AGE-ASSOCIATED DIFFERENCES IN DISCHARGE RATE MODULATION OF HUMAN MOTOR UNITS

by

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ABSTRACT

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The nervous system controls muscle force by modulating motor unit activity, which depends on the integration of synaptic input by the motor neurons. Due to significant remodeling of synaptic inputs and intrinsic properties of motor neurons with advancing age, synaptic integration presumably differs between young and old adults and thereby influences the modulation of discharge rate. The purpose of this dissertation research was to examine the consequences of aging on motor unit discharge characteristics. The first project examined age-related differences in the amount of discharge variability in a hand muscle during index finger abduction tasks. The purpose was to examine the influence of motor unit discharge variability on the force fluctuations in a hand muscle of old adults. The results provided evidence that discharge variability, which was similar for young and old adults, had a significant influence on force fluctuations and that the extent of discharge rate modulation was reduced in old adults. The purpose of the second study was to determine how long motor unit activity could be sustained during a voluntary contraction in humans. The study examined changes in mean motor unit discharge rate and discharge variability for the duration that young and old adults were able to keep a motor unit discharging action potentials. Despite the absence of change in discharge rate for young adults, discharge variability exhibited a marked increase. The duration of motor unit activity in old adults was briefer, yet discharge variability increased to a similar extent as for young adults. The purpose of the third project was to compare the discharge characteristics of motor units recruited during an isometric contraction that was sustained with the elbow flexor muscles by old adults at target forces that were less than the recruitment threshold force of each isolated motor unit. In contrast to motor units in young adults that discharged action potentials either repetitively or intermittently depending on the magnitude of the target-force difference, the motor units of old adults discharged action potentials repetitively regardless of the target-force difference. These results indicate that the rate modulation capabilities of the motor unit pool for biceps brachii are reduced in old adults. The purpose of the fourth study was to compare the discharge characteristics of motor units when recruited during contractions that required young and old adults to maintain a constant elbow angle while supporting a compliant load that was less than the recruitment threshold force of each isolated motor unit. The results indicated that the discharge of motor units in old adults was not modulated by an increase in load compliance, whereas it was in young adults. These findings demonstrate that old adults have a reduced ability to modulate motor unit discharge rate, which must contribute to the decline in motor system performance with advancing age.

Dedication

To my loving wife Stephanie, a lily among thorns.

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Introduction

Movement is a characteristic behavior of humans and animals and great effort has gone into understanding how it is controlled. Even simple movements involve activating an array of muscles, each of which comprises on average several hundred functional units, termed motor units. Each motor unit consists of a motor neuron whose cell body resides in the ventral horn of the spinal cord, the muscle fibers it innervates and the axon that connects the two (Burke 1981; Liddell and Sherrington 1925; Sherrington 1925). The motor unit represents the smallest quantity of muscle the nervous system can control (Eccles and Sherrington 1930). Signals from every part of the nervous system concerned with movement converge onto motor units to enable movement, giving them the distinct role as the "final common pathway" for producing and controlling movement (Sherrington 1925).

The motor output of the spinal cord represents the number of active motor neurons and the rate at which those active motor neurons discharge action potentials. The discharge rate of each motor unit is determined by two factors: *1*) the intrinsic properties of the motor neuron and *2*) the synaptic input received by the motor neuron. Along with the size of the motor neuron, its intrinsic properties influence the integration of the synaptic input and are determined by the characteristics of ion channels embedded in the neuronal membrane (Russo and Hounsgaard 1999). The motor neuron membrane potential is influenced by the conductance to specific ions, which influence the generation of action potentials. One class of ion channels, termed ionotropic receptors, is ligand-gated and responsible for most of the change in conductance of the neuronal membrane (Rekling et al. 2000). For example, the afterhyperpolarization (AHP) period arises from a calcium-gated potassium conductance. This conductance allows potassium ions to flow out of the membrane, moving the membrane potential further below its resting value. The hyperpolarization modulates the ability of the neuron to respond to input, therefore influencing the rate at which the neuron can discharge action potentials. A second class of ion channels, termed metabotropic receptors, is characterized by activating intracellular second messenger pathways (Powers and Binder 2001). For example, the monoamines norepinephrine and serotonin can bind to receptors on the dendrites of motor neurons and induce an inward flow of sodium ions through persistent sodium channels and calcium ions through L-type calcium channels (CaV 1.3). This additional depolarization in the dendrites results in an amplification of the synaptic inputs, thereby manipulating the effects of ionotropic receptors and influence motor neuron discharge characteristics (Alaburda et al. 2002).

Aging is associated with a decline in motor performance that can lead to progressive disability and loss of independence (for review see Doherty, 2003). With regard to age, it is of interest to consider how changes in the intrinsic properties and synaptic inputs of motor neurons can influence motor unit discharge characteristics and alter the capability to modulate discharge rate. For example, the decline in the maximal rate a motor neuron can discharge action potentials appears responsible for the reduced ability of old adults to perform rapid contractions (Klass et al. 2008) and the decrease in motor unit number contributes to the loss of muscle mass and strength (Doherty 2003). The focus of this review is to discuss factors that are known to influence the discharge of motor neurons and how changes in these factors with advancing age can account for the reduction in motor performance in old adults.

Motor unit anatomy

The diversity in anatomical arrangement of motor units endows each muscle with specific characteristics relative to its function. Many aspects of motor unit anatomy influence muscle function, including the size of the motor neuron pool, the number of muscle fibers innervated by each motor neuron, and the organization of the fibers in the muscle.

Motor unit pool

The motor neurons that innervate a single muscle are referred to as a motor neuron pool (Burke et al. 1977; Romanes 1951). As each motor unit comprises a single motor neuron, the terms motor unit pool and motor neuron pool will be used interchangeably. The motor neuron pool for a given muscle is grouped in a 'nucleus' arranged in a column spanning several segments along the longitudinal axis of the spinal cord. A typical motor neuron pool consists of several hundred motor neurons. Generally, the motor unit pools of larger muscles contain more motor units and smaller muscles contain fewer motor units. For example, the size of the motor unit pool has been shown to range anywhere from 10 for small muscles (extensor pollicus longus) to 1,500 for large muscles (triceps brachii; Jenny and Inukai 1983).

