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THE ROYAL SOCIETY

Neuromuscular organization of avian flight muscle: architecture of single muscle fibres in muscle units of the pectoralis (pars thoracicus) of pigeon (*Columba livia*)

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The M. pectoralis (pars thoracicus) of pigeons (*Columba livia*) is comprised of short muscle fibres that do not extend from muscle origin to insertion but overlap 'in-series'. Individual pectoralis motor units are limited in territory to a portion of muscle length and are comprised of either fast twitch, oxidative and glycolytic fibres (FOG) or fast twitch and glycolytic fibres (FG). FOG fibres make up 88–90% of the total muscle population and have a mean diameter one-half of that of the relatively large FG fibres.

Here we report on the organization of individual fibres identified in six muscle units depleted of glycogen, three comprised of FOG fibres and three comprised of FG fibres. For each motor unit, fibre counts revealed unequal numbers of depleted fibres in different unit cross-sections. We traced individual fibres in one unit comprised of FOG fibres and a second comprised of FG fibres. Six fibres from a FOG unit (total length 15.45 mm) ranged from 10.11–11.82 mm in length and averaged ($\pm$s.d.) 10.74 ± 0.79 mm. All originated bluntly (en mass) from a fascicle near the proximal end of the muscle unit and all terminated intramuscularly. Five of these ended in a taper and one ended bluntly. Fibres coursed on average for 70% of the muscle unit length. Six fibres from an FG unit (total length 34.76 mm) ranged from 8.97–18.38 mm in length and averaged 15.32 ± 3.75 mm. All originated bluntly and terminated intramuscularly; one of these ended in a taper and five ended bluntly. Fibres coursed on average for 44% of the muscle unit length. Because fibres of individual muscle units do not extend the whole muscle unit territory, the effective cross-sectional area changes along the motor unit length. These non-uniformities in the distribution of fibres within a muscle unit emphasize that the functional interactions within and between motor units are complex.

Keywords: muscle unit; flight muscle; in-series fibre; muscle fibre morphology

1. INTRODUCTION

Our laboratory is engaged in studies of the neuromuscular basis for wing movements employed by birds during powered flight with focus on two species with contrasting wing morphologies and flight styles, the pigeon (*Columba livia*) and European starling (*Sturnus vulgaris*). In flapping birds, the M. pectoralis produces most of the power during the downstroke required for lift and thrust and the M. supracoracoideus contributes significantly to the execution of wing upstroke (Poore *et al.* 1997). Thus in some respects, the neuromuscular control of the wing during flight might be expected to be less complicated than the multiple muscle systems involved in limb control in non-avian tetrapods. In addition to level flight, however, birds must land, take off and manoeuvre in space, which clearly requires some modulation of individual wing-beat cycles and involvement of additional muscles. We believe an anatomical and functional characterization of the neuronal and contractile components of the flight muscles will ultimately contribute to a general understanding of the diversity of wing shapes and flight styles present in birds and shed light on the evolution of avian powered flight from a therapod reptilian ancestry.

Our most recent findings reveal features of musculoskeletal and neuroanatomical organization that bear directly on the execution of individual wing movements during flight. To illustrate, this essay will address the functional implications for flight of an 'in-series' arrangement of muscle fibres in the M. pectoralis of the pigeon. This example will be set within a functional framework provided by kinematic and electromyographic studies.

(a) *In-series muscle fibres in pigeon pectoralis*

In pigeons the M. pectoralis (pars thoracicus) is composed of two anatomical parts, the sternobrachialis (SB) and the thoracobrachialis (TB), which are separated through most of their length by an aponeurotic central tendon (figure 1). The SB and TB are innervated by separate branches of the brachial plexus and appear to function with some independence during flight (Dial *et al.*

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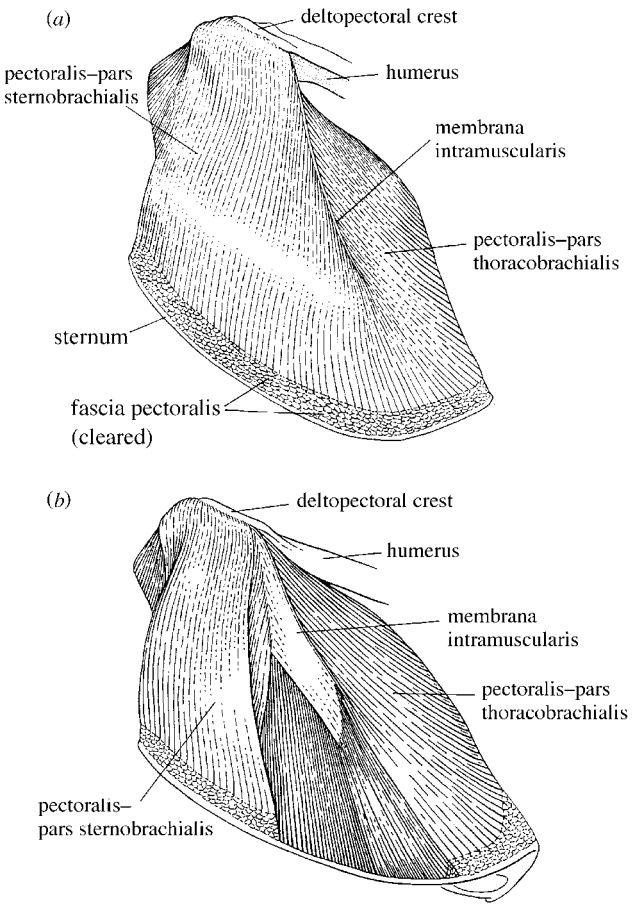

Figure 1. The M. pectoralis (pars thoracicus) of the pigeon *Columba livia* in lateral view (*a*), and cut-away view (*b*). The M. pectoralis consists of the pars sternobrachialis (SB) and pars thoracobrachialis (TB), which are separated by a fascial plate, the membrana intramuscularis. The fascicles of the SB that originate from the furcular shaft anteriorly (clavicular humeral region) and the carina and fascia pectoralis ventrally (sterno-humeral region) are visible. The insertion of these fascicles is complex. The more superficial and anterior fascicles insert onto the ventrolateral surface of the deltopectoral crest; the more caudal fascicles insert onto the ventrolateral surface of the membrana intramuscularis. (After Sokoloff *et al*. 1998.)

