Morphology, Histochemistry, and Function of Epaxial Cervical Musculature in the Horse (*Equus caballus*)

K.S. Gellman,[1]* J.E.A. Bertram,[2] and J.W. Hermanson[1]

[1]*Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14850*
[2]*Department of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, Florida 32306*

ABSTRACT   The semispinalis capitis and splenius muscles of the horse were analyzed for gross morphology, microarchitecture, fiber length, and fiber type. Although these two muscles are similar in size and anatomical position, they are very different from one another in structural design and histochemistry, implying diverse functional roles in the animal's behavior. The histochemical staining profile was limited to two fiber types: slow oxidative and fast glycolytic. The splenius muscle has simple architecture, long fibers, and a 60/40 ratio of SO to FG cross-sectional area. The semispinalis capitis has complex architecture with short-fibered, concentric compartments dorsal to its central tendon and longer-fibered compartments ventrally. The entire dorsal region has an increasing gradient of slow oxidative fiber percentage from caudal to cranial (58–71% SO). In contrast, the ventral region has a decreasing gradient of slow oxidative fibers from caudal to cranial (48–67% FG). These patterns can be interpreted within the context of the cervical musculature during locomotion and posture to indicate the functional advantages of this organization. J. Morphol. 251:182–194, 2002.   © 2002 Wiley-Liss, Inc.

KEY WORDS: horse; muscle; splenius; semispinalis capitis; cervical; muscle histochemistry

The horse, *Equus caballus*, is one of the largest (up to 600 kg), fastest (sustained speeds of over 48 km/h in thoroughbred racehorses) terrestrial mammals. From a comparative biomechanical perspective, the extreme anatomical adaptations used by horses can help elucidate the structure and function of the cursorial locomotory system. The head and neck of the horse, which represent 10% of the total body mass (Buchner et al., 1997), are cantilevered from the trunk of the body with a complex support system that combines passive and active elements. The combined head and neck segment appears to be an essential element of equine gait mechanisms, demonstrating different characteristic oscillations at walk, trot, and gallop that are closely linked to the movement patterns of the limbs.

Previous work (Gellman and Bertram, 2002a,b) investigated the passive contribution of the nuchal ligament to head movement during locomotion. The elastic nuchal ligament, the semispinalis capitis muscle (SS), and the splenius muscle (SP) are the largest structures in the dorsal cervical region. As in many ungulates, the primary and secondary cervical curves of the spine are exaggerated and the dorsal aspect of the neck region is filled in by these three structures. All three anatomical elements have firm attachments to the thoracic vertebrae, cervical vertebrae, and the skull. The elastic nuchal ligament provides both passive support for the head and neck and contributes to locomotion by storing and returning elastic strain energy during head/neck oscillations. Elastic strain energy can provide up to 60% of the work required to raise the head and neck during walking and around 32% of oscillatory work at the trot and gallop. The remainder of work must be supplied by muscular elements, such as the SS and SP. This current study examines the functional characteristics of the muscles that actively raise the head and neck.

Relatively little is known about equine cervical muscles, aside from gross anatomical description (Getty, 1975; Dyce et al., 1996) and some EMG recordings of the splenius (Tokuriki and Aoki, 1991). However, neck muscles have been extensively studied in cats and characterized through architecture, electrical activity, histochemistry, and neuromuscular compartmentalization (Richmond and Abrahams, 1975; Richmond et al., 1978, 1985, 1992; Richmond and Bakker, 1982; Armstrong et al., 1988; Richmond and Armstrong, 1988). Although horses and cats are very different in size and behavior, they

---

share certain features in common as vertebrates and cursorial quadrupedal mammals.

Cervical epaxial muscles have been a topic of great interest historically because of the complexity of their neuromuscular organization and behavior. Over 20 pairs of muscles control head movement in mammals and many of these muscles have potentially similar actions. It has been suggested that this redundancy indicates that the neck is "functionally over-complete" (Pellionisz and Peterson, 1988). However, head positioning is critical to an enormous range of behaviors—visual and auditory orientation, feeding, vestibular function, grooming, predation, defense. An interpretation of the design of the neck in any mammal cannot be determined without thorough investigation of the functional consequences of internal organization and details of the architecture and activity.

The feline biventer cervicis (fBC) muscle is similar to the equine semispinalis capitis, although there are some minor architectural differences in terms of origins, insertions, and compartmentalization. From studies of neural compartmentalization in the fBC it has been suggested (Armstrong et al., 1988; Richmond et al., 1992) that the cranial and caudal portions of the muscle act independently during normal head and neck movement. The feline BC is a pyramidal muscle with oblique, concentric compartments (Fig. 2b). It has been found that each BC compartment has a roughly equivalent functional cross-sectional area, which implies the capacity to generate equivalent amounts of force between compartments (Richmond and Armstrong, 1988). Richmond and Abrahams's (1975) morphological analysis of the fBC suggests that the design strategy of tendinous inscriptions allows long muscles to taper, as toward the head, without compromising parallel fiber architecture. They also found a wide variety of fiber lengths coexisting within compartments, some passing through the tendinous inscriptions and some inserting upon them. It was thought that this arrangement could offer the versatility of peak tension development at different head positions through tensioning the short fibers within specific compartments, while allowing the long perforating fibers to transmit force through the entire muscle. The feline splenius muscle also has a complex architecture, with several incomplete tendinous insertions, whose functional role is unclear. There is a wide variation of individual muscle fiber length between these implied compartments (Richmond and Abrahams, 1975; Richmond et al., 1985).

All of the feline cervical muscles examined display three fiber types: slow oxidative, fast fatigable (glycolytic), and fast fatigue-resistant (Richmond and Abrahams, 1975). The fBC has half slow fibers, with about one-quarter FOG and one-quarter FG fibers. The fSP, in contrast, has more than half fast fatigable fibers and splits the rest between SO and FFR. These profiles seemed consistent with primary EMG

TABLE 1. Experimental subjects

Subject	Sex	Weight (kg)	Age (year)	Breed
A	Male castrate	529.5	2	Dutch warm blood
B	Male castrate	497.7	15	Thoroughbred
C	Male castrate	470.5	6	Thoroughbred

activities measured: tonic support for fBC and phasic activity for fSP (Richmond et al., 1992). There are, however, some apparent contradictions between measured neural patterning, recruitment, and observed motor activity in the long dorsal muscles of the cat neck. Muscles like the fSP were differentially recruited according to activity and neck position, together with arrays of synergistic muscles, which differed for each activity. Clearly, these muscles are not only multiarticular, spanning most of the cervical region, but also multifunctional. The feline electromyographic studies were limited to static postural positions and grooming behaviors and voluntary head turning. Muscle activity was not measured during locomotion.

High concentrations of Golgi tendon organs have been found proximal to the tendinous inscriptions of the BC in the cat (Richmond and Abrahams, 1975; Richmond and Bakker, 1982). This level of sensory feedback for the head/neck position is essential for vestibular and visual function. There is no indication, however, of nerves that are selectively motor or sensory in the feline cervical muscles. Innervating branches of the dorsal cervical nerve roots contain all functional nerve types.

In this study, we examine the gross morphology of the semispinalis capitis and splenius muscles, the architecture and compartmentalization within the muscles, and their fiber type composition. These elements can then be compared and contrasted with analogous cervical muscle function in the cat. Finally, predictions can be made about the functional capabilities of these structures.

MATERIALS AND METHODS
Subjects

All subjects were healthy adult horses euthanized in the Cornell Department of Pathology's Necropsy Service for reasons other than musculoskeletal dysfunction. For histochemistry, samples were taken from nine anatomical sites (eight semispinalis, one splenius) in three animals (Table 1). After fresh muscle samples were taken, the entire muscles were removed for morphologic studies and fixed in 10% formalin. An additional SS muscle from a 200-kg male, castrated, aged, pony was used for morphology only.

