Single and Paired Motor Unit Performance in Skeletal Muscles: Comparison Between Simple and Series-Fibred Muscles from the Rat and the Guinea Pig

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Abstract

We have compared the performance of isolated motor units in a simple parallel fibred rat skeletal muscle with those of a parallel series-fibred guinea pig skeletal muscle. In the rat muscle tension may be delivered efficiently to the tendons since all fibres have tendonous insertions at both ends, while in the guinea pig muscle most fibres terminate intrafascicularly so the pathway for tension delivery includes neighbouring fibres thereby introducing a series compliance. The properties of tension delivery through series fibres is likely to vary as a function of their stiffness (i.e. whether or not they are coactive) and therefore might be altered by motor unit usage patterns. To test this hypothesis we measured the physiological properties of pairs of isolated motor units when stimulated individually and when stimulated together. We found that coactivation of pairs of motor units on average resulted in delivery of more than the sum of the tension of the two contributing units, and that this was greater for the guinea pig than for the rat muscle (19.8% vs. 8.7% increase). The results indicate that tension delivery is modulated by interaction between active and inactive fibres, and that the spatial arrangement of active fibres across and along the muscle belly is a critical determinant of performance in the case of the series-fibred muscle.

Key words: skeletal muscle, motor unit, tension delivery, fibre architecture.

Basic Appl. Myol. 9 (2): 79-87, 1999

The skeletal muscles of small laboratory rodents are predominantly formed of individual fibres running in roughly parallel arrays in which fibres are generally more than half the length of the whole muscle [2], each inserts directly onto tendon at both ends, and in which each receives a single neural contact near its mid-point. Among larger mammals this simple form of neuromuscular organization is much less common and muscles often feature internal tendonous subdivisions, fibres with intrafascicular terminations, various degrees of pinnation, and multiple sites of nerve-muscle contact [9-11, 15, 23, 27, 33]. The unifying design feature of all muscle forms is that they are organised to allow individual fibres to remain relatively short, irrespective of the length of the muscle as a whole. The value of short fibre lengths is the promotion of nearly synchronous activation of sarcomeres at all points along the fibre [16]. Among long parallel fibred muscles, an obvious consequence of the need for short fibres is the presence of fibres with intrafascicular terminations [5, 9-13, 16, 17, 22, 24, 28, 30, 32-35]. Fibres that terminate intrafascicularly are required to deliver their tension to the tendon via an indirect route, likely to include both neighbouring fibres and endomysial connective tissue [22, 33], and this complication introduces a degree of compliance into the pathway that may compromise the delivery of tension [35].

As the basic unit of neuromuscular operation, the motor unit is of particular interest in examination of the way in which series-fibred muscles work. Studies using glycogen depletion to reveal the intramuscular distribution of unit fibres in muscles of this type have shown several types of fibre layout within the muscle belly (illustrated in [35]), but no-one has yet described a motor unit made up of fibres aligned in series along the length of the muscle [6, 21, 25, 26, 29, 35]. This observation highlights the theoretical difficulties described by many workers, namely that the passive stretching of inactive series elements (with consequent storage of some of this energy) and the load of the inactive neighbouring

fibres are likely to reduce the speed and magnitude of force delivery to the tendon [28]. This problem is at its most significant when a single motor unit is activated in isolation. Here, most of the muscle is quiescent and only those fibres contacted by branches of the activated axon will develop tension. In real behaviour, single motor units are rarely activated in isolation. Instead, multiple motor units are activated in a synchronous or overlapping fashion. This type of activity has the potential to create coactive groups of fibres which may interact and modify each other's mechanical properties, particularly if the active fibres are aligned [22].

Several types of interaction between motor units are possible. Alignment of coactive fibres in series along the muscle would minimize compliance, potentially allowing for faster delivery of a greater proportion of the total tension developed. If active fibres were adjacent, then frictional interaction and loading between the two may be minimised. If there were slight discrepancies in timing or amount of tension developed between the two active units, it may be possible for one unit to decrease the efficiency of the other by changing its resting length (and its position on the length-tension curve). Finally, with small and widely dispersed motor units it is possible that two active units may behave independently.

