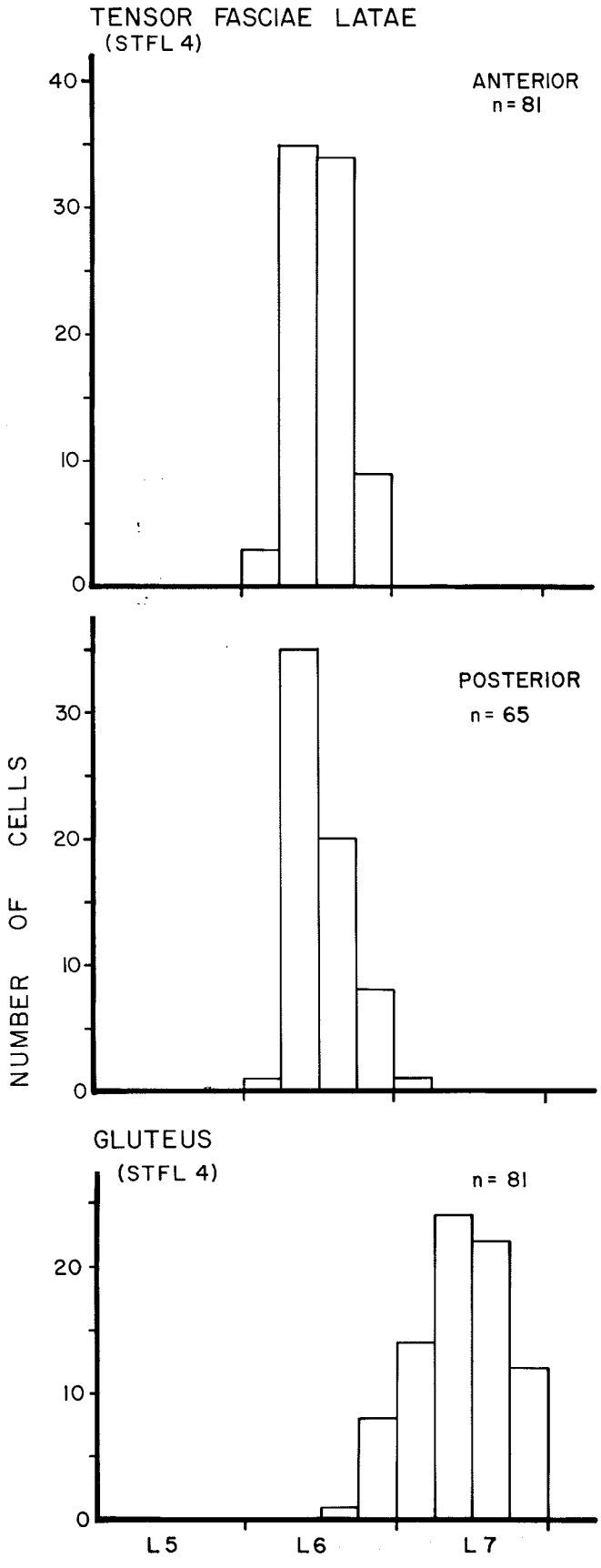


Fig. 5. Rostrocaudal distribution of motoneurons supplying anterior, central, and posterior branches of TFL in two cats (STFL6,7). The location of the L6/L7 segmental boundary is marked by an arrow. The line drawing at the top of each panel shows the composite distribution of all labelled cells; distributions of cells labelled from individual nerve branches are shown below. The histograms (left) show the number of cells in each subpopulation at different rostrocaudal levels, as described in Figure 4.

be found that appeared to contain only FG or HRP reaction product (Figs. 2 and 7); less than 5% of cells appeared to be double-labelled with both HRP and FG. Because HRP labels some cells so heavily, the possibility could not be excluded that the HRP reaction product obscured fluorescence in some double-labelled cells that contained only small amounts of FG. Nevertheless, over 100 FG-labelled cells in each cat (STFL 12-113; STFL 13-133) contained no HRP reaction product.

In both cats, motoneurons supplying the distal branch had slightly more caudal median locations than motoneurons supplying the proximal branch (Fig. 7). In one cat (STFL 12) but not the other (STFL 13), these differences in rostrocaudal distribution were significant statistically ($P < .01$).

In two cats, the locations of labelled cells were studied in cross-sections. As Figure 9 suggests, no obvious differences could be detected in the dorsoventral or mediolateral



distribution of cells supplying different nerve branches, but these differences were not analyzed using quantitative statistical methods.

Tensor fasciae latae

Motoneurons supplying different regions of TFL were also intermingled extensively. Examination of both horizontal (Fig. 5) and cross-sections (Fig. 10) suggested that motoneurons supplying different regions of TFL occupied essentially the same rostrocaudal, dorsoventral, and mediolateral positions within the ventral horn. Furthermore, in most cats, no statistically significant differences were found in the rostrocaudal distribution of motoneurons labelled from different nerve branches. Nevertheless, such differences were detected in a single cat (STFL 6) where they appeared to derive from a slightly more caudal overall distribution of motoneurons supplying the posterior TFL relative to those supplying anterior and central TFL. It is worth noting that the significant differences detectable with the highly sensitive statistical methods used here were very difficult to identify by simple visual inspection (Fig. 5). In two of the cats, no double-labelled cells were recognized but the other two cats had one or two double-labelled cells in the TFL motor nucleus.

DISCUSSION

Methodological considerations

Our ability to draw reliable conclusions about the relative distribution of motoneurons in a single motor nucleus depends on two important methodological capabilities. First is the capability to differentiate two or more populations of motoneurons by using different retrograde tracers in the same animal. Second is the capability to confine each retrograde tracer to motoneurons which have a defined motor-unit territory in the muscle under study.