Beginning in the sixth decade of life, the human neuromuscular system undergoes significant reorganization (Roos et al. 1997). Studies investigating the spinal cords of rats have shown a decrease in the number of motor neurons supplying limb muscles (Hashizume et al. 1988). Human autopsy studies have also shown a reduction in the number of motor neurons in the cervical and lumbar regions of the spinal cord for old adults (Rafalowska et al. 1976; Suzuki et al. 2005; Tomlinson and Irving 1977). There is a more pronounced loss of the largest, higher-threshold motor units in animal studies (Kanda and Hashizume 1989). Other studies support this view by showing an increased proportion of muscle fibers expressing type I myosin in aged animals (Edström and Larsson 1987; Pettigrew and Gardiner 1987), which are innervated by small, lower-threshold motor units (Burke et al. 1971). The pronounced loss of large myelinated axons in the ventral roots of cadavers also suggests the pronounced loss of large motor neurons and their large myelinated axons (Kawamura et al. 1977). In contrast, data from muscle fiber biopsy studies do not support this idea in humans, as some studies found a decrease in the type II fibers, which are innervated by high-threshold motor units (Larsson et al. 1978; Merletti et al. 1992), while others have not (Grimby et al. 1984; Lexell et al. 1986).

To estimate the number of functioning motor units in living young and old adults, a technique called motor unit number estimation has been advanced (McComas et al. 1971). This involves use of electrical stimulation to determine the amplitude of evoked responses for a few single motor units and then divides the average amplitude into the amplitude of a response evoked by a maximal stimulus. The motor unit number estimation technique consistently yields lower estimates for old adults (Brown 1972; Campbell et al. 1973; Doherty and Brown 1993; McComas 1991; 1995; McNeil et al. 2005; Sica et al. 1974). As it is not possible to activate only one motor unit with electrical stimulation, the number of motor units estimated using this technique is consistently lower (biceps brachii, 109) compared with numbers estimated in a study that labeled and counted motor neurons in the primate spinal cord (biceps brachii, 1,051; Jenny and Inukai 1983).

Innervation number

The motor neuron axon branches many times upon entering the muscle and reaches a number of muscle fibers (Kernell 2006). The number of fibers a motor neuron innervates (innervation number) can range from ten fibers in small motor units to several thousand fibers in large motor units (Buchthal and Schmalbruch 1980). The peak force a motor unit can generate is positively associated with its innervation number (Bodine et al. 1988; Kanda and Hashizume 1989; Tötösy de Zepetnek et al. 1992). Therefore, the motor units with the largest innervation numbers are able to generate the largest forces.

The number of muscle fibers belonging to an isolated motor unit can be calculated using the glycogen depletion technique; whereby a motor neuron axon is stimulated repetitively to deplete the intramuscular supply of glycogen in the muscle fibers supplied by the axon (Edström and Kugelberg 1968). The entire muscle is then removed from the animal, cross-sectioned, and histochemically stained to reveal those fibers depleted of glycogen (Bodine et al. 1988; Brandstater and Lambert 1969; Edström and Kugelberg 1968). In a study of the cat medial gastrocnemius, glycogen depletion resulted in small innervation numbers for low-threshold motor units (21 muscle fibers) and large innervation numbers for high-threshold motor units (380 muscle fibers; Burke and Tsairis 1973). These values are also consistent with results obtained from the medial gastrocnemius of the rat (low-threshold motor units: 44 – 77 muscle fibers, high-threshold motor units: 221 – 356 muscle fibers; Kanda and Hashizume 1992). However, it is difficult to obtain cross-sections through the entire muscle that include all of the fibers depleted of glycogen. Therefore, this technique tends to underestimate the actual innervation number (Enoka and Fuglevand 2001).

The average innervation number is obtained by dividing the number of muscles fibers in the muscle by the number of motor neurons innervating that muscle (Feinstein et al. 1955). Muscles primarily concerned with finely grading force have smaller average innervation numbers, whereas muscles that generate high forces have larger average innervation numbers. For example, the first dorsal interosseus (an intrinsic hand muscle) has 119 motor units, approximately 40,500 muscle fibers, and therefore an average innervation number of 340, compared with 579 motor units and an average innervation number of 1,934 for the medial gastrocnemius (Feinstein et al. 1955). These values suggest that this intrinsic hand muscle used for the control of index finger abduction force has, on average, motor units that each produces a small amount of force, whereas the medial gastrocnemius has, on average, motor units that each produces a large force. However, using average innervation number to interpret the control of muscle force seems to be simplistic and the shape and range of the distribution of innervation numbers are likely more important (Enoka and Fuglevand 2001).

The appreciable loss of functioning motor units in old adults is accompanied by an increase in the innervation number of surviving motor units (Roos et al. 1997). When a muscle fiber loses its neural innervation, it will either be re-innervated from a collateral sprout of a surviving motor unit or it will perish (Kanda and Hashizume 1989). Muscle fiber counts from studies using the glycogen-depletion technique have shown an increase in the number of muscle fibers in aged motor units of the cat tibialis anterior (Ansved et al. 1991). In humans, two different measures suggest an increase in the average innervation number of motor units in the muscle of old adults. Macro EMG, a technique that relies on detecting the activity of many muscle fibers active near the recording electrode, has revealed an increase in the innervation number of motor units in the vastus lateralis, tibialis anterior, and the biceps brachii of old adults (de Koning et al. 1988; Masakado et al. 1994; Stålberg et al. 1989; Stålberg and Fawcett 1982). Spike triggered averaging, a technique that extracts the mechanical contribution of a single motor unit from the total muscle force, has yielded smaller average motor unit forces in young (17.4 mN) compared with old adults (29.3 mN; Galganski et al. 1993). The increase in the number of muscle fibers innervated by a single motor neuron with advancing age (Kanda and Hashizume 1989) has important consequences for the motor control of old adults (Galganski et al. 1993).