1987, 1988). When viewed under light microscopy, the SB and TB of pigeons consist of two types of fibres, easily distinguished by histochemical profile and size (Rosser & George 1986; Dial *et al*. 1987). The relatively small, oxidative fibres comprise from 88–90% of the fibre population but have a mean diameter which is one-half that of the relatively large glycolytic fibres (Kaplan & Goslow 1989). We refer to the small, oxidative fibres as fast-oxidative glycolytic (FOG) and the large, non-oxidative fibres as fast-glycolytic (FG) fibres.

In 1987, aware of the presence of these contrasting fibre types, we made electrical recordings from the SB and TB of a pigeon during take-off, sustained flight and landing (Dial *et al*. 1987) that would provide the focus of the studies in our laboratory for several years (figure 2). Note the presence of large spikes during take-off and landing when compared with level flight. Although signal analysis is necessary to identify precise patterns of fibre recruitment from raw electromyographic signals with certainty, it is known that large muscle fibres produce significantly larger spike potentials than small fibres (Olson *et al*. 1968; Goldberg & Derfler 1977). Thus the gradual reduction and loss of large-amplitude spikes during the transition from take-off to level flight, and their reappearance during landing, suggest, respectively, an active derecruitment and subsequent recruitment of motor units consisting of large-diameter muscle fibres during these different flight modes. These patterns, coupled with the earlier comparative fibre-type studies of Talesarus & Goldspink (1978) and *in vivo* glycogen depletion patterns we observed in free-flying pigeons (Welsford *et al*. 1991), contributed to the hypothesis that the relatively large, FG fibres are used for take-off and landing and the small, FOG fibres for uninterrupted flight. It is from this vantage, with interest in gaining a better understanding of FG and FOG fibres, that we initiated studies to anatomically and physiologically characterize isolated motor units in the pigeon pectoralis (Sokoloff *et al*. 1998).

Recently, we reported that the pectoralis of pigeons (*Columba livia*) is composed of short fibres that overlap in-series from muscle origin to insertion and that individual motor units are limited in territory to a portion of the origin-to-insertion muscle length (Sokoloff *et al*. 1998). Muscles composed of short muscle fibres that overlap in-series from muscle origin to insertion were described over 100 years ago for select muscles in human (Froriep 1878), human and cow (Rollett 1856) and frog (Mayeda 1890). Despite this early recognition of in-series fibre organization, only recently has the widespread distribution of this morphology been appreciated and discussed within a functional and/or phylogenetic context (for a review, see Gans & Gaunt 1991; Trotter 1993; Trotter *et al*. 1995).

Indirect investigations of fibre organization via motor endplate distribution patterns reveal the potential for an in-series organization in a variety of architecturally pinnate-, but more often, parallel-fibred muscles in representatives of each of the three classes of amniotes. Direct anatomical studies, via acid digestion and/or glycogen depletion, have provided specific details of the morphology of in-series fibres in relatively few muscles. These studies reveal substantial variation in the *in situ* organization of muscles either specialized for different functions and/or representing different taxa. Because we have not yet successfully tied the variation in fibre organization to function or phylogeny, any general principles governing in-series morphology have remained obscure.

The presence of overlapping, in-series muscle fibres that do not traverse the full length of their associated fascicle(s) has been directly demonstrated in the pectoralis of the quail (*Coturnix japonica*) (Trotter *et al*. 1992) and the pigeon (*Columba livia*) (Sokoloff *et al*. 1998). Of immediate concern are the functional consequences of this fibre arrangement in locomotor muscles of not only birds, but of other tetrapods as well. This question must be addressed initially by determining the intrafibre organization of the motor units within the muscle as well as their innervation pattern. For example, are the fibres organized in-series from the fascicle's origin to insertion innervated by a single motoneuron (figure 3*a*), or are a unit's muscle fibres organized in-parallel, which in turn might require two or more motor units in-series for maximal force transmission (figure 3*b*)?