In the last decade, the methodological tools available to study motoneuronal distribution have become much more powerful because of the introduction of retrograde fluorescent and enzyme tracers that can be used in combination to differentiate populations of motoneurons (Illert et al., '82; Fritz et al., '82, '86a,b; Kuypers and Huisman, '84; Swett et al., '86; Gordon and Richmond, '90). Of the three tracers employed in this study, HRP and FG have already earned a strong reputation for consistency as retrograde tracers in the peripheral nervous system. Previous work has suggested that HRP can label most if not all of the motoneurons whose axons are exposed to it under suitable experimental conditions (Illert et al., '82; Swett et al., '86). FG has been used in fewer studies to date, but our previous experience suggests that it will label at least as many cells as HRP (Gordon and Richmond, '90). Whether FB is equally effective as a retrograde marker is still open to question. Both Illert et al. ('82) and Gordon and Richmond ('90) found a modest reduction in the expected number of FB-labelled cells when comparing HRP and FB-labelled motoneuronal populations. In those studies, it was not clear

Fig. 6. Segmental locations of TFL and gluteus maximus (superficialis) motoneurons. Labelled TFL motoneurons supplying anterior and posterior branches were located primarily in L6, whereas most gluteus motoneurons were found in L7.

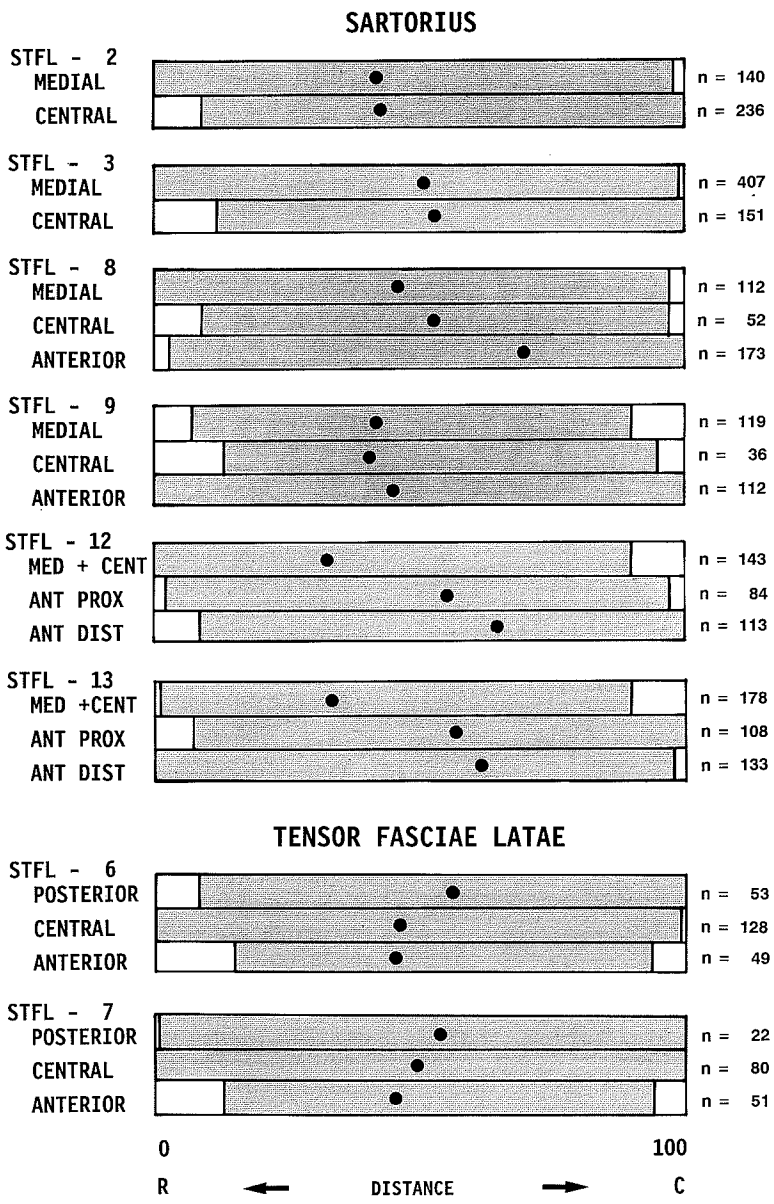


Fig. 7. Distribution of labelled cells in eight spinal cords cut in longitudinal section. In each cat, cell distributions have been normalized by assessing the percentage distance of each labelled cell from the rostral tip of the nucleus (rostralmost cell = 0, caudalmost cell = 100%). Motoneurons supplying different primary branches are represented by labelled grey bands, whose rostral and caudal limits show the locations of the most rostral and caudal cells supplied by that branch. The median value of the labelled subpopulation is indicated by a black dot, and the numbers of labelled neurons in the subpopulation are shown to the right. Double-labelled cells accounted for less than 5% of cells in all cats and have been excluded from these maps. med + cent, medial and central branches taken together; ant. prox, proximally directed branch to anterior SART; ant dist, distally directed branch to anterior SART.

whether the diminished number of cells stained with FB was due to a selective lack of uptake by certain types of motoneurons. In present studies, FB was found to stain both large and small cells in proportions roughly equivalent to those seen with FG or HRP. This suggests that both alpha and gamma motoneurons can transport FB. Further, in this study as in others (Illert et al., '82), the rostrocaudal distribution of cells labelled by FB was similar to that observed using FG or HRP. Nevertheless, to avoid a systematic bias, FB was alternated with other tracers when an identified nerve branch was exposed in sequentially performed experiments.

