JOURNAL OF MORPHOLOGY 191:1–15 (1987)

Distribution and Innervation of Short, Interdigitated Muscle Fibers in Parallel-Fibered Muscles of the Cat Hindlimb

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ABSTRACT The cat hindlimb contains several long, biarticular strap muscles composed of parallel muscle fascicles that attach to short tendons. Three of these muscles — sartorius, tenuissimus, and semitendinosus — were studied by dissecting individual gold-stained fibers and determining the surface distribution of acetylcholinesterase-stained end-plate zones. In each muscle, fascicles were composed of muscle fibers that ran only part of the fascicle length and tapered to end as fine strands that interdigitated with other tapering fibers within the muscle mass. Most muscle fibers measured 2–3 cm in length. Fascicles of muscle fibers were crossed by short transverse bands of endplates (1 mm wide by 1–5 mm long) that were spaced at fairly regular intervals from the origin to the insertion of the muscle. The endplate pattern suggested that the fiber fascicles were organized into multiple longitudinal strips. In the sartorius, the temporospatial distribution of electromyographic (EMG) activity evoked by stimulating fine, longitudinal branches of the parent nerve confirmed that each strip was selectively innervated by a small subset of the motor axons. These axons appeared to distribute their endings throughout the entire length of the fascicles, providing for synchronous activation of their in-series fibers.

Although mammalian muscles can exhibit a remarkable variety of forms (Warwick and Williams, '73), most can be classified as pinnate or parallel according to their fiber orientations (e.g. McMahon, '84; Pierrynowksi and Morrison, '85). Pinnate muscles are usually composed of fibers that are much shorter than the muscle as a whole and run obliquely between two (or more) closely approximated planes of attachment. In contrast, parallel-fibered muscles commonly consist of fiber fascicles running most of the length of the muscle and arranged in sheet-like or strap-like arrays that are parallel to the line-of-pull of the muscle. The functional significance of fascicle arrangements in pinnate versus parallel muscles has been examined theoretically and experimentally in some detail (Gans and Bock, '65; Gans, '82), and it is now well recognized that the architectural arrangement has a significant effect on the length–tension relationship, shortening velocities, and force-developing capabilities of the muscle.

Much less attention has been paid to the differences in muscle fiber organization that may occur within the muscle fascicles themselves. For example, it is often assumed, and occasionally reported (e.g., Sacks and Roy, '82) that the long fascicles of parallel-fibered muscles are comprised of similarly long individual muscle fibers, running the entire length of each fascicle and attached at both ends to the muscle origin and insertion. Such an organization, if it exists, would pose serious problems for the mechanical stability of long muscles. Because conduction velocities in mammalian extrafusal muscle fibers are typically 2–10 m/sec (Eccles and O'Connor, '39; Schwartz-Giblin et al., '84), muscle fibers spanning the 15 cm length of some cat muscles would have propagation times in excess of their twitch rise times. As a result, central portions of long muscle fibers would begin to

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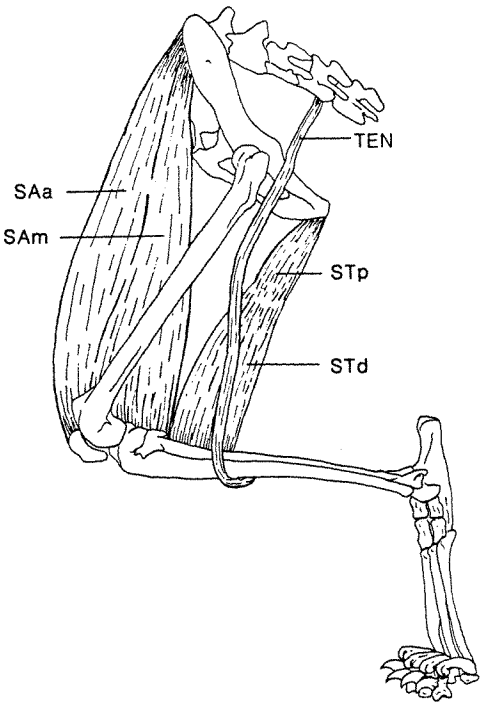

Fig. 1. Anatomical arrangement of the cat hindlimb muscles studied. TEN, tenuissimus; SAa, sartorious pars anterior; SAm, sartorius pars medialis; STp, semitendinosus proximal head; STd, semitendinosus distal head.

contract while their distal portions were still relaxed; this would stretch out the passive sarcomeres at the fiber ends. There appear to be two ways in which long parallel-fibered muscles have been structured to circumvent this problem. A few muscles (including semitendinosus, rectus abdominis, and the neck muscles, biventer cervicis and splenius) are subsectioned by tendinous inscriptions into a number of short, serially-arranged compartments, so that the fascicle lengths are only a fraction of the muscle length as a whole (Bardeen, '03; Cullen and Brodal, '37; Tobias and Arnold, '63; Bodine et al., '82; Armstrong et al., '82; Richmond et al., '85). Other parallel-fibered muscles have no inscriptions, but instead appear to be composed of short muscle fibers that are distributed in serial or overlapping arrays (Bardeen, '03; Huber, '16; Adrian, '25; Cooper, '29; Coërs, '59).

In our study, microdissection techniques were used to analyze the lengths and arrangements of individual, gold-stained muscle fibers from three cat hindlimb muscles — sartorius, tenuissimus, and semitendinosus (Fig. 1). These results have been supplemented by histochemical studies of motor endplate distributions and, in the case of sartorius, by studies of electrically evoked electromyographic (EMG) and muscle-nerve activity resulting from focal microstimulation of nerve branches supplying narrow longitudinal strips of the muscle. The various anatomical, mechanical, and physiological specializations are discussed in relation to the special mechanical properties and control problems of long, parallel-fibered muscles.

MATERIALS AND METHODS

Muscle microdissection

A total of four sartorius, four semitendinosus and eight tenuissimus muscles were dissected from eight adult cats weighing 2.8–4.2 kg, that were killed with an overdose of sodium pentobarbital. The animals had similar skeletal dimensions but differed in obesity. In seven cats, muscles were removed immediately after death, but in a single cat, hindlimb muscles were allowed to go into rigor for 3 hr so that fiber and sarcomere lengths would be stabilized prior to dissection. Muscles were measured and pinned to paraffin blocks. They were immersed in 25% formic acid for 30–240 min, blotted, and impregnated in gold chloride. For individual muscles, the concentration of gold chloride ranged from 0.5–2.0% and exposure times ranged from 1–18 hr. The varied impregnation schedules were employed to provide a selection of muscles with different degrees of staining and fiber strengths; prolonged exposure to gold chloride increases the depth and quality of staining but causes fibers to become brittle. Muscles were blotted and reduced overnight in 25% formic acid, then washed thoroughly in tap water and stored in glycerin for up to 24 months. Prolonged immersion in glycerin softens the connective tissues within muscle and facilitates single fiber dissections.