Muscle fiber organization

The spatial arrangement of the muscle fibers belonging to a motor unit can influence the force they produce (Gans and Bock 1965). The muscle fibers belonging to a motor unit are confined to a limited volume of the muscle (Burke and Tsairis 1973). The size of the area occupied by a single motor unit in the cat tibialis anterior was found to range from 8 to 22% of the entire muscle cross-sectional area (Bodine et al. 1988). In addition, the muscle fibers of a single motor unit are intermingled with muscle fibers of many other motor units (Brandstater and Lambert 1969; Edström and Kugelberg 1968). Consequently, a given area of muscle will contain the muscle fibers of many motor units (Burke 1981). Few muscle fibers extend the entire length of their muscle and as a consequence they transmit their forces to adjacent muscle fibers (Harris et al. 2005; Monti et al. 2001; Street 1983). Because the muscle fibers of motor units are intermingled, the muscle fibers of active motor units will interact with muscle fibers that are not activate. Therefore, changes in the location of muscle fibers belonging to one motor unit will influence the relative amount of interaction among muscle fibers of active and inactive motor units.

At the level of the whole muscle, there are decreases in fascicle length and pennation angle with advancing age (Narici et al. 2003). Following re-innervation of abandoned muscle fibers, the motor units of old cats cover a significantly larger percentage of the whole muscle cross sectional area (Ansved et al. 1991) and there is an increased density of muscle fibers belonging to a single motor unit (Kanda and Hashizume 1989). These changes result in relatively less interaction between the muscle fibers of active motor units with those of inactive motor units. As most muscle fibers do not connect from tendon to tendon, they rely on adjacent muscle fibers and connective tissue to transmit their forces. The inactive adjacent muscle fibers will be passive and contribute to the in-series and/or in-parallel compliance influencing the mechanical properties of activated muscle fibers. Accordingly, ultrasound studies have shown increased muscle and tendon compliance with advancing age (Narici and Maganaris 2006). This increase in compliance has been shown to influence the force transmitted to the skeleton and ultimately the movement of the limb (Monti et al. 2001). Therefore, differences in the neural activation of muscles for old adults may arise from adaptations in muscle architecture with advancing age.

In summary, there are several features of motor unit anatomy that influence muscle activity and define the specific capabilities of skeletal muscle. Age-related changes include a reduction in the number of motor neurons in the spinal cord, an increase in the number of muscle fibers innervated by motor neurons, and changes in the architecture of skeletal muscles.

Motor unit physiology

The generation of action potentials by the motor neuron is determined by the interplay between the intrinsic properties of the motor neuron and the synaptic input received by the motor neuron. This section will cover critical sources of synaptic input to the motor neuron and several intrinsic properties that influence the generation of action potentials.

Synaptic input

Each motor neuron generates action potentials based on synaptic input received from several sources, such as afferent feedback from the periphery, local interneurons, and higher brain centers (Rekling et al. 2000). A single motor neuron receives 50,000 to 140,000 synaptic inputs that each release neurotransmitter that either directly (ionotropic receptors) or indirectly (metabotropic receptors) opens ion channels embedded in the cell membrane (Ornung et al. 1998). The primary distinction between ionotropic and metabotropic classes is that action of ionotropic inputs results in immediate opening of ion channels, whereas metabotropic inputs activate a cascade of intracellular events that change the response of the motor neuron to subsequent ionotropic inputs (Heckman et al. 2009). This section will summarize important sources of ionotropic and metabotropic synaptic inputs onto the motor neuron pool.

Afferent feedback

The role of afferent feedback is to provide the central nervous system with information on the current state of the systems it controls. A predominant source of feedback from the periphery is transmitted via muscle spindle Ia and group II afferents (Brown and Fyffe 1981). The Ia afferent transmits information about the rate of change in length of intrafusal muscle fibers and in response, generate excitatory postsynaptic potentials by releasing glutamate directly (ionotropic) onto motor neurons supplying the extrafusal muscle fibers of that same muscle (Ornung et al. 1995). The feedback from the Ia afferent is wide spread, with each single Ia afferent arising from a given muscle sending a branch to each one of the motor neurons supplying that same muscle, as well as projecting to supraspinal centers (Mendell and Henneman 1968). It is also known that Ia afferents from one muscle can project to the motor neuron pool of another muscle. For example, the Ia afferents from brachioradialis can influence the discharge of motor units for the biceps brachii (Barry et al. 2008). This example of a connection between muscles demonstrates how the activation of muscles that cross the same joint can be modulated at the spinal level. The central nervous system can modulate the release of neurotransmitter at the presynaptic terminal through the activation of local interneurons (Maluf and Enoka 2005). For example, the reflex responsiveness of the stretch reflex is enhanced when a limb supports a compliant load compared with exerting force against a rigid force transducer (Akazawa et al. 1983). This enhanced responsiveness for contractions that involve a compliant load has been accompanied by reduced presynaptic Ia inhibition when compared with tasks involving a rigid force transducer (Baudry and Enoka 2009).

The responsiveness of several reflex pathways declines with age, such as those involving the activity of the Ia afferent (Baudry et al. 2010; Boxer et al. 1988; Butchart et al. 1993; Earles et al. 2001; Kallio et al. 2010; Kido et al. 2004; Morita et al. 1995). Reductions in Ia afferent function with age may result from impaired synaptic transmission (Boxer et al. 1988), an overall reduction in the Ia afferent volley (Falco et al. 1994; Rivner et al. 2001), and apparent changes in Ia presynaptic inhibition (Baudry et al. 2010). Additionally, just as injury to a peripheral nerve will result in a permanent reorganization of Ia afferent connections onto the motor neuron pool

(Alvarez et al. 2010), it can be expected that age-associated changes in Ia afferent function could result in similar reorganization.