In summary, synchronous activation of several units could result in alteration of the characteristics of tension delivery: compliance, frictional and loading interactions may all change. This theory has been tested (directly and indirectly) on several occasions [1, 3, 4, 7, 14,18, 31]. In general, the finding is that synchronous coactivation of several units results in the delivery of more tension than could be predicted by calculating the algebraic sum of the individual components. This has previously been explained (and dismissed) as being due to the existence of polyneuronal innervation [1, 14], to "mechanical factors" [1], or due to overcoming "frictional forces" [7]. Although one study has examined the phenomenon in two different muscles and found this type of tension summation to be present to different extents in those muscles [7], no study has attempted to investigate this type of functional interaction in muscles of different architectural types, and then to correlate the results with the muscle's known morphological characteristics.

One would predict that non-linear force summation should vary as a function of muscle architectural type. In the simple non series-fibred muscles typical of small rodent leg muscles the performance improvements on multi unit stimulation should be modest, since all fibres have direct aponeuroses on both tendons and series compliance will be low. In the series-fibred muscles typical of larger experimental mammals motor unit performance should vary as a function of whether series-fibres are synchronously active (stiff) or inactive (compliant), with tension delivery being more efficient through stiff fibres.

In this paper we examine the phenomenon of non-linear force summation in two muscles of different architectural types: 1) a simple parallel fibred muscle (the rat soleus) in which fibres run tendon-to-tendon and in which there is a single zone of nerve contact; and 2) a parallel series-fibred muscle (the guinea pig sternomastoid) featuring short fibres with intrafascicular terminations, and four zones of nerve contact [5]. Our goal was to see whether non-linear tension summation between coactive motor units varied as a function of motor unit architectural properties.

Materials and Methods

The main goals of this study required direct comparison of the physiological properties of isolated motor units in muscles of different architectural types. Specifically, we wished to compare units of a parallel series-fibred muscle (which we refer to as "series-fibred") with those of a parallel non-series-fibred muscle (which we call "simple"). After detailed investigation of the architectural forms of convenient, well circumscribed muscles in lab mammals, it became apparent that a cross-species comparison would be required. The rat and mouse provided no convenient series-fibred muscles [2], while the guinea pig provided no convenient simple muscles [23]. Other selection criteria included ease of dissection with motor nerve accessible and intact, muscle shape (relatively thin and flat is best for survival in vitro), length and shape of tendon available for attachment to recording apparatus, and force generating potential (we wanted two muscles likely to produce similar tension at a magnitude to allow accurate recording from isolated motor units). The muscles that best met these needs were the rat soleus (simple) and guinea pig sternomastoid (series-fibred). We used a twitch stimulation paradigm to overcome the complications of different myosin isoforms and fusion frequencies between the two muscles, to optimize our ability to see the role of series compliance without the complication of "pretensioning" by earlier impulses (as would be the case with a tetanic stimulation regime), to avoid the complications of post-tetanic potentiation which may have become important with the required stimulation protocol [20], and to facilitate direct comparison with similar published twitch data.

Animals and tissues

Experiments were done on adult female albino guinea pigs, and adult female Wistar rats. Guinea pigs were killed with an intravenous overdose of Saffan (alphaxalone, alphadolone acetate, Pitman-Moore NZ Ltd). The upper chest and neck regions of the animal were shaved and an incision made from just below the manubrium to the base of the jaw line. The flaps of skin

were pinned back to expose the neck structures. Blunt dissection was used initially to expose the branches of the second to fourth cranial nerves and the spinal accessory nerve, which innervate the sternomastoid. These nerves travel in union towards the rostro-lateral portion of the muscle. The nerve was cut prior to the point of bifurcation close to the muscle, and the short muscle tendons were then cut close to their sites of attachment on the sternum and the mastoid process. The entire nerve muscle preparation was then removed to an oxygenated (95% O_2/5% CO_2) saline solution containing (mM): NaCl 120; KCl 4.5; $CaCl_2$ 4.0; $MgSO_4$ 1.0; $NaHCO_3$ 25; BES 5.0; H_2O_2 0.003%; heat inactivated serum 2.0%. Tissues were continuously superfused with this solution at room temperature for the duration of the experiment.