Each stained muscle was subdivided into muscle bundles measuring about 1–3 mm in the transverse plane and running from muscle origin to insertion. From a sampling of these bundles were dissected 10–50 muscle fibers to assess the quality of staining in each specimen. The preliminary dissections established that all three hindlimb muscles were predominantly composed of muscle fibers less than 4.0 cm in length and these had typical interfascicular terminations (cf. Richmond et al., '85). However, it was not possible from

these random dissections of fiber bundles to discern whether regional differences existed in muscle–fiber length or patterns of termination. Thus, systematic fiber dissections were carried out in two sartorius, one semitendinosus, and two tenuissimus muscles whose muscle fibers showed sufficient tensile strength that fascicles could be microdissected into their component fibers with little fiber breakage. Every effort was made to analyze the features of each fiber encountered in sequence during the teasing process to minimize the possibility that fibers were selected in a nonrandom manner. Because of the nongaussian distributions of fiber lengths, we calculated both means and medians of various populations, although these were never significantly different.

A similar number of fibers in all muscles was further studied at the light microscopic level by mounting the fibers in glycerin on glass slides. In each muscle, sarcomere lengths for some fibers were determined by directly measuring the distance spanned by ten sarcomeres using a ×100 oil immersion objective and a calibrated eyepiece graticule, then calculating the mean sarcomere length. Measurements of sarcomere length were repeated at 8–20 sites along the length of each single fiber. Sarcomere length was used to normalize fiber lengths to correct for shortening during rigor and differences in animal size.

Endplate staining

The right tenuissimus, semitendinosus, and sartorius muscles were dissected and removed from three additional pentobarbital-anesthetized cats weighing 2.7–5.0 kg. (The contralateral limbs were used for the evoked potential studies described below; the animals were sacrificed while still anesthetized.) The muscles were immediately placed in acetylcholinesterase incubating medium (Lojda et al., '79) for 8–12 hr. They were then rinsed in tap water and placed in a differentiating solution of 5% ammonium sulfide for 5 min. Endplate zones appeared as short, dark bands (Fig. 8). Following a second tap water rinse, the muscles were fixed in 10% formalin for 8–24 hr and then stored in glycerin. Prolonged muscle storage was avoided because staining quality is known to deteriorate with time. Photographs of the muscles were taken with a 4 × 5 inch Nikon Multiphot camera. For semitendinosus, the relatively thick fascial sheath resulted in low and uneven photographic contrast; hence, endplate distribution is shown as an anatomical drawing made from a combination of these photographs and the actual stained specimen (Fig. 8).

Evoked potential mapping

The sartorius muscle was selected for electrophysiological studies because its intramuscular nerve branches are clearly visible and readily accessible on the inner surface of this broad, thin muscle. In three pentobarbital-anesthetized animals, the left sartorius muscle was surgically exposed and reflected to expose its nerve supply and the adjacent saphenous nerve. Both electrical stimulation and recordings were made with hand-held electrodes consisting of closely spaced (2–3 mm) bipolar balls or nerve hooks formed on the end of 0.010 inch platinum wire. To activate fine intramuscular nerve branches, the ball electrodes were positioned directly on the visible nerves. Biphasic, controlled-current pulses (typically 0.1–0.5 mA at 0.1 msec phase duration) were generated by a photoisolated stimulator and balanced biphasic pulse generator (similar to Bak Electronics, Inc., BSI-2 and BPG-1 models, respectively). Recordings of evoked potentials were made from main nerve trunks and from the surfaces of various muscles using a transformer–coupled differential amplifier with 100–10,000 Hz bandwidth (similar to Micro Probe, Inc., model ADT-1). A Tektronix oscilloscope and camera were used to record multi-trace sweeps that were synchronized to the stimuli, which were repeated at about 1-sec intervals (see Fig. 9).

During the dissection of the various thigh muscle layers, electrical stimuli at a high intensity (1–10 mA) were directed to any structure that appeared to contain a nerve bundle; any twitch response was palpated manually to determine its apparent origins. Electrical recordings from all potentially involved muscles were used to determine the presence of a local contraction, as opposed to a mechanically transmitted motion from another structure. Latency was used to differentiate reflex responses (latency 8–12 msec) from direct motor axon activation (latency 1–5 msec) and from direct muscle fiber activation (latency less than 1 msec) to identify positively those nerve branches that contributed direct motor innervation.

TABLE 1. Muscle fiber lengths

Muscle	Sarcomere length ± SD	Number	Fiber lengths (cm) Locus	(n)	Mean ± SD	Median	Scaled[1] (mean ± SEM)
Sart. Med. A	2.2 ± .33	(143)	prox	(60)	2.4 ± 0.6	2.3	2.5 ± 0.08
			mid	(170)	2.4 ± 0.6	2.3	2.5 ± 0.05
			dist	(63)	2.2 ± 0.6	2.2	2.3 ± 0.08
Sart. Ant. A	(See above)		prox	(46)	2.0 ± 0.5	2.0	2.1 ± 0.07
			mid	(124)	2.2 ± 0.5	2.2	2.3 ± 0.04
			dist	(40)	2.4 ± 0.7	2.3	2.4 ± 0.16
Sart. Med. B	1.9 ± .21	(68)	prox	(73)	2.0 ± 0.6	1.8	2.4 ± 0.07
			mid	(207)	2.1 ± 0.5	2.0	2.5 ± 0.03
			dist	(85)	2.1 ± 0.7	2.0	2.5 ± 0.08
Sart. Ant. B	(See above)		prox	(42)	1.9 ± 0.5	1.8	2.3 ± 0.08
			mid	(99)	1.9 ± 0.3	1.8	2.3 ± 0.03
			dist	(38)	1.8 ± 0.5	1.8	2.2 ± 0.08
Ten. C	1.9 ± 0.11	(96)	mid	(59)	2.1 ± 0.4	2.0	2.5 ± 0.05
Ten. D	2.5 ± .24	(99)	mid	(67)	2.0 ± 0.5	1.9	1.8 ± 0.06[2]
			dist	(28)	3.0 ± 0.6	3.0	2.8 ± 0.11[2]
ST Prox. E	2.0 ± .15	(150)	span	(118)	2.1	2.1	2.4[2]
			short	(72)	1.5 ± 0.2	1.5	1.7 ± 0.02[2]
ST Dist. E	(See above)		prox	(73)	2.1 ± 0.6	2.1	2.4 ± 0.07
			mid	(68)	2.1 ± 0.4	2.2	2.4 ± 0.05
			dist	(81)	1.9 ± 0.6	1.7	2.2 ± 0.07

[1]Normalized to sarcomere length of 2.3 μm.
[2]See text for explanation.

RESULTS

Muscle microdissections

In all muscles, the most striking observation was the preponderance of short muscle fibers that had "intrafascicular terminations" (Bardeen, '03) as summarized in Table 1. Fibers in central portions of the muscle characteristically tapered at both ends to fine strands with submicron diameters (Fig. 2). These strands were closely applied to the surfaces of neighboring fibers. Commonly, the tapered end of one fiber was juxtaposed alongside a second fiber that tapered in the opposite direction, so that the two adherent fibers appeared to form a single long strand that could be divided into its constituents only by careful teasing. Fibers at the origin and insertion of the muscle were attached at one end to the tendon in a typical rounded-off junction typical of musculotendinous attachments, but at the other end they tapered in the same manner as the fibers in central muscle regions.