Local interneurons

Interneurons reside in the spinal cord, next to motor neurons and exert a powerful influence on the generation of action potentials (Rekling et al. 2000). Most is known about three types of interneurons: primary afferent depolarizing (PAD) interneurons, Renshaw cells, and Ia inhibitory interneurons. The central nervous system can depress the release of glutamate by presynaptic terminals of Ia afferents by activating PAD interneurons (Meunier and Pierrot-Deseilligny 1989). This action, known as Ia presynaptic inhibition, falls under the metabotropic class and is one of the best-characterized spinal pathways in animals and humans. In addition to these PAD interneurons, motor neurons also receive recurrent axon collaterals from other motor neurons innervating the same or synergistic muscles (Cullheim and Kellerth 1978; Cullheim et al. 1977). Recurrent collaterals stimulate the release of GABA and glycine by the Renshaw cell (ionotropic receptors), which result in inhibitory inputs on the motor neuron (Schneider and Fyffe 1992). Despite extensive investigation, the role of recurrent inhibition in motor control is still unclear (Windhorst 1996). Suggested roles for recurrent inhibition include reducing the variability in discharge times by interacting with the afterhyperpolarization period (Mattei et al. 2003; Rossi et al. 2003), and contributing to task related muscle coordination at the same or different joints (Katz et al. 1993). The reciprocal Ia inhibitory interneuron is responsible for reciprocal Ia inhibition between antagonist pairs of muscles (Hultborn 2006). In this pathway, the Ia afferent of the agonist muscle spindle projects onto the reciprocal Ia inhibitory interneuron, which then makes connections with 20% of the entire motor neuron pool of the antagonist muscle (Burke et al. 1971). The function of the reciprocal Ia inhibition pathway is to connect inhibition of an antagonist muscle to activation of an agonist muscle during movements that involve flexion and extension about a joint.

Presynaptic inhibition can also modify reciprocal Ia inhibition and age-related differences in presynaptic inhibition are likely to account for changes in reciprocal Ia inhibition with advancing age (Earles et al. 2001). Recurrent inhibition at rest has been found to remain unchanged with age in humans (Chalmers and Knutzen 2004). This suggests that age associated differences in motor unit discharge rate are the result of changes in other factors other than recurrent inhibition (Rossi et al. 2003). There appear to be reductions in reciprocal Ia inhibition with advancing age (Kido et al. 2004). These reductions are believed to contribute to an enhancement in coactivation of agonist-antagonist muscle pairs in old adults (Darling et al. 1989), but a convincing link has not been established (Hortobágyi et al. 2006).

Higher brain centers

Spinal motor neurons receive numerous projections from higher brain centers, which have a pronounced influence on their activity. Ionotropic inputs include monosynaptic connections via the corticospinal tract onto motor neurons and are believed to be important in the control of voluntary movement (Binder et al. 1998; Dum and Strick 1996; Riddle et al. 2009). Indirect connections include the vestibulospinal tract, fundamental for balance control (Fukushima et al. 1979; Shinoda et al. 1988), and rubrospinal tracts, which are involved in providing volitional commands (Burke et al. 1970; Endo et al. 1975; Hongo et al. 1969). Studies have shown reductions in the amount of synaptic input received by motor neurons from the corticomotor tract with age (Eisen et al. 1996; Oliviero et al. 2006).

The primary metabotropic sources of innervation to the motor neuron pool, arising from the brain stem, are the monoaminergic pathways. Axons that release serotonin and norepinephrine originate in the brain stem (caudal raphe nucleus for serotonin and locus coeruleus for norepinephrine) and terminate in the spinal cord, releasing serotonin and norepinephrine (Heckman et al. 2003; Heckman et al. 2009; Hultborn et al. 2004). These monoamines are the main neuromodulators of motor neuron activity and their modulatory effects on the generation of motor neuron action potentials will be discussed in detail later in this chapter (c.f., persistent inward currents). The literature on changes in the metabotropic inputs with age is sparse, but an age-related degeneration of serotonin axons has been observed in the lumbosacral region of the spinal cords of old rats (Johnson et al. 1993). Although this reduced amount of serotonin released onto the motor neuron implies reduced neuromodulation, it has been shown in cases of chronic spinal cord lesions that motor neuron have an increased excitability (Bennett et al. 2001; Harvey et al. 2006c; Li et al. 2007) possibly due to an increase in serotonin receptor sensitivity to residual endogenous monoamines (Harvey et al. 2006a; b). It is therefore possible that motor neurons of old animals and humans are more susceptible to the effects of neuromodulation.

Intrinsic properties of motor units

The intrinsic properties influence the manner in which synaptic inputs are integrated and transformed into the final motor output. Additionally, the actions of neuromodulators can change the intrinsic properties of motor neurons.

Afterhyperpolarization period

The afterhyperpolarization (AHP) period is the length of time following an action potential that the motor neuron membrane potential is more negative than its resting value. The AHP period is caused by the slow closing of calcium-gated potassium channels, creating a prolonged outward movement of potassium ions (Barrett et al. 1980). The ability of the motor neuron to generate successive action potentials without an increase in inward current is reduced during this period. The AHP period is considered to be an important intrinsic mechanism for determining the minimal and maximal rate of repetitive discharge rate of motor neurons (Eccles and Lundberg 1958; Kernell 1965b). The AHP duration is negatively correlated with motor neuron size, being longest in small motor neurons (Eccles et al. 1957; Zwaagstra and Kernell 1980). The AHP has also been shown to correlate positively with input resistance (Zengel et al. 1985) and negatively associated with the amount of current needed to generate an action potential (Carp 1992; Zengel et al. 1985).