Morphology

Fixed specimens were used to identify functionally important compartments of the SS muscle by

Fiber length: mean (SD) mm, n = 2			
S-1	20.0 (7.1)	90.0 (0)	S-2
S-3	42.5 (3.5)	107.5 (17.7)	S-4
S-5	65.0 (7.5)	135.0 (7.1)	S-6
S-7	112.0 (17.7)	160.0 (0)	S-8
sp	215.0 (7.1)		

[1]Segments S-1 through S-8 are the compartments of the semispinalis capitis (SS) muscle, as illustrated in Figure 2a.
[2]The splenius muscle is not compartmentalized in the horse. Segments S-1, S-3, S-5, and S-7 are listed at the left, S-2, S-4, S-6, and S-8 at the right.

isolating connective tissue divisions and innervation from individual cervical nerves. Careful dissection traced the branching of the dorsal rami from their origin at the cervical intervertebral foramina, near the muscle attachment on the articular facets. An intact SS specimen was dissected along the connective tissue partitions to visualize the three-dimensional relationship of the presumed compartments. Eight sampling areas for histochemistry and morphometry were chosen, four dorsal and four ventral to the central tendon, based on the changing morphology of the compartments from cranial to caudal ends.

Morphometry

Muscle fiber bundles in the intact fixed muscle specimens were measured using calipers (Table 2). Then the intact muscles were subjected to a controlled digestion in dilute nitric acid (5–15%) until connective tissue attachments were compromised and individual fiber bundles could be teased away from the compartments. These fiber bundles were separated and placed in a solution of 50% glycerol until soft and pliable. They were then separated, under a dissecting microscope, to individual muscle fibers that were examined for continuity between attachments and measured for length. An average of 30 fiber bundles was measured for each segment and an average of 20 individual fibers was separated to evaluate continuity through the length of the compartment.

Histochemistry

Samples were obtained from each of the functional regions of the splenius and semispinalis muscles. Muscle samples (1 cm³) were removed from the animal within 60 min of euthanasia, mounted with 5% gum tragacanth onto cork, snap-frozen in isopentane cooled to about −150°C in liquid nitrogen, and stored at −85°C. Serial sections were cut on a cryostat for comparative histochemical assay. A rat diaphragm standard was used as a control throughout. Sections from each muscle, mounted on glass slides, were stained for alpha-glycerophosphate dehydrogenase (GPD) to assess glycolytic potential (Wattenberg and Leong, 1960) and for nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) to assess oxidative capacity (Novikoff et al., 1961). Tissues stained for GPD were incubated in 0.20 M phosphate buffer containing 9.3 mM GPD, 1.2 mM Nitro blue tetrazolium, and 2.3 mM menadione for 45 min at 37°C. Tissues stained for NADH-TR were incubated in Trizma buffer (Sigma Chemical Co., St. Louis, MO; pH 7.4, 0.20 mM) containing 1.4 mM NADH and 2.5 mM Nitro blue tetrazolium for 30 min at 37°C. All sections were rinsed in deionized water, dehydrated in acetone, and mounted with glycerogel. For myosin-ATPase staining, the protocols were modified from Brooke and Kaiser (1970). Sections on slides were preincubated for 5 min in a 0.2 mM barbital acetate buffer (pH 4.3, 4.4, 4.5) or preincubated for 10 min in a glycine buffer solution (20 mM glycine, 74 mM NaCl, 38 mM CaCl), pH 10.3, at 37°. These samples were then further incubated for 30 min at 37° in sodium barbitol buffer solution (1.4 mM ATP, 18 mM CaCl2, pH 9.4). They were then processed sequentially with solutions of 1% CaCl$_2$, 2% CoCl$_2$, and 1% ammonium sulfide before dehydration in 70%, 80%, 95%, and 100% ethanol, then cleared with xylenes. Coverslips for slides were mounted with Permount (Fisher Scientific, Fair Lawn, NJ) or GVA mounting medium (Zymed, San Francisco, CA). The GVA provided better preservation of ATPase stain results.

Images magnified (×150) with an Olympus photomicroscope were viewed and captured with a digital video processor. For each muscle compartment of each specimen, 750–1,200 fibers were counted and classified as type I or type IIa, using mATPase sections with 4.4 pH acid preincubation (Brooke and Kaiser, 1970), based on comparison with alkaline phosphatase preincubation results and serial NADH-TR and α-GPD reacted samples.

Using a digital imaging program (NIH Image, v. 1.61), the average cross-sectional area of each fiber type in each segment was determined by measuring 60 fibers of each. Cross sectional area between FG and SO fibers within segments was compared using a two-sample Student's *t*-test, with $P = 0.05$ considered significant (Table 3). Fiber cross sectional areas were compared between the compartments of the semispinalis muscle using a two-way ANOVA and Tukey's (HSD) multiple range test (Table 4) (Statistix for Windows, Analytical Software, Tallahassee, FL, 1996).

Fiber type in muscles and identified compartments within muscles were profiled in two ways. First, the percentage of type I (slow oxidative) and type II (fast glycolytic) fibers was calculated based on the fiber count for each segment. Second, to determine a functional cross-sectional profile the fiber count was multiplied by the mean cross-sectional area for each fiber type. The mean values from the three animals and standard deviations are pre-

TABLE 3. Fiber cross-sectional area of SS and SP

Cross-sectional fiber area: mean (SD) μm^2, n = 3

	SO[1]	FG[2]	SO	FG	
S-1	3,511 (1,328)	3,901 (1,020)*	3,081 (1,419)	2,924 (1,070)	S-2
S-3	2,420 (1,009)	2,810 (991)*	2,780 (1,193)	3,100 (1,349)*	S-4
S-5	3,296 (1,252)	3,300 (1,140)	2,786 (1,159)	2,772 (907)	S-6
S-7	2,582 (1,054)	2,450 (832)	2,825 (1,152)	2,954 (914)	S-8
sp	2,773 (1,253)	2,890 (896)			

[1,2]SO and FG are slow oxidative and fast glycolytic, respectively. Segments S-1, S-3, S-5, and S-7 are listed at the left, S-2, S-4, S-6, and S-8 at the right.
*Significant ($P = 0.05$) difference between mean area of slow and fast fibers within compartment.

sented in Tables 4 and 5. Statistical comparisons of fiber percentages between segments were not made because the sample size (three specimens) was too small to determine whether the distribution of values was normal (Gaussian).

RESULTS
Muscle Morphology and Architecture

The principal anatomical components of the dorsal neck in the horse are shown in Figure 1. The nuchal ligament originates from the first four thoracic dorsal spinous processes, runs cranioventrally, and inserts on the skull and cervical vertebrae. The SS muscle is oriented in the craniodorsal direction, originating on the cervical and cranial thoracic vertebrae and inserting on the nuchal crest of the skull (Fig. 1a). The SS is a complexly structured muscle, with multiple innervation and numerous connective tissue insertions. The muscle is pyramidal in shape, with the compartments narrowing toward the insertion on the skull. Cranially, there is a strong, focal tendon that spreads into a wide aponeurosis inserting on the skull. This tendon extends two-thirds the length of the muscle. The dorsal region consists of oblique bands, arranged concentrically (Fig. 2a), with relatively short fibers oriented parallel to the dorsum of the neck. Each of the dorsal compartments sends tendinous slips along the dorsal border to the insertion on the skull. The ventral region has longer fibers that pass from the central tendon to the articular processes of the cervical and thoracic vertebrae (C2–T4). Ventral and caudal to the tendon, fiber bundles are oriented between their cervical attachments and the central tendon, at angles becoming more acute cranially, giving an indirect line of action between the cervical vertebrae and the skull.

The different compartments of the SS, as defined by their tendinous inscriptions, appear to be innervated by the dorsal branches of their most proximal spinal nerves (Fig. 3). The same nerves, crossing the central tendon, innervate both the dorsal and ventral regions of the semispinalis muscle. One branch, composed of elements from spinal nerves C5 and T2, travels cranially along the deep surface of the muscle, parallel to the central tendon, toward the head, before arborizing in the dorsalmost parts of the muscle at the level of the fourth and fifth cervical vertebrae. The splenius muscle is innervated primarily by branches from spinal nerves C6, C7, and T2. These perforate through the semispinalis discretely at the cranial tendinous border of segment S-5, to the deep (medial) aspect of the splenius. A branch of C3 may be continuing on to innervate the cranial aspect of the rhomboideus cervicis muscle.

