Distribution of Motor Unit Force in Human Extensor Digitorum Assessed By Spike-Triggered Averaging and Intraneural Microstimulation

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Keen, Douglas A. and Andrew J. Fuglevand. Distribution of motor unit force in human extensor digitorum assessed by spike-triggered averaging and intraneural microstimulation. J Neurophysiol 91: 2515–2523, 2004. First published January 14, 2004; 10.1152/jn.01178.2003. A peculiar aspect of the muscular organization of the human hand is that the main flexors and extensors of the fingers are muscles that each give rise to four parallel tendons that insert on all the fingers. It has been hypothesized that these multi-tendoned muscles are comprised of functional compartments, with each finger controlled by a discrete population of motor units. The purpose of this study was to determine the force distribution across the four fingers for motor units in human extensor digitorum (ED), a multi-tendoned muscle that extends the fingers. The force distribution was assessed by spike-triggered averaging and intraneural microstimulation for 233 and 18 ED units, respectively. A selectivity index from 0 (force equally distributed across the fingers) to 1.0 (force concentrated on a single finger) was used to quantify the distribution of motor unit force across the four digits. The mean selectivity index was high for ED motor units assessed with intraneural microstimulation (0.90 ± 0.28) and was significantly greater than that obtained with spike-triggered averaging (0.38 ± 0.14). Therefore it is likely that each finger is acted on by ED through a discrete population of motor units and that weak synchrony between motor units in different compartments of ED may have contributed to the appearance of spike-triggered average force on multiple fingers. Moreover, the high selectivity of motor units for individual fingers may provide the mechanical substrate needed for highly fractionated movements of the human hand.

INTRODUCTION

The remarkable dexterity of the human hand derives in large part from the ability to move fingers relatively independently of one another. Many species of nonhuman primates are also capable of fractionated finger movements enabling the manipulation of objects in highly skilled tasks (Fragaszy 1998). There is general agreement that this well-developed capacity to move the digits independently in humans and other primates is not so much a consequence of specialized mobility of the joints (Wood Jones 1941) but rather appears related to the existence of direct connections between the motor cortex and motor neurons supplying the muscles of the hand (Bennett and Lemon 1996; Bortoff and Strick 1993; Clough et al. 1968; Heffner and Masterson 1975; Lawrence and Hopkins 1976; Lawrence and Kuypers 1968; Phillips and Porter 1964; Porter and Lemon 1993).

An additional factor that has received less attention, but nevertheless might have a substantial influence on the capacity to move the digits independently, relates to the organization of the muscular apparatus through which neural commands are transformed into movements. For instance, the primary finger extensors and flexors are extrinsic muscles that give rise distally to parallel tendons that insert onto multiple digits. Consequently, activation of these muscles might be expected to produce movements in several digits rather than produce individual movements. It is possible, however, that these muscles are comprised of distinct functional compartments, with each compartment controlled by a separate set of motor units. Accordingly, force produced by motor units in multi-tendoned muscles should be highly selective for particular digits. Surprisingly, motor unit force in such a muscle in the monkey was only moderately selective for individual tendons (Schieber et al. 1997).

Knowledge of how motor unit force in humans is distributed across multiple tendons is crucial for understanding the neuromotor control strategies used to perform finger movements. Therefore the goal of this study was to evaluate how force developed by single motor units in a multi-tendoned muscle, the human extensor digitorum (ED), is distributed across the fingers. This was accomplished using two techniques, each with its own advantages and limitations. One technique, spike-triggered averaging, extracts the average force transient associated with the discharge of motor units from the whole muscle force signal generated during voluntary contraction. This method is straightforward to implement enabling several units to be sampled in an experiment. It is strictly valid, however, only when other motor units discharge independently of the reference unit—a condition that may be rarely satisfied. Indeed, motor units within (Datta and Stephens 1990; Nordstrom et al. 1992; Schmied et al. 1993) and across certain muscles (Brenner et al. 1991; Gibbs et al. 1995) may exhibit significant short-term synchrony due to the presence of branched, common, last-order synaptic inputs to motor neurons (Kirkwood and Sears 1978). The other method, intraneural microstimulation of single motor axons, is technically demanding but is not influenced by the activity of other units. We found that spike-triggered averaging resulted in the appearance of motor unit force on multiple fingers. In contrast, intraneural microstimulation produced force almost exclusively on single digits. This suggests that most muscle fibers innervated by a single motor axon insert on a single ED tendon and that weak synchrony between motor units in different compartments of ED (Keen and Fuglevand 2004) may lead to the appearance of spike-triggered average force on multiple fingers.
METHODS

A total of 48 experiments was performed on the right ED muscle in 21 healthy human volunteers (10 women and 11 men; age, 20–42 yr). Twenty-two experiments involved spike-triggered averaging, and 26 experiments measured the distribution of motor unit force across the fingers by intraneural microstimulation of single motor axons. The experimental procedures were approved by the Human Investigation Committee at the University of Arizona. All subjects gave their informed consent to participate in the study.

