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RESEARCH ARTICLE

Frances J.R. Richmond · Kan Singh
Brian D. Corneil

Marked non-uniformity of fiber-type composition in the primate suboccipital muscle obliquus capitis inferior

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Abstract Obliquus capitis inferior (OCI) is a monoarticular suboccipital muscle linking the transverse process of the atlas (C1) to the spinous process of the axis (C2). Histochemical analysis of fiber-type composition showed that the muscle has a marked gradient of fiber-type distribution in which type I fibers comprise 95–100% of fibers in the deepest region but less than 10% of fibers in the superficial layer. Step-like changes in fiber-type proportions occurred between groups of fascicles. In most instances the boundaries between these fascicles did not exhibit different perimysial features from those fascicles with similar fiber-type proportions. OCI contained large numbers of muscle spindles, which were concentrated in deep regions rich in type I fibers. The degree of nonuniformity in fiber-type distribution seen in OCI is unusually large when compared with patterns described in other primate muscles, and has implications for the way that the muscle is studied anatomically and physiologically.

Key words ATPase · Neck · Rhesus monkey · Muscle spindle · Compartmentalization

Introduction

The functional properties of a mammalian muscle are dictated in large part by its fiber composition. Most mammalian muscles contain a heterogeneous mixture of fiber types, whose relative proportions are thought to reflect the overall glycolytic and oxidative capabilities of the muscle. Thus the relative proportions of the three main fiber types present in muscle (type IIb, equivalent to fast-glycolytic fibers, type IIa or fast-oxidative glycolytic fibers, and type I or slow-oxidative fibers) are often estimated to gain insight into the relative roles of different muscles in phasic or tonic motor activities (Burke 1981). However, the intramuscular distribution of different fiber types may not be uniform. In feline and rodent muscles, where most detailed studies have been carried out, gradients of fiber-type distribution are often found in complex or compartmentalized muscles (e.g., Gonyea and Ericson 1977; English and Letbetter 1982; Balice-Gordon and Thompson 1988), and even simple muscles can have higher concentrations of fast fibers on one or more muscle edges (e.g., Richmond and Abrahams 1975; Selbie et al. 1993).

The distribution of fiber types in primate muscles has not been studied as systematically as in quadrupedal species. Studies in human muscles have generally relied on small biopsy samples taken from a restricted muscle region (e.g., Bagnall et al. 1983; Ford et al. 1986; Simoneau and Bouchard 1989). Such an approach is appropriate only when muscles have an approximately even distribution of fiber types across their breadth and width. In the few monkey and human muscles that have been examined more extensively (e.g., McIntosh et al. 1985; Acosta and Roy 1987), varying degrees of nonuniformity in fiber-type composition have been reported. For example, little compartmentalization of fiber types has been identified in extrinsic finger muscles of monkeys (Maurer et al. 1995), but significant nonuniformity of fiber-type distribution was seen in two parts of flexor carpi ulnaris that were separated by a central tendon (McIntosh et al. 1985). Nevertheless, in most primate muscles studied to date, differences in proportions were relatively modest; all fiber types were represented, albeit in different relative proportions in different muscle parts. In this paper we report a much steeper gradient of fiber-type distribution in a small suboccipital muscle with no gross anatomical indications of compartmentalization. The presence of such a large gradient has methodological implications for anatomical and electromyographic studies.

F.J.R. Richmond (✉) · K. Singh · B.D. Corneil
MRC Group in Sensory-Motor Neuroscience,
Department of Physiology, Queen's University,
Kingston, Ontario, Canada K7L 3N6
e-mail: fjr@biomed.queensu.ca
Tel.: +1-613-545-2806, Fax: +1-613-545-6840