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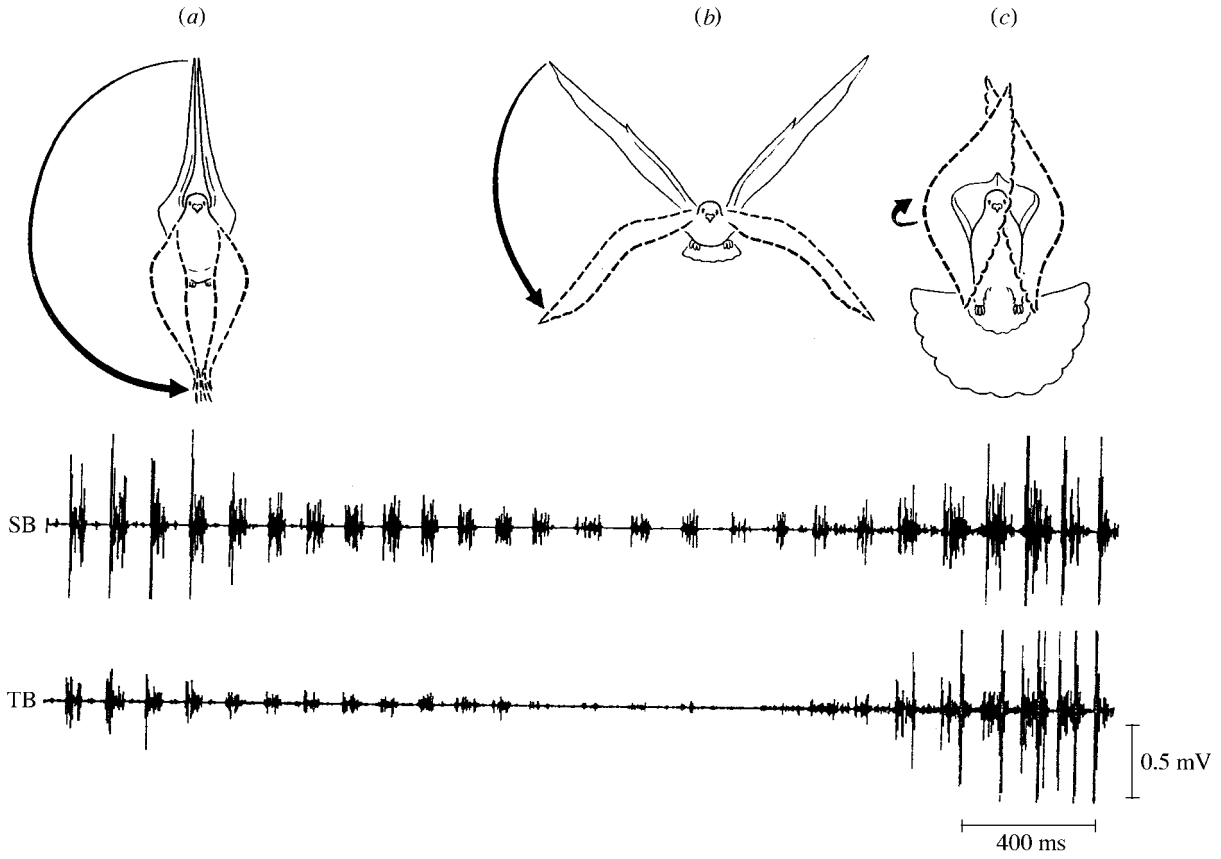

Figure 2. Differential muscle fibre activity during pigeon flight. Wing-beat amplitude and corresponding electromyographic activity. (*a*) Acceleration following release from hand, large wing-beat amplitude, presence of large-amplitude spikes. (*b*) Fast flight, small wing-beat amplitude, reduction of large-amplitude spikes. (*c*) Landing, large wingbeat amplitude, reappearance of large-amplitude spikes. Large-amplitude spikes in the sternobrachialis (SB) and thoracobrachialis (TB) heads of the pectoralis early and late in the sequence are suggestive of the activity of the large, fast glycolytic fibres. (After Dial *et al*. 1987.)

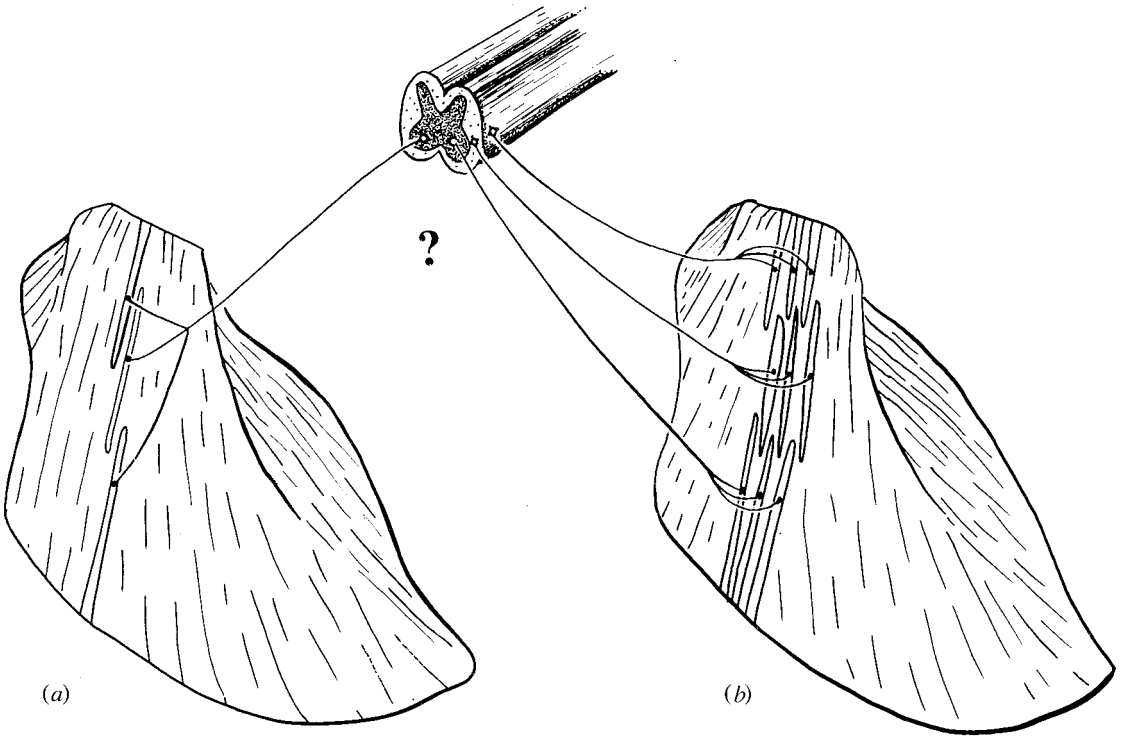

Figure 3. Possible organization of motor units within the M. pectoralis. Within the pigeon pectoralis, the muscle units comprised of fibres arranged in-series may be organized either in-parallel with one another (*a*), or in-series (*b*). One purpose of the present study was to determine which organization is the 'norm'.

