Comparison of contractile responses of single human motor units in the toe extensors during unloaded and loaded isotonic and isometric conditions

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Leitch M, Macefield VG. Comparison of contractile responses of single human motor units in the toe extensors during unloaded and loaded isotonic and isometric conditions. J Neurophysiol 114: 1083–1089, 2015. First published June 3, 2015; doi:10.1152/jn.00121.2015.—Much of the repertoire of muscle function performed in everyday life involves isotonic dynamic movements, either with or without an additional load, yet most studies of single motor units measure isometric forces. To assess the effects of muscle load on the contractile response, we measured the contractile properties of single motor units supplying the toe extensors, assessed by intraneural microstimulation of single human motor axons, in isotonic, loaded isotonic, and isometric conditions. Tungsten microelectrodes were inserted into the common peroneal nerve, and single motor axons (n = 10) supplying the long toe extensors were electrically stimulated through the microelectrode. Displacement was measured from the distal phalanx of the toe with either an angular displacement transducer for the unloaded (i.e., no additional load) and loaded (addition of a 4-g mass) isotonic conditions or a force transducer for the isometric conditions. Mean twitch profiles were measured at 1 Hz for all conditions: rise time, fall time, and duration were shortest for the unloaded isotonic conditions and longest for the isometric conditions. Peak displacements were lower in the loaded than unloaded isotonic conditions, and the half-maximal response in the loaded condition was achieved at lower frequencies than in the unloaded isotonic condition. We have shown that the contractile responses of single motor units supplying the human toe extensors are influenced by how they are measured: twitches are much slower when measured in loaded than unloaded isotonic conditions and slowest when measured in isometric conditions.

Comparison of contractile responses of single human motor units in the toe extensors during unloaded and loaded isotonic and isometric conditions.

During voluntary contractions the central nervous system controls motor output by two mechanisms: 1) recruitment, whereby units are progressively recruited in order of size from smallest to largest, the smallest units generating the smallest forces or displacements, and 2) rate-coding, by which an increase in the firing rate of active motoneurons will cause an increase in force or displacement, at least within the linear range over which the motoneurons are operating. This transformation of motoneuron discharge into force or movement is a fundamental feature by which the nervous system controls the skeletal muscles that subserve voluntary and involuntary movements (Heckman and Binder 1991). The introduction of intraneural microstimulation of single human motor axons has allowed one to assess the contractile response of single motor units to controlled trains of stimuli, specifically to examine the utility of rate-coding to control force production and the influence of fatigue on the force-frequency response. Motor axon microstimulation has been used to measure the contractile properties of single motor units in the thenar muscles acting on the thumb (Thomas et al. 1990, 1991a, 1991b, 2006; Westling et al. 1990), the long and short extensor muscles acting on the toes (Leitch and Macefield 2014; Macefield et al. 1996), and the long and short flexor muscles acting on the fingers (Fugl-Evand et al. 1999; McNulty et al. 2000).

We have been increasingly interested in how rate-coding operates during voluntary contractions. It is known that human motoneurons do not fire in a regular fashion when volitionally activated, but rather display variability of around 25% (as measured by the coefficient of variation). This physiological variability is known to fall as mean firing rate increases (Stålberg and Thiele 1973). It also has been shown that much of this variability is derived from variations in central drive rather than variations in synaptic input from muscle afferents (Macefield et al. 1993). Physiological variability in a spike train features short and long interspike intervals, and, given that preceding a train of regular pulses with a short interspike interval (“initial doublet”) augments force production by a single motor unit (Macefield et al. 1996), we had reasoned that the presence of discharge variability throughout a train of stimuli would augment the contractile response. Using microstimulation of single human motor axons supplying the long toe extensor muscles, we recently demonstrated that incorporating physiological variability into trains of stimuli produced an increase in contractile response, both before and after muscle fatigue (Leitch and Macefield 2014). Curiously, we noticed that the twitches, measured as angular displacements of a toe in isotonic conditions, were much briefer than those recorded from the same muscles in an earlier study that measured the contractile response as isometric force: the mean twitch rise time for 14 single motor units was 44.5 ± 7.6 ms in isotonic conditions (Leitch and Macefield 2014) yet 74.8 ± 3.9 ms in for 19 motor units in isometric conditions (Macefield et al. 1996).