Muscle anatomy

The ED originates from the lateral epicondyle of the humerus and from aponeuroses situated between the wrist extensor muscles (Wood Jones 1941). In the distal third of the forearm, the tendons destined for the fingers emerge from a relatively indistinguishable muscle mass where they pass under the extensor retinaculum at the wrist and continue across the dorsum of the hand. In this region, the tendons of ED are linked together by bands of connective tissue called the juncturae tendineae. The tendons then cross the metacarpophalangeal (MCP) joints of digits 2–5 and insert via a complex arrangement into the bases of the proximal, middle, and distal phalanges. The human ED and its homologue in the monkey, extensor digitorum communis, are innervated by 4–8 branches of the radial nerve (Abrams et al. 1997; Serlin and Schieber 1993). A muscle closely associated with the ED, the extensor digiti minimi (EDM), arises with ED from a common origin, runs medially to and is often fused with ED along most of its length, and inserts into the fifth digit. Digit 5 commonly receives tendons from both ED and EDM. In many cases, however, the tendon from ED is absent, and digit 5 receives a tendon only from EDM (von Shroeder et al. 1990; Wood Jones 1941). As far as we are aware, it is not possible to distinguish activation of EDM from activation of the digit 5 compartment of EDM. For brevity, therefore, we refer to the ED-EDM muscle complex simply as ED.

Experimental arrangement

Details of the experimental arrangement have been provided in previous reports (Keen and Fuglevand 2003, 2004). For both sets of experiments, subjects were seated in a dental chair with their right elbow and wrist supported and immobilized. The hand was held in a position midway between fully supinated and fully pronated with the thumb pointing upward. With the hand in this orientation, gravity had little effect on the extension force produced by the fingers. The MCP joints were maintained at a joint angle of ~90° by metal cuffs that encircled the proximal interphalangeal joints and were attached to separate force transducers via lightweight cables. The length of each cable was adjusted at the beginning of the experiment so that each digit was preloaded in this flexed position. In the spike-triggered averaging experiments, each finger was preloaded with a force of ~2 N. In the series of experiments involving microstimulation, each finger was preloaded with a force of ~400 mN. Less preload was used for the microstimulation experiments because of the increased sensitivity of the force transducers. The fingers were flexed to lengthen ED and thus optimize the force output of this muscle.

Force recording

Extension force of the digits was measured by four force transducers (Grass Instruments, Warwick, RI). For the experiments that involved spike-triggered averaging, the force transducers had a range of 0–5 N, with a sensitivity of 780 mN/mV. More sensitive force transducers with a range of 0–1 N and a sensitivity of 156 mN/mV were used for the intraneural microstimulation experiments. The force transducers were mounted in a custom built manipulandum that allowed each transducer to be aligned with the proximal interphalangeal joint of the appropriate finger. The force signals were amplified (×1,000; World Precision Instruments, Sarasota, FL) and displayed on an oscilloscope.

Spike-triggered averaging

Motor unit action potentials were recorded with sterilized tungsten microelectrodes (1- to 5-μm tip diam, 250-μm shaft diam, ~20 kΩ impedance postinsertion at 1,000 Hz; Frederick Haer, Bowdoinham, ME) inserted into ED for the experiments that involved spike-triggered averaging. A surface electrode (Ag–AgCl, 4 mm diam) attached to the skin overlying the radius served as a reference electrode. Intramuscular EMG signals were amplified (×1,000), band-pass filtered (0.3–3 kHz), displayed on an oscilloscope, and routed to an audio amplifier. Subjects performed weak isometric extension of all four fingers to activate ED while the microelectrode was manipulated until the action potentials of a single motor unit could be clearly identified. Once a motor unit was identified, subjects sustained a weak contraction of ED and attempted to maintain the unit discharging at a low rate (8–10 Hz). The intramuscular EMG signal and extension force of each finger were recorded for 3 min or until the motor unit could no longer be clearly discriminated. The extension force of each digit during this task was usually <1 N in excess of the resting tension. The subject received visual and auditory feedback on the discharge of the motor unit and 1–2 min of rest between recordings. After each recording, the microelectrode position was readjusted, which occasionally included removal of the microelectrode and reinsertion at a new site until the action potentials of presumably a new motor unit could be identified. Successive trials were performed for up to 2 h.