Here we describe a primarily in-parallel architectural arrangement of individual fibres within pectoralis motor units. Our findings also reveal that individual pectoralis motor units are composed of fibres of differing length; thus motor unit cross-sectional area, and the effect of a unit on adjacent muscle fibres, is not equivalent at all levels of the motor unit. We also report differences in the structure of motor units comprised of FG versus FOG fibres that may reflect on their specific functional role during flight.

2. METHODS

(a) *Muscle unit isolation and depletion*

We used eight adult pigeons (250–350 g) of either sex in acute studies of muscle units. All experiments followed a protocol approved by the Brown University Animal Care and Use Committee, in accordance with the rules for animal welfare mandated by the US National Institutes of Health. For at least five days prior to surgery, birds were injected with 25 ml of a 10% dextrose solution subcutaneously. Birds were anaesthetized with ketamine (60 ml mg kg^{-1} IM) and xylazine (6.0 ml mg kg^{-1} IM) with supplemental doses administered as needed. Details of our methods for the surgical exposure of the pectoralis muscle–nerve and functional isolation of the motor units are given in a recent paper (Sokoloff *et al*. 1998). Briefly, we intubated and ventilated the bird with a mixture of humidified oxygen (20%) and nitrogen (80%) and performed a laminectomy over cervical roots 10, 11, and 12 on the left side. In six birds, after transection of each root, axons were functionally isolated by ventral root dissection. We progressively split nerve sections into filaments until a single alpha motor axon was functionally isolated. To prevent incidental depletion of multiple muscle units during the functional isolation, we limited stimulation of nerve filaments to less than 30 times at twitch strength. Control experiments (two birds) of whole nerve stimulation failed to produce muscle fibre glycogen depletion following 50 trains of tetanic stimuli (80 Hz, 1 s train duration). Following the characterization of the unit's mechanical properties, we stimulated the filament once a minute for 1–3 hours (40–80 Hz, 330 ms duration) to deplete the muscle unit of its glycogen stores.

All data were digitally recorded (10 MHz^{-1}) and stored on disk for off-line analysis on a Nicolet 400 series waveform acquisition system.

(b) *Muscle unit histochemistry*

Following euthanasia, we removed the left pectoralis muscle in its entirety for histochemical analysis. We traced and measured the left pectoralis muscle and cut it into 13–20, 10 mm^3 blocks. Care was taken to preserve the orientation of tissue. We also removed tissue samples from the right pectoralis as a control. Blocks were frozen in isopentane at −160 °C and stored at −70 °C. Cryostat cross-sections of 30 µm thickness were cut at −20 °C, mounted directly onto coverslips and placed in cooled Carnoy's fixative preparatory to staining for glycogen (Schiff-periodic acid). Every 50–100 sections, three 12 µm sections were reacted for reduced nicotinamide adenine dinucleotide diaphorase (NADH-D) to allow the determination of oxidative capacity. Coverslips were dehydrated, covered with permount, and mounted on slides.

We identified muscle fibres from photographic prints of NADH-D and glycogen reactions. Fibres of the pectoralis fall into two categories relative to size and reaction to NADH-D: (i) those with a mean diameter of 33.5 µm and a dark reaction with NADH-D, the FOG fibres, and (ii) those with a mean diameter of 78.9 µm and a light to moderate reaction with NADH-D, the FG fibres.

(c) *Reconstruction of single fibres*

We analysed glycogen-stained slides under brightfield microscopy. Each depleted fibre was identified as white in a field of fuschin fibres. We calculated the origin-to-insertion length of each muscle unit as 30 µm multipled by the number of sections with depleted fibres, and we determined the number of depleted fibres in section sampled throughout the motor unit length. Precise reconstruction of a large number of single fibres was difficult because of the muscle's complex architecture; fibres were often orientated at an angle relative to the cut surface of the tissue block, which made it particularly difficult to trace a fibre from one block to the next. Nevertheless, we were able to reconstruct six depleted fibres from a unit comprised FOG fibres and six depleted fibres from a unit comprised FG fibres. In addition to fibre length and position in the entire motor unit field, we noted the mode of origin and termination of each fibre (i.e. whether blunt or tapered).

3. RESULTS

The location and physiological properties of the six motor units used in this study are described in Sokoloff *et al*. (1998, figs 9–13). Four relatively distinct regions of the pectoralis exist, three in the SB and one in the TB, which differ in location, fascicle orientation and fascicle length. Three of the motor units comprised FG fibres and one comprised FOG fibres, which were located in the clavicular–humeral region and two comprised FOG fibres, which were located in the sternal–humeral region. An example of the general location within the pectoralis of one FG unit and one FOG unit (FG1 and FOG1, respectively, of Sokoloff *et al*. (1998)) is depicted in figures 4–5 as is the spatial distribution of the fibres of each unit sampled at three levels taken, 15–25%, 40–60%, and 75–85%, along the origin-to-insertion length.

All fibres of all motor units we isolated illustrated a stable ATPase reaction with an alkaline preincubation (pH 10.4) (Rosser & George 1986; Kaplan & Goslow 1989). The FOG fibres had a strong reaction with NADH-D whereas the FG fibres did not. Differences in the organization of FG versus FOG units are apparent for the FG1 and FOG1 units (figure 6). Glycogen-depleted FOG fibres are located throughout individual fascicles and are relatively dispersed (figure 6*a*). In contrast, FG fibres tend to be located along the fascicular borders and are more tightly arranged (figure 6*b*).

All six pectoralis motor units are confined to a subvolume of the muscle, and most course for only a portion of the origin-to-insertion length of the muscle region in which they are situated. Histograms of the innervation ratio (the number of depleted muscle fibres), measured at different proximo-distal levels of the motor unit, indicate that not all muscle fibres in a motor unit extend the full length of the motor unit territory. In general, more fibres are observed at either the origin or mid-level of the unit (figure 7). This is the case for both FG and FOG motor units.