Muscular contractions can be categorized into isometric, isotonic, and loaded isotonic movements. This is based on the forces generated, muscle length, and dynamic shortening. Isometric contractions involve a muscle generating force without changing in length. For example, when the hand grips an object, the interphalangeal joints do not change their angles, but muscles acting on the digits generate forces sufficient to maintain grip. In isotonic contractions muscle tension remains constant despite a change in muscle length, such as when gesticulating with the hand. Loaded isotonic contractions occur when muscles can shorten (concentric) or lengthen (eccentric) while still generating force, such as when weight lifters are doing bicep curls: initially, the movements are concentric;
however, as the forces generated by the downward load exceed those that can be generated by the muscle, the contraction will become eccentric. Most studies conducted in this field have focused on isometric movements (Milner-Brown et al. 1973), and few studies have examined the biomechanical differences between isometric, isotonic, and loaded isotonic conditions. Though the study of muscles under isometric conditions has been pivotal in our understanding between the frequency of stimulus and forces produced, particularly with respect to microstimulation of single motor axons (Fuglevand et al. 1999; Leitch and Macefield 2014; Macefield et al. 1996, 1999; McNulty et al. 2000; Thomas et al. 1990, 1991a, 1991b, 2006; Westling et al. 1990), with one exception all of these studies have measured force production of single motor units in isometric conditions. Indeed, ours was the only study to have measured the contractile responses of single human motor units in isotonic conditions, and as noted above, twitch durations were considerably shorter in isotonic than isometric conditions (Leitch and Macefield 2014).

Studies in experimental animals have shown that the twitch contraction time can predict the tension-frequency (t-f) relationship of a motor unit: the slower the twitch, the lower the frequencies required in the steep region of the t-f curve (Kernell et al. 1975). It has also been shown that slow-twitch units summate more readily than fast-twitch units, and thus the activation rate required to produce half-maximal force is often lower (Botterman et al. 1986; Kernell et al. 1983). Furthermore, experiments conducted by Jewell and Wilkie (1960) showed similar results. They studied the isolated whole sartorius muscle of the frog and observed shorter twitch durations during isotonic conditions than in isometric conditions. There is no doubt that the animal work has been pivotal in our understanding of the mechanical properties of muscles, but we were curious to see whether the same could be found in single human motor units, obviously when studied in intact conditions. We used the long toe extensor muscles, which have long tendons, a feature we had previously noted accounts for the longer isometric twitch durations of these muscles compared with muscles with short tendons, such as the intrinsic muscles of the hand (Fuglevand et al. 1999; Macefield et al. 1996, 1999; McNulty et al. 2000).

The primary purpose of the current study was to characterize the contractile responses of the same motor unit in the toe extensor muscles of humans in three conditions, unloaded isotonic, loaded isotonic, and isotonic conditions, to determine the frequencies required to generate half the maximal contractile response (measured as angular displacement or isometric force) and to define the frequency at which motor units are fully fused during the different conditions. We activated one and only one motor axon, which was subjected to all three recording conditions. So, whereas the muscle fibers supplied by a given motoneuron clearly determine the twitch properties of the motor unit (Scott et al. 2001), we are specifically interested in how different mechanical conditions affect the contractile responses of a single motor unit. As noted above, other studies have only examined the half-maximal force during isometric conditions (Fuglevand et al. 1999; Macefield et al. 1996, 1999; McNulty et al. 2000; Thomas et al. 1990, 1991a, 1991b, 2006; Westling et al. 1990), and to our knowledge, no studies have compared the contractile responses of single human motor units in unloaded isotonic, loaded isotonic, and isometric conditions.