Rats were killed by cervical dislocation. The skin covering the distal hindlimb was removed, the achilles tendon cut, and the gastrocnemius muscle group reflected away to expose soleus, which was carefully removed with as much of its nerve and tendons as possible. The muscle was maintained and used in a saline solution identical to that described above for guinea pigs.

Experimental preparation

For each animal, muscles from both sides were removed to a Sylgard (Dow Corning) lined Perspex chamber and viewed under a dissecting microscope. Muscles were pinned to the base of the recording chamber, cleaned of excess fat and connective tissue, and elevated slightly from the base of the chamber to maximize surface area in contact with the perfusion solution and minimize contact with the dish. Care was taken to ensure that all muscles were well aligned and that they pulled along their long axis directly in line with the point of attachment to the force transducer. The sternal end of sternomastoid and the proximal end of soleus were immovably anchored to the dish, while the other tendon was firmly attached to a length of fine tungsten wire (TW-56, Warner Instruments, Connecticut, USA) in turn attached to a sensitive isometric force transducer (Harvard Apparatus, Massachusetts, USA). The recording setup was designed with low-compliance couplings in mind. The transducer has a displacement of 0.1 mm for a 1 gram applied force. The muscle was coupled to the transducer at one end with zero compliance low mass tungsten wire, the other end immovably fixed with tungsten pins to the firm base of the dish. Force transducer output went through a MacLab bridge amplifier (AD Instruments, Australia), to a MacLab 4 (AD Instruments) and finally to an Apple Macintosh LCIII computer running Scope software (AD Instruments) for display and analysis.

Single motor units were stimulated by teasing the cut end of the nerve into individual or small groups of filaments, and drawing one of these filaments into the tip of a close fitting glass suction electrode. The silver wires of the suction electrode were connected to an isolated stimulator (Type 2533, Devices Instruments, Welwyn Garden City, England). This process was completed twice on each nerve, so that two separate motor axons could be activated together or in isolation. Stimulator triggering was achieved using MacLab and Scope. Initially, the whole nerve was stimulated and resulting tension was recorded. Muscle length was then adjusted to give the maximal tension (not shown), and the muscle was held at this length for the remainder of the experimental protocol.

Motor unit isolation and activation

Axons were stimulated with single lms square wave pulses ranging from 0.01-5.00 V. Motor units were isolated by stimulating each teased nerve filament with increasing intensity to determine whether the branch comprised one or more axons. Increments in tension on increasing stimuli indicated recruitment of more than one axon in the suction electrode. In these cases it was essential to confirm isolated stimulation of a single unit, which was achieved by careful adjustment of stimulus parameters until a minimal, reproducible, indivisible all-or-nothing twitch resulted. This process was repeated for each suction electrode until we were sure that in each we were activating a single motor axon.

Data gathering

Each unit was stimulated to give a single twitch response and the magnitude and time to peak of tension recorded. After stimulation of each motor unit in isolation both motor units were activated synchronously and the same parameters measured. The arithmetic sum of the tensions produced by each unit activated in isolation was compared with the tension produced when the two units were activated in unison. We also compared twitch time to peak tension by calculating the average of the two times when stimulated in isolation with the twitch time to peak tension of the double unit stimulation. From these figures we calculated a rate of tension delivery for each unit and unit pair. This was done by dividing peak tension by twitch time to peak tension.

Statistical analysis was done using InStat 2.02 (Graphpad Software Inc.), the particular statistical test employed being indicated with the significance values in the text.