The equine splenius, lying lateral to the semispinalis capitis, is a simple muscle, with no tendinous inscriptions (Fig. 1b). Its caudal aponeurosis inserts, with that of the rhomboideus cervicis, onto the thoracodorsal fascia on the dorsal midline at the level of T3–T5, while its cranial aponeurosis joins with that of the longissimus capitis muscle (not shown) and inserts lateral to the nuchal crest. The fibers appear to span the entire length of the muscle and were found to be approximately 220 mm long in the animals studied. In contrast, the fibers seen in the SS are shorter and vary between compartments (Table 2). When SS fiber bundles were separated into individual muscle fibers, approximately 10% of muscle fibers were found to have tapered ends shorter than

TABLE 4. Fiber cross-sectional area of SS, comparison of all fibers within compartments

Cross-sectional fiber area: mean (SD) μm^2, n = 3

S-1	3,706 (1,198)[a]	3,005 (1,263)[b]	S-2
S-3	2,609 (1,017)[c,d]	2,940 (1,281)[b]	S-4
S-5	3,298 (1,195)[e]	2,779 (1,039)[b,c,d]	S-6
S-7	2,516 (951)[d]	2,886 (1,047)[b,c]	S-8

a, b, c, d, and e represent groups in which the means are not significantly different from one another. Rejection level = 0.05. Segments S-1, S-3, S-5, and S-7 are listed at the left, S-2, S-4, S-6, and S-8 at the right.

TABLE 5. Fiber type % of SS and SP: fiber count only

Percentage by count: mean (SD), n = 3

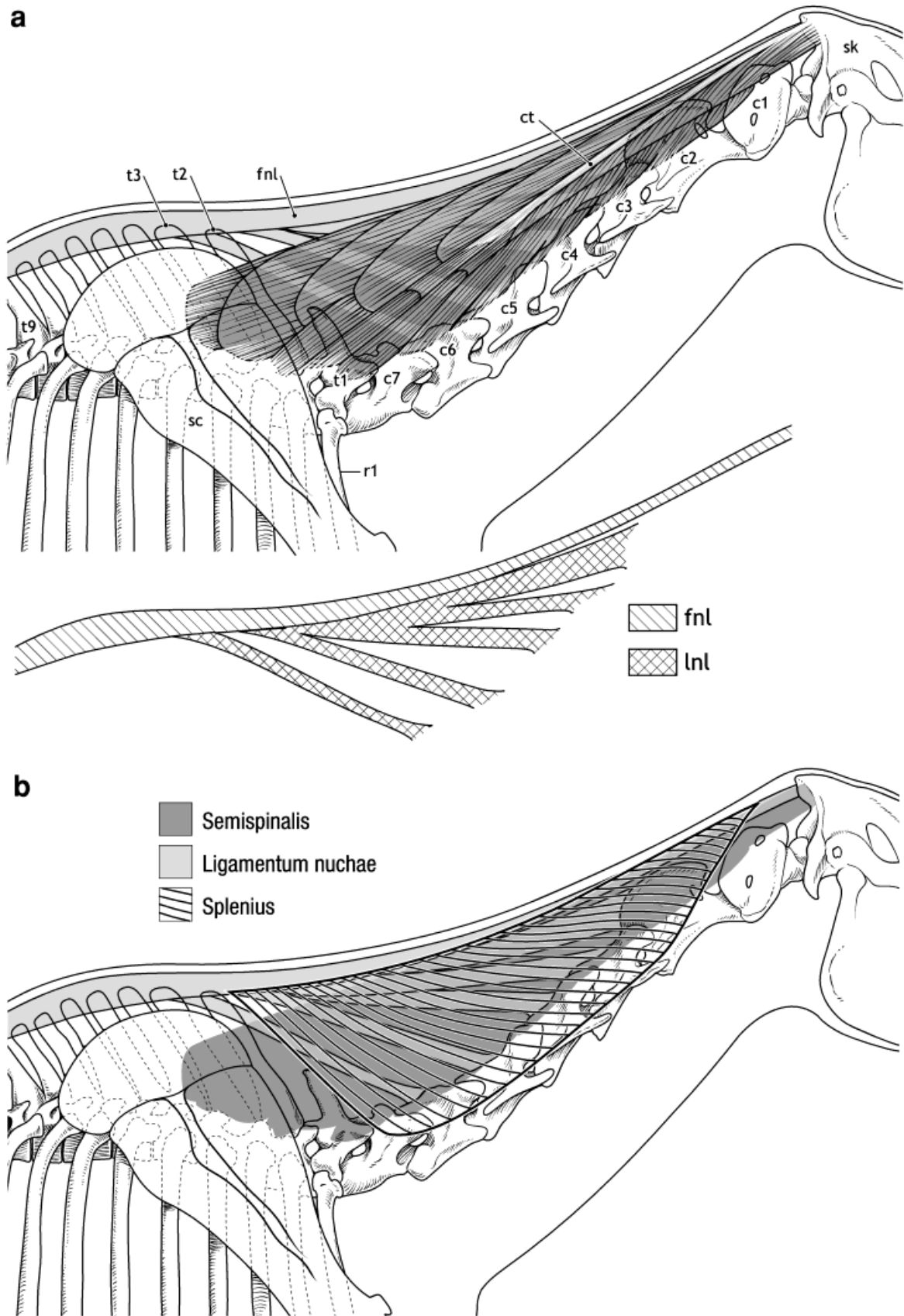|	SO	FG	SO	FG	
S-1	73.1 (17)	26.9	31.5 (14.5)	68.5	S-2
S-3	63.9 (10.7)	36.1	37.7 (7.4)	62.3	S-4
S-5	60.6 (5.5)	39.4	44.5 (13.8)	55.5	S-6
S-7	60.1 (5.9)	39.9	49.6 (5.8)	50.4	S-8
sp	61.7 (5.1)	38.3			