Intraneural microstimulation

Tungsten microelectrodes with 2–3 mm of insulation removed from the tip were inserted into each of the four compartments of ED to record the EMG responses to intraneural microstimulation. Placement of the EMG electrodes in the different compartments of ED was verified based on the magnitude of forces developed on each finger in response to electrical stimulation (100–300 μA, delivered at 1 Hz). Such intramuscular stimulation in ED usually causes force to develop predominately on one digit only (Keen and Fuglevand 2003). At some stimulation sites, however, similar levels of force were developed on two fingers. Those sites were assumed to be near a boundary between two compartments. In those cases, the position of the electrode was adjusted until force was selectively developed on one digit. Four surface electrodes (4 mm diam, Ag–AgCl) attached to the skin overlying the radius served as reference electrodes for the intramuscular EMG electrodes. EMG signals were amplified (×1,000), band-pass filtered (0.3–1 kHz), and displayed on oscilloscopes.

To estimate the path of the radial nerve that innervates ED, a hand-held bipolar electrode consisting of two saline-soaked felt pads (inter-electrode distance of ~2 cm) was used initially to deliver stimuli along the lateral distal surface of the upper arm. Stimuli consisted of 4- to 8-mA constant current pulses (1-ms duration) delivered at 1 Hz. The electrode was oriented with the cathode distal and the anode proximal and initially situated about 10 cm above the lateral epicondyle of the humerus. The electrode was moved in small steps proximally and distally to determine the cathode site that evoked the greatest extension force in the fingers for a given amount of current. This site was marked on the skin with ink and was used as a guide for the subsequent insertion of the intraneural microelectrode. The entire area was cleansed with alcohol, and an insulated tungsten microelectrode was inserted through the skin in the upper arm at the site determined by surface stimulation.

The electrode was slowly advanced 3–5 cm in an attempt to penetrate the radial nerve while negative current pulses (15–20 μA, 0.2 ms, 1 Hz) were delivered through the electrode. A surface elec-
trode attached to the skin overlying the lateral aspect of the upper arm served as the reference electrode. At a given insertion site, repeated advances of the microelectrode were often made using slightly different angles in the transverse plane for each penetration. Often multiple insertions of the electrode at different sites were required until weak stimulation evoked twitches of ED. If twitches or paresthesias could be elicited at stimulus currents <15 μA, it was assumed that the electrode had penetrated the nerve.

The criteria used to determine whether a single ED motor axon could be selectively activated in response to intraneural stimulation were based on those described previously (Fuglevand et al. 1999; Macefield et al. 1996; Westling et al. 1990). First, the position of the electrode was gently manipulated while low-intensity current pulses (~15 μA) were delivered through the electrode until a site was found that elicited extension force on one or more of the fingers and EMG responses from at least one compartment of ED. Once such a site was found, stimulus intensity was reduced and increased gradually from a subthreshold level until all-or-none responses were observed to occur concurrently in the EMG and force signals. Then, if EMG and force responses remained stable over a clearly discernible range of stimulus intensities above threshold, referred to as the safety margin (>1–2 μA), the site was considered as one suitable for activating single motor axons supplying ED. Once a site was identified that yielded unitary responses, the motor axon was stimulated at 1 Hz for ~1 min. Motor axons were also stimulated at 20 Hz for 2 s to evoke larger forces and provide better signal-to-noise ratio than individual twitches.

In both types of experiments, extension force of each finger, and intramuscular EMG signals were digitally sampled at ~2.5 and 18.5 kHz/channel, respectively, using the Spike2 data acquisition and analysis system (Cambridge Electronics Design, Cambridge, UK). Current pulses were also sampled (20 kHz) by monitoring the voltage drop across a known resistance placed in series with the output of the constant-current stimulating device.

**Data analysis**

Data were analyzed off-line using Spike2 and custom-designed software for all of the experiments. Motor unit discrimination, necessary to estimate motor unit force by spike-triggered averaging, was accomplished using a template-matching algorithm based on waveform shape and amplitude. An event channel representing the timing of discharges of accepted action potentials for a motor unit was generated. The event channel was used as a trigger to send a brief time segment (140 ms, 15 ms pretrigger) of each force channel to an averaging algorithm (Stein et al. 1972; Stephens and Usherwood 1977). The resulting spike-triggered average of each force channel provided an estimate of the force transient contributed by the reference motor unit to each of the fingers. Twitches evoked by intraneural microstimulation were analyzed from the ensemble average of ~10 responses. For responses obtained both with spike-triggered averaging and intraneural microstimulation were analyzed from the ensemble average of ~10 responses. For responses obtained both with spike-triggered averaging and intraneural microstimulation at 1 Hz, peak force and time to peak force (contraction time), measured from the initial rise in force, were determined from the force profile detected on each finger. Half-relaxation time was measured from twitches obtained with microstimulation but not from spike-triggered averages because the relaxation phase of these responses were often distorted. Total motor unit force was calculated as the sum of the peak forces across all fingers. Unweighting of preloaded flexion force was treated as a negative force. Motor unit contraction time was calculated as the mean contraction time for the four fingers. If a motor unit produced no force on a digit, the contraction time for that digit was omitted.