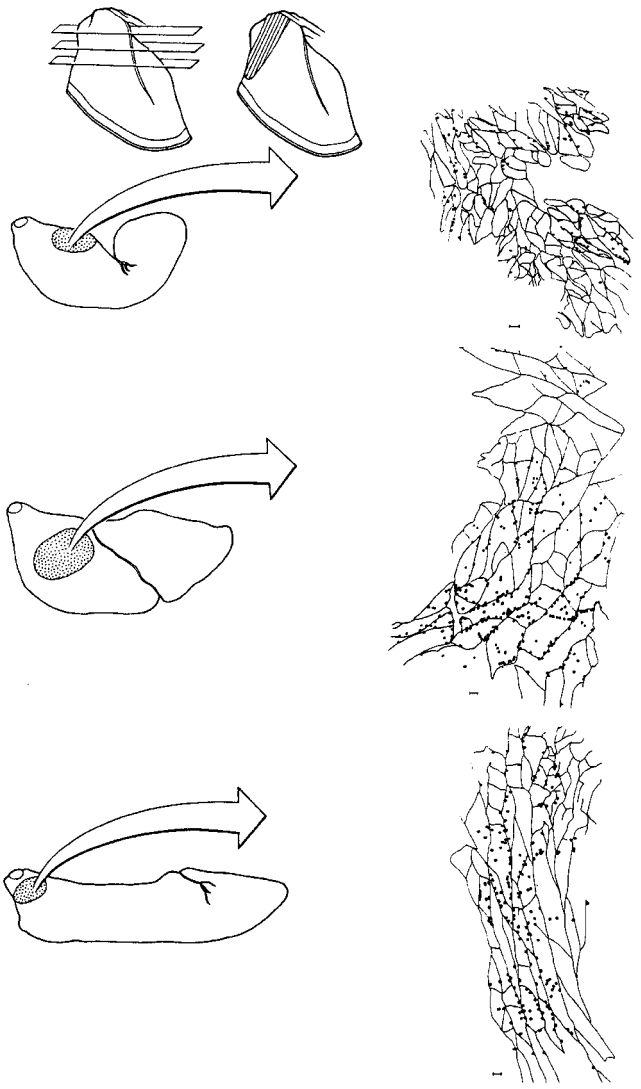

Figure 4. Organization within the pigeon SB of a muscle unit comprised of FG fibres. The two top icons represent the left M. pectoralis in lateral view (see figure 1). The top left icon depicts the cross-sectional levels at which the muscle unit fibres are viewed (shown in the three paired traces below) and the top right icon depicts the origin-to-insertion extent of the unit (shaded). The three sets of illustrations below each show the location of motor unit fibres within a cross-section. The whole muscle is indicated in cross-section (left); the arrow points to an expanded view (right) showing the corresponding fibres of the unit (dots), in association with adjacent fascicles. This unit is located in the clavicular–sternal region. The fascicles containing this unit are spatially confined not only along an origin-to-insertion axis, but in the anteroposterior and mediolateral dimensions as well. Typical of FG fibres, a close association with the fascicle boundaries is seen. Although some fibres of this unit attach distally to the furcula and proximally to the deltopectoral crest, the largest number of fibres are found midway along its proximodistal extent. This unit possesses innervation ratios of 118, 304, and 191 at the three levels. Scale bars, 500 µm. (After Sokoloff *et al*. 1998.)

We traced six fibres depleted of their glycogen from the FG1 unit and six fibres from the FOG1 unit in their entirety to determine the exact location of individual fibres within the motor unit. Some of these fibres are identified in cross-section in figure 6, and correspond to the identified fibres

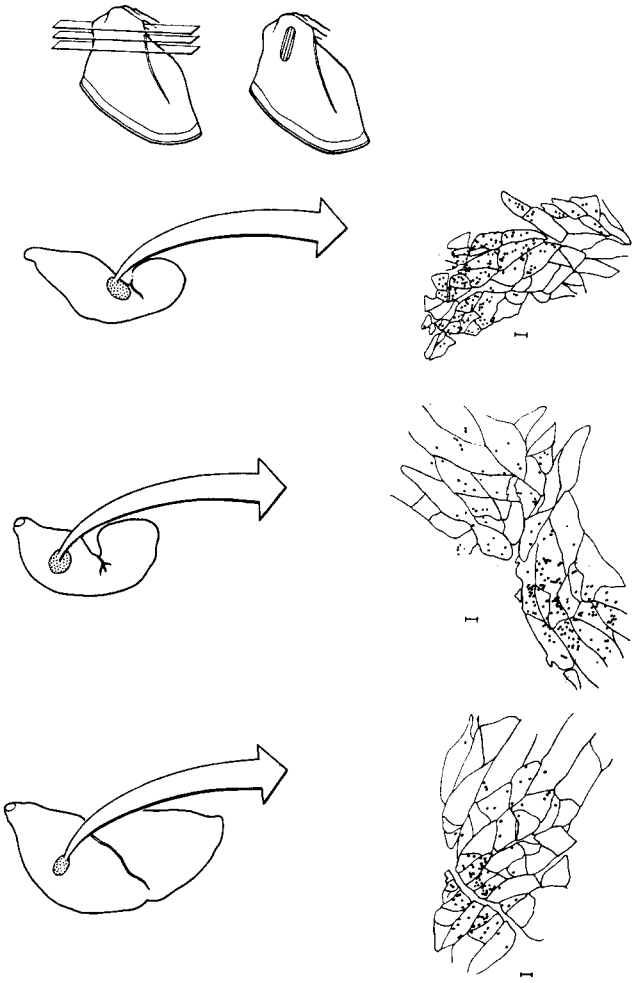

Figure 5. Organization within the pigeon SB of a muscle unit composed of FOG fibres. Layout and abbreviations as in figure 4. This unit is located in the sternal–humeral region and possesses a territory 30% of the origin-to-insertion length of its associated fascicles. Typical of the FOG units, the fibres tend to be located in the centre of fascicles and away from the fascicular boundary. Scale bars, 500 µm. (After Sokoloff *et al*. 1998.)