Fig. 1. Head and neck anatomy. **a**: Structures of the dorsal neck in an alert head position in *Equus caballus*: cranial thoracic and cervical vertebrae (solid lines), nuchal ligament, both funicular and lamellar (light gray shading), semispinalis capitis muscle (darker gray shading). Note central tendon and elaborate compartmentalization of semispinalis muscle. Muscle fiber direction in solid lines. ct, central tendon of semispinalis muscle; c1–c7, cervical vertebrae; fnl, funicular nuchal ligament; lnl, lamellar nuchal ligament; r, rib; sc, scapula; sk, skull; t1–t9, thoracic vertebrae. **b**: Splenius muscle overlay: note simple fiber architecture, more ventral and lateral vertebral attachments, and relationship to nuchal ligament and semispinalis muscle. Cranial and caudal attachments are by aponeurosis to lateral occipital crest and dorsal midline fascia respectively.
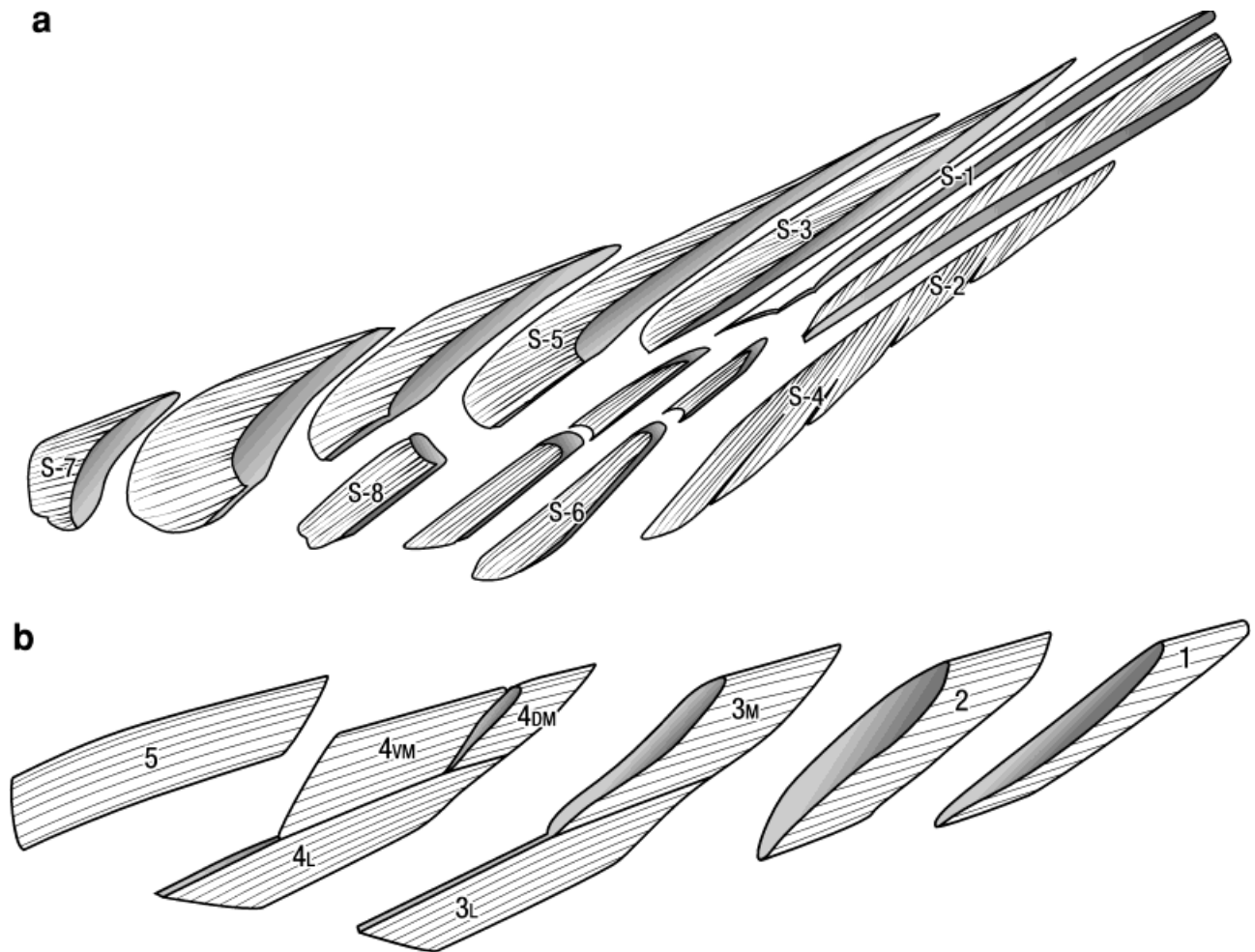
Fig. 2. Architecture of equine semispinalis capitis and feline biventer cervicis. **a**: Exploded view of right semispinalis capitis in *Equus caballus*. Individual compartments (segments), as defined by tendinous inscriptions, are separated in this view, to show fiber direction and obliquity. Compartments (S-1–S-8) are labeled as per histochemical sampling. Position is as seen in situ, in an alert head position, with the head on the right. Note the concentric dorsal compartments, with concavity toward the head. **b**: Exploded view of right biventer cervicis in *Felis cattus* (not to same scale as equine muscle). This muscle is analogous in cat to semispinalis capitis muscle in horse. Note multiple compartmentalization, pyramidal overall shape, and oblique compartment orientation. Dorsal region has concentric compartments, but concavity is away from the head. Ventral compartments (3L, 4L, 5) have longer fibers and are oriented toward vertebral attachments at cervicothoracic junction. (From Richmond and Armstrong, 1988.)

the fiber bundles. All tapered ends were located halfway between their segment's tendinous insertions, although the fiber bundles were different lengths between segments.

Muscle Histochemistry

The SS and SP in the adult horse are composed of only two muscle fiber types (Fig. 4). Type SO fibers stained positively after acidic preincubation and mATPase reactions but negatively after alkaline preincubation and mATPase reactions. The type SO fibers reacted strongly for NADH-TR but not for α-GPD. In contrast, type FG fibers reacted strongly after alkaline preincubation and mATPase reactions and after reaction for α-GPD. These FG fibers were nonreactive after acidic preincubation and mAT-

Pase reactions and were weakly stained following the NADH-TR reactions. All classifications were in agreement with our results for rat diaphragm fibers (which include type SO and FOG fibers) as well as other equine muscles studied. Some of the longer-fibered segments in the SS (S-4, S-6) show a few intermediate (IIa/FOG) fiber types, but the number of these cells was not statistically significant, representing less than 1% of all fibers observed.

Using the SS central tendon to delineate six ventral and seven dorsal divisions, eight functional regions were chosen to represent the muscle for fiber typing (Fig. 2a). The most cranial segments, S-1 (dorsal) and S-2 (ventral), are located immediately caudal to the attachment on the skull. S-7 (dorsal) and S-8 (ventral), are from the most caudal muscle compartments, near T-2 and the cervical–thoracic
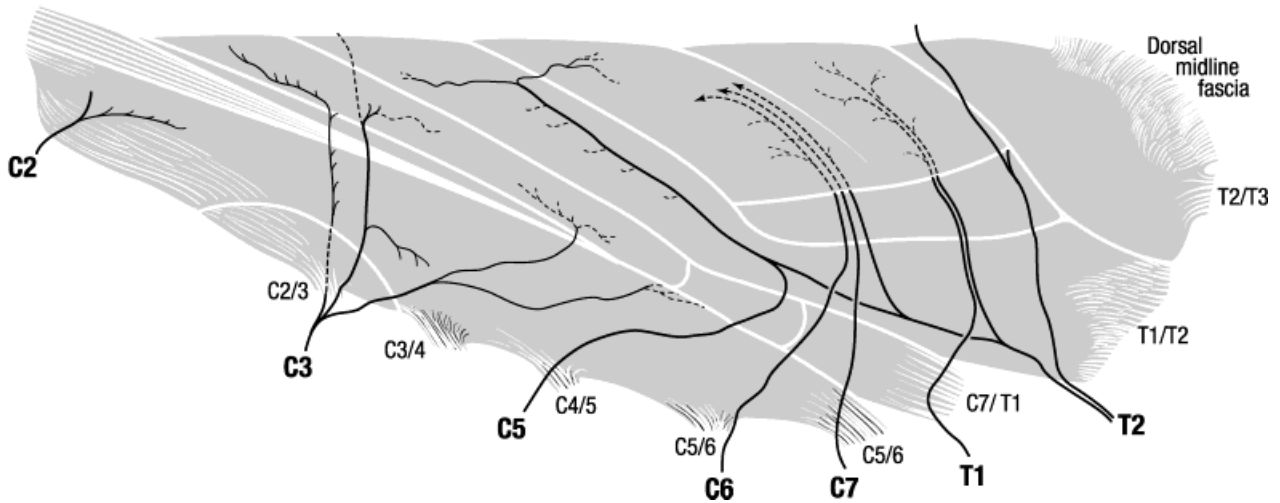
Fig. 3. Schematic view of semispinalis capitis innervation. Medial view of right semispinalis capitis muscle in *Equus caballus*, showing pathways of cervical spinal nerves arborizing through the muscle compartments. Position is ex vivo, and so more contracted than for in situ view. Most regions are innervated by their most proximal spinal nerve, labeled (in bold) as C2 through T2. Branches from C3 and T2 exit the muscle dorsally, presumably to innervate the rhomboideus cervicis muscle. A conjoined branch from C6, C7, and T2 emerges on the lateral surface to innervate the splenius muscle. In this specimen, spinal nerve C4 was not observed.