**METHODS**

*General procedures.* Successful experiments were performed on healthy subjects, 6 male and one female, the majority of whom were 18–23 yr of age (1 subject was 53 yr). Experiments were conducted under the approval of the Human Research Ethics Committee of the University of Western Sydney. Subjects provided informed written consent. The participants were seated with the knee flexed to 120°. The foot was fixed onto a rigid footplate and a Velcro strap (Velcro hook and loop fasteners) firmly attached over the tarsometatarsal joint of the foot so as not to interfere with dorsiflexion of the relevant toes. The thigh was supported by a vacuum cast, preventing any movement at the knee during the experimental procedures. The common peroneal nerve was located at the fibula head by delivering cathodal pulses via a 2-mm surface probe (2–10 mA, 0.2 ms, 1 Hz; Stimulus Isolator, ADInstruments, Sydney, Australia) Once the optimal site for stimulating the nerve was located, a high-impedance tungsten microelectrode (Frederick Haer, Bowdoin, ME) was inserted into the skin. Constant-current cathodal pulses (0.02–1.00 mA, 0.2 ms, 1 Hz) were delivered through the microelectrode, and the microelectrode was manually adjusted to search for a motor fascicle supplying either extensor hallucis longus (EHL), extensor digitorum longus (EDL), or extensor digitorum brevis (EDB). Electromyogram (EMG) was recorded by attaching two surface Ag-AgCl electrodes over the following muscles: tibialis anterior, the peronei, or EDB. One electrode was affixed over the muscle belly and one over the tendon. Signals were amplified (gain 1,000×; BioAmplifier, ADInstruments) and then filtered (10 Hz–1 kHz) and recorded (2 kHz sampling) on a computer-based data acquisition and analysis system (PowerLab 16 SP hardware and LabChart Pro 7 software; ADInstruments). Twitch parameters were measured using the Peak Parameters feature of LabChart software. Isotonic and loaded isotonic contractile properties were measured using a highly sensitive angular displacement transducer (24 μVV° sensitivity; Panlab, Barcelona, Spain) placed over the nail of the stimulated toe: either digit 1 for EHL or digits 2–5 for EDL. Displacement was measured from the distal phalanx of the activated toe, with a bandwidth of DC to 100 Hz, and sampled at 200 Hz. Loaded isotonic conditions were produced by adding a 4-g mass (40 mN) to the displacement transducer. Contrac tive force (DC–100 Hz; 200-Hz sampling) was measured using an isometric force transducer (model 1030; UFL Morro Bay, CA) placed over the nail of the digit.

*Recording and stimulation protocol.* An appropriate intrafascicular site was established when microstimulation generated a small twitch and a reproducible EMG potential, where an increase in current within a small safety margin failed to recruit an additional motor axon. These currents would typically be between 4–14 μA, depending on the diameter of the axon and the well-defined safety margin, typically 2–4 μA, over which increases in current failed to change either the twitch amplitude or EMG profile; at this point, we were stimulating a single motor axon. The motor axon was then stimulated with a set of 10 pulses (1 Hz) at the threshold current to measure the twitch properties of the motor unit. Fusion properties were calculated to determine the point at which full fusion had occurred. As described previously (Macefield et al. 1996), they were calculated as the peak-tough difference (computed either from the displacement or force transducer) and were measured in each condition, starting at 2 Hz (to which the peak-tough difference was normalized) until full fusion had occurred in each of the three conditions; a peak-tough difference of zero means that the tetanic contraction was fully fused.