Sartorius

The two sartorius muscles (A and B) that were systematically dissected had a similar structural appearance. Once specimen was taken from the cat whose limbs were allowed to go into rigor prior to histological processing (muscle A) and was slightly longer than the muscle dissected and histologically processed immediately after death (maximum fascicular span = 11.5 vs. 10 cm respectively). However, both muscles had a similar structural appearance. The medial part of the muscle appeared as a thin, flat sheet whereas the anterior part of the muscle was thickened. Fascicular bundles from the most medial part of sartorius were always longer than bundles from anterior sartorius [medial: anterior measurements = 11.5:9 cm (muscle A), 10:7 cm (muscle B)].

Figure 3 shows schematically the arrangement of two sets of fibers dissected from single fascicles of anterior and medial sartorius. The interdigitations were not confined to any special regions. For any path from origin to insertion, tension would have to be conveyed through a minimum of approximately four to five serially arranged fibers. Microdissections of sartorius muscles A and B yielded total populations of 506 and 544 unbroken fibers respectively. Most fibers ranged in length between 1.0 and 3.0 cm (sartorius A, 519/544, 95%; sartorius B, 468/506, 92%). The remaining 63 of 1050 fibers in the two populations were longer than 3.0 cm but only seven of these long fibers were found to span sartorius from its tendon of origin to its tendon of insertion.

In addition to the large sample of unbroken fibers we found 16 fibers in the two muscles that were longer than 4 cm but were broken during microdissection. These fibers poten-

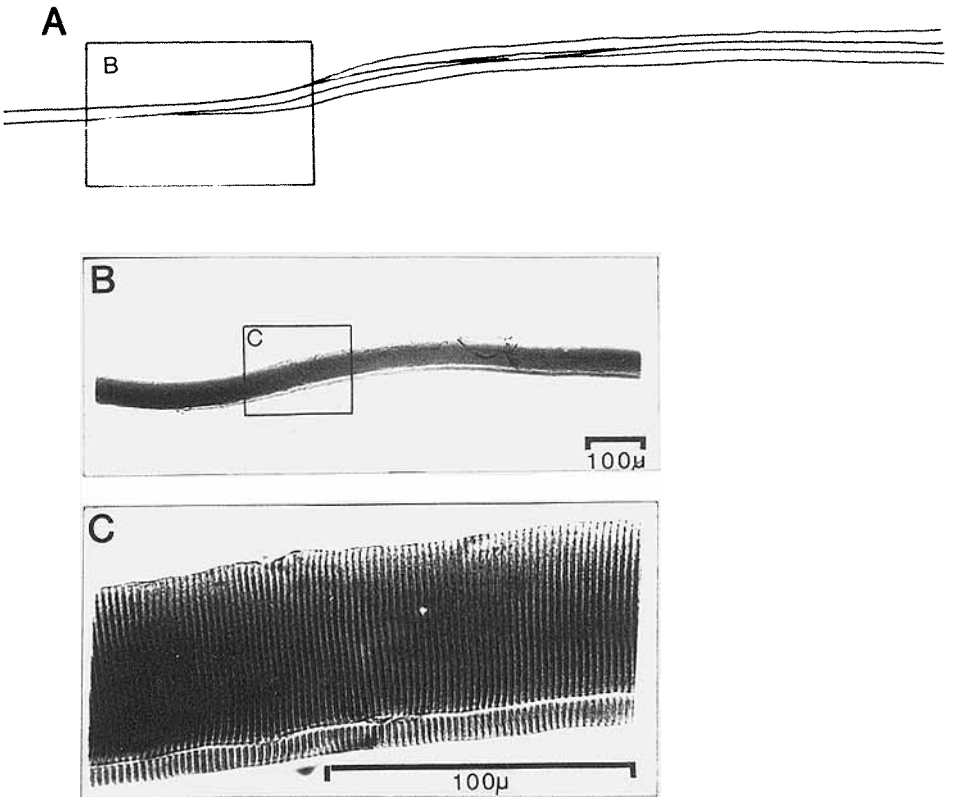

Fig. 2. Sketch (A) and photomicrographs show the arrangements of typical tapered fibers (medial sartorius) at their sites of apposition. The same region is depicted at three different magnifications in A, B, and C. In C note the registry of sarcomeres in the two fibers. No special attachments or other structures related specifically to the tapered free ends could be resolved by the light microscope.

tially might have increased our sample of fibers spanning from origin to insertion but would not greatly change the general observation that the muscles were composed chiefly of shorter fibers.

Fibers with tapers at both ends made up the largest proportion of dissected fibers. Fibers that tapered in both directions were not significantly different in length than fibers that were attached to tendinous tissue at one muscle end or the other (Table 1). In addition, no statistically significant difference was observed in the lengths of fibers composing medial versus anterior sartorius (Fig. 4), although both medial sartorius muscles had small numbers of unusually long fibers not seen in the anterior part. Seventeen of the dissected fibers inserted into Golgi tendon organs with typical afferent innervation that was well-stained. These fibers ranged between 1.4–2.4 cm in length and tapered at their intramuscular ends to terminate as tapered strands like neighboring fibers with conventional tendinous attachments (Fig. 5).

Sarcomere lengths usually fell into a narrow range of values over the length of each single fiber. Occasional differences of greater than $\pm 10\%$ along or between fibers probably resulted from inordinate stretching during dissection. Sarcomere lengths generally measured less than 2.5 μm (Table 1), although four instances were found in which sarcomeres had been stretched to over 3 μm. In one instance, these stretched sarcomeres were found along the main shaft of a fiber, whereas in three other instances they were observed in the tapered fiber ends. In preparations where tapered fibers were left in their associations with adjacent fibers, the sarcomere spacings of tapered strands remained in register with those of the adjacent fibers against which they were apposed (Fig. 2C).

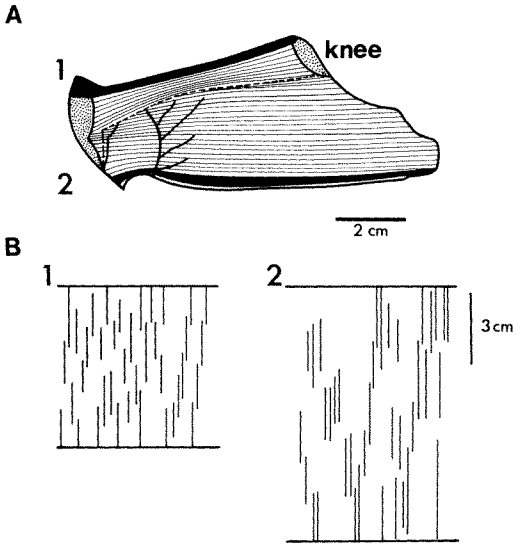

Fig. 3. Fiber arrangements in two sets of extrafusal fiber bundles from different regions of the sartorius muscle. (A) Line drawing of the ventral surface of sartorius following "en bloc" staining with gold chloride. The fiber organization in two strips from anterior and medial muscle parts (1 and 2 respectively) are illustrated. (B) Typical distribution of dissected fibers in muscle fascicles from anterior (1) and medial (2) muscle regions. Fibers are shown schematically as black lines whose lengths and location with respect to the muscle origin and insertion are drawn to scale. These fibers constitute only a fraction of the fibers dissected in each strip.