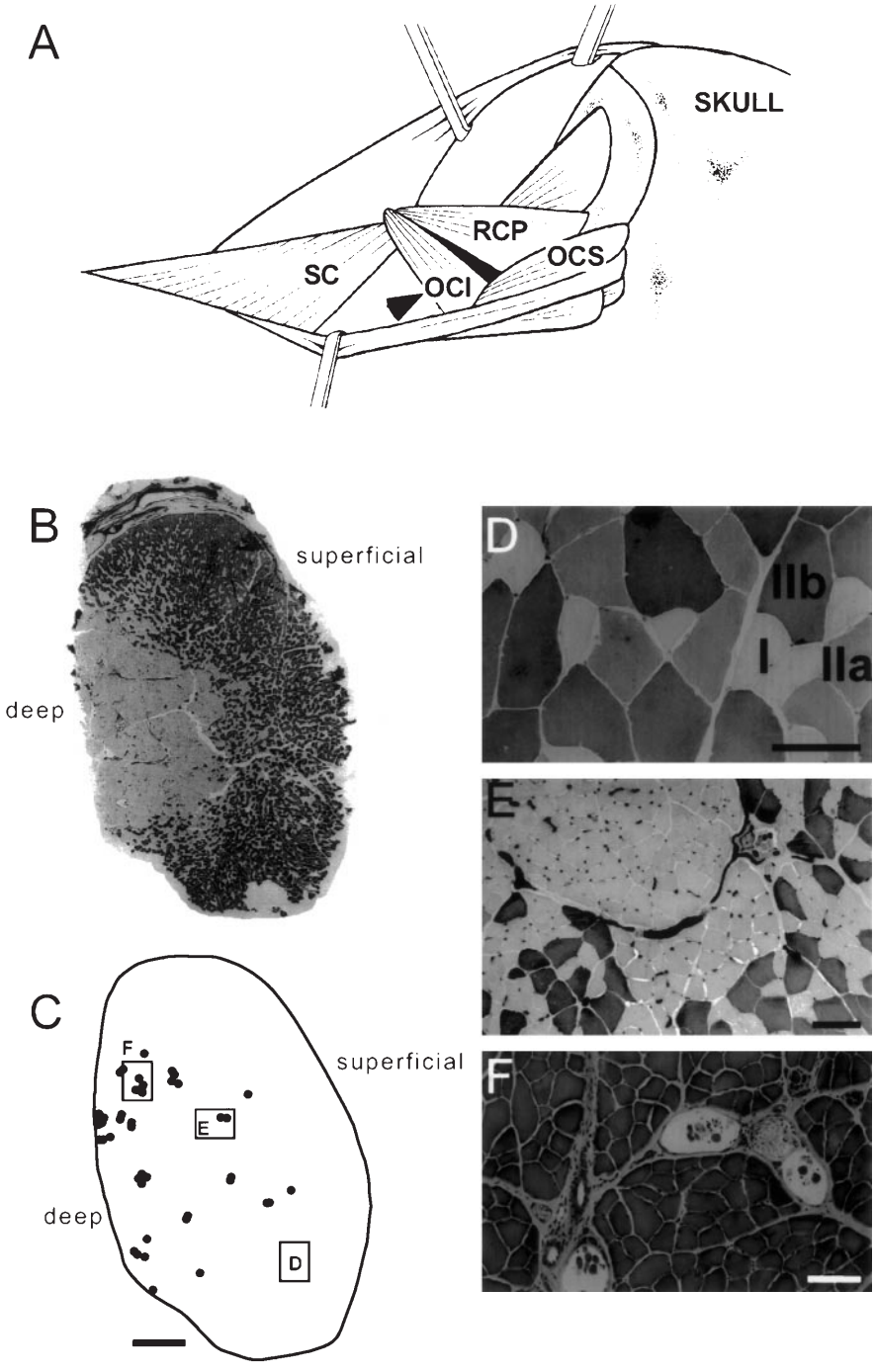

Fig. 1A–F Distribution of fast and slow fiber types in obliquus capitis inferior. The line drawing **(A)** shows the structure of obliquus capitis inferior (*OCI*) in relation to other deep neck muscles. The *arrow* illustrates diagrammatically the transverse level of section from which the photomicrographs were taken. At low power **(B)**, an obvious gradient of staining intensity reflects the increased concentration of type I fibers in the deep regions of the muscle. Darkly staining type IIa and b fibers are located only in more superficial regions. On **C** are also shown the approximate sites represented in higher power photomicrographs **(D–F)** chosen to illustrate features of histochemistry and receptor distribution. **D** Shows the relative staining intensities of different fiber types. **E** Shows the nature of a typical border between regions containing different fiber-type proportions. Typical muscle spindles, stained with hematoxylin and eosin, are shown in **F** (*RCP* rectus capitis posterior major, *OCS* obliquus capitis superior, *SC* semispinalis cervicis). *Bars* 1 mm **(C)**, 100 µm **(D–F)**

Materials and methods

Three rhesus monkeys (two male, one female, weights 6.3–7.5 kg) were anesthetized with ketamine hydrochloride (10 mg/kg, i.m.). They were then put to death with an intravenous overdose of sodium pentobarbital. All experimental procedures were carried out according to the guidelines of the Canadian Council of Animal Care and were approved by the Queen's University Animal Use Committee. Some of the monkeys had been previous subjects in chronic electrophysiological studies of the superior colliculus or in studies of reproductive hormone cycling that did not