Force produced by intraneural stimulation at 20 Hz was measured as the average force during the middle second of stimulation relative to a baseline force for each finger. Occasionally the baseline force drifted during the 2 s of stimulation. Therefore to account for the changing baseline, force was measured immediately before and after the 2 s of stimulation. The baseline force was taken as the slope between these two points and force was calculated relative to this baseline. Total motor unit force in response to 20 Hz stimulation was calculated as the sum of the force for all fingers.

A selectivity index derived by and described in detail by Schieber et al. (1997) was used to quantify the distribution of motor unit force across the four digits. The selectivity index is calculated from the relative force produced on each of the fingers as selectivity index = d/d_{max}, where

\[ d = \sqrt{\frac{\sum (\tau_i - \tau_{\text{avg}})^2}{\sum (\tau_i)^2}} \]

and \( d_{\text{max}} = \sqrt{\frac{\sum (\tau_i)^2}{\sum (\tau_i)^2}} \)

where \( \tau_i \) is equal to one divided by the number of tendons emanating from the muscle (in the present case, \( \tau_i = 0.25 \)), and \( \tau_{\text{avg}} \) represents the fractional force (finger force/total force) for each finger, \( i \). A selectivity index of one represents a motor unit that transmits all of its force to only one finger, whereas a selectivity index of zero indicates a motor unit that distributes its force evenly across all fingers. A preferred finger was designated for each motor unit based on the digit to which the unit exerted the most force.

A one-way ANOVA was used to compare the selectivity index across fingers. A Student’s t-test was used to compare selectivity index values and contractile properties obtained by intraneural microstimulation of single motor axons to spike-triggered averaged responses of single motor units. Values are reported as means ± SD with a probability of 0.05 selected as the level of statistical significance.

**RESULTS**

This paper reports the contractile properties and force distribution across the four fingers for 233 motor units based on spike-triggered averaging and for 18 motor units activated by intraneural microstimulation of single motor axons in human ED. The small number of units recorded with microstimulation primarily was due to the difficulty in identifying intraneural sites that fulfilled the criteria for selective stimulation of ED motor units. Also, potentially useful sites were occasionally lost due to inadvertent movement of the microelectrode when subjects made small postural adjustments. For sites at which we able to successfully stimulate single motor axons, the average activation threshold and safety margin were 9.4 ± 7.2 and 6.8 ± 3.7 μA, respectively.

For the experiments using spike-triggered averaging, 10.6 ± 4.5 motor units were recorded on average per experimental session. The mean number of events used for spike-triggered averaging was 1072 ± 592 with a range of 52–3,194. A sample recording of ~8 s extracted from a 3-min record is shown in Fig. 1A. The discharge times of the unit were identified and used to plot the instantaneous frequency as shown above the intramuscular EMG trace. Spike-triggered averaging of the four force channels was utilized to obtain an estimate of the force transients exerted by the motor unit on each finger. An example of spike-triggered averaged forces from a single ED motor is shown in Fig. 1B. This unit produced most force on digit 4, a comparable amount of force on digit 3, and less force on digits 2 and 5. Therefore the spike-triggered average force...
delivery of the 10 stimuli shown in Fig. 2 from digit 4. The EMG and force responses associated with the stimuli nevertheless evoked distinct EMG and force responses. Despite the presence of low-level background EMG activity, on an expanded time scale for digits 2–5, microstimulation of a single motor axon is shown in Fig. 2A, with a relatively low selectivity index of 0.34. For this unit, the force was primarily exerted on digit 3, with some force also evident on digits 4 and 5, resulting in a selectivity index of 0.86. EMG responses to intraneural stimulation in this case were isolated to the D3 compartment of ED. Figure 3C depicts a unit (same unit as shown in Fig. 2) that produced ~60 mN of force on digit 4, a modest level of force on digit 3, and a slight, although detectable, force response on digit 5. This unit, in response to 20-Hz stimulation, had a selectivity index of 0.91.

Intraneural microstimulation at 20 Hz was successfully applied in 12 of 14 motor units and was not attempted in four units. Examples of the force records and superimposed EMG responses from four different ED units during 2-s trains of stimulation at 20 Hz are shown in Fig. 3. Each of these four examples was obtained in a different experimental session. For the unit depicted in Fig. 3A, ~20 mN of force was produced on digit 2 with little force developed on the other digits, resulting in a selectivity index of 0.92. Intramuscular EMG responses were detected only in the D2 compartment of ED. In Fig. 3B, force was primarily exerted on digit 3, with some force also evident on digits 4 and 5, resulting in a selectivity index of 0.86. EMG responses to intraneural stimulation in this case were isolated to the D3 compartment of ED. Figure 3C depicts a unit (same unit as shown in Fig. 2) that produced ~60 mN of force on digit 4, a modest level of force on digit 3, and a slight, although detectable, force response on digit 5. This unit, in response to 20-Hz stimulation, had a selectivity index of 0.91.