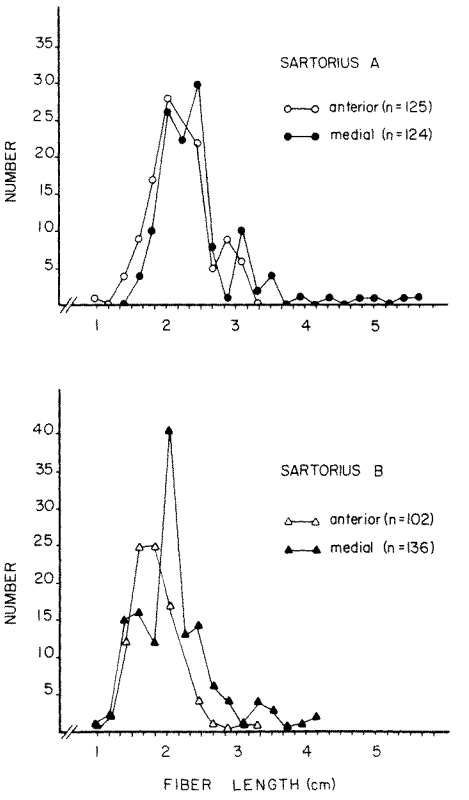

Fig. 4. Lengths of fibers sampled from the most medial strips of sartorius compared to those in its thickened anterior part. Sartorius (A) was set in rigor before staining whereas (B) was processed immediately, resulting in greater shortening.

Although sarcomere length varied in both dissected muscles, the mean sarcomere length in muscle A (which was allowed to go into rigor prior to processing) was longer than that of fibers in muscle B (Fig. 6B). This observation correlates with the shorter muscle fiber lengths found in sartorius B (Fig. 6A), and suggests greater shrinkage in its fibers. Table 1 shows the results of normalizing the median muscle fiber lengths measured in various parts of all muscles, using a standard sarcomere length of 2.3 μm. When so normalized, the mean fiber length for all parts of both sartorius muscles was statistically the same (within the standard error of the mean).

Tenuissimus

Tenuissimus is a long fragile, strap-like muscle that poses special problems for staining and dissection because it is easily overimpregnated and thus made brittle. Nevertheless, populations of 97 and 95 unbroken fibers were dissected from two tenuissimus muscles measuring 12.0 cm (tenuissimus C) and 14.5 cm (tenuissimus D) after staining. None of these fibers spanned the muscle from origin to insertion and most fibers measured 3 cm or less (tenuissimus C, 93/97 fibers; tenuissimus D, 80/95 fibers). We found that 19 of 192 unbroken fibers in the combined fiber sample were longer than 3.0 cm. However, these numbers may slightly underestimate the incidence of longer fibers since five additional fibers were dissected that ran for distances of between 2.5–4.8 cm but were broken before their terminations could be reached. In tenuissimus D, fibers inserting at the distal tendon were significantly longer than those composing the belly of the muscle, with normalized median lengths of 3.3 cm (SEM = $\pm$0.11, n = 28) versus 1.7 cm (SEM = $\pm$0.06, n = 67), respectively. In tenuissimus C, the distal end

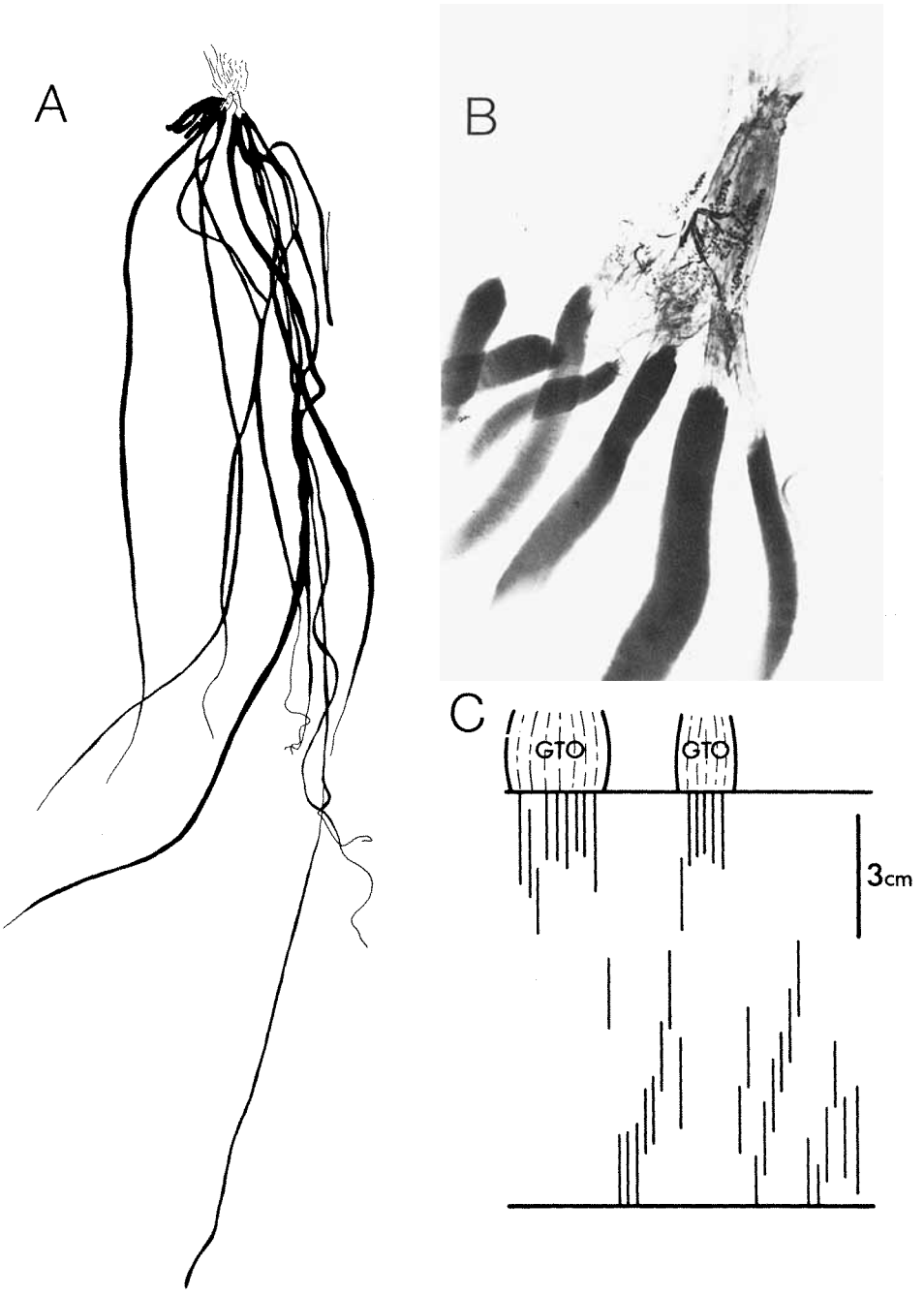

Fig. 5. Structural relationships for a Golgi tendon organ dissected from sartorius. (A) Line drawing of the GTO (arrow) and its intact complement of muscle fibers. All of the fibers were shorter than 3.0 cm. (B) Magnified photomicrograph of the GTO drawn in A. (C) Schematic to show the relative lengths of some muscle fibers inserting into the GTO in A (left) compared to fiber lengths in a second nearby tendon organ (right) and in other parts of the same muscle strip. The origin of the muscle is designated by the top horizontal line. (See Fig. 3 for further explanation of the format).