appear to affect neck musculature. Obliquus capitis inferior (OCI, also known as obliquus capitis posterior; Szebenyi 1969) was removed within 3 h after death. Because OCI is short (<2 cm), each muscle could be mounted as a single block onto a cryostat chuck. Blocks were frozen in liquid nitrogen, warmed to –20°C, and cut into 16-µm sections at levels between the belly and the cranial insertion of the muscle. Adjacent sections were stained with hematoxylin and eosin and for adenosine triphosphatase (ATPase) activity following formalin fixation and alkali preincubation at pH 10.4 for 4 min (Guth and Samaha 1970).

Muscle fibers were classified as type IIb, type IIa, and type I according to their characteristic dark, moderate, and lightly ATPase-staining profiles, respectively. The use of ATPase histochemistry to differentiate fiber types was chosen because of its well-established reliability in discriminating fiber types in monkey muscles (e.g., Bagnall et al. 1983; McIntosh et al. 1985). Less than

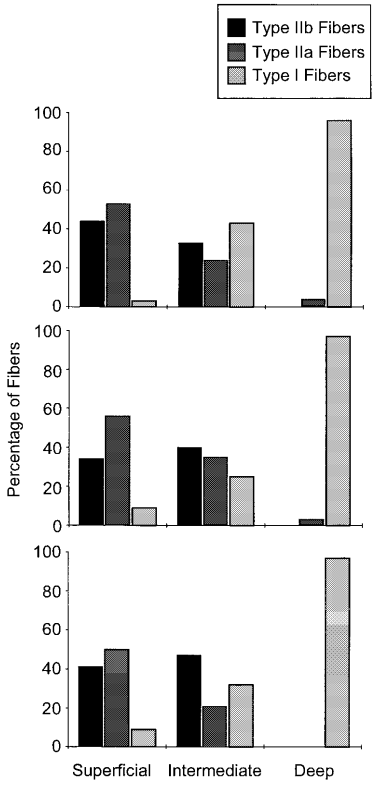

Fig. 2 Relative proportions of different fiber types in deep, intermediate, and superficial regions of OCI in three different animals. Note that different fiber types are distributed in a similar gradient across the muscle of each animal

5% of muscle fibers were found to be difficult to classify with confidence in well-stained sections. Relative numbers of the different fiber types were counted in at least 3 territories of 100 adjacent fibers on the superficial surface, the intermediate region and deep (vertebral) surface in each muscle. The relative percentage of each fiber type was determined for the superficial, intermediate, and deep regions from the cumulative counts in each region.