The other factor that will limit uptake of tracer is the damage or obstruction of individual axons during surgical procedures and nerve exposure. This problem has been discussed elsewhere in depth (Swett et al., '86; Gordon and Richmond, '90). In a few experiments (e.g., SART 8,9; central branch), relatively few motoneurons were found to be labelled compared to other experiments. However, it is not clear if these variable numbers reflect a failure of

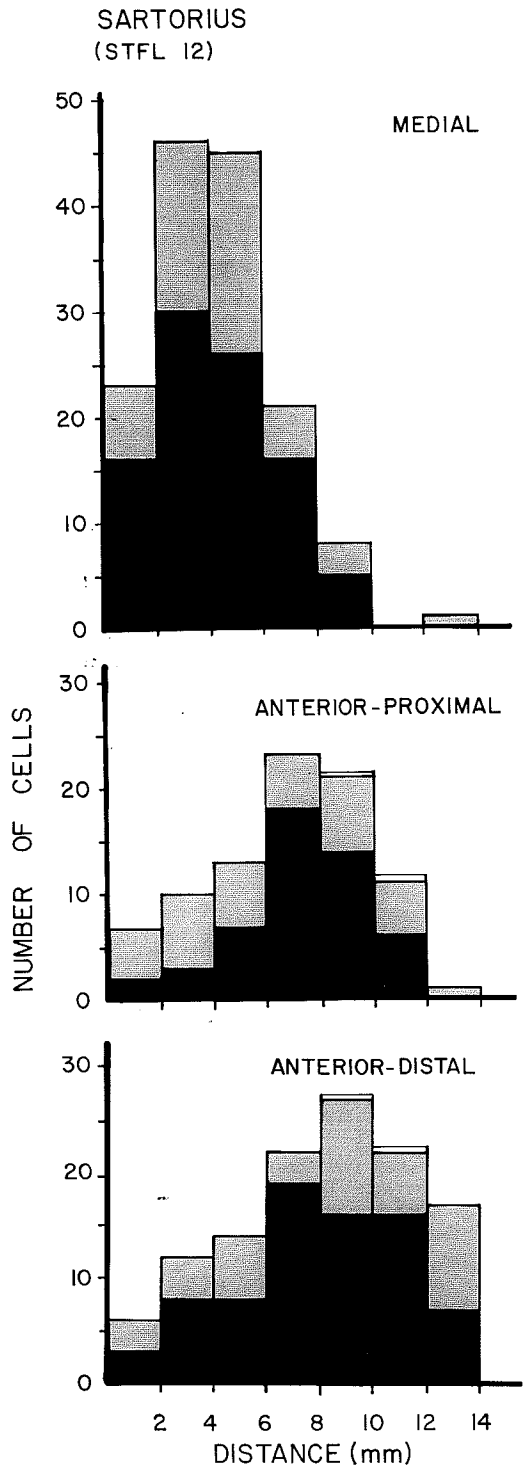


Fig. 8. Distribution of SART motoneurons in the cat with the greatest topographical separation of medial and anterior motoneurons. In each column, dark bars represent single-labelled cells with mean equivalent diameters greater than 35 μm. Grey bars represent single-labelled cells smaller than 35 μm, open bars represent clearly double-labelled cells. Locations are expressed as the distance of each cell from the rostral end of the motor nucleus.

transport in some axons. Regional nerve branches were found to vary in calibre and likely contained different numbers of motor axons. The presence of only a small number of labelled cells might simply reflect a relatively small content of motor axons in a regional nerve branch from one cat compared to another. Problems with tracer uptake did not seem to pose a major obstacle to successful retrograde transport in the present work. In most experiments, the numbers of labelled motoneurons in SART motor nuclei were similar to or greater than those reported by Romanes ('51) using chromatolytic methods (274 cells).

Anterior sartorius: More than one compartment?

Previous EMG studies have provided strong evidence that discrete subvolumes of TFL or SART receive their motor innervation from different primary branches of their muscle nerve (Loeb et al., '87; Pratt and Loeb, '91; Chanaud et al., '91b). This organization into "neuromuscular compartments" (English and Letbetter, '82) can be exploited in studies of motoneuronal distribution, by applying tracers to cut nerve branches known to supply specific muscle regions. Exposure of a cut nerve effectively circumvents the problems of spurious cell labelling that can be encountered when various tracers are injected into adjacent regions of muscle (Richmond et al., '78; Haase and Hrycyshyn, '85, '86). Further, the method of nerve-branch exposure should reveal the presence of motoneurons whose axons split and run in two different nerve branches because their somata should contain two different tracers.

In the present study, there was a very low incidence of double-labelled cells. This finding was perhaps not surprising when tracers were placed on nerve branches supplying different, in-parallel compartments of SART. Previous studies have shown that EMG activity produced by stimulating a single SART branch is recorded in a long, narrow strip of fibers with well-demarcated edges as might be expected if motor-unit territories were confined between sharp intramuscular borders (Loeb et al., '87). However, relatively few double-labelled cells also appeared to be present when proximally and distally directed branches in anterior SART were exposed to separate tracers. Prior to undertaking these experiments, we had assumed that anterior SART constituted a single compartment, and many motoneurons would have axon branches in both the proximally and distally directed limbs of its muscle nerve. HRP and FG had been used specifically on the anterior-proximal and anterior-distal branches because the two tracers were felt to offer the best combination for detecting double-labelled cells. Even with this combination, we could not be certain that all double-labelled cells would be detected. HRP labelling was intense and we could not guarantee that it would not obscure low levels of fluorescence in a few double-labelled motoneurons. Nevertheless, in a similar study of neck-muscle motoneurons, double-labelled cells were readily detected (Gordon and Richmond, '91). Further, the fact that more than 100 cells were labelled only with FG and not with HRP suggests that more than 100 motor units are served entirely by axons that entered anterior SART by way of its proximally-directed nerve branch. The relative paucity of double-labelled cells provides evidence that anterior SART may have two separate subsets of motor units that are supplied respectively from the proximally directed or the distally directed branch. In contrast, only a small