The inaccessibility of human motor neurons leaves us with little direct information on the characteristics of the human AHP. One indirect approach involves plotting the relation between the mean and standard deviation of interspike intervals obtained from motor unit recordings and using an inflection point in the data as an estimate of the AHP duration (Liang et al. 2010; Piotrkiewicz 2001; Piotrkiewicz et al. 2007). However, a simulation of synaptic current applied to cat motor neurons found the method of determining the AHP based on variability of interspike intervals to be inaccurate (Powers and Binder 2000). An alternative technique, named interval death rate analysis (Matthews 1996), has gained wider acceptance with recent efforts to establish reliability for this technique (MacDonell et al. 2007). Briefly, the interval death rate analysis technique involves the application of a mathematical transform to the interspike interval histograms generated from a single train of motor unit discharge times. The ultimate result of this transform is the time constant, which estimates the duration of the AHP during voluntary contractions in humans (Matthews 1996).

The afterhyperpolarization duration has been shown to increase with advanced age in cat phrenic and cat and rat lumbar motor neurons (Cameron et al. 1991; Engelhardt et al. 1989; Kalmar et al. 2009), and remain unchanged in another study of cat lumbar motor neurons (Morales et al. 1987). Although limited to low threshold motor units, a study using the inflection point analysis technique found that the AHP increases with age in human motor neurons (Piotrkiewicz et al. 2007). In the only study to use the interval death rate analysis to compare young and old adults, it was shown that old adults had increased AHP durations compared with young adults (Christie and Kamen 2009b). Therefore it seems that there is an increase in the AHP duration with advancing age, possibly due to a reduced physical activity with advancing age (Christie and Kamen 2009b; Cormery et al. 2005).

Input resistance

The input resistance of a neuron determines how much its membrane potential will change in response to an applied current. It is quantified experimentally with injected currents but is taken to represent the response to synaptic current *in vivo* (Kernell 1966). According to Ohm's Law ($\Delta V = IR$), a neuron with a larger input resistance (R_{in}) will experience a larger change in membrane potential (ΔV) in response to a given current (I) than a neuron with a smaller input resistance. Accordingly, experimental data show that smaller neurons have a larger input resistance, resulting in a greater change in membrane potential in response to a given synaptic input current (Fleshman et al. 1981; Zengel et al. 1985). Input resistance depends on the size of the neuron and the density of resting ion channels and small motor neurons have a greater density of resting ion channels than large motor neurons (Burke et al. 1982; Gustafsson and Pinter 1984; Kernell and Zwaagstra 1981). Therefore, differences in input resistance between small and large motor neurons may reflect differences in density of resting ion channels associated with cell size (Gardiner 1993).

Rheobase current

The amount of current needed to reach voltage threshold for discharging an action potential is quantified as the rheobase current and is a measure of intrinsic excitability. There exists a smaller range of values for the voltage threshold of motor neuron excitation as compared with the range in rheobase values within a given motor neuron pool (Carp 1992; Pinter et al. 1983). This mismatch suggests that variations in rheobase are largely explained by variations in input resistance. Additionally, the range of values of input resistance is similar to that for rheobase. The amount of current required to reach threshold varies systematically for motor neurons of different size, with smallest neurons being easiest to excite (Zengel et al. 1985). Rheobase current is associated with input resistance, making it functionally relevant to the characteristics of the motor unit.

Input resistance and rheobase current have been found to increase with advancing age in animal experiments (Chase et al. 1985; Kalmar et al. 2009; Morales et al. 1987). The positive relation between input resistance and rheobase current observed in young animals is maintained in aged animals (Chase et al. 1985; Kalmar et al. 2009; Morales et al. 1987). This implies that either the input resistance and rheobase current of the same motor neuron decreases with age, or the selective loss of larger, high-threshold motor neurons biases the sampling of smaller, low-threshold motor neurons. In either scenario, it seems that the excitability of motor neurons is enhanced with age. This enhanced intrinsic excitability may be a compensatory adaptation to the way the motor neuron pool responds to other changes in the central nervous system, such as a reduction in descending corticospinal projections (Eisen et al. 1996).

Persistent inward currents

A persistent inward current (PIC) is a slowly inactivating depolarizing inward current mediated either by voltage-gated calcium or sodium channels (Hounsgaard et al. 1988; Kiehn and Eken 1997; Schwindt and Crill 1977). The calcium PIC is slow to activate and deactivate, whereas the sodium PIC is relatively fast (Heckman et al. 2009; Lee and Heckman 1999). When activated by neuromodulatory transmitters, such as serotonin and norepinephrine, the targeted metabotropic receptors initiate an intracellular cascade resulting in the amplification of synaptic input by providing an inward current. This inward current builds onto concurrently active ionotropic input effectively amplifying the input received (Johnson and Heckman 2010). The slow inactivation of these channels allows a period of depolarization (plateau potential) even when the excitatory synaptic input is removed. If sufficient, the prolonged elevation of membrane potential can support sustained discharge of action potentials. Initial studies found that providing an inhibitory input could inactivate PICs (Schwindt and Crill 1980).

The precise functional role of persistent inward currents is unclear, but has been suggested to include a mechanism of gain modulation and a means to reach higher levels of the current-frequency curve (Cushing et al. 2005). The activation of PICs may also be helpful in situations when motor neurons need to be active constantly for long periods of time, such as during postural control or prolonged isometric contractions (Elbasiouny et al. 2010).

Conversely, the activity of the monoaminergic pathways is reduced during sleep (Jacobs et al. 2002), evident by the decreased discharge rate of serotonin neurons in the cat brain stem during slow wave sleep and ceased discharging entirely during rapid eye movement sleep (Jacobs et al. 2002). Suppression of monoaminergic activity during sleep is consistent with the proposed role of this neuromodulators during arousal and motor behavior (Heckman et al. 2009).