in figure 8. Six fibres from FOG1 (unit length 15.45 mm) ranged from 10.11 to 11.82 mm in length and averaged 10.74 ± 0.79 mm (figure 8, left). FOG fibres coursed on average for 70% of the muscle unit length. Six fibres from FG1 (unit length 34.76 mm) ranged from 8.97 to 18.38 mm in length and averaged (±s.d.) 15.32 ± 3.75 mm (figure 8, right). FG fibres thus coursed on average for 50% of motor unit length. All fibres originated bluntly, but differences in the mode of termination of fibres were related to fibre type. Five of the six FOG fibres tapered whereas only one of the FG fibres tapered. All other traced fibres terminated abruptly in a blunt ending.

4. DISCUSSION

Sokoloff *et al*. (1998) illustrated that within the M. pectoralis (pars thoracicus) of pigeons, a high percentage of fascicles are attached at one end to a connective tissue sheet rather than to bone. Within a fascicle, most fibres are attached bluntly at their origin or insertion and traverse into the muscle belly to end intrafasicularly in a taper; hence, they are organized

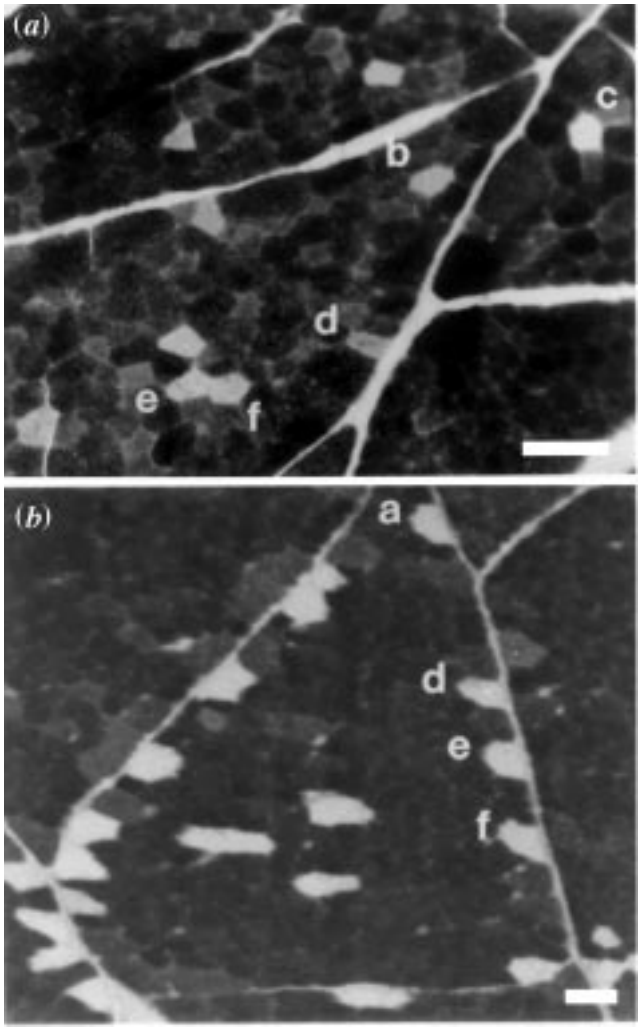

Figure 6. Photomicrographs of glycogen-depleted muscle fibres (white) from a FOG muscle unit (*a*, FOG1) and an FG muscle unit (*b*, FG1) showing the predominant location of FG fibres along connective tissue fascicles. Photomicrographs also identify the individual fibres that were traced in multiple tissue sections to determine the architecture of single fibres within each muscle unit. (*a*) Depleted fibres from FOG1 in muscle unit cross-section at 30% of muscle unit origin-to-insertion length (see left arrow in figure 8). Fibres (b), (c), (d), (e) and (f), identified here in cross-section, are illustrated in their entirety in figure 8. Scale bar, 100 µm. (*b*) Depleted fibres from FG1 in cross-section taken at 79% of muscle unit origin-to-insertion length (see right arrow in figure 8). Fibres (a), (d), (e) and (f), shown here in cross-section, are illustrated in their entirety in figure 8. Scale bar, 100 µm. Note differences in magnification of (*a*) and (*b*).