For comparison, an example of responses to intraneural microstimulation of a single motor axon is shown in Fig. 2A. Despite the presence of low-level background EMG activity, stimuli nevertheless evoked distinct EMG and force responses from digit 4. The EMG and force responses associated with the delivery of the 10 stimuli shown in Fig. 2A are superimposed on an expanded time scale for digits 2–5 in Fig. 2B. The time scale for the EMG responses is briefer than for the force responses, and the stimulus artifacts are denoted with arrows. The baseline force detected at stimulus delivery has been subtracted from each force trace. The superimposed force traces for digit 4 demonstrate consistent twitch transients that were associated with EMG responses detected only in the digit 4 compartment of ED. Smaller and more variable force responses were recorded on digits 2, 3, and 5.

The average responses to the 10 stimuli are shown at higher magnification in Fig. 2C. Because of the limited number of responses included in the averages (5–10), it was not uncommon for the average profiles to indicate moderate force on some digits (e.g., digit 2, Fig. 2C), despite the absence of obvious force transients in the individual force traces (Fig. 2B). This was particularly the case when the baseline force exhibited marked fluctuations. Notwithstanding this limitation, the selectivity index calculated from the average force responses of this unit to intraneural microstimulation (Fig. 2C) was 0.61, nearly double the selectivity of that determined for the unit shown in Fig. 1 using spike-triggered averaging.

FIG. 1. Brief sample of force and intramuscular EMG data taken from a 3-min record (A) and example spike-triggered average forces (B). Bottom 4 traces in A are the simultaneous force recordings for digits 2–5. Directly above the force records is the intramuscular EMG trace recorded with a tungsten microelectrode, depicting the discharge of a single unit. Discharge times were identified and used to plot the instantaneous frequency that is displayed in the top trace. Spike-triggered averaging was used to determine force transient on each finger associated with discharge times of a motor unit. This motor unit displayed a characteristic broad distribution of force across the digits and consequently had a low selectivity index value of 0.34.

FIG. 2. Sample force and EMG data during 1-Hz stimulation of a single motor axon. A: stimuli from intraneural microstimulation of a single motor axon at 1 Hz are depicted in the bottom trace. Directly above this are the simultaneous force recordings for digits 2–5. Distinct twitch responses temporally associated with delivery of stimuli can be seen on digit 4. B: the force records are the intramuscular EMG recordings from the D2-D5 compartments of ED. C: superimposed EMG and force responses for digits 2–5 for the 10 stimuli shown in A are represented on an expanded time scale. Arrows denote stimulus artifacts detected in each compartment. Superimposed force traces show consistent twitch responses for D4, with smaller and more variable responses for the other digits. C: average (n = 10) responses shown on expanded scale. For this unit, the force was relatively selective for digit 4, with a selectivity index of 0.61.
No significant correlation between contraction time and motor unit force was found for the population of motor units obtained by spike-triggered averaging. Nor was any significant correlation between motor unit twitch force and either contraction or half-relaxation times found for the units stimulated intraneurally at 1 Hz. These findings are consistent with those reported previously for motor units in other human muscles using intraneural microstimulation (Fuglevand et al. 1999; Macefield et al. 1996; Thomas et al. 1990b).

**Force distribution**

The mean selectivity index for motor units characterized by spike-triggered averaging was 0.38 ± 0.14, with a range of 0.09–0.79. In contrast, the mean selectivity index for motor units activated with intraneural microstimulation at 1 Hz was 0.73 ± 0.32, with a range of 0.21–1.49. Selectivity was even higher based on the force produced in response intraneural microstimulation at 20 Hz in the 12 units successfully activated in this way (mean selectivity index, 0.92 ± 0.21; range, 0.64–1.35). Because the responses to 20-Hz stimulation were less variable than twitch forces, analysis of selectivity index was based on responses to 20-Hz stimulation if available for a motor unit.

A histogram comparing the force distribution of ED motor units assessed by intraneural microstimulation and spike-triggered averaging according to selectivity index is shown in Fig. 4. The mean selectivity of 18 motor units (12 at 20 Hz) assessed by intraneural microstimulation was 0.90 ± 0.28. In contrast, the selectivity index for motor units obtained with spike-triggered averaging was significantly lower, with a mean value of 0.38 ± 0.14 (P < 0.01). Therefore, while the contractile force of motor units obtained with spike-triggered averaging and intraneural microstimulation were similar, their patterns of force distribution across the fingers were strikingly different.