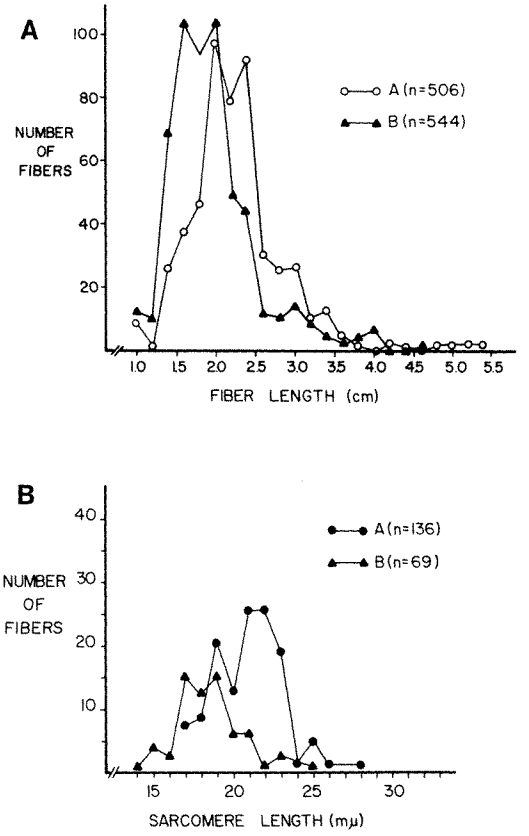

Fig. 6. (A) Lengths of extrafusal fibers dissected from two gold-stained sartorius muscles (designated as A and B). The distribution of fiber lengths was similar for both muscles, but the mean fiber length of muscle A, which had been allowed to go into rigor prior to staining, was significantly longer than that of muscle B ($p = 0.012$, two-tailed T-test). (B) Range of sarcomere lengths measured in fibers sampled from different regions throughout sartorius A and B. The longer sarcomere lengths in muscle A suggest that less fiber shrinkage occurred, and may thus account for the longer lengths of its muscle fibers when compared to sartorius B (see Table 1).

of the muscle was too brittle to permit systematic fiber sampling.

Semitendinosus

Dissections were carried out in a single semitendinosus muscle measuring 8.5 cm in total length following gold impregnation. Semitendinosus is divided into two serially arranged compartments of muscle fibers joined together by a tendinous inscription. On the ventral surface of the muscle, we found a bundle of muscle fibers less than 1 mm thick that ran superficially to the in-

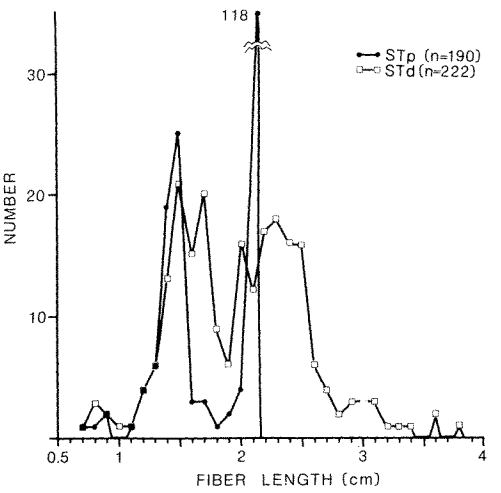

Fig. 7. Fiber length distributions for all fibers from STp and STd heads, showing bimodal distributions for both. The broken peak for STp represents the total number of fibers spanning from origin to inscription; it is positioned at the mean length for the five bundles from which fibers were dissected.

scription (not previously reported), and thus was not divided into two serially arranged subsections. A total of 451 unbroken fibers were obtained from semitendinosus. Of these, 190 fibers were taken from the shorter proximal muscle compartment in which fiber bundles ranged between 1.7–2.4 cm in length. Most individual fibers in the bundles ran the entire distance from the origin to the inscription, but 72 of 190 fibers were shorter and had only one end anchored to a tendinous attachment. Note the resulting bimodal distribution in Fig. 7.

In the longer distal compartment of semitendinosus, fiber bundles spanned distances of 3.4–5.4 cm between the inscription and the tendon of insertion. The large majority of these fibers (233/241 fibers) ran only a part of the fascicle length. As in sartorius and tenuissimus, fibers located at the ends of the compartment were attached at one end to collagen, while they tapered at the other end to a fine strand. Other fibers in central muscle regions were tapered at both ends. No significant difference was found between the lengths of fibers with one tendinous attachment and those with two tapered ends (Table 1).

Figure 7 shows the distributions of fiber lengths from the whole proximal head and from the whole distal head. Interestingly, *both* populations were bimodal, with concen-

trations of fibers around 1.5 cm and 2.4 cm instead of the unimodal peaks at around 2.0 cm seen in sartorius and tenuissimus. However, there was a distinct lack of fibers extending *almost* but not completely from origin to insertion, in favor of a large number of fibers extending about two-thirds of the way across the muscle from either the origin or the inscription. There was a similar but less markedly bimodal distribution in the much longer distal head, with all three classes of fibers (connected to inscription or insertion or neither) tending to be shorter than 1.7 cm or longer than 2.0 cm.

Endplate staining

Figure 8 shows photographs or sketch reconstructions of the surfaces of all three muscles. Darkly stained bands are acetylcholinesterase-positive zones presumably associated with the motor endplates of innervating motoneurons. In all three muscles, these zones are scattered across most of the muscle surface, as would be required to innervate the short muscle fibers distributed at various positions throughout the muscle. Some regions appear to contain multiple strips of bands; within each strip the longitudinal interval between bands is fairly regular but offset from the adjacent strips. In sartorius, a line parallel to the fascicles traverses five to seven endplate bands, similar to the number of muscle fibers required to traverse the muscle (Fig. 3). However, in tenuissimus and semitendinosus the intervals between endplates are much shorter than the muscle fibers, suggesting a more complex interdigitation of muscle fibers and their innervation.

Electrical mapping

The nerve to sartorius enters the muscle on its internal surface about one-fifth of the distance from origin to insertion and gives off a series of proximally and distally directed longitudinal branches (grossly visible) as it courses from the medial edge to the anterior edge of the muscle (see sketch in Fig. 9). Electrical stimulation slightly above threshold applied to any of these longitudinal branches produced a synchronous twitch that appeared to extend along the length of the muscle but did not spread laterally. The stimulus strength had to be increased several-fold before the contraction changed in character, spreading medially and/or laterally to adjacent strips.

Bipolar recordings during low-intensity stimulation of a single branch confirmed that there was short-latency electrical activity throughout a narrow longitudinal strip of muscle fibers extending over the entire length of the muscle. This was true for nerve branches that coursed proximally as well as distally to the axis of the main nerve. When the recording electrodes were displaced more than about 3 mm to either side of this strip (approximately half the distance between the visible nerve branches), the amplitude of the recorded potentials fell rapidly to zero.