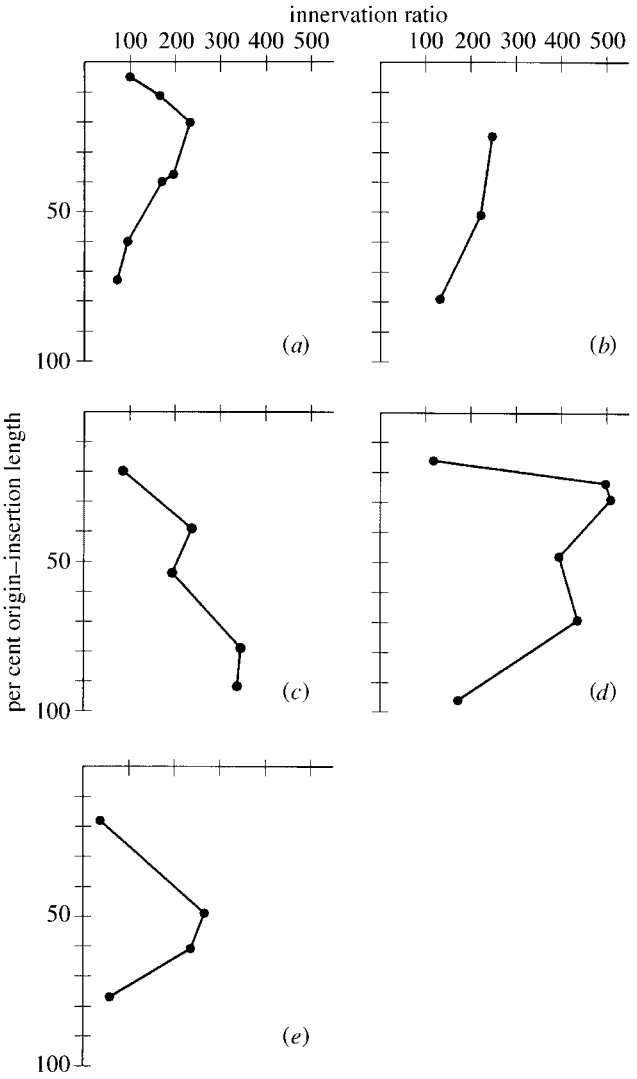

Figure 7. Histogram of the number of fibres (the innervation ratio) sampled at different cross-section levels for five units, three comprised of FG fibres and two comprised of FOG fibres. Note maximum number of fibres located either near origin or mid-levels of motor units. (*a*) FOG1; (*b*) FOG2; (*c*) FG1; (*d*) FG2; (*e*) FG3.

in-series. Histochemical–physiological associations of isolated muscle units reveal two populations of contrasting mechanical properties and peripheral organization. Muscle units composed of relatively large FG fibres, in contrast to the units composed of relatively small FOG fibres, produce higher forces, possess faster twitch contraction times, and are more fatigable. Compared with FOG units, FG units are longer, possess higher innervation ratios and are more densely organized (i.e. they comprise a larger percentage of fibres of the same type present in the cross-section of motor unit territory).

The principal additional finding shown in this study is that individual muscle fibres do not traverse the entire muscle unit but are limited to a percentage of proximo-distal length. Individual fibres of each unit are arranged more in-parallel than in-series and seldom traverse the entire origin-to-insertion distance. Reconstruction of fibres *in situ* also demonstrates that pectoralis muscle units may be comprised of fibres of different length. As a result, the effective force generated by a motor unit will not be equal throughout its entire length and muscle units in the pigeon pectoralis cannot be considered simply as scaled up versions of individual fibres. These non-uniformities in the distribution of fibres within a muscle unit suggest that the functional interactions within and between motor units are complex. Further, in the pigeon pectoralis, muscle units cannot be fully characterized by a single maximal innervation ratio.

The present results also reveal differences in the internal organization of FG versus FOG motor units. On

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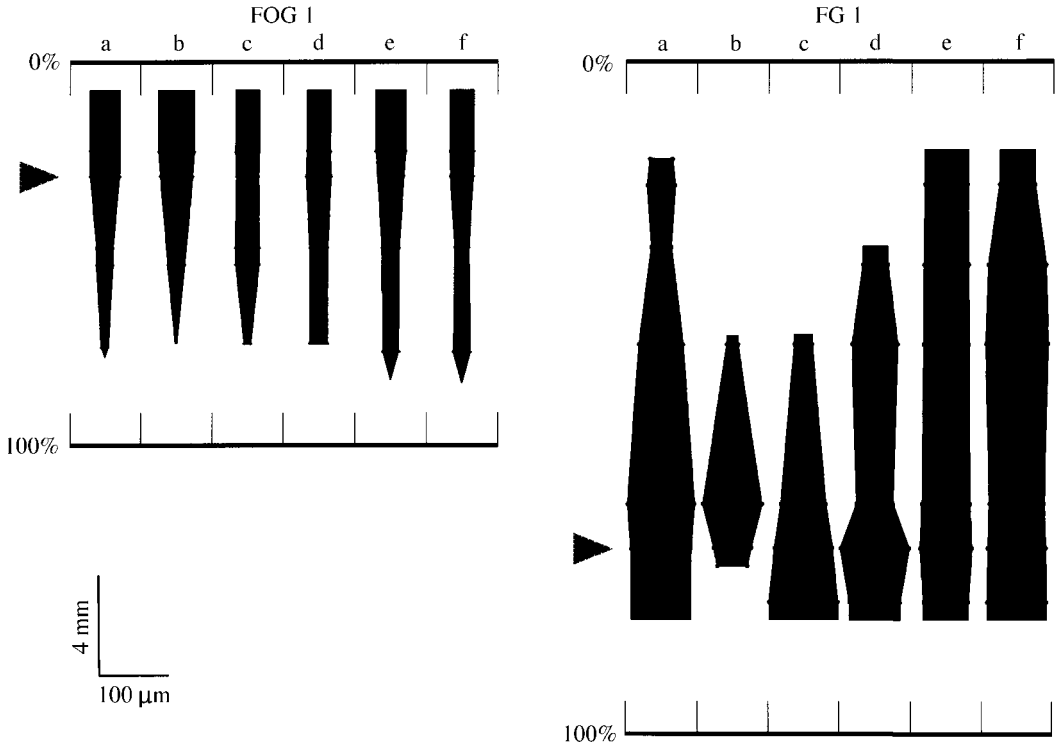

Figure 8. Illustration of individual depleted fibres from motor unit FOG1 and FG1. Carrots on each fibre indicate sections in which the diameter of the depleted fibre was measured. Fibres are located relative to overall motor unit length; the proximal (0%) and distal limits (100%) of each motor unit are indicated by horizontal bars. Arrows to the side of each muscle unit correspond to the sections shown in figure 6. Note that individual fibres course for 44% of FG unit length, but 70% of FOG unit length.

average fibres in FG muscle units coursed for only 44% of unit territory whereas fibres in FG units coursed for 70%. This finding suggests that the longer length of FG versus FOG muscle units is due in part to differences in the arrangements of FG versus FOG fibres within each type of unit and not solely to the greater length of FG fibres. These type-related differences in muscle unit architecture are supported by consideration of fibre length and unit length measures: although FG fibres are on average 1.5 times longer than FOG fibres, territories of FG muscle units are on average twice as long as territories of FOG units (Sokoloff *et al*. 1998). Relative to FOG units, fibres of FG units are organized more in-series.