junction, respectively. The intermediate pairs of compartments are spaced relatively equidistant along the length of the muscle. Splenius samples were taken primarily from the center of the muscle belly, but several additional samples were taken from the caudal and cranial and the superficial and deep extremes to evaluate whether there were regional differences in fiber type composition, as seen in the feline splenius (Richmond and Abrahams, 1975). No apparent differences in fiber type percent-

ages were observed between the different parts of the splenius muscle. Table 3 gives the average cross-sectional areas for each muscle type in each SS segment and the splenius. Differences in fiber areas between fast and slow fibers are seen only in compartments 1, 3, and 4. Table 4 compares the mean cross-sectional areas (combining FG and SO fiber areas) between compartments. No meaningful pattern in the grouping of the means was observed. This may be due, in part, to the small number of
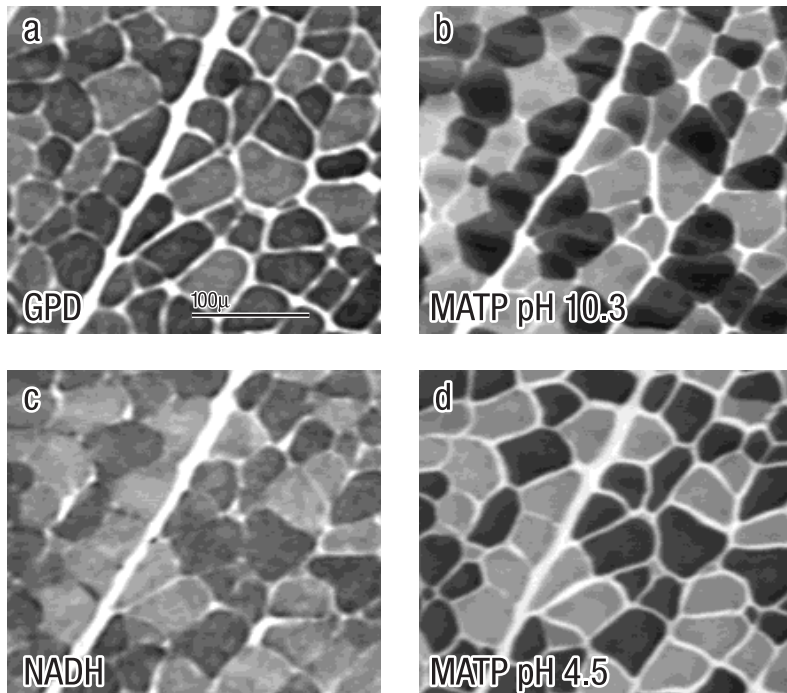

Fig. 4. Comparative histochemistry (GPD, NADH, acid mATPase, basic mATPase). Serial sections cut from semispinalis compartment S-5 in *Equus caballus*, with differing histochemical treatments as labeled. Note only two fiber types: slow oxidative and fast glycolytic. Slow fibers are darker in GPD (**a**) and myosin (**b**) ATPase pH 10.3, lighter in NADH (**c**) and myosin (**d**) ATPase pH 4.5. Scale bar in GPD = 100 µm.

*TABLE 6. Fiber type % of SS and SP: fiber
count × cross-sectional area*

Percentage by area: mean (SD), n = 3

	SO	FG	SO	FG	
S-1	70.8 (16.4)	29.2	33.0 (17.7)	67.0	S-2
S-3	60.8 (9.6)	39.2	34.6 (9.3)	65.4	S-4
S-5	60.1 (0.5)	39.9	44.1 (13.1)	55.9	S-6
S-7	57.8 (7.9)	42.2	52.1 (5.4)	47.9	S-8
sp	59.4 (2.9)	40.6			

animals and large standard deviation found in the mean cell area for each compartment. Tables 5 and 6 show the fiber type distribution of the eight SS regions and the SP as percentages of the total counted fibers and as percentages of the functional cross-sectional area of the muscles, respectively. It can be seen that the percentage of SO fibers increases cranially in the dorsal region and decreases cranially in the ventral region (Fig. 5). This creates two opposing gradients of fiber types between the dorsal and ventral region. Although statistical comparisons of fiber type distribution between segments would be inappropriate with a sample size of three, all of the animals examined (including several not reported here because of incomplete regional sampling) showed a clear difference between fiber type profiles in the dorsal and ventral regions of the SS muscle, and all showed the trend to increase SO fibers in the dorsal cranial part of the muscle and decrease SO in the ventral cranial region.

DISCUSSION

The histochemistry and morphology of the equine cervical epaxial muscles are unusual. Most adult mammalian skeletal muscle is composed of at least three fiber types: slow-twitch, oxidative (SO); fast-
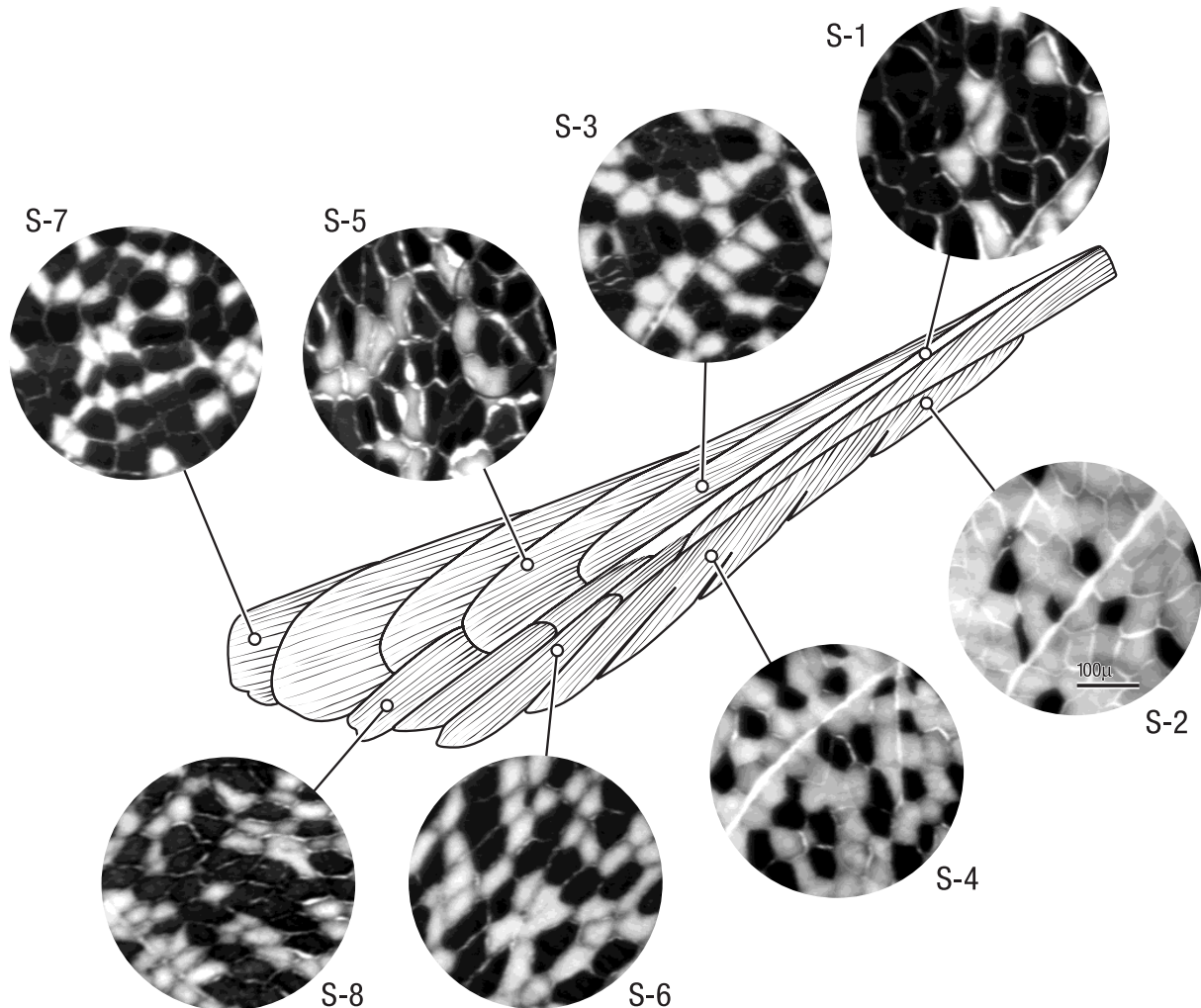