Results

Results are derived from the study of 99 pairs of sternomastoid motor units isolated from the muscles of 10 guinea pigs, and 64 pairs of soleus motor units isolated from the muscles of 5 rats. The uncomplicated and routine methodology gave reliable and reproducible results. Muscle preparations survived in vitro for the duration of the experiment (4-8 hours) with no diminution of re-

sponse. We have kept the preparation active and in good condition in other experiments for up to 24 hours. Magnitude and timing of twitch contractions were measured directly from the computer trace using the MacLab marker and cursor facilities. In spite of the major difference in architectural form of the two muscles, isolated motor units of the rat soleus and the guinea pig sternomastoid delivered similar tensions in response to single stimuli (Table 1). Examination of the data plots presented in Figure 1 shows that the two muscles generated results which were qualitatively very similar (the average percent tension increase being the only difference), supporting our view that the species and myosin isoform differences between the two muscles are of no consequence for the parameters being compared in this study.

Twitch tension summation

Guinea pig

The average isolated sternomastoid motor unit delivered 1.78 nN of force with a twitch time to peak tension of 52.3 ms in response to a single suprathreshold stimulus of the motor axon (Table 1). When pairs of units were stimulated synchronously there were three possible outcomes: they delivered either the same, more, or less tension than the arithmetic sum of that produced when the two units were stimulated in isolation. We defined "the same tension" as: having a tension on paired activation within 2% of the summed tension on single unit activation, and 8% of specimens fell within this range. Those having a more than 2% increase in tension comprised 71% of specimens (n = 70). These positively interacting unit pairs delivered an average 34% tension increase on double unit activation. The remaining 21% of specimens (n = 21) generated a greater than 2% reduction in tension on coactivation, with a mean decline of -21%. The biggest declines in tension on coactivation appeared when total combined tension was at the low end of the observed range (Fig. 1), suggesting it is a feature of the interaction of small motor units. For the group as a whole, paired unit stimulation produced 19.8% more tension than the sum of the same two units stimulated individually. Traces of individual examples of unit pairs showing positive, negative, or no interaction appear in Figure 2 A-C.

Table 1. Twitch measurements for isolated rat and guinea pig motor units.

	Rat	Guinea Pig
Single unit twitch force mean±SD (mN)	1.75±1.2	1.78±1.5
Paired stimulation force mean±SD (mN)	3.62±2.0	4.12±2.7
Average % increase paired stimuli mean±SD	8.7±30.9	19.8±35.3
Single twitch ttp mean±SD (ms)	74.3±11.6	52.3±12.3
Paired stim. ttp mean±SD (ms)	72.7±1 1.3	49.8±10.6
Rate of tension delivery mean±SD (mN/ms)		
positively interacting	4.8±2.4	10.7±8.5
non-positively interacting	5.4±2.9	6.1±6.9

Rat

The average isolated rat soleus motor unit delivered 1.75 nN of force with an average twitch time to peak tension of 74.3 ms in response to a single stimulus of the motor axon (Table 1). On paired activation of rat units 8% of specimens fell within 2% of the sum of the individual values, with 64% (n = 41) producing a greater than 2% increase, and 28% (n = 18) giving a more than 2% reduction in tension. The mean tension increase among the 41 positively interacting units was 23%, while the mean decrease among the 18 negatively interacting units was -22%.

Paired unit stimulation produced an average increase in delivered tension of 8.7% for the group, and this was significantly (two-tailed P value = 0.0416, unpaired t test) lower than the 19.8% increase for the population of guinea pig units. Traces of individual examples of unit pairs showing positive, negative, or no interaction appear in Figure 2 D-F.

Rate of tension development

We measured twitch time to peak tension for isolated and paired unit stimulation and found no difference between these two measures (Table 1), despite the delivery of larger amounts of tension on paired unit acti-

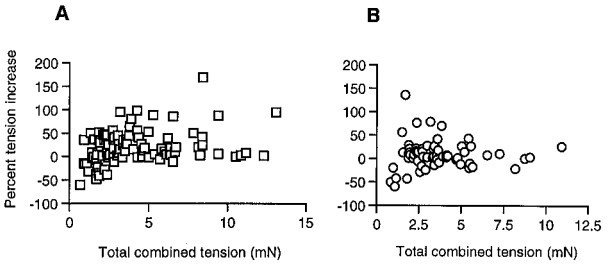

Figure 1. Scatter plots showing the percent change in tension delivered on combined unit activation as a function of total combined tension. Percent increase is calculated as recorded tension on synchronous coactivation divided by sum of individual unit tensions, multiplied by 100. Each symbol represents one pair of units. A) Guinea pig sternomastoid units, average tension increase is 19.8%. B) Rat soleus units, average tension increase is 8.7%. Tension increase on paired unit activation was significantly greater for the guinea pig than for the rat (P = 0.0416).