(a) *Relation to other studies*

Direct studies of in-series fibres reveal substantial variation in the *in situ* organization of muscles either specialized for different functions and/or representing diverse taxa. With respect to the two fundamental parameters of absolute fibre length and fibre length as a percentage of overall fascicle length, for example, fibre lengths in the neck muscle (responsible for head retraction) of a turtle (*Pseudemys scripta*) range from 4 to 6 mm (36–62% fascicle length) (Callister *et al*. 1992), whereas fibres in the pectoralis muscle (wing depression) of the quail (*Coturnix japonica*) range from 8.8 to 33.2 mm (20–79% fascicle length) (Trotter *et al*. 1992). In the human sartorius, a relatively long, architecturally parallel bifunctional muscle, fibres range from 6 to 20 mm (12–40% fascicle length) (Heron & Richmond 1993), whereas in cat tibialis anterior, an ankle dorsi-flexor of unipinnate architecture, fibres range from 8.7 to 58 mm (65–100% fascicle length) (Ounjian *et al*. 1991). We are in agreement with Ounjian *et al*. (1991) and Gans & Gaunt (1991) that, in the absence of further comparative studies framed within specific functional–phylogenetic contexts, these data preclude generalizations about absolute and relative fibre length from one in-series muscle to the next within as well as across species.

Based on single fibre dissections, Trotter and colleagues have provided considerable empirical morphological data for in-series muscle fibres and have proposed testable models to explain how in-series fibres function (for a review, see Trotter 1993; Trotter *et al*. 1995). They propose that force produced by an active in-series muscle fibre is transferred to adjacent fibres (active or passive) through shear interactions mediated by an endomysial connective tissue network. The geometry of the muscle fibre, in particular the extent and mode of fibre termination, thus influences the effective transfer of force between muscle fibres (Eldred *et al*. 1993; Trotter *et al*. 1995).

In addition to individual muscle fibre morphology, models of force production must consider the organization of individual muscle fibres into motor units, because this organization determines the functional combinations of fibres during muscle activation. Thus, in an actively contracting muscle of in-series design, fibre interactions do not occur at random but are mediated by the organization of fibres into motor units and by the particular pattern of motor unit activation. Intra- as well as inter-

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spatial relationships of fibres of different muscle units will influence a muscle unit's force profile (Ounjian *et al*. 1991; Trotter *et al*. 1995).

Our finding that pectoralis motor units are comprised of muscle fibres distributed asymmetrically within a muscle unit is similar to that of several other studies of vertebrate skeletal muscle. In the cat anterior sartorius, for example, although muscle unit territories extend the origin-to-insertion length of the muscle, individual fibres do not extend the full length of the muscle unit (Loeb *et al*. 1987; Smits *et al*. 1994). The largest number of fibres (highest innervation ratio) is observed either in the proximal or distal end of the muscle unit. Smits *et al*. (1994) also found larger innervation ratios for FG (mean 550) versus FOG (mean 156) and SO (mean 190) units. An uneven distribution of innervation ratios at different proximo-distal levels also exists in the cat tibialis anterior muscle (Roy *et al*. 1995); in this muscle, the majority of fast twitch muscle fibres (both fatigue resistant and fatigable) do not extend the full length of the muscle unit territory (Ounjian *et al*. 1991; Roy *et al*. 1995). Additionally, an uneven distribution of innervation ratios at different proximo-distal levels is suggested in figures of depleted units from the cat medial gastrocnemius (Burke & Tsairis 1973) and cat lateral gastrocnemius (English & Weeks 1984) muscles.

Differences in muscle unit organization between pigeon pectoralis and other muscles are also evident. For example, although FG fibres are the longest fibres and have the largest percentage of blunt terminations in both turtle neck retractor muscles (Callister *et al*. 1992) and the pigeon pectoralis, in cat tibialis anterior it is the slow oxidative fibres and not the fast twitch fibres that are generally longest, extend for the greatest percentage of fascicle length, and possess blunt origins and terminations (Ounjian *et al*. 1991).

(b) *Functional implications*

The generation of force in a muscle of in-series design is a consequence of both the mechanical mechanisms of force transfer between fibres, and the innervation and activation of muscle fibres by the nervous system. The functional significance and neural control of in-series muscles in general is not clearly understood (Trotter *et al*. 1995). Sokoloff *et al*. (1998) considered the general organization of FG and FOG motor units in pigeon pectoralis in the context of fibre length, fibre morphology and fascicle length, histochemical–physiological correlations, *in vivo* power production, neuron size and motor unit type, motor unit recruitment order, and functional compartmentalizaton of motor units for flight. Differences in these features between motor units comprised of FOG versus FG fibres supported the hypothesis that these two motor unit types are differentially activated during flight. The present findings, that the muscle units comprised of FG fibres are organized more in-series than those comprised of FOG fibres, enable us to extend the discussion of type differences in force generation within and across motor units in the pigeon pectoralis.

An on-average longer length of individual FG fibres means that an FG unit will probably have more sarcomeres in-series than a FOG unit, and thus may be capable of contributing force over a longer muscle length change (i.e. high power) such as occurs during the large-amplitude wing movements during take-off and landing (Dial *et al*. 1987; Dial & Biewener 1993). In this respect, the pigeon pectoralis is organized like the turtle neck retractor muscle, in which the longer FG fibres are thought to provide relatively greater speed of contraction and minimize in-series compliance (Callister *et al*. 1992).

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