Fig. 5. Semispinalis schematic histochemistry. Graphical representation of semispinalis capitis histochemistry in *Equus caballus*. All sections are mATPase, acid preincubation at pH 4.5. Note dorsal compartments (1, 3, 5, 7) demonstrate an increasing gradient of slow fibers (dark) toward the head (right), and ventral compartments (2, 4, 6, 8) show an increasing gradient of fast (light) fibers toward the head. See Tables 5 and 6 for percentages of fast and slow fibers by compartment. Scale bar = 100 μm.

twitch oxidative/glycolytic (FOG); and fast-twitch glycolytic (FG). In most locomotory muscles, a variety of fiber types allows flexibility of function, in terms of velocity and force production. This would allow the animal to optimize either for speed or economical usage, depending on the situation (Rome et al., 1988). Myofibers are found in differing proportions depending, presumably, on the function of the individual muscle. Other constraints, such as ontogeny, evolution, or neural patterning may also play an important role in determining the relationship between the motor unit and a muscle's fiber type composition (Burke and Edgerton, 1975; Burke, 1981). The existence of these unusual two-fiber type muscles in the horse may be an extreme adaptation to their comparatively large size and speed. However, contraction activity in vivo can be modified by other means than the physiologic capabilities of fiber type (Herzog, 2000). It may be that neural recruitment and compartmentalization play a larger role than previously assumed in determining functional capability for these equine muscles.

The morphology of these muscles is clearly relevant to their function as well. The traditional interpretation of muscle function, as seen in single plane rotation (hinge-type) joints, has been that the contracting muscle pulls a tendon attached to the far side of a joint, applying a moment to the joint and causing a rotation in its position, often changing the orientation of the distal segment. Several criteria are necessary for this functionality: the muscle must contract quickly enough to move the joint within the required time frame for limb advancement, it must be able to achieve a length change greater than the tendon's length change (i.e., uncrimping of collagen fibers plus strain), and the tendon attaching the muscle to the distal bone must be stiff enough to transmit the tensile force generated by the muscle (Biewener, 1998). This ultimately generates power for locomotion. However, many muscle–tendon complexes do not fit this power transmission paradigm. Although all vertebrate tendons are made of similar collagenous material, their behavior in vivo is defined by both material and structural properties together: long thin tendons are more compliant than short thick ones (Wainwright et al., 1982; Proske and Morgan, 1987; Bertram and Marsh, 1998). Some multipinnate muscles, such as the forelimb superficial digital flexor muscle in the horse, have extremely short, slow fibers and long, compliant tendons. The contraction distance of these short fibers, sometimes less than 5 mm long, is less than the uncrimping of the long tendon under load, and much less than the 5–10% strain of the fully loaded tendon. This muscle design might be ineffective for moving a joint, but could economically produce force to resist and modulate the stretch of its associated tendon, storing elastic strain energy to be released in a later part of the gait cycle (Cavagna et al., 1977; Alexander and Bennet-Clarke, 1977; Dimery et al.,

1986; Alexander, 1988; Wilson et al., 1991; Biewener and Baudinette, 1995; Bertram and Marsh, 1998).

Unlike humans, whose heads are on top of a spinal column, quadrupeds have a horizontal spinal orientation from which the head and neck extend, like a cantilevered beam. The bending of the spinal beam under gravitational forces creates an opportunity to store elastic strain energy in the soft and bony tissues of the spine (Alexander et al., 1980). The structural anatomy of the cervical region is dominated by three types of tissues: bony elements (vertebrae and skull), connective tissue (the elastic nuchal ligament), and muscle (the dorsal cervical musculature). While the rigid bony elements and the elastic nuchal ligament offer passive support for the weight of the head and neck, it has been shown that passive support alone can not account for the range of head movement observed during locomotion (Dimery et al., 1985; Gellman and Bertram, 2002b). The anatomic position of both the semispinalis and splenius muscles is suitable for raising or resisting lowering the head.

In the horse, despite parallel, redundant orientation and complex architecture, the histochemical profile of the SS and SP muscles suggests that they are not easily categorized as force-producing and power-generating muscles, respectively. The splenius, with its simple, strap-like architecture and long fibers seems morphologically designed to be a power-producing muscle, functioning to raise the head during locomotory oscillations. However, we find that it is predominantly composed of slow oxidative fibers (59%), implying that postural support is also a large part of its function. Its lateral position, as well as its more lateral attachment on the skull, may increase its effectiveness for unilateral head maneuvers, as seen in the cat (Richmond et al., 1992).

Being the most superficial of the dorsal cervical muscles, the equine splenius has been studied in vivo with transcutaneous and surface electromyography (Tokuriki and Aoki, 1991; Robert et al., 1998). These studies suggest several functional characteristics. First, a focal motor point is described (Robert et al., 1998), where a single strong signal was found by back stimulation for surface placement of their electrodes on the splenius muscle. This suggests that, consistent with its simple architecture, splenius activation is simultaneous for the entire muscle. Second, it was found (Tokuriki and Aoki, 1991) that the splenius has tonic bilateral activity in the standing animal, confirming its postural support role, presumably dominated by slow oxidative muscle fiber activity.

During locomotion, it was found that the splenius exhibits bilateral activity during each forelimb stance (Tokuriki and Aoki, 1991; Robert et al., 1998). Kinematic data for walking and trotting standardbreds (Gellman and Bertram, 2002b) show that the head and neck are at their lowest position in the

oscillation cycle at mid-stance for each forelimb placement. Splenius activation, at the walk, typically begins before the head oscillation minima, and continues until after the head has begun rising. At the trot, the signal begins at the instant of foot contact and stops at mid-stance (Tokuriki and Aoki, 1991). On this basis, it seems likely that the muscle is acting to decelerate the head/neck complex, which is falling passively from gravity and the inertia of the vertical motion as the direction of travel changes during the foot contact oscillation.

For this deceleration, it might be possible to utilize an isometric, holding contraction, since this could maximize the muscular force available for decelerating the head/neck complex, according to the muscle force–velocity curve (Hill, 1922). Since in this circumstance the muscle simply resists applied load and does not need to shorten, velocity of contraction would be irrelevant and slow twitch muscle fibers could be used. This would be an economical strategy, since myosin cross-bridges do not have to be broken and reformed in isometric contraction. Once the head and neck are decelerated, the strain energy stored in the nuchal ligament (and perhaps the semispinalis muscle as well) helps to re-elevate the system in the second half of the oscillation. Analysis of the mechanical work done at the cervicothoracic joint during head/neck oscillations indicates that the transition between downward and upward motion requires the greatest power input (Gellman and Bertram, 2002b).

The splenius may also be stiffening and stabilizing the neck from the impact of the ground reaction forces. Changing the stiffness of the neck may be especially important at the trot, since the bouncing oscillation frequency of the body may exceed the natural frequency of the head and neck system in its normal configuration, causing an interference harmonic. The role of natural frequency and resonance in locomotion will be discussed further with regard to the function of the semispinalis.

Less detailed information is available in the literature regarding splenius activity at the gallop. It appears that the activity is asymmetrical in duration between sides, consistent with the asymmetric nature of the limb activity at the gallop (Tokuriki and Aoki, 1991). The splenius is active during the entire stance phase and portions of swing on the side of the trailing forelimb, but only fires from mid-swing to mid-stance on the side of the leading forelimb. The transition between the lowering and the raising phase of the head/neck oscillation cycle takes place within both these periods, so it is possible that the splenius is decelerating the head/neck complex at the gallop also.