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**Contractile properties**

The contractile force of the motor units assessed by spike-triggered averaging and intraneural microstimulation was similar. The mean total force for the 233 motor units studied using spike-triggered averaging was 11.4 ± 9.7 mN (range, 0.5–53.8 mN), similar to and not statistically different from the mean total twitch force of 13.8 ± 10.3 mN (range, 2.7–37.7 mN) for the 18 motor units stimulated intraneurally at 1 Hz. These force values are comparable to that obtained for human ED by Monster and Chan (1977), with a mean spike-triggered average force estimated from their Fig. 8 to be ~14 mN. Twelve of the motor units in the present study were also stimulated at 20 Hz, and the average total force in response to 20-Hz stimulation was 34.9 ± 26.5 mN (range, 9.9–102.3 mN).

Contraction times for motor units recorded using spike-triggered averaging were significantly briefer (P < 0.01) than those obtained by intraneural microstimulation, with mean values of 43.2 ± 8.6 (range, 11.3–80.3) and 50.7 ± 11.5 ms (range, 26.3–76 ms), respectively. Mean half-relaxation time for the units studied with intraneural microstimulation was 56.6 ± 10.9 ms (range, 40.5–74 ms). Half-relaxation time could not be reliably determined using spike-triggered averaging.
The 18 units characterized using microstimulation were obtained from 7 of the 13 subjects that participated in microstimulation experiments. Eight of these units were recorded from one subject (who participated in multiple sessions), three units were recorded from two subjects each, and one unit was recorded from each of four subjects. No statistically significant difference was found between the mean selectivity index for the 8 units recorded from the one subject and the 10 units recorded from the other subjects.

Six of the 18 units assessed with microstimulation exhibited selectivity index values > 1.0. Of these six, three were units that exerted most of their force on digit 5. As mentioned above, selectivity index values > 1.0 can occur if motor unit activity causes unloading of force in some digits (treated as a negative value in calculation of selectivity; e.g., Figure 3D). Unloading was not seen in spike-triggered average responses.

Motor units were categorized according to the digit on which they produced the greatest force, referred to as the preferred finger. Based on this classification, 39, 93, 63, and 38 motor units, whose force was estimated by spike-triggered averaging, were designated to have preferred fingers of digits 2–5, respectively. There was no statistical difference in the selectivity index across motor unit groups based on preferred finger (mean selectivity index of motor units for preferred fingers of digits 2–5 was 0.4 ± 0.15, 0.4 ± 0.12, 0.37 ± 0.16, and 0.37 ± 0.13, respectively). Because no difference in selectivity index was found between digits, the data were combined across fingers. The grouped data showed a weak but significant correlation between motor unit force and selectivity ($P = 0.003$, $r = -0.19$), with stronger motor units tending to have lower selectivity indices. Overall, these data indicate that selectivity did not vary across fingers but was negatively correlated, in a modest way, to spike-triggered average motor unit force.

For the data obtained by stimulation of single motor axons, 6, 6, 1, and 5 motor units were designated as the preferred fingers of digits 2–5, respectively. There was no statistical difference in the selectivity index across motor unit groups based on preferred finger. The mean selectivity index of motor units for preferred fingers of digits 2–5 was 0.85 ± 0.29, 0.83 ± 0.26, 0.91, and 1.04 ± 0.33, respectively. Because no difference in the selectivity index was found, the data were combined across digits. In contrast with the data obtained with spike-triggered averaging, these data showed no significant correlation between motor unit force and selectivity. The absence of a significant correlation in data collected by intraneural microstimulation may have been partly due to the small sample size.

DISCUSSION

The main finding of this study was that forces elicited by intraneural microstimulation of single motor axons supplying human ED were significantly more concentrated on individual digits compared with force responses obtained with spike-triggered averaging. Stimulation of single motor axons provides a relatively direct means to assess motor unit contractile properties. Spike-triggered averaging, on the other hand, extracts the contractile response associated with the discharge of a single unit from a force signal comprised of the contributions of many units. It is a method, therefore, that is indirect and is also susceptible to systematic errors (Calancie and Bawa 1986; Kirkwood 1979). Consequently, we interpret these results obtained with microstimulation as those most representative of the physiological properties of motor units in ED. Those results suggest that most muscle fibers comprising single motor units in ED insert on just one tendon and thereby transmit force principally to one finger. The ED, therefore, can be considered as a muscle composed of four distinct populations of motor units, each of which controls a separate digit.