Following the single twitches, trains of 10 pulses were delivered at frequencies ranging from 2 to 30 Hz, delivered in an ascending order at 2-Hz increments. The interval between each train was ~1.5 s, and ~1.5 min elapsed between the different conditions (isotonic, loaded isotonic, and isometric). This allowed sufficient time to either add
load during the loaded isotonic movements or change to the force transducer for the isometric conditions. These regular trains produced a standard contractile-response curve, allowing us to compare the contractile responses produced by regular trains of stimuli at a range of frequencies (2–30 Hz). This procedure was always conducted in the same order: first, the isotonic responses to 10 pulses at 1 Hz, followed by 10 pulses from 2 to 30 Hz; second, loaded isotonic responses to 10 pulses at 1 Hz, followed by 10 pulses from 2 to 30 Hz; and last, the isometric responses to 10 pulses at 1 Hz, followed by 10 pulses from 2 to 30 Hz. All statistical analyses were performed using Prism 6 software (GraphPad, San Diego, CA). Values are means ± SE.

RESULTS

Microstimulation of single motor axons was successful for 10 motor units in the long toe extensor muscles: 5 motor axons supplied EDL, and 5 supplied EHL. All motor units were studied in three conditions: unloaded isotonic (measured as angular displacement), loaded isotonic (measured as angular displacement with the addition of a 4-g mass), and isometric (measured as force), with the exception of one unit that was lost before the isometric trial was run.

Twitch properties. Twitch duration parameters for the individual motor units, measured at 1 Hz, are provided for the isotonic, loaded isotonic, and isometric conditions in Table 1. Repeated-measures one-way ANOVA with Tukey’s multiple comparison test was used to compare mean durations across the nine units studied in all three conditions. Mean rise time (time to peak of twitch) was 39.3 ± 4.7 ms for the unloaded isotonic condition, increasing to 44.1 ± 4.9 ms for the loaded isotonic condition and increasing further to 54.2 ± 6.9 ms for the isometric condition [F(1,080, 8.639) = 6.566; P < 0.05]. Likewise, twitch fall times increased from unloaded isotonic (131.2 ± 10.0 ms) to loaded isotonic (148.7 ± 10.7 ms) and isometric (166.4 ± 8.2 ms) conditions [F(1,972, 15.77) = 23.81; P < 0.0001]. Finally, as expected, total twitch durations increased progressively from unloaded isotonic (170.5 ± 12.1 ms) to loaded isotonic (192.9 ± 13.9 ms) and isometric (220.6 ± 13.8 ms) conditions [F(1,520, 12.16) = 31.38; P < 0.0001]. These data are shown graphically in Fig. 1.

Frequency-response curves. Trains of 10 pulses were delivered at frequencies ranging from 2 to 30 Hz. At each frequency, the peak displacement was normalized to the value at 30 Hz in the unloaded condition; in the isometric conditions, peak force was normalized to the force at 30 Hz. Experimental records for one motor unit, recorded in unloaded isotonic conditions, are shown in Fig. 2A; data from the same unit in loaded isotonic conditions are illustrated in Fig. 2B. Trains of 10 pulses were delivered from 2 to 30 Hz, in 2-Hz increments; for clarity, only the sections extending from 4 to 16 Hz are shown. Peak displacement was seen to be significantly lower in the loaded than unloaded isotonic conditions, and the frequency required to generate half of the maximal response in the loaded condition was achieved at lower frequencies than in the unloaded isotonic condition for all 10 units. Data for one unit are shown graphically in Fig. 3. It can be seen that 50% of the maximal displacement was generated at around 11 Hz for the unloaded condition, whereas in the loaded condition a frequency closer to 15 Hz was required to generate the same displacement. However, when we normalized the responses to the peak displacement in each condition (no load and with the 4 mN load), it can be seen in Fig. 3B that lower frequencies were required to generate 50% of the maximal responses. Even lower frequencies were required to generate half of the maximal force for this same motor unit.