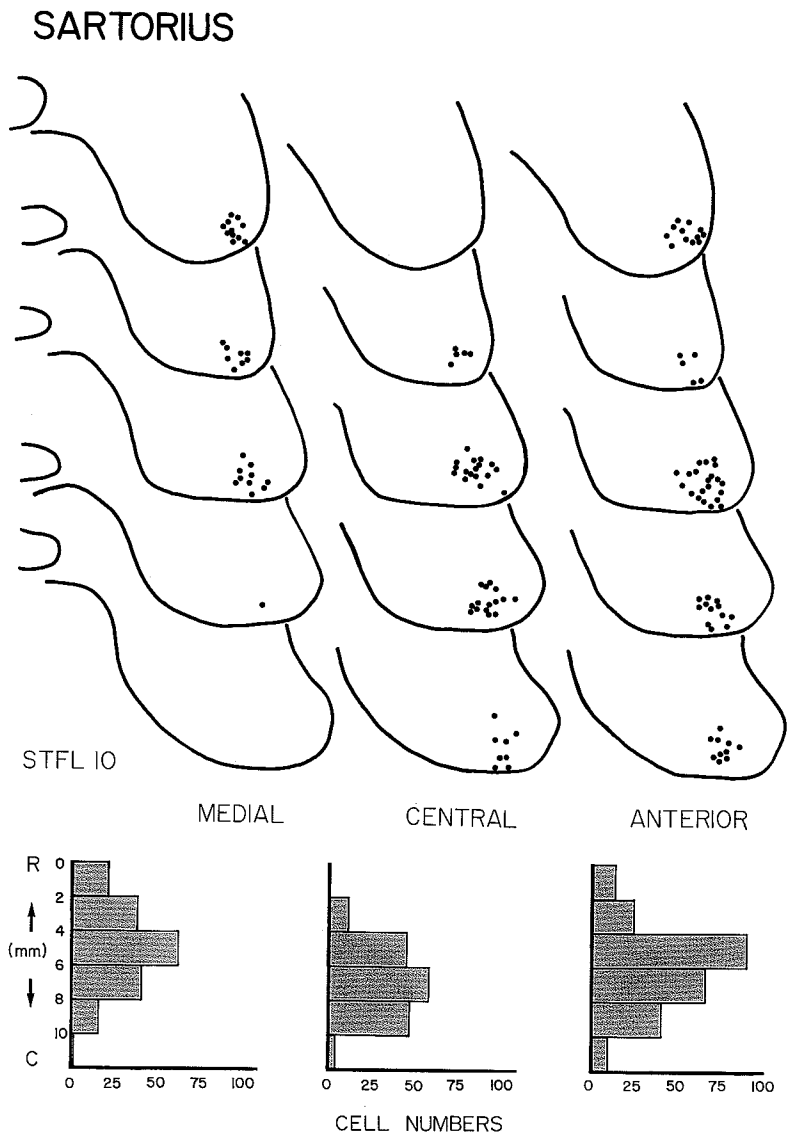


Fig. 9. Distribution of labelled motoneurons (shown as dots) at different rostrocaudal levels of the SART motor nucleus. Representative cross-sections are approximately 2 mm apart. Onto each cross-section has been mapped the cells found in eight consecutive 40 μm sections. The histograms below show the total number of cells contained in each 2 mm block of tissue from the rostral to the caudal pole of the nucleus.

number of motor units appear to be supplied by motor axons that branch and run both proximally and distally.

The functional significance of proximally and distally directed nerve branches in anterior SART is not yet understood. In preliminary experiments, we have confirmed the observations of Loeb et al. ('87) that stimulation of either the proximally or distally directed branch evokes at least some short-latency (M-wave) EMG activity along the entire length of anterior SART (Thomson et al., '90). However, contractions produced by stimulating the individual branches were not symmetrical; reflective markers at the midpoint of anterior SART moved toward the origin of the muscle during contractions elicited by stimulating the proximally directed branch but moved toward its insertion following stimulation of the distally directed branch (Thomson et al., '90). Further, repetitive stimulation of the proximally directed branch to anterior SART produced glycogen depletion in most of its proximal muscle fibers but only a minority of its distally located fibers. A mirror-image pattern of depletion was observed when the distally directed branch was stimulated. The results of both neuronal-labelling and physiological studies suggest that the proximal and distal parts of anterior SART may be organized as two interdigitating subvolumes of motor units whose fibers are arranged largely but not exclusively in-series.

In-series organization of motor units has been reported in certain other long muscles, including the limb muscle semitendinosus (ST) (English and Weeks, '87) and the neck muscles, splenius (Richmond et al., '85), and biventer cervicis (Armstrong et al., '88). The in-series arrangement may complicate the neural control of anterior SART as it does for the other in-series muscles because effective development of tension would seem to depend upon the coordinated recruitment of separate subpopulations of motoneu-