There are two main limitations associated with the use of spike-triggered averaging to characterize the contractile properties of motor units. First, partial fusion of twitches is inevitable at the lowest rates of discharge that can be maintained during voluntary contraction. This causes twitch amplitude and contraction time to be underestimated (Andresen and Bar-On 1983; Calancie and Bawa 1986; Nordstrom et al. 1989; Taylor et al. 2002; Thomas et al. 1990a). Second, the presence of synchronized activity between the target unit and other units exaggerates spike-triggered averaged responses (Kirkwood 2001; Taylor et al. 2002). Because fusion tends to reduce the amplitude of spike-triggered averages and synchrony tends to augment them, these two factors may partially offset one another (Taylor et al. 2002; Thomas et al. 1990a). Consistent with this idea, we found similar mean forces for motor units assessed by spike-triggered averaging and by stimulation of motor axons at 1 Hz, a technique not affected by fusion or synchrony.

The influence of synchrony on spike-triggered averaging was of particular concern regarding the assessment of motor unit force distribution across the fingers. Recently, we found strong synchrony for motor unit pairs within compartments of ED, less but significant synchrony for motor unit pairs in neighboring compartments, and weaker synchrony for motor-unit pairs in nonadjacent compartments (Keen and Fuglevand 2004). Accordingly, motor units that reside in other compartments and that exert force on other fingers will discharge simultaneously with the unit under study by spike-triggered averaging more often than expected due to chance. Such synchrony, therefore, likely contributed to the appearance of spike-triggered average forces on other fingers giving the false impression that motor unit forces in ED were broadly distributed.

The most significant limitation of the data obtained with intraneural microstimulation is the small sample size. In 26 experiments carried out using this procedure, we were able to isolate and characterize twitch properties for only 18 motor units. This low yield relates to the difficulty in placing a microelectrode at a site within a nerve where weak stimulation activates only one motor axon supplying a target muscle (Fuglevand et al. 1999; Westling et al. 1990). Furthermore, because minute changes in microelectrode position can cause unitary responses to deteriorate, recordings obtained with intraneural microstimulation tended to be unstable. Consequently, for some motor units, we were able to record responses for only a limited time, and the number of twitches used to obtain average responses was relatively small (5–10). This factor, coupled with low signal-to-noise ratio, meant that force measurements derived from 1-Hz microstimulation were susceptible to random errors (Fig. 2, B and C). Although the
use of 20-Hz stimulation helped to improve signal-to-noise ratio, individual values of selectivity index should be interpreted cautiously. Nevertheless, the mean selectivity index obtained with microstimulation was nearly 2.5 times greater than that obtained with spike-triggered averaging and had a value close to 1.0. This finding suggests that each finger is acted on by more-or-less discrete populations of motor units.

There is the possibility that the lower selectivity observed with spike-triggered averaging compared with microstimulation might have arisen due to systematic differences in the population of units sampled by the two methods. The distortions caused by fusion and synchrony make it difficult to compare the two samples on the basis of force amplitude or contraction time. Nevertheless, the sample of units obtained with spike-triggered averaging probably consisted mostly of low-threshold motor units, because motor unit activity was recorded only during weak contractions. There is little evidence to suggest, however, that low-threshold weak motor units are necessarily less selective than higher-threshold strong motor units. Indeed, data from this study and from motor units recorded from the monkey extensor digiti quarti et quinti (Schieber et al. 1997) indicate that weaker motor units tend to be slightly more selective than stronger units. Consequently, it seems unlikely that the lower selectivity of units studied with spike-triggered averaging was due to the preponderance of low-threshold units sampled with this method.

The high selectivity of ED motor units for individual fingers may seem surprising given the presence of the juncturae tendinum, a system of connective tissue bands that link together the distal tendons of ED. In a recent study, we investigated the function of the juncturae tendinum in distributing force across the fingers (Keen and Fuglevand 2003). Force developed on each finger was recorded in response to intramuscular stimulation from multiple locations within ED. This was done both under passive loading of ED and when subjects superimposed a voluntary contraction on the passive load. Force responses to intramuscular stimulation were highly selective for individual digits and were not affected by the superimposition of voluntary muscle contraction. These findings suggested that the juncturae tendinum play only a minor role in distributing force across the fingers. Instead, the juncturae tendinum may primarily serve to prevent lateral subluxation of ED tendons over the MCP joints (Saldana and McGuire 1986). Furthermore, the absence of an effect of loading on selectivity suggests that the disparity in selectivity between spike-triggered average responses and those obtained with microstimulation was probably not due to differences in the passive loading conditions used for the two types of experiments.