Fusion-frequency relationships. Fusion properties were analyzed for the three conditions. As with the 1-Hz pulses, at 2 Hz the twitches were still distinct; there was no twitch summation because the frequencies were too low. As the frequency increased, individual twitches started to superimpose, and the degree of fusion increased with frequency, reaching a maximum at 14–16 Hz. At low frequencies, from 2 to 6 Hz, there was no significant correlation between frequency and fusion across all three conditions because at these low frequencies there was little to no twitch summation. However, from 8 to 16 Hz, the degree of fusion increased, more so for the loaded isotonic and isometric conditions (Table 2). This shows that during a loaded isotonic (or isometric) condition, fusion occurs at lower frequencies than in an unloaded condition. This is shown graphically in Fig. 4.

In the unloaded isotonic condition, the stimulation frequency required for the twitches to fully fuse was higher than in the other conditions, presumably because the twitches were shorter in the unloaded conditions. At 10 Hz, the twitches were fully fused in the isometric conditions, but in the unloaded isotonic condition, the degree of fusion was only 69.0 ± 3.8% of maximum. At this same frequency,

<table>
<thead>
<tr>
<th>Unit</th>
<th>Muscle</th>
<th>Rise Time, ms</th>
<th>Fall Time, ms</th>
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<tr>
<td></td>
<td></td>
<td>Unloaded isotonic</td>
<td>Loaded isotonic</td>
</tr>
<tr>
<td>1</td>
<td>EDL</td>
<td>32.8</td>
<td>35.6</td>
</tr>
<tr>
<td>2</td>
<td>EDL</td>
<td>28.3</td>
<td>27.2</td>
</tr>
<tr>
<td>3</td>
<td>EDL</td>
<td>26.8</td>
<td>31.4</td>
</tr>
<tr>
<td>4</td>
<td>EHL</td>
<td>32.7</td>
<td>41.2</td>
</tr>
<tr>
<td>5</td>
<td>EHL</td>
<td>69.2</td>
<td>72.5</td>
</tr>
<tr>
<td>6</td>
<td>EDL</td>
<td>29.4</td>
<td>37.8</td>
</tr>
<tr>
<td>7</td>
<td>EHL</td>
<td>38.3</td>
<td>38.9</td>
</tr>
<tr>
<td>8</td>
<td>EDL</td>
<td>42.5</td>
<td>53.9</td>
</tr>
<tr>
<td>9</td>
<td>EHL</td>
<td>53.7</td>
<td>58.7</td>
</tr>
<tr>
<td>10</td>
<td>EHL</td>
<td>21.6</td>
<td>41.1</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>37.5 ± 14.4</td>
<td>43.8 ± 13.8</td>
<td>54.2 ± 20.8</td>
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</tbody>
</table>

Twitch parameters were measured for 10 single motor units in loaded and unloaded isotonic conditions and for 9 single motor units in isometric conditions. For each unit the mean value (±SD) of 10 pulses was calculated for either extensor digitorum longus (EDL) or extensor hallucis longus (EHL).

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addition of the 4-g load increased the degree of fusion to 85.6 ± 5.3%.

**DISCUSSION**

Intraneural microstimulation of single human motor axons was employed to assess the contractile properties of individual motor units in the long toe extensor muscles in unloaded isotonic, loaded isotonic, and isometric conditions, with respect to both twitch duration and frequency response. Most studies on single motor units, in both experimental animals and humans, have measured contractile response as isometric force. Previous research has shown that there is a sigmoidal relationship between frequency of stimulation and the force produced (Bigland and Lippold 1954; Cooper and Eccles 1930) whereby an increase in frequency beyond 30 Hz fails to increase force.

It also has been shown that individual motoneurons fire within the linear range of the force-frequency response curve, between 8 and 20 Hz (Macefield et al. 1996).