The morphology and histochemistry of the equine semispinalis capitis imply differential function between its dorsal and ventral regions, and possibly between its caudal and cranial regions as well. This would be consistent with functional studies of the feline biventer cervicis and anterior sartorius, where a single muscle activation can result in shortened fibers cranially and lengthened fibers caudally (Armstrong et al., 1988; Scott et al., 1992). In cadaver horses, ex vivo, a large length change can be observed in the SS central tendon as the neck is manipulated to a lowered position. As seen in Figure 5, the muscle fibers closest to the skull attachment have the highest percentage of slow oxidative fibers. Segment S-1, which is located within the curve of the tendon itself, had 92% slow fibers in one specimen. The fiber length also becomes shorter in the dorsal regions closer to the skull. This combination of long, narrow tendon, slow contractile activity, and short fiber length suggests a muscle designed to facilitate energy storage in the tendon, rather than to generate power: the short fibers cannot contract sufficiently to transmit tension along the tendon to power joint movement. In this way, the dorsal aspect of the SS could contribute to elastic strain energy storage, similar to the passive nuchal ligament that is stretched when the head is lowered and returns strain energy to help raise the head.

There has been a great deal of interest generated in recent years by muscles with in-series architecture, whether the fibers taper interfascicularly or are separated by horizontal septae (Bodine et al., 1982; English and Weeks, 1987; Loeb et al., 1987; Trotter, 1990; Gaunt and Gans, 1992; Heron and Richmond, 1993; Westneat et al., 1993; Roy et al., 1995). The lack of single contractile units spanning from origin to insertion raises the question of how force is transmitted across the muscle. For muscles whose in-series fibers terminate interfascicularly, such as the human sartorius or gracilis, it is suggested that shear forces across the endomesial connective tissue of the tapered ends play an important role. However, examples of connective tissue septae oriented horizontally or obliquely to the origin/insertion line are found in many muscles as well: the feline splenius and biventer cervicis, the equine SS, and the rectus abdominus of many species. It is also a pervasive morphological feature of fish myomeres. Connective tissue divisions can facilitate neuromuscular compartmentalization for differential function along a long muscle, such as the cranial and caudal regions of the long neck muscles. Or, in the fish locomotion model, in-series compartments can be an integral part of the undulatory locomotion cycle. Alternatively, interfascicular terminations in a long muscle might allow for faster synchronous activation along the long axis of the muscle by splitting it into smaller conduction units (Armstrong et al., 1988; Heron and Richmond, 1993).

Another possibility is that the dorsal muscle region of the SS could be morphologically analogous to the concentrically arranged compartments of fish myomeres. Recent studies have suggested that the myomeres play an important role in swimming locomotion by modulating the stiffness of the fish's body,

matching the natural frequency of the body axis oscillations to the desired swimming velocity (McHenry et al., 1995; Long and Nipper, 1996; Long, 1998). This allows the fish to exploit its body morphology and environment while using metabolic resources economically. Although the head/neck oscillations of the horse represent a very different type of mechanical system, the overall harmonics of the animal's body and its interaction with the ground during locomotion are still an essential factor in musculoskeletal design. Different gaits will require oscillations of varying frequencies, and it is likely to be biomechanically advantageous to "tune" the stiffness of the system to the optimal frequency. Recent work on limb muscle mechanics has suggested that the interaction of muscle harmonics with ground reaction forces may play a larger role in locomotion than previously thought (Nigg-Benno and Liu-Wen, 1999; Wilson et al., 1991; Blickhan et al., 2000; Wilson and McGuigan, 2000).

The ventral compartments of the SS appear to have a different function. The long fibers and predominantly fast twitch fiber type of the cranial region are consistent with our expectations of a power generating muscle. The line of force, however, is puzzling. Each compartment attaches on the central tendon and inserts on the articular processes of sequential cervical vertebrae. With the neck in an upright position, there does not seem to be any point to shortening these muscle fibers, since the body of the neck cannot compress. However, if the neck is lowered, as for feeding from the ground, the central tendon will be maximally stretched. In this configuration, the ventral muscle segments align with the central tendon and the cervical spine, and active shortening would help raise the neck and head.

Functional in vivo studies are needed to verify these suggested functions during locomotion and other head movement. Although the SS is deep to the splenius, the simple architecture of the SP, and the discrete tendinous bands of the SS, make it possible to locate different semispinalis compartments for insertion of EMG electrodes and sonomicrometer crystals using diagnostic ultrasound.

The importance of an individual muscle's architecture to its functional activity is a subject of great interest to muscle biologists (Gans and Bock, 1965; Gans and Gaunt, 1991; Richmond, 1998). Many highly pinnate muscles have been previously classified "postural" muscles, because their predominantly SO fibers were not thought capable of the fast repetitive motions required by gait activity. Recent work indicates that the contractile properties of muscle fibers in dynamic circumstances may differ substantially from the assumed properties based on observation of the static muscle (Herzog, 2000). This can extend even to the case where the dynamic activity at the fiber level can be paradoxical to the whole muscle behavior. These "postural" muscles have the capacity to play an important role in loco-

motion through interactions with passive musculoskeletal elements. It is critically important for the horse to limit its investment of metabolic energy in locomotion because the mass-specific metabolic rate is less for large animals than small ones (Kleiber, 1932). This limitation makes it difficult for larger animals to deliver adequate metabolic energy to their limbs to directly power locomotion. It has been shown that for large, fast animals, aerobic muscle power can only account for a small fraction of the mechanical work performed at higher velocities (McMahon, 1984; Taylor, 1994; Minetti et al., 1999). It must be assumed that other energy-saving mechanisms, such as elastic strain energy storage and release from associated structures, are contributing the remainder, allowing these animals to travel rapidly over long distances.

The phenomena of parallel and seemingly redundant muscle systems is not uncommon in biological design. We believe it represents both a safety factor (in case of injury) and an optimization for economical usage of energy resources, which is a critical factor for large, cursorial animals. Grouping these muscle tendon complexes together can provide greatly enhanced functional versatility overall, while streamlining individual muscle design.

We have found that the equine dorsal cervical musculature appears to have multiple functional capabilities, contributing to postural support, locomotory oscillations, and head positioning. The splenius, in addition to providing static postural support and turning the head, functions during locomotion to resist lowering of the head/neck complex. The multiple neuromuscular compartmentalization of the semispinalis capitis allows for differential function between its dorsal and ventral regions. The semispinalis exhibits a unique gradient of fiber types, where SO fibers become more concentrated cranially in the dorsal regions and less concentrated cranially in the ventral ones. We interpret this as indicating that the dorsal region of the semispinalis capitis provides passive support, stiffening, and modulation of the central tendon's spring-like qualities, while the ventral region is capable of generating muscular power to raise the head from a lowered position.

ACKNOWLEDGMENTS

LITERATURE CITED

Alexander RMcN. 1988. Elastic energy stores in running vertebrates. Am Zool 24:85–94.

Alexander RMcN, Bennet-Clark HC. 1977. Storage of elastic strain energy in muscle and other tissues. Nature 265:114–117.

Alexander RMcN, Dimery NJ, Ker RF. 1980. Elastic structures in the back and their role in galloping in some mammals. J Zool Lond (A) 207:467–482.

Armstrong JB, Rose PK, Vanner S, Bakker GJ, Richmond FJR. 1988. Compartmentalization of motor units in the cat neck muscle, biventer cervicis. J Neurophysiol 60:30–45.

Bertram JEA, Marsh RL. 1998. Introduction to the symposium: muscle properties and organismal function: shifting paradigms. Am Zool 38:697–703.

Biewener AA. 1998. Muscle function in vivo: a comparison of muscles used for elastic energy savings versus muscle used to generate mechanical power. Am Zool 38:703–717.

Biewener AA, Baudinette RV. 1995. In vivo muscle force and elastic energy storage during steady-speed hopping of tammar wallabies (*Macropus eugenii*). J Exp Biol 98:1829–1841.

Blickhan R, Meier P, Seyfarth A, Wagner H. 2000. Properties of the muscle tendon unit and system dynamics (Abstract). Proc Soc Exp Biol, March 2000.

Bodine SC, Roy RR, Meadows DA, Zernicke RF, Sacks RD, Fournier M, Edgerton VR. 1982. Architectural, histochemical and contractile characteristics of a unique biarticular muscle: the cat semitendinosis. J Neurophysiol 48:192–201.