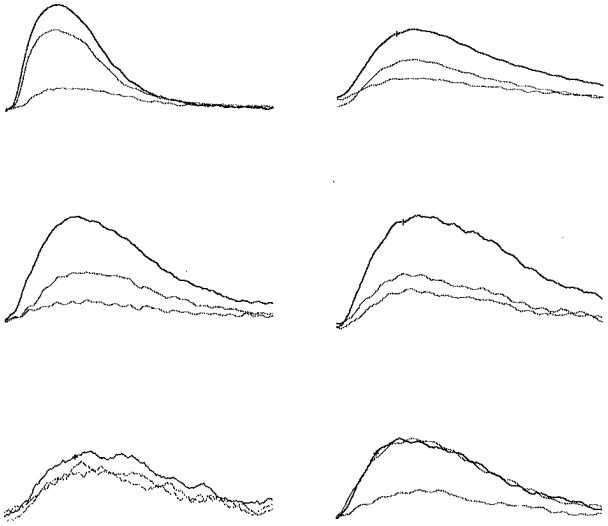

Figure 2. Traces of motor unit tensions from each outcome category. Tension (vertical axis) is displayed against time (200 msec shown in the left column, 300 msec in the right). In each case the greatest tension is that recorded on synchronous coactivation of two motor units, while the two smaller tensions are those recorded by each of the two units in isolation. Guinea pig sternomastoid units (left column). Top, no interaction between units. Unit 1 = 2.24 nN, unit 2 = 8.33 nN (sum 10.57 nN), synchronous coactivation = 10.53 nN. Centre, positive interaction. Unit 1 = 1.00 nN, unit 2 = 2.53 nN (sum = 3.53 nN), synchronous coactivation = 6.54 nN (85% increase). Bottom, negative interaction. Unit 1 = 0.56 nN, unit 2 = 0.50 nN (sum = 1.06 nN), synchronous coactivation = 0.89 nN (16% decrease). Rat soleus units (right column). Top, no interaction between units. Unit 1 = 5.99 nN, unit 2 = 2.72 nN (sum 8.71 nN), synchronous coactivation = 8.74 nN. Centre, positive interaction. Unit 1 = 5.08 nN, unit 2 = 3.56 nN (sum = 8.64 nN), synchronous coactivation = 10.93 nN (27% increase). Bottom, negative interaction. Unit 1 = 2.68 nN, unit 2 = 7.77 nN (sum = 10.45 nN), synchronous coactivation = 8.18 nN (22% decrease).

vation. This suggested that the rate of tension delivery might have been higher on paired unit activation. We wished to know whether this was the case and whether it was influenced by the type of interaction between the two units. The datasets were split into positively interacting and non-positively interacting groups (no interaction plus negative interaction). The rate of tension delivery for guinea pig sternomastoid units which interacted positively (10.7 mN/ms) was significantly higher (two tailed P value = 0.0148, unpaired t-test) than the rate for non-positively interacting units (6.1 mN/ms, Table 1, Fig. 3). By contrast, the same method of analysis applied to the rat soleus data showed no significant difference (two-tailed P value = 0.4394, unpaired t-test) between positively interacting (4.8 mN/ms) and non-positively interacting (5.4 mN/ms) unit pairs (Table 1, Fig. 3).