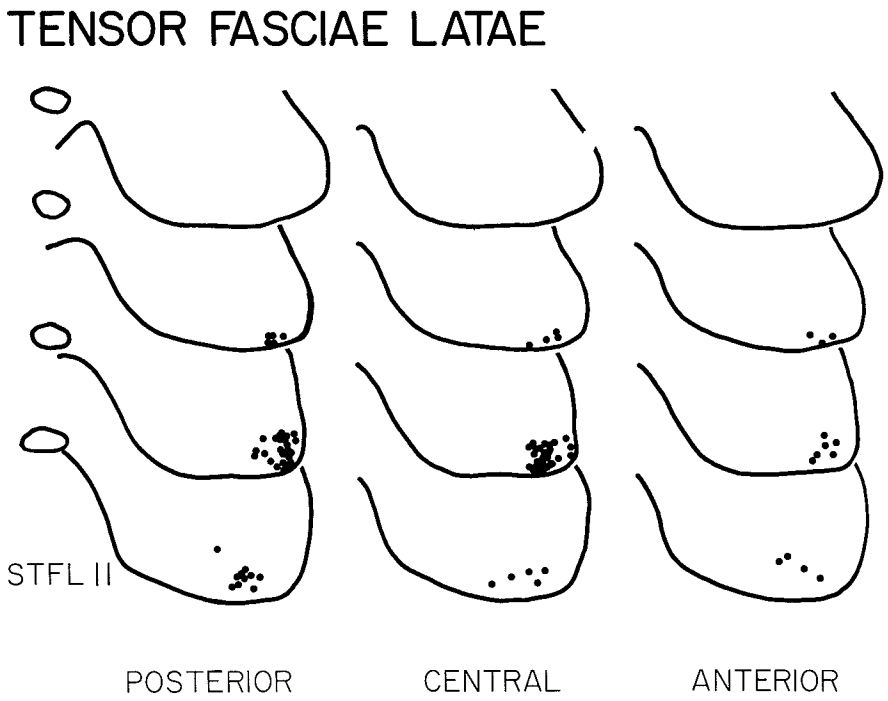


Fig. 10. Distribution of labelled motoneurons at different rostrocaudal levels of the TFL motor nucleus. Representative cross-sections are 2 mm apart; cells (shown as dots) were mapped from eight consecutive sections at each interval. No differences were obvious in the rostrocaudal, dorsoventral, or mediolateral locations of cells labelled from different nerve branches.

rons supplying the two ends of the muscle. Present studies show that the motoneurons supplying anterior-proximal and anterior-distal SART are intermingled in the same territory and thus are positioned topographically to receive similar inputs. However, despite their close anatomical relationship, the two sets of motor units may not always be recruited according to the same patterns. As early as 1910, Sherrington suggested that the two ends of anterior SART do not participate equally in flexion and crossed-extension reflexes. During flexion reflexes, he reported that only the proximal part showed obvious contractions; during crossed extension reflexes, the distal part of anterior SART contracted, whereas the proximal part relaxed. More recently, Hoffer et al. ('87) have reported that anterior SART contains two "task groups" of motor units that are recruited according to different patterns during locomotion. However, both of these "task groups" appear to participate as flexors in the flexion reflex produced by large diameter cutaneous afferents (Fig. 5 in Loeb et al., '87). It remains to be seen whether the two "task groups" of motoneurons have any relationship to the anatomically defined subpopulations of motoneurons shown here to supply anterior SART by way of proximally and distally directed branches.

Topographical differentiation within motor nuclei

Tensor fasciae latae and sartorius have been described as "mechanically heterogeneous" muscles because contractions of different, in-parallel compartments result in different mechanical actions (Chanaud et al., '91b). Their biomechanical heterogeneity is paralleled by differentiation in their patterns of recruitment during a range of normal behaviors (Hoffer et al., '87; Pratt and Loeb, '91; Pratt et al., '91; Chanaud et al., '91b). However, present findings suggest that such a clear-cut peripheral pattern of compartmentalization cannot be recognized in the topographical organization of motoneurons in the spinal cord. Motoneurons supplying functionally distinct muscle regions were found to be intermixed extensively throughout the length of SART and TFL motor nuclei, and only in SART could we demonstrate consistent but modest differences in the relative densities (but not extents) of motoneurons from one end of the nucleus to the other.

Topographical differences have been reported previously in motor nuclei of other architecturally complex muscles, but the magnitudes of such differences are highly variable. At one extreme are neck muscles such as biventer cervicis, whose in-series compartments are supplied by motoneurons in separate segmentally ordered subnuclei (Richmond et al., '78). At the other extreme is semitendinosus, a hindlimb muscle with two in-series compartments whose motoneurons are thought to be intermingled extensively (Letbetter and English, '81). In the present work, the intermingled organization of motoneurons supplying TFL appeared to be as indiscriminate as that reported for ST. However, the situation in SART was more complicated because motoneurons supplying different in-parallel strips showed a modest but consistent variation in density along the length of the nucleus. Motoneurons with shorter axons that supplied the most medial parts of SART tended to occur more rostrally, whereas motoneurons whose axons ran further to supply progressively more anterior parts of the muscle were concentrated more caudally. The factors responsible for this topographical gradient are probably rooted in embryology and development and are still poorly understood. As one speculation, we might postulate that

the topographical organization relates in some way to the progressive delay in maturation known to occur developmentally from the rostral to the caudal end of the spinal cord (Cameron et al., '89). If some rostrally located motoneurons were to send axons into SART prior to caudally located motoneurons, they might have a preferential opportunity to supply the most immediately available muscle fibers which would be along the medial edge. Motor axons arriving slightly later would have to grow further in order to establish comparable territories in the still-denervated anterior regions. Thus, gradients in motoneuronal location could, in theory, occur because of anatomical considerations rather than any particular functional rationale. Rostrocaudal gradients reflecting the length of the muscle-nerve pathways can be found elsewhere in the lumbosacral cord: motoneurons supplying distal muscles tend to lie more caudally than those of proximal muscles (cf. Sherrington, 1892; Romanes, '51), and motoneurons supplying distal compartments of a single muscle tend to lie more caudally than those supplying its proximal compartments (Iliya and Dum, '84; Hardman and Brown, '85; Weeks and English, '85, '87; Laskowski and Sanes, '87).