Brooke MH, Kaiser KK. 1970. Three "myosin adenosine triphosphatase" systems: the nature of pH lability and sufhydryl dependence. J Histochem Cytochem 18:670–672

Buchner HHF, Savelberg HH, Schamhardt HC, Barneveld A. 1997. Inertial properties of Dutch warmblood horses. J Biomech 30:653–658.

Burke RE. 1981. Motor units: anatomy, physiology, and functional organization. In: Brookhart JM, Mountcastle VB, editors. Handbook of physiology. Bethesda, MD: Am Physiol Soc section 1. The nervous system, vol 2. Motor control, part 2, p 345–422.

Burke RE, Edgerton VR. 1975. Motor unit properties and selective involvement in movement. Exerc Sport Sci Rev 3:31–81.

Cavagna GA, Heglund NC, Taylor CR. 1977 Mechanical work in terrestrial locomotion, two basic mechanisms for minimizing energy expenditure. Am J Physiol 233:R243–R261.

Dimery NJ, Alexander RMcN, Deyst KA. 1985. Mechanics of the ligamentum nuchae of some artiodactyls. J Zool Lond (A) 206: 341–351.

Dimery NJ, Alexander RMcN, Ker RF. 1986. Elastic extension of leg tendons in the locomotion of horses *(Equus caballus)*. J Zool Lond (A) 210:415–425.

Dyce KM, Sack WO, Wensing CJ. 1996. Textbook of veterinary anatomy. Philadelphia: WB Saunders.

English AW, Weeks OI. 1987. An anatomical and functional analysis of cat biceps femoris and semitendinosis muscles. J Morphol 191:161–175.

Gans C, Bock WJ. 1965. The functional significance of muscle architecture: a theoretical analysis. Ergeb Anat Entwick 38: 115–142.

Gans C, Gaunt AS. 1991. Muscle architecture in relation to function. J Biomech 24:53–65.

Gaunt AS, Gans C. 1992. Serially arranged myofibers: an unappreciated variant in muscle architecture. Experimentia 48: 864–868.

Gellman KS, Bertram JEA. 2002a. The equine nuchal ligament. 1. Structural and material properties. Vet Comp Orthop Traum (in press).

Gellman KS, Bertram JEA. 2002b. The equine nuchal ligament. 2. Passive dynamic energy exchange in locomotion. Vet Comp Orthop Traum (in press).

Getty R (ed.). 1975. Sisson and Grossman's the anatomy of the domestic animals, 5th ed. Vol. 2. Philadelphia: WB Saunders.

Heron MI, Richmond FJR. 1993. In-series fiber architecture in long human muscles. J Morphol 216:35–45.

Herzog W. 2000. In vivo mechanics and function of selected cat hindlimb muscles during locomotion and movement (Abstract). Proc Soc Exp Biol, March 2000.

Hill AV. 1922. The maximum work and mechanical efficiency of human muscles and their most economical speed. J Physiol 56:19–41.

Kleiber M. 1932. Body size and metabolism. Hilgardia 6:315–353.

Loeb GE, Pratt CA, Chanoud CM, Richmond FJR. 1987. Distribution of innervation of short interdigitated muscle fibers in parallel fibered muscles of the cat hindlimb. J Morphol 191: 1–15.

Long JH Jr. 1998. Body stiffness in swimming eels. Am Zool 38:771–792.

Long JH Jr, Nipper KS. 1996. The importance of body stiffness in undulatory propulsion. Am Zool 36:678–694.

McHenry MJ, Pell CA, Long JH Jr. 1995. Mechanical control of swimming speed: stiffness and axial wave form in undulating fish models. J Exp Biol 198:2293–2305.

McMahon TA. 1984. Muscles, reflexes and locomotion. Princeton, NJ: Princeton University Press.

Minetti AE, Ardigo LP, Reinach E, Saibene F. 1999. The relationship between mechanical work and energy expenditure of locomotion in horses. J Exp Biol 202:2329–2338.

Nigg-Benno M, Liu-Wen. 1999. The effect of muscle stiffness and damping on simulated impact force peaks during running. J Biomech 32:849–856.

Novikoff AB, Shin W, Drucher J 1961. Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. J Biophys Biochem Cytol 9:47–61.

Pellionisz AJ, Peterson BW. 1988. A tensorial model of neck motor activation. In: Peterson BW, Richmond FJR, editors. Control of head movement. New York: Oxford University Press. p 178–186.

Proske U, Morgan DL. 1987. Tendon stiffness: methods of measurement and significance for the control of movement. A review. J Biomech 20:75–82.

Richmond FJR. 1998. Elements of style in neuromuscular architecture. Am Zool 38:729–742.

Richmond FJR, Abrahams VC. 1975. Morphology and enzyme histochemistry of dorsal muscles of the cat neck. J Neurophysiol 38:1313–1321.

Richmond FJR, Armstrong JB. 1988. Fiber architecture and histochemistry in the cat neck muscle, biventer cervicis. J Neurophysiol 60:46–59.

Richmond FJR, Bakker DA. 1982. Anatomical organization and sensory receptor content of soft tissues surrounding upper cervical vertebrae in the cat. J Neurophysiol 48:49–61.

Richmond FJR, Scott DA, Abrahams VC. 1978. Distribution of motoneurons to the neck muscles, biventer cervicis, splenius and complexus in the cat. J Comp Neurol 181:451–464.

Richmond FJR, MacGillis DRR, Scott DA. 1985. Muscle-fiber compartmentalization in cat splenius muscles. J Neurophysiol 53:868–885.

Richmond FJR, Thomson DB, Loeb GE. 1992. Electromyographic studies of neck muscles in the intact cat. I. Patterns of recruitment underlying posture and movement during natural behaviors. Exp Brain Res 88:41–58.

Robert C, Valette JP, Denoix JM. 1998. Surface electromyographic analysis of the normal horse locomotion: a preliminary report. In: Lindnor A, editor. Conference on Equine Sports Medicine and Science.

Rome LC, Funke RP, Alexander RMcN, Lutz G, Aldridge H, Scott F, Freadman M. 1988. Why animals have different muscle fiber types. Nature 335:824–827.

Roy RR, Garfinkel A, Ounjian M, Payne J, Hirahara A, Hsu E, Edgerton VR. 1995. Three dimensional structure of cat tibialis anterior motor units. Muscle Nerve 18:1187–1195.

Scott SH, Thomson DB, Richmond FJR, Loeb GE. 1992. Neuromuscular organization of feline anterior sartorius. II. Intramuscular length changes and complex length-tension relationships during stimulation of individual nerve branches. J Morphol 213:171–183.

Taylor CR. 1994. Relating mechanics and energetics during exercise. In: Jones JH, editor. Comparative vertebrate exercise physiology: unifying physiological principles. San Diego: Academic Press. p 182–213.

Tokuriki M, Aoki O. 1991. Neck muscle activity in horses during locomotion with and without a rider. In: Persson, S, Lindholm A, Jeffcott L, editors. Equine exercise physiology 3. Davis, CA: ICEEP Publications. p 146–150.

Trotter JA. 1990. Interfiber tension transmission in series fibered muscles of the cat hindlimb. J Morphol 206:351–361.

Wainwright SA, Biggs WD, Curry JD, Gosline, JM. 1982. Mechanical design in organisms. Princeton, NJ: Princeton University Press.

Wattenberg LW, Leong JL. 1960. Effects of coenzyme Q_{10} and menadione on succinate dehydrogenase activity as measured by tetrazolium salt reduction. J Histochem Cytochem 8:296–303.

Westneat MW, Hoese WH, Pell CA, Wainwright SA. 1993. The horizontal septum: mechanisms of force transfer in locomotion of scombrid fishes (Scombidae, Pericformes). J Morphol 217: 183–204.

Wilson AM, McGuigan MP. 2000. The role of the digital flexor muscles in the equine limb (Abstract). Proc Soc Exp Biol, March 2000.

Wilson GJ, Wood GA, Elliott BC. 1991. Optimal stiffness of series elastic components in stretch-shorten cycle activity. J Appl Physiol 70:825–833.