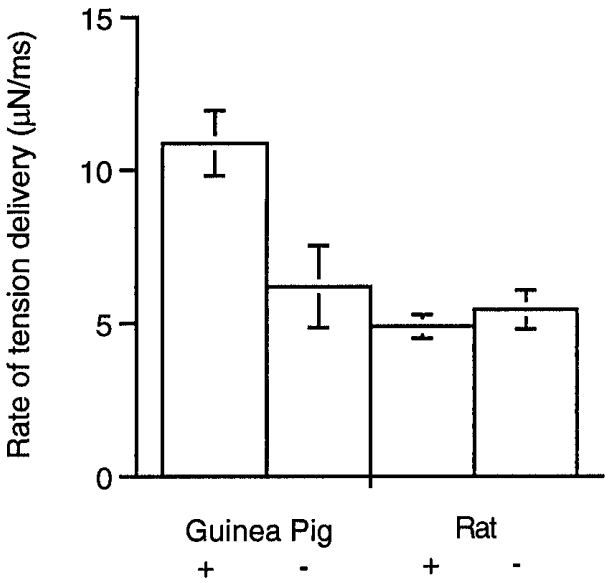

Figure 3. The rate of tension delivery on paired motor unit activation. Data are split according to whether the unit pair showed a positive interaction (+) or a negative or no interaction (-) on coactivation. For the guinea pig sternomastoid, unit pairs showing positive interaction had a mean rate of tension development of 10.7 mN/ms, significantly (P = 0.0148) higher than that of the negatively interacting group (mean 6.1 mN/ms). For motor units of the rat soleus, positively interacting (mean 4.8 mN/ms) and negatively interacting (5.4 mN/ms) groups were not different (P = 0.4394).

Discussion

Our main observation is that when stimulated synchronously, many pairs of motor units deliver tension more efficiently than when stimulated in isolation. Further, we report that this occurs to a greater extent in the series-fibred guinea pig sternomastoid than in the simple rat soleus. This finding highlights the need to consider both muscle architectural type and realistic motor unit activation patterns in any analysis of tension delivery.

Transfer of tension from sarcomeres to extracellular matrix

Sarcomeres are the sites of tension generation within muscle fibres. Most sarcomeres must transmit the force they generate directly to their series and parallel neighbours, eventually to be applied laterally across the sarcolemma and to the extracellular matrix via large struc-

tural proteins [22]. Some tension is transmitted along the fibre to be applied across the sarcolemma at the myotendonous junction [24, 32-35]. In relatively simple muscles such as the rat soleus, individual fibres terminate proximally and distally with a myotendonous junction [2] so that tension is delivered either directly to the tendon or indirectly via the endomysial connective tissue. Tension delivery may be compromised with this layout due to stretching of the connective tissue of the tendon and endomysium (forces which are stored by the compliant elastic tissue rather than transmitted directly as would occur through a stiff coupling), due to friction and shear stress at interfaces between active and inactive fibres, and due to the load imposed by adjacent inactive fibres.

In series-fibred muscles such as the guinea pig sternomastoid, the same mechanisms for transfer of tension from the sarcomeres to the tendons/extracellular matrix will exist with the added complication of series compliance due to one or more myofibres between the point of intrafascicular termination of the fibre and the tendon to which the force must ultimately be applied [9-13, 16, 17, 24, 28, 30, 32-35]. The main goal of this study was to examine how reduction in this series compliance by stiffening (activating) the intervening fibres impacted on motor unit performance.

Enhancement of motor unit performance by paired unit coactivation

Awareness of the presence of compliance in series-fibred muscles raises a number of questions regarding the efficiency of tension delivery from active fibres, and an obvious way to address this is to compare motor unit performance in a situation which highlights the theoretical problems (i.e. isolated motor unit stimulation) with one which simulates a realistic usage pattern (double unit stimulation). We found that significant amounts of tension were not immediately delivered to the tendon (illustrated schematically in Fig. 4), and that some of this was "recovered" by: 1) a reduction in friction, shear stress and loading between adjacent active and inactive fibres (the more active fibres there are, the more likely it is that adjacent fibres will be synchronously active); 2) to the "sharing" of the pretensioning of the connective tissue of the tendons and the extracellular matrix (the more tension developed, the less compliant will be the connective tissue and the extracellular matrix); and 3) to a reduction in the number of inactive series fibres in the case of the series-fibred muscle. In the series-fibred muscle, we predicted that impediments to tension delivery on isolated unit activation may be larger than for the simple muscle due to series compliance and found this to be the case (two-tailed P value = 0.0416, unpaired t-test).