Figure 9 shows recordings made at 12 sites spaced 6 mm apart along such a typical longitudinal strip. Latencies of EMG activity were all about 2–3 msec except at the distal end of the muscle. The EMG potentials recorded from progressively more distal sites alternated between leading negative waveforms and leading positive waveforms. In each trace, the more proximal electrode of the bipolar pair was connected to the positive input of the differential amplifier. Thus a leading-positive triphasic waveform would correspond to an action potential propagating in a distalward direction, and a leading-negative waveform should indicate propagation proximally. Some of the traces show distinctly multiphasic waveforms with long latency components (e.g. third and seventh from the top). The distalmost three traces do not reverse polarity but show triphasic, leading-positive traces at increasing latency. (The distalmost record was obtained near the musculotendinous junction, where the mechanical action of the twitch tended to displace the electrode from electrically active tissue, resulting in poor superimposition of these traces.)

The line drawing in Figure 9 shows a muscle- and nerve-fiber arrangement that could give rise to the observed waveforms. We have interpreted the polyphasic waveforms as arising from the interaction of potentials in two sets of overlapping muscle fibers, one set with a motor endplate band relatively close to the recording electrodes and another set with a different endplate zone located farther from the recording electrodes and on their opposite side. This pattern of endplate organization would presumably result in short latency EMG activity propagated in one direction in the first set of fibers, and longer-latency components propagated in the opposite direction by the second set of fibers. The effects of conduction delay in the muscle fi-

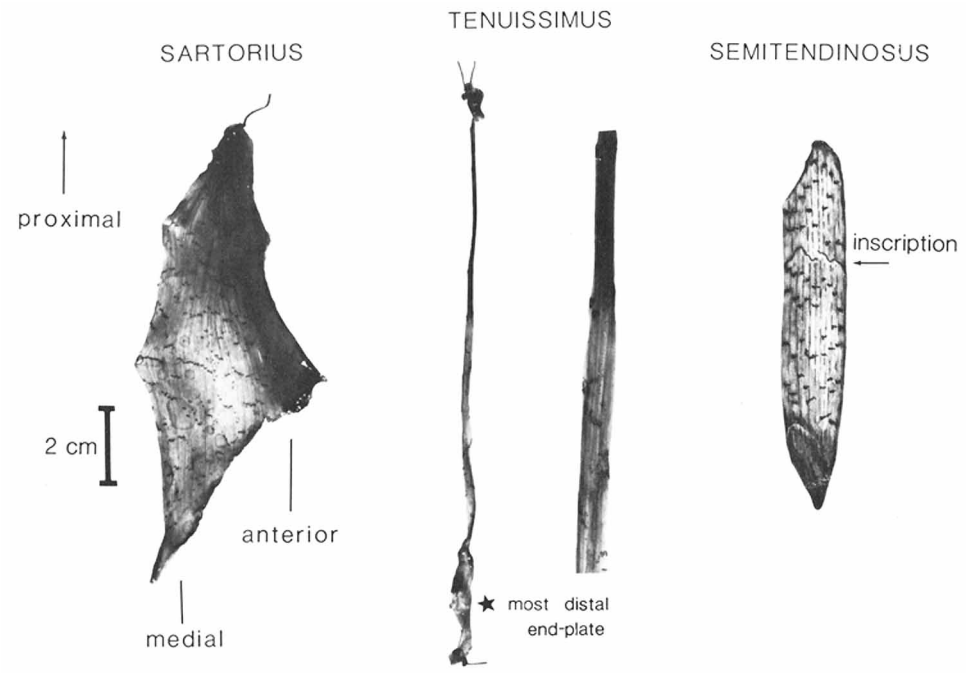

Fig. 8. Dissecting-microscope views of the muscles stained for acetylcholinesterase (short dark bands in loosely organized columns extending across the length of each muscle); photographs of sartorius and tenuissimus; sketch of semitendinosus.

bers can be observed without occlusion by other waveforms in the distalmost two tracings.

The nerve bundle distributions to sartorius muscle from the femoral nerve trunk were found to vary in the gross anatomical dissections. To explore this further, we used focal stimulation and recording techniques on small nerve branches to reveal the details in two animals. Figure 10 shows the pattern of nerve branching visible on the internal surface of each muscle and confirmed by these experiments. In cat CP22, the anterior part of the muscle, SAa, was innervated exclusively by the main nerve to sartorius (solid lines), which diverged from the common femoral nerve at about the same level as the bifurcation of the saphenous nerve. However, sartorious was also supplied by two small branches that diverged quite proximally from the saphenous nerve (dashed lines). Stimulation of the distalmost branch, S3, evoked EMG activity along the medialmost edge of the muscle; stimulation of branch S2 evoked activity in the rest of SAm between the medial edge and SAa. Recordings made along the length and width of this section of the muscle during stimulation of S2 produced a pattern similar to that evoked by stimulating a single intramuscular branch (Fig. 9). However, the pattern of alternating waveform polarity was less clear. The S2 branch gave rise to two adjacent, parallel intramuscular branches, which presumably had innervation points at different positions along the length of the muscle. Thus small lateral displacements of the recording electrodes resulted in complex, changing polyphasic patterns.

The motor innervation of sartorius in CP23 was more complex than in CP22. The main sartorius nerve produced twitches throughout the SAa and along the medialmost edge of SAm but not in the intervening zone. The intervening region could be recruited by stimulating a single, large branch of the saphenous nerve that originated somewhat more distally than the motor branches of the saphenous nerve that were noted in CP22. Stimulation of various branches shown in the sketch produced short latency (less than 0.5 msec) action potentials in either the sartorius nerve (R1) or motor branch of the saphenous nerve (R2), but not both.

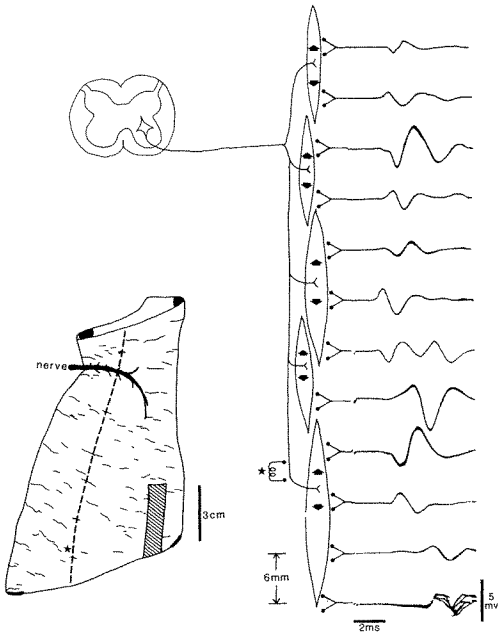

Fig. 9. Set of EMG recordings made at various positions along the course of one longitudinal nerve branch on the inside surface of SAm (dashed line in inset sketch) with stimulus applied near the distal end of the branch (star). The schematic diagram of muscle fiber arrangement and alpha motoneuron innervation points is an interpretation consistent with the polarities and latencies of the various EMG waveforms (see text).