It should be noted that the larger mean value of selectivity index obtained in response to microstimulation was due in part to the existence of units that exhibited unloading of force on one or more digits leading to selectivity index values >1.0. Such unloading has been observed previously in response to stimulation of single motor units in multi-tendoned forelimb muscles of the cat and monkey (Schieber et al. 1997) and in response to intramuscular stimulation of single compartments in human ED (Keen and Fuglevand 2003). It has been suggested that such unloading might be caused by the development of tension by some motor units on the proximal aponeurosis and origin of muscle fibers that insert on other tendons (Schieber et al. 1997). Also, based on a mechanical model, we suggested that unloading could occur in one tendon in response to active tension developed in a neighboring tendon if the two tendons are interconnected by an obliquely oriented juncturae tendinum arising proximally on the unloaded tendon and distally on the neighboring active tendon (Keen and Fuglevand 2003). Such an arrangement is most often observed between the tendons inserting on digits 4 and 5, with the juncturae tendinum originating proximally on the digit 4 tendon and inserting distally on the digit 5 tendon (von Schroeder et al. 1990). Consistent with the model and anatomical observations, unloading of force in response to intramuscular stimulation (Keen and Fuglevand 2003) or microstimulation (this study) was most commonly found on digit 4 for stimulation sites that evoked most force on digit 5.

If we had assigned a selectivity index value of 1.0 for all cases in which selectivity exceeded 1.0, as was done by Schieber et al. (1997), the mean selectivity index for microstimulation would have been 0.83 ± 0.19. This value is still quite large and more than double than that obtained with spike-triggered averaging. Unloading was not seen in spike-triggered averages, presumably because the relatively small magnitude of unloading was superseded by larger positive forces developed by motor units in neighboring compartments that were synchronized to the trigger unit.

The human ED, therefore, can be considered as a muscle consisting of four neuromuscular compartments, with each compartment comprising a relatively distinct region of muscle fibers innervated by a subpopulation of motor neurons (English et al. 1993). Numerous examples of such compartmentalization have been described (see reviews by English et al. 1993; Serlin and Schieber 1993; Windhorst et al. 1989). Compartmentalization has been demonstrated based on direct evidence in which muscle fibers of single motor units are shown to be confined to circumscribed muscle regions (Armstrong et al. 1988; English and Weeks 1984), inferred from the aggregation of specific muscle fiber types in different regions of a muscle (Chanaud et al. 1991; English and Letbetter 1992; Wang and Kernell 2001), or suggested from the existence of multiple primary nerve branches that supply different portions of a muscle (Abrams et al. 1997; English and Weeks 1984; Schieber et al. 2001).

An important issue intertwined with the presence of neuromuscular compartments relates to whether or not compartments, such as those comprising the multi-tendoned muscles of the hand, can be differentially activated (Kilbreath and Gandevia 1994; Windhorst et al. 1989; Zatsiorsky et al. 2000). Numerous behavioral studies have demonstrated that the fingers do not move (Häger-Ross and Schieber 2000; Robinson and Fuglevand 1999) or generate force (Danion et al. 2003; Reilly and Hammond 2000; Zatsiorsky et al. 2000) entirely independently of one another. Such lack of independence appears to be due, at least in part, to activity in muscle compartments that act on fingers adjacent to the one intended for movement (Kilbreath and Gandevia 1994; Reilly and Schieber 2003). Consistent with these observations, we have shown, using cross-correlation analysis of the discharge times of pairs of motor units, that last-order synaptic inputs appear to diverge to supply motor neurons innervating different compartments of ED (Keen and Fuglevand 2004). Therefore inadvertent movement of other fingers when attempting to move a single finger is likely partly associated with relatively expansive terminal projections of descending inputs (Asunuma et al. 1979; Buys and von Schroeder 1990).
al. 1986; Fetz and Cheney 1980; Shinoda et al. 1981) that limit the ability to activate selectively motor neurons supplying individual compartments of extrinsic finger muscles (Kilbreath and Gandevia 1994; cf. Danion et al. 2003). It is important to note, however, that last-order synaptic inputs to ED motor neurons are not uniformly distributed across the entire pool, but instead appear to predominately (although not exclusively) supply subsets of motor neurons innervating specific finger compartments of ED (Keen and Fuglevand 2004). Presumably, ED motor units acting on different fingers can be activated somewhat differentially to facilitate movements of individual fingers.

This study represents the first systematic investigation of the distribution of motor unit force in a multi-tendoned human muscle using intraneural microstimulation. Other investigators have examined the force distribution of single motor units in multi-tendoned muscles in nonhumans by surgical isolation and stimulation of motor axons. Fritz et al. (1992) showed that the forces developed by motor units in the cat extensor digitorum communis are broadly distributed with a low average selectivity index (~0.14). Schieber et al. (1997) found the distribution of motor unit force in extensor digiti quinti of the macaque monkey to be moderately selective, with a mean selectivity index of 0.57. These values stand in contrast to the high degree of selectivity observed in this study for human extensor digitorum motor units (mean selectivity index of 0.90). Therefore the progressively enhanced capacity for fractionated movements in forelimb digits ascending the evolutionary scale from cats to monkeys to humans may not depend only on the extent and pattern of pyramidal-tract termination on spinal motor neurons (Heyner and Masterson 1975), but may also relate to the selectivity of motor unit force for individual digits.

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REFERENCES