Results

OCI is a short, parallel-fibered muscle that spans from the spinous process of the axis to the lateral process of the atlas (Fig. 1A). All OCI muscles examined in this study contained a mixture of type I and II fibers that were distributed asymmetrically from the superficial to the deep surface of the muscle (Fig. 1). Type II fibers predominated on the superficial surface (Fig. 2). Most fascicles of fibers that bordered the superficial edge of the muscle contained only one or two type I fibers and a few fascicles contained none at all. Beneath the surface, fiber-type composition changed in a graded manner, so that fascicles had more equal proportions of the three fiber types only a few millimeters deep to the muscle surface (Fig. 1D). The relative proportion of type I fibers increased, often in one or more abrupt steps, with muscle depth. Typically, fascicles containing a relatively even mixture of fiber types lay juxtaposed to fascicles containing mainly or only type I fibers (Fig. 1E). Thus, the muscle appeared to have a histochemical division into two parts (Fig. 1B). However, the apparent borders were not marked by any consistent increase in the thickness of perimysial connective tissues. In most muscles, the deepest regions contained a small proportion (<5%) of fast fiber subtypes (Fig. 2), but some fascicles contained no fast fibers (Fig. 1B,E).

Muscle spindles were distributed disproportionately in the deep regions of muscle cross sections analyzed here (Fig. 1). Between 19 and 42 muscle-spindle profiles could be identified in single cross sections from different muscles. None of these spindles were located in the superficial one-third of the cross section and most were found in the deepest one-third. Many of the spindles occurred in complexes, as described previously in suboccipital muscles of cats and man (Bakker and Richmond 1981, 1982).

Discussion

The present study draws attention to a compelling nonuniformity of fiber-type distribution in a structurally simple muscle. Modest gradients in the distribution of fiber types have been reported previously in several forelimb and hindlimb muscles of the monkey (McIntosh et al. 1985; Roy et al. 1984; Acosta and Roy 1987), but the depth of this gradient was unexpected. Type I fibers were found to account for all or nearly all of the fibers in deep regions but less than 10% of fibers in superficial fascicles of OCI. In previous studies of mammalian muscles, it has been relatively rare to find fascicles composed exclusively of type II or type I fibers. Of particular note have been feline soleus, composed exclusively of type SO (type I) fibers (Ariano et al. 1973), and caudofemoralis, composed almost exclusively of fast-fiber subtypes (Ariano et al. 1973; Brown et al. 1998). In addition, Yokoyama (1982) noted that longissimus muscles of monkeys contained a region composed exclusively of fast fibers. However, to our knowledge, no muscles have been reported previously that contain fascicles composed almost exclusively of type I fibers in one part and type II fibers in another. Typically, muscles have more modest gradients in which the relative content of type I fibers varies by less than 50% (McIntosh et al. 1985; Acosta and Roy 1987), even when the most differentiated regions are compared.

One other muscle that has an unusually steep gradient of fiber types is OCI in the cat (Richmond et al. 1988). In feline OCI, the gradient appears to be somewhat less extreme, because fascicles on both surfaces contain at least a few fibers of all three types. Nevertheless, a similar pattern is seen in which slow (type I) fibers are rare on the superficial muscle surface but predominate more deeply. The presence of steep fiber-type gradients in OCI muscles of phylogenetic classes as differentiated as carnivores and primates suggests a strong conservation of this organizational pattern. It remains to be determined

whether a similar kind of gradient will be found in human OCI. Gradients of fiber-type distribution have been reported in a few human muscles but systematic studies of intramuscular patterns are relatively rare because of the difficulties in obtaining and preparing large whole muscles (cf. Henriksson-Larsen et al. 1983; Lexell et al. 1983; Travnik et al. 1995).

The presence of fiber-type nonuniformities in muscles such as OCI has significant implications for experimentalists. Unless such gradients are recognized and taken into account when collecting data, they can introduce an unanticipated bias into the experimental results. It is difficult to assess the fiber-type composition of muscle unless all parts can be examined systematically. When the analyses rely on biopsy samples of muscle, it can be particularly difficult to ensure that presumably matched samples from different subjects or sides of a single subject are not taken from different muscle depths or compartments. In OCI, for example, a few millimeters difference in the location of a sampling site could result in a difference in estimated slow-fiber proportions exceeding 50%. Sampling differences may explain why biopsied vertebral muscles on different sides of the same monkey or human have been found to have large differences in fiber-type composition even when sampling sites were considered to be identical (Bagnall et al. 1983; Ford et al. 1986).