The pattern of topographical organization in SART is perhaps most comparable to that reported previously for lateral (LG) or medial gastrocnemius (MG) where topographical relationships between "compartment nuclei" have been examined in some detail (Weeks and English, '85, '87). In these muscles, motoneurons supplying different in-parallel compartments were also intermixed throughout the motor nucleus, but their relative densities varied from the rostral to the caudal pole of the nucleus. However, previous studies of topographical patterns in hindlimb motor nuclei were conducted using only HRP, and patterns of organization could only be assessed indirectly by comparing the distribution of motoneurons supplying the whole muscle on one side of the cat with that of motoneurons supplying a defined part of the muscle on the other side of the cat. With such methods, only one subpopulation of motoneurons can be labelled in a single experiment and the subtle differences between two intermingled subpopulations become more difficult to gauge. Further, patterns of topographical organization can vary from cat to cat, and these differences will confound statistical analyses when highly sensitive methods such as the Mann-Whitney U-test are used to compare data from more than one animal. To illustrate this problem, we compared the distribution of motoneurons supplying anterior SART in STFL8 and STFL9 (Fig. 5), by normalizing the locations of the cells according to the length of the SART motor nucleus as a whole and then comparing the two populations using the Mann-Whitney U-test. By such an approach, significant differences ($P < .01$) could be identified in the distributions of the two subpopulations, even though the two nerve branches had the same innervation target.

Considerations for motor control

Studies of topography have attracted interest not merely for the anatomical information that they impart. For many years, neuroscientists have questioned whether topographical factors may be important functionally, in providing cues for the appropriate organization of various motoneuronal inputs (Wyman, '73, Wyman et al., '74; Kandou and Kernell, '89; Lüscher and Vardar, '89; see also Windhorst et al., '89). Recent anatomical reports of nonrandom motoneuronal distribution within single motor nuclei have also drawn attention to the role that might be played by topography in developing appropriate neural connections. Both Iliya and Dum ('84) and Weeks and English ('85) have postulated that asymmetries in the rostrocaudal distribution of compartment subnuclei might be responsible for the differences in primary spindle (Ia) afferent connectivity that have been found in the motor nuclei of some hindlimb muscles such as MG (Lucas and Binder, '84; Lucas et al., '84; Vanden Noven et al., '86). Support for this notion has also come from electrophysiological studies of triceps surae motoneurons (Clamann et al., '85; Lüscher and Vardar, '89; Lüscher et al., '89), which have shown that excitatory postsynaptic potentials (EPSPS) of single Ia fibers decrease as a function of increasing rostrocaudal distance between the entry point of the fiber and the location of its target motoneuron. Because LG motoneurons tend to lie rostrally to MG motoneurons, Lüscher et al. ('89) argued that the differences in connectivity from heteronymous to homonymous motoneurons could be attributed primarily to their topographical organization. They concluded that "structural and topographical factors are major determinants for the development of connections in the spinal cord. At least for the close synergists studied here, species-related cellular recognition processes cannot explain the overall differences in homonymous and heteronymous connectivity."

However, the findings presented here challenge the notion that topographical differences are responsible for selective recruitment patterns arising at least for some types of neural input. It is difficult to see how the axons responsible for the specialized recruitment of TFL could use topographical cues to establish their appropriate circuitry because cell bodies serving functionally distinct parts of TFL are intermixed completely. Even in the SART motor nucleus, where modest topographical gradients can be detected between medial and anterior subpopulations, extensive intermingling would ensure that an incoming axon would encounter motoneurons supplying both anterior and medial SART at almost any level of the SART motor nucleus. Unless additional mechanisms were available to differentiate motoneurons with different targets, one would expect EMG activity in medial and anterior SART to be much less selective than it is. This is not to say that some particular classes of input, such as monosynaptic Ia input, might not organize themselves using a topographically based mechanism. The presence of topographically weighted inputs might even be expected to occur in situations where no other form of "species recognition" or "motor learning" is required to ensure the appropriate action of the input under study. Topographical weighting does not seem to explain, however, the distinct differences in heteronymous Ia connectivity that have been reported for medial and anterior SART. Eccles and Lundberg ('58) found that Ia inputs from rectus femoris excited motoneurons supplying anterior SART but commonly inhibited those supplying medial SART. In contrast, only motoneurons supplying medial SART could be excited by Ia inputs from gracilis. These observations led Eccles and Lundberg ('58) to state: "It is remarkable that motoneurons supplying two separate portions of the same muscle differ so fundamentally that one group is excited and the other inhibited by rectus volleys and yet in many other respects they have a common pattern of innervation."

The clustering of motoneurons into specific motor nuclei as identified by Romanes ('51) may be related to several different processes involving the development of the spinal

cord and its connections with the concurrent differentiation of mesoderm into groups of skeletal muscles. Some of these organizations may persist into adulthood and may be correlated, albeit loosely, with the strength of some synaptic inputs such as those of Ia systems. However, other patterns of input appear to have selectivities that transcend spinal topography. The mechanisms governing this connectivity remain to be identified. The search for unifying principles of neuromuscular organization and control seems to be facing a dilemma: as anatomical and functional methods have been improved in resolution and extended to a number of diverse muscles, a diminishing proportion of the available data can be explained by our current hypotheses. Mechanically heterogeneous muscles like SART and TFL may prove to be excellent models in which to study the organizing principles for descending and segmental connections that may be arranged according to nontopographical rules.

ACKNOWLEDGMENTS

We thank Janet Creasy, Desirée Reesor, and Karen Smith for their assistance with experiments and data analysis, Dr. N. Freedman, C. Engstrom, and P. Gross for advice on statistics, and Dr. V.C. Abrahams, and Dr. P.K. Rose for their useful suggestions. This work was supported by the Medical Research Council of Canada.

LITERATURE CITED

Armstrong, J.B., P.K. Rose, S. Vanner, G.J. Bakker, and F.J.R. Richmond (1988) Compartmentalization of motor units in the cat neck muscle, biventer cervicis. J. Neurophysiol. *60:*30–45.

Burke, R.E. (1981) Motor units: Anatomy, physiology and functional organization. In V.B. Brooks (ed.): Handbook of Physiology, Section I: Nervous System II. Bethesda, MD: American Physiological Society, pp. 345–422.