In the few studies that have compared motor neuron discharge in response to intracellular currents between young and old animals, it seems that the proportion of lumbar motor neurons that demonstrate persistent inward currents increases with age (Bae et al. 2008; Kalmar et al. 2009), which does not appear to be the case in humans. For example, the incidence of self-sustained discharge in response to plateau potentials is similar between young and old adults in the tibialis anterior muscle (Kamen et al. 2006). When a paired motor-unit technique (Gorassini et al. 1998) was performed in a small hand muscle of young and old adults, there were no differences in the estimated strength of persistent inwards currents between young and old adults (Jesunathadas et al. 2010). As the incidence of persistent inward currents vary between muscles in the same animals (Cotel et al. 2009), the role of persistent inward currents in human aging requires further investigation in a variety of muscles. An additional challenge exists in the difficultly in translating these findings in animal preparations to human motor unit discharge patterns.

Accommodation

Accommodation describes the increased time and amount of current needed to reach threshold for generating action potentials with an decrease in the rate of depolarizing current injection (Araki and Otani 1959). This response is likely caused by sodium channel inactivation

(Meunier and Borejsza 2005). Sodium channels typically inactivate after 5 to 10 ms, which at slow enough rates of current summation increases the amount of current required to reach threshold. The presence of accommodation has been linked to the size of the motor neuron, such that smaller motor neurons do not exhibit accommodation whereas larger motor neurons do (Burke and Nelson 1971).

No studies have compared the presence of accommodation in young and old animals. Researchers have suggested that intermittent motor unit discharge activity could be attributed to the presence of accommodation (Miller et al. 1996). However, this interpretation is based on the observation that intermittent discharge in cat motor neurons injected with depolarizing current was most prevalent in high-threshold motor neurons. It remains to be seen what the effect of advancing age is on motor neuron accommodation. The association between intermittent discharge and high-threshold motor units may be altered with age, as recent data demonstrates the repetitive discharge of high-threshold human motor units in the biceps brachii (Pascoe et al. 2010).

Discharge characteristics

The force exerted by a muscle during a voluntary contraction depends on the number of motor units that are activated (motor unit recruitment) and the rate at which they discharge action potentials (rate coding; Adrian and Bronk 1929). Upon recruitment, a motor unit will begin discharging action potential at a low rate producing a series of twitch responses in the muscle fibers belonging to that motor unit (Kernell 2006). At high enough discharge rates, these twitches overlap and summate to produce greater force by the motor unit (Macefield et al. 1996;

Thomas et al. 1991). Activation of motor units is influenced by recruitment threshold, minimal discharge rate, maximal discharge rate, and discharge variability.

Recruitment of motor units

Recruitment threshold is the force at which a motor unit is active and begins discharging action potentials during a voluntary contraction. The order in which motor units are recruited is relatively fixed (Burke 1981; Desmedt and Godaux 1977a; Lindsley 1935; Smith 1934). In early studies, investigators observed that the amplitude of the signals that appeared in their recordings progressively increased from small to large (Denny-Brown and Pennybacker 1938; Seyffarth 1940). Further study revealed that the primary factor determining the order of motor unit activation is motor neuron size, as indicated by cell surface area, such that smaller neurons are recruited earlier than larger neurons (Cope and Pinter 1995; Desmedt and Godaux 1977b; Henneman 1957; Henneman and Olson 1965; Kernell 1966; Mendell 2005). This reproducible finding is known as Henneman's size principle.

There are three functional advantages to ordering motor unit recruitment by motor neuron size. First, the smallest motor units experience the least reduction in force production during continuous stimulation (i.e., most fatigue resistant), which is important for low intensity contractions performed throughout the day. Second, determining recruitment order at the level of the spinal cord reduces the cortical burden (Henneman 1979). Third, this arrangement permits small force increments to occur before larger increments, allowing for more precise and graded control of muscle at lower forces. As there are many more small motor neurons within a given motor neuron pool, most of the motor units are recruited at low forces (Elek et al. 1992; Grillner and Udo 1971; Milner-Brown et al. 1973). In addition, the upper limit of motor unit recruitment for most muscles is approximately 85% of maximal force (De Luca et al. 1982; Kanosue et al. 1979; Kukulka and Clamann 1981; Van Cutsem et al. 1997) and in some hand muscles is approximately 60% of maximal force (De Luca et al. 1982; Milner-Brown et al. 1973; Moritz et al. 2005; Spiegel et al. 1996; Thomas et al. 1986). Forces beyond the upper limit of motor unit recruitment are produced solely by changes in discharge rate alone.

Although motor units in old adults are activated in accordance with the size principle (Fling et al. 2009), a shift in the distribution of motor unit recruitment thresholds to lower forces has been observed (Erim et al. 1999; Fling et al. 2009; Klass et al. 2005b). This shift may be observed due to a selective loss of high threshold motor units, causing the upper limit of recruitment of tibialis anterior to be lower in old adults (~64%) compared with that of young adults (~90%; Klass et al. 2008). A reduced upper limit of recruitment suggests that a greater proportion of the force capacity of the muscle arises from changes in discharge rate (Connelly et al. 1999). However, maximal discharge rates are also reduced (Kamen et al. 1995; Rubinstein and Kamen 2005) and Connelly et al. (1999) have suggested that a change in the contractile properties of old adult muscle fibers enhances the ability of force generation at lower rates. Therefore, it is equally likely that the observed reduction in upper limit of recruitment arises from a sampling bias toward lower threshold units in old adults.

Minimal discharge rate

The minimal discharge rate of a motor neuron refers to the lowest rate at which it can discharge action potentials repetitively. The minimal discharge rate is approximately equal to the reciprocal of the AHP duration in anesthetized animals (Kernell 1979; 1965b). During voluntary contractions in humans, typical minimal discharge rates range from 5 to 8 pulses per second (pps) for low-threshold motor units, but seem to be higher (10 to 23 pps) for high-threshold motor units (Gydikov and Kosarov 1974; Kudina and Alexeeva 1992; Moritz et al. 2005; Søgaard et al. 1996; Spiegel et al. 1996; Van Cutsem et al. 1997).