Heterogeneity in fiber-type distribution must also be taken into account when designing experimental protocols for electromyographic recording or intramuscular stimulation. This problem was well illustrated in previous EMG studies of OCI in chronically instrumented cats implanted with two different types of EMG electrodes to record muscle activity during head turning. Recordings made using epimysial patch electrodes that were implanted on the superficial muscle surface were characterized by large, short bursts of activity during vigorous head movements such as head shaking, whereas those made using hook electrodes in the deeper part of the muscle had longer bursts, a smaller dynamic range, and easily recognized periods of sustained activity when the cat held certain head postures. These results were taken as evidence that the deeper slow region of feline OCI has a more postural role, in keeping with the greater fatigue resistance known to be associated with slow fibers (Richmond et al. 1988). In the monkey, EMG analyses to date also suggest that OCI may have a dual role during head movement. It produces a phasic burst of activity at the onset of a rapid horizontal movement (in the head-free animal) or attempted movement (in the head-fixed animal), followed by a lower level of tonic activity as long as the head is held in an eccentric posture (Corneil et al. 1996). These two types of activity may reflect the differential recruitment of fast and slow regions of the muscle, respectively. However, if EMG signals from only one region were available, one might question whether the role defined for the muscle would be skewed based on interpretations of the apparent strength of phasic versus tonic activity in the fibers closest to the electrodes.

The present study was not undertaken to study receptor organization in OCI. Nevertheless it was difficult to ignore the large number of muscle-spindle profiles that crowded the deepest layers of the muscle. Exceptionally high densities of muscle spindles have been identified previously in human and feline suboccipital muscles, including feline OCI (Cooper and Daniel 1963; Bakker and Richmond 1981, 1982). The strong association of spindles with slow-fiber regions of OCI is consistent with previous reports that muscle spindles are typically concentrated in muscle regions richest in slow fibers (e.g., Yellin 1969; Botterman et al. 1978; Richmond and Stuart 1985). We noted that the deep surface of OCI containing the majority of muscle spindles adhered closely to underlying vertebral bones, and could be damaged easily when the muscle is removed. Thus, we were not convinced that even the large number of profiles that we counted were fully representative of spindle numbers in this muscle.

The results of the present study underline the significant complexities that can exist even in architecturally simple muscles. It is not uncommon in the literature to represent muscles by a single ratio of fiber-type proportions and on that basis to define the muscle as fast or slow. However, for a muscle such as OCI, such a designation is impossible. The muscle instead appears as if it were two functional modules laminated together so that the muscle can produce both bursts of force needed for fast phasic movements and lower maintained force needed to maintain eccentric head postures.

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References

Acosta L Jr, Roy RR (1987) Fiber-type composition of selected hindlimb muscles of a primate (cynomolgus monkey). Anat Rec 218:136–141

Ariano MA, Armstrong RB, Edgerton VR (1973) Hindlimb muscle fiber populations of five mammals. J Histochem Cytochem 21:51–55

Bagnall KM, Ford DM, McFadden KD, Greenhill BJ, Raso VJ (1983) A comparison of vertebral muscle fiber characteristics between human and monkey tissue. Acta Anat 117:51–57

Bakker DA, Richmond FJR (1982) Muscle spindle complexes in muscles around upper cervical vertebrae in the cat. J Neurophysiol 48:62–74

Bakker GJ, Richmond FJR (1981) Two types of muscle spindles in cat neck muscles: a histochemical study of intrafusal fiber composition. J Neurophysiol 45:973–986

Balice-Gordon RJ, Thompson WJ (1988) The organization and development of compartmentalized innervation in rat extensor digitorum longus muscle. J Physiol 398:211–231