Burke, R.E., P.L. Strick, K. Kanda, C.C. Kim, and B. Walmsley (1977) Anatomy of medial gastrocnemius and soleus motor nuclei in cat spinal cord. J. Neurophysiol. *40:*667–680.

Cameron, W.E., H. Fang, B.S. Brozanski, and R.D. Guthrie (1989) The postnatal growth of motoneurons at three levels of the cat neuraxis. Neurosci. Lett. *104:*274–280.

Chanaud, C.M., and J.A. Macpherson (1991) Functionally complex muscles of the cat hindlimb. III. Differential activation within biceps femoris during postural perturbation. Exp. Brain Res. (in press).

Chanaud, C.M., C.A. Pratt, and G.E. Loeb (1991a) Functionally complex muscles of the cat hindlimb. II. Mechanical and architectural heterogeneity in the biceps femoris. Exp. Brain Res. (in press).

Chanaud, C.M., C.A. Pratt, and G.E. Loeb (1991b) Functionally complex muscles of the cat hindlimb V. The roles of histochemical fiber-type regionalization and mechanical heterogeneity in differential muscle activation. Exp. Brain Res. (in press).

Clamann, H.P., E. Henneman, H.-R. Lüscher, and J. Mathis (1985) Structural and topographical influences on functional connectivity in spinal monosynaptic reflex arcs in the cat. J. Physiol. (London) *358:*483–507.

Eccles, J.C. (1964) The Physiology of Synapses. Springer-Verlag: New York, pp. 246–254.

Eccles, R.M., and A. Lundberg (1958) Integrative pattern of Ia synaptic actions on motoneurones of hip and knee muscles. J. Physiol. (London) *144:*271–298.

English, A.W., and O.I. Weeks (1987) An anatomical and functional analysis of cat biceps femoris and semitendinosus muscles. J. Morphol. *191:*161–175.

English, A.W., and W.D. Letbetter (1982) Anatomy and innervation patterns of cat lateral gastrocnemius and plantaris muscles. Am. J. Anat. *164:*67–77.

Fritz, N., M. Illert, and P. Reeh (1982) Location of median and ulnar motornuclei in the cat. Neurosci. Lett. *30:*103–108.

Fritz, N., M. Illert, and P. Reh (1986a) Location of motoneurones projecting to the cat distal forelimb. II Median and ulnar motor nuclei. J. Comp. Neurol. *244:*302–312.

Fritz, N., M. Illert, and P. Saggau (1986b) Location of motoneurons projecting to the cat distal forelimb. I. Deep radial motornuclei. J. Comp. Neurol. *244:*286–301.

Gerfen, C.R., and P.E. Sawchenko (1984) An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: Immunohistochemical localization of an axonally transported plant lectin, *phaseolus vulgaris* leucoagglutinin (PHA-L). Brain Res. *290:*219–238.

Gordon, D.C., and F.J.R. Richmond (1989) Organization of motor nuclei supplying intervertebral muscles of the cat revealed by multiple labelling. Proc. Soc. Neurosci. *15:*695 (Abstract).

Gordon, D.C., and F.J.R. Richmond (1990) Topography in the phrenic motoneuron nucleus demonstrated by retrograde multiple-labelling techniques. J. Comp. Neurol. *292:*424–434.

Gordon, D.C., and F.J.R. Richmond (1991) Distribution of motoneurons supplying dorsal suboccipital and intervertebral muscles in the cat neck. J. Comp. Neurol. *304:*343–356.

Haase, P., and A.W. Hrycyshyn (1985) Labeling of motoneurons supplying the cutaneous maximus muscle in the rat, following injection of the triceps brachii muscle with horseradish peroxidase. Neurosci. Lett. *60:*313–318.

Haase, P., and A.W. Hrycyshyn (1986) On the diffusion of horseradish peroxidase into muscles and the "spurious" labeling of motoneurons. Exp. Neurol. *91:*399–403.

Hardman, V.J., and M.C. Brown (1985) Spatial organization within rat motoneuron pools. Neurosci. Lett. *60:*325–329.

Henneman, E., G. Somjen and D.O. Carpenter (1965a) Functional significance of cell size in spinal motoneurons. J. Neurophysiol. *28:*560–580.

Henneman, E., G. Somjen, and D.O. Carpenter (1965b) Excitability and inhibitibility of motoneurons of different sizes. J. Neurophysiol. *28:*599–620.

Hoffer, J.A., G.E. Loeb, N. Sugano, W.B. Marks, M.J. O'Donovan, and C.A. Pratt (1987) Cat hindlimb motoneurons during locomotion. III. Functional segregation in sartorius. J. Neurophysiol. *57:*554–562.

Huisman, A.M., H.G.J.M. Kuypers, and C.A. Verburgh (1982) Differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in cat and monkey. Prog. Brain Res. *57:*185–217.

Iliya, A.R., and R.P. Dum (1984) Somatotopic relations between the motor nucleus and its innervated muscle fibers in the cat tibialis anterior. Exp. Neurol. *86:*272–292.

Illert, M., N. Fritz, A. Aschoff, and H. Holländer (1982) Fluorescent compounds as retrograde tracers compared with horseradish peroxidase (HRP). II. A parametric study in the peripheral motor system of the cat. J. Neurosci. Methods *6:*199–218.

Kandou, T.W.A., and D. Kernell (1989) Distribution of activity within the cat's peroneus longus muscle when activated in different ways via the central nervous system. Brain Res. *486:*340–350.

Kuypers, H.G.J.M., and A.M. Huisman (1984) Fluorescent neuronal tracers. In S. Fedoroff (ed.): Advances in Cellular Neurobiology. Volume 5. Orlando, FA: Academic Press, Inc., pp. 307–340.

Laskowski, M.B., and J.R. Sanes (1987) Topographic mapping of motor pools onto skeletal muscles. J. Neurosci. *7:*252–260.