**Twitch properties and biomechanical considerations.** Dorsiflexion of the toes was measured during loaded and unloaded isotonic movements by using a highly sensitive angular displacement transducer placed on the nail of the activated toe. Twitch properties in unloaded conditions showed a shorter rise time and fall time, and hence a shorter total twitch duration, than in the loaded condition, which could be explained purely on a biomechanical basis. As was first identified by Hill (1951), tendon compliance affects the twitch contraction time. Because there is no load (other than the mass and hence inertia of the toe itself) associated with the isotonic condition, we would expect to see greater peak displacement and a much faster rise time and fall time, resulting in a shorter twitch duration. These faster twitch durations would agree with the fact that the tendon is able to move more readily than during loaded conditions, in which tendon compliance must be taken up before movement can occur. Addition of a load to the isotonic condition resulted in significantly longer twitch durations, due to a combination of both increased rise time and fall time. Conversely, when we consider the biomechanics of isometric conditions, the tendon will lengthen as the muscle shortsens. A way of visualizing this is to imagine the movement of a rubber band from a fixed point. If we consider the rubber band to act as the tendon and the fixed point as the toe, then when the muscle shortsens during an isometric contraction the rubber band would lengthen before any buildup of force at the fixed end. Clearly, the reduced displacement in the loaded condition can be explained by the downward force produced by the 4-g mass (40 mN); the added mass would also make the twitches more sluggish, slowing both the rise time and fall time. Although the mass used in these experiments would not be enough to have any effect on whole muscle, the contractile responses we are measuring are only produced by the few muscle fibers supplied by the single motor axon; we had previously shown that single motor units in the long toe extensors produce mean twitch forces of 20 mN (Macefield et al. 2000). Isometric conditions showed a further increase in rise time, fall time, and twitch duration. Both loaded isotonic and isometric conditions displayed much slower and longer twitches than during unloaded conditions.

**Fusion properties of the toe extensor muscles.** Between 8 and 12 Hz, the degree of fusion was significantly different in the unloaded isotonic, loaded isotonic, and isometric conditions, whereas full fusion did occur in all conditions between 10 and 16 Hz. It is well documented that human motor units begin firing at 7–8 Hz (Hannerz 1974; Petajan 1981). Furthermore, microstimulation of single motor axons supplying the toe extensors has shown that no further increase in
contractile response occurs beyond 16–18 Hz (Leitch and Macefield 2014; Macefield et al. 1996). Therefore, we suggest that the fusion values found in the current study are physiologically relevant within the range of 8–16 Hz because this is the primary range over which motoneurons supplying the human toe extensor muscles fire during volitionally generated contractions.

**Functional consequences.** Many movements conducted by humans in everyday life involve isotonic movements, either unloaded or with the addition of load. While there is no doubt that isometric contractions are essential in human function, such as in stabilizing and static grip, isotonic contractions have been understudied and are clearly important. The current research has contributed significant new knowledge on the displacement-frequency relationship during loaded and unloaded isotonic conditions of single motor units in human toe extensor muscles and provided further information on the force-frequency relationships of the same motor units during isometric conditions. We have shown that twitches are slower and longer during loaded isotonic conditions, and even more so in isometric conditions. We have shown that lower frequencies are required to generate 50% of the contractile response of a muscle in isometric conditions, with significantly higher frequencies being required in isotonic conditions. By understanding how single motor units operate in different biomechanical conditions, we can more closely emulate the patterns of neuronal activity needed to produce appropriate contractile responses in different conditions.

As noted above, it is known that in the “primary range” of operation, human motoneurons fire within the linear range of the force-frequency curve; stimulating at frequencies below 8 Hz or above 18 Hz fails to result in an increase in contractile response of the activated motor unit (Leitch and Macefield 2014; Macefield et al. 1996). However, it has been reported that when rapid movements occur which require ballistic contractions, such as throwing or kicking a ball, then motoneurons can fire at greater rates when stimulated with higher-amplitude currents. This is known as the “secondary range” (Kernell 1965; Schwindt 1973). Although the secondary range is of physiological importance, it was beyond the scope of the current study, in which we focused on stimulating single motor axons within the primary range of motoneuron firing.