Botterman BR, Binder MD, Stuart DG (1978) Functional anatomy of the association between motor units and muscle receptors. Am Zool 18:135–152

Brown IE, Satoda T, Richmond FJR, Loeb GE (1998) Feline caudofemoralis muscle: muscle fibre properties, architecture, and motor innervation. Exp Brain Res 121:76–91

Burke RE (1981) Motor units: anatomy, physiology, and functional organization. In: Brooks VB (ed) The nervous system (Handbook of physiology, sect 1, vol II: Motor control, part 1). Am Physiol Soc, Bethesda, MD, pp 345–422

Cooper S, Daniel PM (1963) Muscle spindles in man; their morphology in the lumbricals and the deep muscles of the neck. Brain 86:563–586

Corneil BD, Loeb GE, Richmond FJR, Munoz DP (1996) EMG activity in dorsal neck muscles of the rhesus monkey during head movements and in different head positions. Soc Neurosci Abst 22:2036

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

Ford DM, Bagnall KM, McFadden KD, Reid DC (1986) A comparison of muscle fiber characteristics at different levels of the vertebral column in the rhesus monkey. Acta Anat 126: 163–166

Gonyea WJ, Ericson GC (1977) Morphological and histochemical organization of the flexor carpi radialis muscle in the cat. Am J Anat 148:329–344

Guth L, Samaha FJ (1970) Procedure for the histochemical demonstration of actomyosin ATPase. Exp Neurol 28:365–367

Henriksson-Larsen KB, Lexell J, Sjöström M (1983) Distribution of different fibre types in human skeletal muscles. I. Method for the preparation and analysis of cross-sections of whole tibialis anterior. Histochemistry 15:167–178

Lexell J, Henriksson-Larsen KB, Sjöström M (1983) Distribution of different fibre types in human skeletal muscles. 2. A study of cross-sections of whole m. vastus lateralis. Acta Physiol Scand 117:115–122

Maurer JJ, Singer MA, Schieber MH (1995) Fiber type composition of morphologic regions in the macaque multitendoned finger muscles. Acta Anat 154:216–223

McIntosh JS, Ringqvist M, Schmidt EM (1985) Fiber type composition of monkey forearm muscle. Anat Rec 211:403–409

Richmond FJR, Abrahams VC (1975) Morphology and distribution of muscle spindles in dorsal muscles of the cat neck. J Neurophysiol 38:1322–1339

Richmond FJR, Stuart DG (1985) Distribution of sensory receptors in the flexor carpi radialis muscle of the cat. J Morphol 183:1–13

Richmond FJR, Bakker DA, Stacey MJ (1988) The sensorium: receptors of neck muscles and joints. In: Peterson BW, Richmond FJR (eds) Control of head movement. Oxford University Press, New York, pp 49–62

Roy RR, Bello MA, Powell PL, Simpson DR (1984) Architectural design and fiber-type distribution of the major elbow flexors and extensors of the monkey (cynomolgus). Am J Anat 171: 285–293

Selbie WS, Thomson DB, Richmond FJR (1993) Suboccipital muscles in the cat neck: morphometry and histochemistry of the rectus capitis muscle complex. J Morphol 216:47–63

Simoneau J-A, Bouchard C (1989) Human variation in skeletal muscle fiber-type proportion and enzyme activities. Am J Physiol 257:E567–E572

Szebenyi ES (1969) Atlas of *Macaca mulatta*. Associated University Press, New Jersey, pp 44–223

Travnik L, Pernus F, Erzen I (1995) Histochemical and morphometric characteristics of the normal human vastus medialis longus and vastus medialis obliquus muscles. J Anat 187: 403–411

Yellin H (1969) A histochemical study of muscle spindles and their relationship to extrafusal fibre types in the rat. Am J Anat 125:31–37

Yokoyama Y (1982) Analyses on the fiber compositions of the lumbar back muscles in mammals. J Jpn Orthopaed Assoc 56:579–594