Letbetter, W.D., and A.W. English (1981) The relationship between peripheral intramuscular "compartments" and spatial arrangement of biceps femoris and semitendinosus motor nuclei in the cat lumbar spinal cord. Proc. Soc. Neurosci. *7:*557 (Abstract).

Lichtman, J.W., S. Jhaveri, and E. Frank (1984) Anatomical basis of specific connections between sensory axons and motor neurons in the brachial spinal cord of the bullfrog. J. Neurosci. *4:*1754–1763.

Loeb, G.E., C.A. Pratt, C.M. Chanaud, and F.J.R. Richmond (1987) Distribution and innervation of short, interdigitated muscle fibers in parallel-fibered muscles of the cat hindlimb. J. Morphol. *191:*1–15.

Lucas, S.M., and M.D. Binder (1984) Topographic factors in distribution of homonymous group Ia-afferent input to cat medial gastrocnemius motorneurons. J. Neurophysiol. *51:*50–63.

Lucas, S.M., T.C. Cope, and M.D. Binder (1984) Analysis of individual Ia-afferent EPSPs in a homonymous motoneuron pool with respect to muscle topography. J. Neurophysiol. *51:*64–74.

Lüscher, H.-R., C. Stricker, E. Henneman, and U. Vardar (1989) Influences of morphology and topography of motoneurons and muscle spindle afferents on amplitude of single fiber excitatory postsynaptic potentials in cat. Exp. Brain Res. *74:*493–500.

Lüscher, H.-R., and U. Vardar (1989) A comparison of homonymous and heteronymous connectivity in the spinal monosynaptic reflex arc of the cat. Exp. Brain Res. *74:*480–492.

Mesulam, M.-M. (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. Cytochem. *26:*106–117.

Pratt, C.A., W.J. Yee, C.M. Chanaud, and G.E. Loeb (1984) Organization of the cat sartorius motoneuron pool. Proc. Soc. Neurosci *10:*629 (Abstract).

Pratt, C.A., C.M. Chanaud, and G.E. Loeb (1991) Functionally complex muscles of the cat hindlimb. IV. Intramuscular distribution of movement command signals and cutaneous reflexes in broad, bifunctional thigh muscles. Exp. Brain Res. (in press).

Pratt, C.A., and G.E. Loeb (1991) Functionally complex muscles of the cat hindlimb. I. Patterns of activation across sartorius. Exp. Brain Res. (in press).

Richmond, F.J.R., D.R.R. MacGillis, and D.A. Scott (1985) Muscle-fiber compartmentalization in cat splenius muscles. J. Neurophysiol. *53:*868–885.

Richmond, F.J.R., D.A. Scott, and V.C. Abrahams (1978) Distribution of motoneurones to the neck muscles, biventer cervicis, splenius and complexus in the cat. J. Comp. Neurol. *181:*451–463.

Romanes, G.J. (1951) The motor cell columns of the lumbo-sacral spinal cord of the cat. J. Comp. Neurol. *94:*313–363.

Schmued, L.C., and J.H. Fallon (1986) Fluoro-Gold: A new fluorescent retrograde axonal tracer with numerous unique properties. Brain Res. *377:*147–154.

Sherrington, C.S. (1892) Notes on the arrangement of some motor fibres in the lumbo-sacral plexus. J. Physiol. (London) *13:*621–772.

Sherrington, C.S. (1910) Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. J. Physiol. (London) *40:*28–121.

Siegel, S. (1956) Nonparametric Statistics. New York: McGraw-Hill Book Co., pp. 95–158.

Sperry, R.W. (1963) Chemoaffinity in the orderly growth of nerve fiber patterns and connections. Proc. Natl. Acad. Sci. U.S.A. *50:*703–710.

Stuart, D.G., T.M. Hamm, and S. Vanden Noven (1988) Partitioning of monosynaptic Ia EPSP connections with motoneurons according to neuromuscular topography: Generality and functional implications. Prog. Neurobiol. *30:*437–447.

Swett, J.E., R.P. Wikholm, R.H.I. Blanks, A.L. Swett, and L.C. Conley (1986) Motoneurons of the rat sciatic nerve. Exp. Neurol. *93:*227–252.

Thomson, D.B., S.H. Scott, F.J.R. Richmond, and G.E. Loeb (1990) Complex motor unit architecture of anterior sartorius muscle in the cat. Proc., First World Congress of Biomechanics (Abstract).

Vanden Noven, S., T.M. Hamm, and D.G. Stuart (1986) Partitioning of monosynaptic Ia excitatory postsynaptic potentials in the motor nucleus of the cat lateral gastrocnemius muscle. J. Neurophysiol. *55:*569–586.

Weeks, O.I., and A.W. English (1985) Compartmentalization of the cat lateral gastrocnemius motor nucleus. J. Comp. Neurol. *235:*255–267.

Weeks, O.I. and A.W. English (1987) Cat triceps surae motor nuclei are organized topologically. Exp. Neurol. *96:*163–177.

Windhorst, U., T.M. Hamm, and D.G. Stuart (1989) On the function of muscle and reflex partitioning. Behav. Brain Sci. *12:*629–681.

Wyman, R.J. (1973) Somatotopic connectivity or species recognition connectivity? In R.B. Stein, K.G. Pearson, R.S. Smith and J.B. Redford (eds.): Control of Posture and Locomotion. New York: Plenum Press, pp. 45–53.

Wyman, R.J., I. Waldron, and G.M. Wachtel (1974) Lack of fixed order of recruitment in cat motoneuron pools. Exp. Brain Res. *20:*101–114.

Zwaagstra, B., and D. Kernell (1981) Sizes of soma and stem dendrites in intracellularly labelled α-motoneurones of the cat. Brain Res. *204:*295–309.