Our main finding is that, on average, coactivation of series-fibred motor units results in greater tension re-

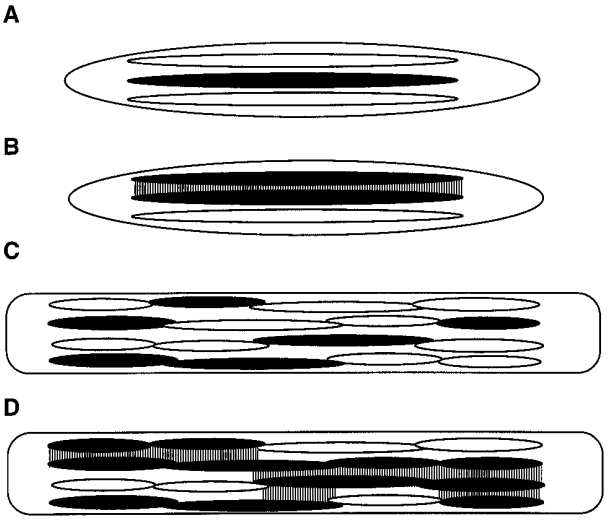

Figure 4. A schematic representation of the model developed to account for the current results. Simple (A, B) and series-fibred (C, D) skeletal muscles are represented. Individual fibres are represented within muscle outlines. Active (stiff, non-compliant fibres) are shaded, while inactive fibres, compliant connective tissue and tendons are shown in white. In A and B, the simple muscle is considered to comprise a parallel array of fibres that span the entire distance between tendons, embedded in a connective tissue matrix. When an isolated motor unit is activated (shaded fibre in A) the load imposed by adjacent inactive fibres and compliant tissues may compromise tension delivery to the tendon. When two units are synchronously coactivated (second f1bre shaded in B) there may be a reduction in the number of active-inactive fibre interfaces (shaded with vertical lines) and consequent reduction in the inactive fibre load resulting in more efficient delivery of tension to the tendon. In C and D, the series-fibred muscle is represented in a similar fashion, showing four sets of fibres connected in series. The fibres of each motor unit are spread across and along the muscle belly in a way that resembles the patterns found in real muscles of this type. When an isolated motor unit is activated (shaded fibres in C) the connective tissue compliance and load due to adjacent inactive f1bres will compromise tension delivery as for the simple muscle, but the additional compliance of the inactive series fibres (white) further compromises delivery of tension to the tendon. When a pair of units are coactivated (second unit shaded in D) there is stiffening of some of the previously inactive series fibres which makes them more efficient intermediaries in the tension delivery pathway. In this situation, some improvement in tension delivery may occur by the same mechanism as outlined for the simple muscle (shaded with vertical lines as in B), with additional improvement due to stiffening of some or all of the inactive series elements.

covery than for simple muscles (19.8 vs. 8.7%). Close analysis of the data shows that this is largely due to a higher proportion of positively interacting specimens (and the average magnitude of those interactions) among the series-fibred muscles. For the series-fibred muscle, 71% of specimens interacted positively with a mean improvement in tension delivery of 34%, while for the simple muscle 64% of pairs gave improvements on paired activation, at an average level of 23%. On the negative side, 21% of series-fibred unit pairs produced an average 21% decline in tension delivery, and 28% of simple pairs produced a 22% decline. We believe these differences relate to the architectural forms of the two muscles, as outlined above. Due to the layout of the series-fibred muscle and the likely spatial relationship of unit fibres across and along the muscle belly (see [35]), isolated unit activation is likely to be less efficient than for the simple muscle. With the greater tension losses to series compliance in this layout, also comes the increased potential for tension recovery with the less compliant two-unit activation protocol; and this is what we found.