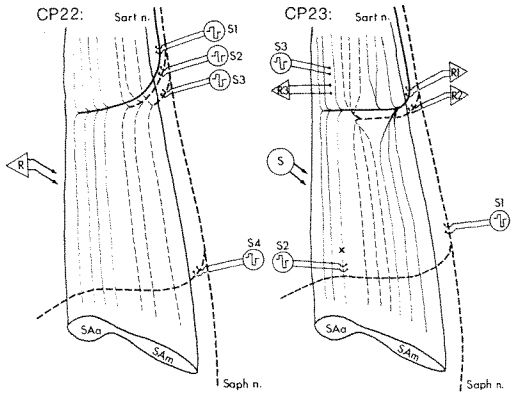

Fig. 10. Internal surfaces of left sartorius muscles from two cats and representative stimulation/recording experiments. CP22 shows four nerve stimulation sites S1–4 and roving bipolar ball electrode R used to record at various sites on muscle surface. CP23 shows stimulus sites S1–3 and roving stimulus S plus bipolar nerve hook recordings R1 and R2 and muscle surface recording R3. Cross mark site of nerve cut. See text for results.

Both CP22 and CP23 had a distal branch of the saphenous nerve that crossed the surface of the sartorius muscle without giving off any apparent muscular branches. Stimulation of S4 in CP22 and S1 in CP23 produced a reflex twitch in the deep thigh musculature with a latency of about 10 msec, but there was no short latency EMG indicative of an M-wave in any of the anterior thigh muscles. In CP23, the reflex appeared to cause visible movement in the proximal portion only of the sartorius muscle. However, EMG recordings made with a bipolar probe revealed no activity in any part of sartorius. Rather, the mechanical twitches were found to originate exclusively in the posterior half of tensor fascia lata, which originates in part from the internal fascia of sartorius near the pelvis (and which is held taut by the fascia lata). EMG activity with a latency of about 10 msec was confined to this small but powerful, deep muscle.

Additional dissection was undertaken in CP23 to determine if the finest branches of SAa derived from motoneurons supplying the entire length of this part of the muscle, as shown for SAm in Figure 9. Stimulation of a fine, distal intramuscular branch S2 (elevated on hook electrodes) evoked synchronous, short latency activity along the entire length of the SAa. After cutting this branch proximally at the cross, only the distal response remained. In another test, ball electrodes S3 were used to stimulate the surface of the proximal part of the SAa muscle between two visible nerve branches. At strengths just below threshold for a coordinated response over the length of the muscle, it was occasionally possible to elicit a very localized twitch in the proximal part of the muscle only. However, EMG recording at site R3 revealed that these twitches were accompanied by localized electrical activity with a latency of less than 0.5 msec, indicative of direct muscle fiber activation, whereas nerve activation always produced EMG latencies of greater than 1.0 msec.

DISCUSSION

A consistent picture emerges from all three methods employed here. These three long, parallel-fibered muscles are composed primarily of short muscle fibers arranged in series. Their motor supply is widely distributed over the length of the muscle and gives rise to multiple endplate zones, whereas pinnate muscles typically have a single, obliquely oriented band of endplates servic-

ing their relatively short fibers (Galvas and Gonyea, '80). At least in some cases, individual motor axons branch to create muscle unit territories that are long and narrow, suggesting that the longitudinally oriented branches of the muscle nerves and their staggered strips of endplates may correspond to functional entities of motor control.

Historical perspective

The organization described here is in agreement with that proposed by Adrian ('25) for the cat tenuissimus and by Cooper ('29) for cat sartorius. Further, it suggests a physiological basis for previous observations of distributed endplates in semitendinosus (English, '85) and other long muscles (Coërs, '59; Adams and MacKay, '60; Karnovsky and Roots, '64). There has been a history of occasional reports that long, parallel muscles in such varied sites as the abdominal musculature of dogs and the limb muscles of roosters are composed of short, in series fibers (Bardeen, '03; Huber, '16; Van Harreveld, '47). However, the observation is not readily made from the usual cross-sectional histology of muscles because only a relatively small percentage of total fiber length is tapered, resulting in a correspondingly low incidence of small diameter profiles, which may be dismissed as intrafusal or atrophic fibers. In fact, it seems likely that most long, uninscripted muscles are composed of short, in-series fibers, yet the many mechanical, developmental, neural, and control implications of this architecture and its variations have been neglected, even in comprehensive reviews of muscle morphology (e.g. Gans, '82). Thus it seems useful to examine these features specifically and to speculate on their possible significance.

Architectural features of parallel muscles

Limits on fiber length

Action potentials in muscle fibers propagate by nonsaltatory conduction similar to that of unmyelinated nerve fibers. Preliminary studies of single unit conduction velocity in tenuissimus muscle (Chanaud et al., '85) agree with predictions that this velocity is slow, approximately 3 m/sec. A 12-cm muscle fiber innervated at its midpoint would experience a delay of 20 msec in the spread of myoelectrical activity to the ends. This delay is on the same order as the twitch rise-time (Close, '72; Brody, '76; Lev-Tov et al., '84). The inverted-U shape of the length/tension curve suggests that this delay could cause a disruptive mechanical instability. As an inactive region of muscle fiber is stretched past its optimal length, its ability to generate active force declines. Thus, when the activation finally arrives at the fiber ends, the ends might already be pulled into a mechanically disadvantageous and perhaps even damaging overextension of their sarcomeres.

It seems likely that the time course of the "active state" (as reflected in the twitch tension rise-time) must impose a limit on the length of muscle fiber that will be mechanically stable. However, the rise-time of tension that is typically measured at the muscle ends under isometric conditions will be only a weak indicator of the dynamics of intrafiber force generation under more diverse (and more normal) kinematic conditions (Joyce et al., '69). Most of the muscle fibers studied in these various hindlimb muscles had only a small range of lengths when normalized to the same sarcomere spacing, which is consistent with the notion of some general constraint on fiber length. However, details such as the bimodal distribution of fiber lengths in semitendinosus (Fig. 7) and the presence of fibers that are even shorter than the already short, inscripted compartments of some neck muscles (Richmond et al., '85) indicate the need for more data relating structure to function.

Balance of tension in fascicles

Breaking up a long muscle fascicle into many short, in-series muscle fibers potentially poses an even greater instability problem if the activation level of the fibers at one end exceeds that of the fibers at the other end. The EMG recordings reported here (Figs. 9, 10) confirm the indirect demonstration of Adrian ('25) that at least some motor axons supplying muscle fibers at one end of such a fascicle have branches that innervate muscle fibers throughout the entire length of the fascicle. Preliminary studies of single units in tenuissimus suggest that this is a general property of all of its motor units and that their innervated fibers occupy a similar cross-sectional area at all positions along the length of the fascicle (Chanaud et al., '85). Because the conduction velocity in the branches of alpha motoneurons is at least 20 times faster than the conduction velocity of the muscle fibers, this innervation scheme provides for a simultaneous and equal active tension generation over the length of the fascicle for each twitch of each motor unit. (However, muscles are known in which motor unit domains extend only partly across

the length of uninscripted fascicles (Richmond et al., '85).

Sherrington (1894) reported a class of flexor reflexes whose mechanical action appeared to be restricted to the proximal end of the cat sartorius. However, the electrical mapping of saphenous nerve reflexes described here suggests that the sartorius was simply conveying passively the mechanical effects actually generated in the underlying tensor fascia lata. Visual observations of mechanical responses during flexor reflexes could easily misidentify this source, which is confined to the anterior part of tensor fascia lata, a small but strong and architecturally complex muscle.