The current research also has functional implications for therapies such as functional electrical stimulation (FES), whereby muscles weakened or paralyzed by stroke or spinal cord injury are electrically activated in an attempt to improve rehabilitation. Most improvements to this technique have involved advances in technology, programming, and implementation (Lee et al. 1999). FES involves continuous activation of the paretic or paralyzed muscle with regular trains of stimula-
tion. Our previous study (Leitch and Macefield 2014) showed that by incorporating variability into the trains of stimuli, which emulates the variability exhibited by volitionally activated motoneurons, we can generate greater contractile responses. We hypothesized that incorporating variability into the stimulation trains used in FES would optimize the contractile responses and overcome some of the decrements associated with fatigue. In the current study we have shown that twitch and fusion-frequency properties also differ as a function of load on the muscle. Accordingly, if we are able to determine the point at which full fusion occurs in different biomechanical conditions of the muscle, we can control the contractile behavior based on the task at hand. Therefore, if a loaded isotonic movement is required during FES, we now know that lower frequencies are required to achieve full tetanic fusion, that it will reach 50% of its maximal response at lower frequencies than in an unloaded condition, and that even lower stimulation rates are required to generate isometric forces. This would mean that the rate-coding behavior of human motoneurons can be optimized to the biomechanical requirements to produce the appropriate contractile response. Clearly, further research must be conducted in nonisometric conditions to further elucidate control mechanisms at the single motor unit level.

Conclusions. We have shown, for the first time, that the contractile response of single human motor units in the long toe extensor muscles, as assessed by intraneural motor axon microstimulation, are affected by the environment in which the motor unit contracts. In unloaded isotonic conditions twitches are briefer, and fusion occurs later, than in the presence of a load. Twitch durations are the longest, and fusion frequencies the lowest, in isometric conditions. We believe these differences allow the skeletomotor neurons to utilize rate coding and adjust the firing rates to the biomechanical conditions in everyday tasks.

Fig. 3. Frequency-response curve for a single motor unit. A: absolute angular displacements in unloaded (open symbols) and loaded (filled symbols) isotonic conditions. B: normalized displacements and isometric force for the same unit shown in A.

Table 2. Percentage of fusion in single motor units during unloaded isotonic, loaded isotonic, and isometric conditions

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Unloaded isotonic</th>
<th>Loaded isotonic</th>
<th>Isometric</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>30.6 ± 4.0</td>
<td>37.1 ± 4.3</td>
<td>30.6 ± 6.4</td>
</tr>
<tr>
<td>6</td>
<td>50.2 ± 4.3</td>
<td>52.1 ± 3.9</td>
<td>51.5 ± 5.9</td>
</tr>
<tr>
<td>8</td>
<td>60.6 ± 4.8</td>
<td>67.2 ± 4.1*</td>
<td>80.7 ± 6.6+</td>
</tr>
<tr>
<td>10</td>
<td>69.0 ± 3.8</td>
<td>85.6 ± 5.3†</td>
<td>100.0 ± 0.0§</td>
</tr>
<tr>
<td>12</td>
<td>87.7 ± 4.5</td>
<td>96.9 ± 3.1*</td>
<td>100.0 ± 0.0‡</td>
</tr>
<tr>
<td>14</td>
<td>98.2 ± 1.8</td>
<td>100.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td>16</td>
<td>100.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for percentage of fusion in single motor units (n = 9) during unloaded isotonic, loaded isotonic, and isometric conditions. *P < 0.05; †P < 0.0001 for differences between unloaded isotonic and loaded isotonic conditions; ‡P < 0.01; §P < 0.0001 for differences between unloaded isotonic and isometric conditions.

Fig. 4. Fusion properties of the single motor units for all 3 conditions. There was no fusion at 2 Hz, but fusion progressively increased with frequency.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.L. and V.G.M. performed experiments; M.L. and V.G.M. analyzed data; M.L. and V.G.M. interpreted results of experiments; M.L. and V.G.M. pre-
pared figures; M.L. drafted manuscript; M.L. and V.G.M. edited and revised manuscript; M.L. and V.G.M. approved final version of manuscript; V.G.M. conception and design of research.

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