A variety of methods can been used to measure the minimal discharge rates during voluntary contractions. In one method, a series of discrete force targets are presented for the subject to match and maintain for 3 to 10 s. The minimal rate is taken from the discrete target just above the target force in which there is no motor unit discharge (Barry et al. 2007; Moritz et al. 2005). In a second method, the rate taken from a window of time centered about at the time of recruitment during a linear increase in force (e.g., ramp contraction) yields a similar minimal discharge rate to that obtained from the discrete contractions (Barry et al. 2007; Moritz et al. 2005). Another common method requires subject to progressively lower the discharge rate of a single motor unit, presented as audio feedback, until the rate is regular but as low as can be achieved. The force at which the lowest rate occurs can be taken as the minimal discharge rate and is typically lower than for discrete or ramp contractions (Barry et al. 2007; Moritz et al. 2005; Spiegel et al. 1996).

The minimal discharge rates achieved by motor units of varying recruitment threshold force are similar between young and old adults (Barry et al. 2007). Because of the relation between minimal discharge rate and the duration of the AHP period, this finding is supported by the observation of no change in AHP duration in aged animals (Morales et al. 1987). No study on human motor units has revealed consistently lower minimal discharge rates in old adults as would be predicted by the suggested increase in the AHP period. This lack of difference indicates that the minimal discharge rate is not responsible for the age-related changes in motor performance.

Maximal discharge rate

The maximal discharge rate of a motor neuron refers to the highest frequency a motor unit can discharge action potentials. Typical values range from 20 to 60 pps during gradual increases in isometric muscle force (Hannerz 1974; Monster and Chan 1977; Seki et al. 2007; Van Cutsem et al. 1998; Van Cutsem et al. 1997), but instantaneous rates as high as 100 to 200 pps have been obtained during ballistic contractions (Desmedt and Godaux 1977a; Klass et al. 2008; Van Cutsem and Duchateau 2005). Furthermore, maximal discharge rates observed in the soleus, first dorsal interosseus, and the biceps brachii are less for low-threshold motor units (Barry et al. 2007; Gydikov and Kosarov 1974; Moritz et al. 2005; Oya et al. 2009). A recent study indicated a strong association between a decline in maximal discharge rates of tibialis anterior motor units and the capacity to perform rapid dorsiflexion torques (Klass et al. 2008).

Various methods have been used to measure the maximal discharge rates during voluntary contractions in humans. One method is an extension of the discrete target method mentioned above. In this method, subjects match a series of discrete target forces for 3-5 s and the discharge rate is taken from the ensuing plateau in force. Maximal discharge rate is then estimated when discharge rate remains constant despite subsequent increases in target force (Barry et al. 2007; Moritz et al. 2005). In a second method, the performs a maximal isometric voluntary contraction while a microelectrode is used to record the intramuscular EMG (Dalton et al. 2010). The EMG obtained during both methods is subject to contamination by an increased interference in the signal due to the overlap of several concurrently active motor units (Keenan et al. 2004; Keenan et al. 2006).

Maximal discharge rates were observed to be lower in old adults compared with young adults during maximal contractions of a small hand muscle (Kamen et al. 1995), biceps and triceps brachii (Dalton et al. 2010), vastus lateralis (Kamen and Knight 2004), and tibialis anterior (Christie and Kamen 2009b; Connelly et al. 1999; Rubinstein and Kamen 2005). The finding that maximal motor unit discharge rates did not differ between young and old adults in the soleus suggests that reductions with advancing age are muscle dependent (Dalton et al. 2009). When tracking a single motor unit through a range of forces, the peak discharge rates observed were significantly lower in old adults (Barry et al. 2007). Reduced maximal discharge rates could be caused by a prolongation of the AHP period with age (Kernell 1965b), which is supported by direct measurements in animals and estimates in humans (c.f., afterhyperpolarization above). The functional implications for reduced maximal discharge rates have been observed in various tasks. First, reduced maximal discharge rates was associated with significantly reduced maximal dorsiflexion torque in old adults (Connelly et al. 1999). Second, reduced rate of dorsiflexion torque development in old adults was also attributed to a reduction in maximal discharge rates (Klass et al. 2008).

Discharge variability

The discharge rate of a motor unit during a voluntary contraction is not constant, but exhibits a degree of variability. At low discharge rates (long interspike intervals) the standard deviation of discharge rate is high and declines as the discharge rate increases (Clamann 1969; Matthews 1996; Person and Kudina 1972; Tokizane and Shimazu 1964). When discharge variability is normalized to the mean interspike interval (coefficient of variation), discharge variability is highest at recruitment and subsequently drops off as force increases above recruitment (Moritz et al. 2005). Additionally, the shape of the interspike interval histogram can be used to characterize motor unit discharge variability (Clamann 1969). During voluntary isometric contractions, the shape of the histogram has been found to be positively skewed at low discharge rates and then to become normal with an increase in discharge rate (Barry et al. 2007; Clamann 1969; Person and Kudina 1972; Poliakov et al. 1995; Tokizane and Shimazu 1964). The shift from positive skew to normal occurs relatively rapidly, occurring just above force levels about motor unit recruitment force (Barry et al. 2007).

It has been shown that synaptic noise, defined as random fluctuations in membrane potential, is the major contributor to variability in interspike intervals (Calvin and Stevens 1968; Stein et al. 2005). Synaptic noise originates from inherent spontaneous neurotransmitter release, random sources of excitation (Calvin and Stevens 1968), and stochastic opening of voltage-gated ion channels (White et al. 2000). Synaptic noise is likely to produce discharge variability by introducing fluctuations in the AHP as it approaches the threshold for depolarization. These fluctuations have the largest probability for eliciting discharge at low rates, when membrane potential is just below the threshold for generating action potentials (Matthews 1996; Person and Kudina 1971; Stein et al. 2005). A functional role for synaptic noise can be seen when the amount of synaptic noise increases during sustained contractions. This progressive increase has been suggested to occur as an attempt to produce more short interspike intervals (<20 ms), which have been shown to provide a transient increase in force (Burke et al. 1976; Christie and Kamen 2006b).