Distribution of tension in fascicles

At any position along a fascicle, fibers belonging to a single muscle unit will presumbly constitute only a small, spatially distributed fraction of the total number of fibers seen in cross-section. If the exact position of any single fiber is random, then the odds will be low that its tapered intramuscular end will be connected specifically to another fiber of the same unit. This would tend to cause a microscopic version of the same sort of instability considered in the previous section. There are two mechanically stable solutions to this potential problem.

One possibility would be that muscle fibers of a single motor unit make specific connections with each other and not with fibers of other motor units. To achieve this kind of architectural specificity would require developmental processes with much more detailed specificity of innervation than has yet been proposed. We know that muscle fibers are polyinnervated during fetal development (O'Brien et al., '78). One speculation might be that they lose this pattern in a manner that is coordinated by patterns of active tension development. Alternatively, muscle fibers might originally span the entire muscle length, with subsequent spread of single axon endplates and transverse fiber splitting producing the adult pattern. The small number of muscle fibers that we noted to span the muscle length (sartorius and distal semitendinosus) may represent a residual population from this process.

A more likely alternative to specific end-to-end fiber termination is that the tension generated by each muscle fiber is conveyed more generally to a diffuse network of epimysial connective tissue rather than specifically onto a single adjacent fiber. Such a matrix has been described (Borg and Caulfield, '80; Rowe, '81), although Barrett ('62) argued in favor of the more specific end-to-end linkage. Precise reconstructions of single unit domains using glycogen depletion are underway (as has been done in pinnate muscles, e.g. Burke and Tsairis, '73) to determine the nature of these fiber-to-fiber connections. Such studies should also be helpful in understanding differences in the spacings of endplate regions along single longitudinal strips, which may relate to differences in the degree of fiber interdigitation and overlap that are possible variables in this general architectural scheme.

Conservation of fiber length

Parallel-fibered muscles generally occur where long skeletal lever arms provide for large mechanical advantage and long stroke lengths. Thus they are the converse of pinnate-fibered muscles, which achieve comparable stroke lengths in the muscle fibers by mechanically amplifying the typically short stroke length of the whole muscle (Gans, '82). However, the muscles studied here are notable for having such long lever arms that the physiological range of muscle lengths, if conveyed unattenuated to their constituent muscle fibers, would cause them to work over unusually large regions of the length/tension and velocity/tension relationships of sarcomeres. If, as suggested in the previous section, the individual fibers insert diffusely into an epimysial matrix, then it is possible that this matrix has elastic properties that would change the way the stretch is distributed within the muscle. This could permit the interdigitated fibers to slide with respect to each other, so that overall changes in muscle length would not necessarily be reflected in proportional changes of fiber or sarcomere length. A preliminary report of the sarcomere intervals measured in the tenuissimus and sartorius muscles under various conditions and lengths suggests that there may be considerable interfiber motion (Rindos et al., '84). Such an architectural feature could provide yet another variable suitable for adapting the mechanical characteristics of different muscles to the very different kinematic conditions under which they work (Loeb, '85).*

*Note added in proof: Unusually broad length/tension curves have been reported in such parallel-fibered cat hindlimb muscles [Prosé, L.P. (1985) De Functionele Stabiliteit van de Knie van de Kat, Ph.D. diss., Faculteit van de Geneeskunde, Vrije Universiteit Brussel, Brussels, Belgium; Otten, E., work in preparation].

Motor control of series compartments

Some muscles spanning long distances are grossly divided by inscriptions into separately innervated compartments, e.g. the two heads of semitendinosus (Bodine et al., '82) and the multiply inscripted neck muscles (Richmond et al., '85). Thus, there must exist neural control solutions to the problem of maintaining mechanical stability across series structures. Gross inscriptions may simplify the problem by removing the requirement for stability at the single fascicle level and requiring only that the activation of all the fascicles so tied together be equal on each side of the inscription. However, this begs the question of why some long muscles are compartmentalized in series, some employ motor units distributed over the length of the muscle, and some have a combination of both (e.g. semitendinosus). Further, as noted earlier, motor unit domains do not necessarily extend across the entire length of uninscripted compartments (Richmond et al., '85). It seems likely that there have been evolutionary trade-offs between the mechanical properties desired of each muscle and the amount of neural circuitry required to cope with the emergent complexities of control.

Motor control of parallel compartments

The compartmentalization of sartorius into longitudinal strips, each of which is innervated exclusively and entirely by one subpopulation of motoneurons, could simplify two control problems inherent in broad sheet-like muscles that have widely distributed attachments. First, it would provide a reasonably small domain over which the forces generated by the fibers of each unit would have to be evenly distributed. Second, it would separate the motor units on the basis of the gradually changing mechanical action that occurs from medial to anterior across the broad insertion of the muscle. All parts of sartorius contribute to hip flexion, but the knee action changes from flexion to neutral to extension across the muscle. Retrograde labeling studies of the motoneurons innervating the various parts of this muscle indicate that they are distributed along a rostral-caudal gradient in the spinal motor nucleus (Pratt et al., '84) that corresponds loosely to the medial-to-anterior sequence of nerve branches within sartorius. The concept of parallel compartments with either specialized recruitment (English, '84) or localized proprioceptive feedback (Binder and Stuart, '80) requires at the least that the motor axons confine their terminal branches to such anatomically defined subregions of the muscle. However, in practice, the comparaments have been defined by more proximal divisions of the main muscle nerve. The variability and complexity of such branches (as illustrated in Fig. 10) suggests some caution in interpreting these manifestations of compartmentalized motor control. The intramuscular organization of motor pools may be different from and perhaps more orderly than that expressed in the peripheral nerves.

The role of architecture in the specialization of mammalian muscles

Mammalian limbs generally contain many more individual muscles than actually required to control the degrees of freedom provided by their joints. Anatomists have categorized muscles simply by their joint actions, thus forming what are known as "synergistic groups." However, this term imputes a functional organization for which evidence is often lacking. It also overlooks readily apparent differences in skeletal lever arms and connective tissue architecture. We would suggest that the multiplicity of mammalian muscles may be a consequence of the specialization of many of them to perform well only for kinematically restricted tasks (e.g. large vs. small excursions, active lengthening vs. active shortening). At the filament and sarcomere level, mammalian muscles appear to be much less specialized than invertebrate or lower vertebrate muscles (see Hoyle, '83), but this may reflect metabolic rather than mechanical constraints. Higher vertebrates must learn to use their muscles in a large number of kinematically diverse situations, for which the variety of architectural forms noted here may provide a useful range of mechanical characteristics. Thus, the behavioral diversity of higher vertebrates as compared to lower animals may be served not by a smaller number of less specialized muscles but rather by a larger number of more specialized muscles. Quantitative studies of relative muscle recruitment in a wide variety of tasks have begun to reveal the predicted functional specialization within synergistic groups (O'Donovan et al., '82; Abraham and Loeb, '85; Abraham et al., '85). The consequences for the neural circuitry that controls these diverse muscles remain as significant challenges to the theoretical and experimental branches of sensorimotor control physiology.

ACKNOWLEDGMENTS

The authors thank O. Morris, D. Reesor, J. Crocket, and M. Manley for assistance with dissections and preparation of figures, and J. Kraft for processing the manuscript.

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