Non-interacting and negatively interacting pairs

A lack of change in motor unit performance on coactivation may be relatively easily explained if each motor unit occupies a relatively small proportion of the muscle volume, if the two selected units have no fibres in close proximity to one another either across or along the muscle belly, and if onset of tension development between the two is truly synchronous. In such situations the two units will fail to interact on coactivation and each behave as though stimulated in isolation, and 8% of both rat and guinea pig samples fell into this category.

The third group of specimens in our dataset was those pairs of units that impacted negatively on each other's function. In this situation the tension delivered by one or both units appeared to have declined in consequence of two-unit stimulation. A similar outcome has been seen by others [7, 31]. The likely explanation for this phenomenon relates to the ability of one active unit to change the length of the fibres of the other unit to a sub-optimal position on the length-tension curve. This may occur, for instance, if a fast and a slow unit are coactivated. The higher rate of cross-bridge formation among the fast fibres will promote tension development and sarcomere shortening (even under nominally isometric whole-muscle conditions) slightly in advance of the coactive slow fibres. Active shortening among some fibres will result in changes in length of their inactive neighbours, potentially away from their optimal resting length and resulting in the production of less tension by the slower of the two units. This must occur between adjacent fibres as they are bound together by extracellular matrix connections and cannot change length completely independently of one another [19]. Of course, this phenomenon could also work in the opposite way to contribute to improvements in tension delivery, if the slower (or slightly later) activated unit is brought to a more favourable position on its length tension curve by the action of its neighbours. Slight variations in axon conduction velocity between units of different types may also contribute to negative interactions, driving a further asynchrony in onset of tension development between the coactive units. In addition, in experiments such as this, the muscle is held at what we determine to be nominally its "optimal" length for tension delivery. Previous work suggests that optimal length is fibre-type dependent [8], so it is likely that any single length will be optimal for some fibre types and sub-optimal for others. Each of these explanations is consistent with a motor unit size and type-specific interaction, and there is good evidence that motor unit type and recruitment order has a critical bearing on the nature of the interaction [31].

Close examination of the data presented in Figure 1 shows that the most negatively interacting units delivered tension at the low end of the observed range (0.5-2.5 nN). This suggests that the effect is greatest between pairs of small units, or when one unit contributes little tension (average single unit twitch magnitude is 1.75 nN). This result is not inconsistent with recent observations that negative interaction has a degree of fibre type specificity [31].

Enhancement of rate of tension delivery

For any given myosin isoform, a fibre's rate of length change is proportional to the number of sarcomeres connected in series, with higher rates being correlated with higher numbers of sarcomeres (longer fibres). The series alignment of coactivated fibres from different units would have the effect of increasing the number of synchronously active sarcomeres connected in series, effectively creating a single long fibre and should therefore result in an increase in the rate of length change by that "contractile unit". Faster rates of change in fibre length are likely to result in faster rates of delivery of tension. Note that this length-dependent change is independent of myosin isoform and does not involve change in rate or amount of tension developed at the sarcomere level. According to our hypothesis, enhancement of performance by coactivation of unit pairs is brought about partially by the creation of aligned series of synchronously active fibres, while those pairs which do not show functional enhancement are unlikely to have their fibres aligned in series. To test this, we examined rate of delivery of tension among the groups, and found a significant increase among the positively interacting units of the series-fibred guinea pig sternomastoid (Fig. 3). This is an important finding as it indicates that unit pairs that interact positively are more likely to form aligned

series of fibres along the muscle's length. Contrasting this result with that of the non-series rat muscle, there is no set of stimulus conditions which can increase the number of active sarcomeres aligned in series in this muscle so there should be no improvement in rate of tension delivery on paired unit activation. Indeed, we find this to be the case (Fig. 3) with the rate of tension delivery between positively and negatively interacting units being the same. To confirm this we are currently mapping the spatial distribution of motor unit fibres in the various categories by glycogen depletion, and the results of these experiments will be reported elsewhere.

Acknowledgements

This work was supported by grants from the New Zealand Health Research Council, Lottery Health, and the Otago Medical Research Foundation. We thank Assoc. Prof. A.J. Harris and Prof. D.A. Fischman for their helpful comments on the manuscript. The protocol had the approval of the University of Otago Animal Ethics Committee.

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