Researchers have suggested a functional link between the level of discharge variability and the fluctuations in force during isometric contractions at varying intensities (Kornatz et al. 2005; Laidlaw et al. 2000; Tracy et al. 2005). When key features of discharge variability were incorporated into a computer model of motor unit rate coding and recruitment of an entire motor unit pool, the ability of the model to reproduce experimental results was dramatically improved

(Barry et al. 2007; Moritz et al. 2005). Changes in the amount of discharge variability have implications for altering the performance of the motor system in regards to maintaining a constant force.

Many studies have investigated the age-related changes in discharge variability. Early studies proved inconclusive, with some reporting greater discharge variability in old adults (Kornatz et al. 2005; Laidlaw et al. 2000; Tracy et al. 2005), whereas others found similar levels in young and old adults (Galganski et al. 1993; Semmler et al. 2000; Vaillancourt et al. 2003). Due to the limitation of recording from a few motor units of the motor unit pool, researchers made an effort to systematically quantify discharge activity across a broad range of forces for both low- and high-threshold motor units (Barry et al. 2007; Moritz et al. 2005). These studies revealed similar levels of discharge variability in young and old adults. An important observation from the studies by Barry et al. (2007) and Moritz et al. (2005) was the existence of a rapid drop from high levels of discharge variability at recruitment (~30%) to low levels of discharge variability at forces above recruitment (~13%). It is likely that the conflicting results from earlier studies emerged from the failure to record the activity of the same motor unit across a range of forces. Although the findings of Barry et al. (2007) demonstrate that there is no difference in the intrinsic discharge variability between young and old adults during brief isometric contractions, longer contractions that are influenced by changes in synaptic input (e.g., increased descending drive during fatigue) may lead to greater discharge variability in old adults.

Conclusions

The information obtained from motor unit recordings offers a unique insight into how muscles are controlled, and therefore how human movement is produced. The output from the spinal cord comprises the number of active motor units and rate at which they discharge action potentials. As these two factors are the product of the interaction between the synaptic input received by motor neurons and the intrinsic properties, the characterization of discharge rate modulation in humans can provide insight into the integration of these two factors and how they change with age.

With advancing age there are substantial reductions in the functional range of the motor system. These reductions can be attributed to changes in the anatomy of motor units including a reduction in the number of functional motor units, an increase in innervation number of surviving motor units, and an increase in the density and area of motor units undergoing re-innervation. Changes in the intrinsic properties of motor neurons include an increased input resistance, decreased rheobase, and a prolonged AHP period. It is yet to be determined if there are changes in the accommodation of current thresholds with advancing age. Changes in the discharge characteristics of motor units with age include a shift to lower recruitment thresholds of the motor unit pool, a reduced upper limit of motor unit recruitment, and reduced maximal discharge rates. The consequence of these changes may reduce the performance capabilities (e.g., maximal force, rate of torque development, and maintaining constant force) of the motor system of old adults.

Chapter II

Rate coding is compressed but variability is unaltered for motor units in a hand muscle of old adults

Abstract

The discharge of single motor units (*n* = 34) in the first dorsal interosseus muscle and the fluctuations in force during steady contractions were measured across a range of index finger abduction forces in old adults (77.1 ± 6.9 yr, *n* = 20). These results were compared with previously reported data on 38 motor units from young adults (25.7 ± 5.7 yr). Both minimal and peak discharge rates increased with recruitment threshold, but the strength of the relations was notably weaker for the old adults. Minimal discharge rates were similar for young and old adults ($P = 0.77$), whereas peak discharge rates were lower for old adults ($P < 0.01$). Consequently, the range of rate coding for each motor unit was substantially less for the old adults (7.1 pps) compared with the young adults (12.1 pps, $P < 0.01$). However, the variability in motor unit discharge times was similar for young and old adults; the coefficient of variation of the interspike intervals was similar at recruitment (young: 27.1%, old: 25.4%, $P = 0.39$) and declined with an increase in discharge rate (young: 14.2%, old: 13.2%, $P = 0.21$). Furthermore, the fluctuations in force during steady isometric contractions (2-95% of maximal force) were similar for young and old adults, except that the relative variability at the lower force was greater for the old adults. A computational model of motor unit recruitment and rate coding incorporated the experimental observations and was able to match the measured and simulated values for force steadiness across the operating range of the muscle.

Introduction

As an explanation for the decline in motor performance that accompanies advancing age, a number of studies focused on the motor output from the spinal cord by recording motor unit discharge (Christie and Kamen 2006a; Erim et al. 1999; Galganski et al. 1993; Kamen and Roy 2000; Kamen et al. 2006; Laidlaw et al. 2000; Semmler et al. 2000; Soderberg et al. 1991; Tracy et al. 2005; Vaillancourt et al. 2003) and by assessing the intrinsic properties of motor neurons (Engelhardt et al. 1989; Morales et al. 1987). The results indicated that the change in some performance capabilities, such as the maximal strength of a muscle, is not influenced by changes in motor unit activity. For example, although peak discharge rates are less during maximal contractions in old adults (Kamen et al. 1995), contractile speed is slower and this enables greater twitch fusion at lesser rates (Connelly et al. 1999) and old adults do not exhibit a conspicuous deficit in voluntary activation (De Serres and Enoka 1998; Klass et al. 2005a). Rather, the decrease in the peak force that can be achieved by old adults during a maximal isometric contraction is largely attributable to the loss of muscle mass (Frontera et al. 2000a; Frontera et al. 2000b; Trappe et al. 2003).

However, the motor unit pool does experience considerable remodeling with advancing age that results in fewer, but larger, functioning motor units in a muscle (Campbell et al. 1973; Gutmann and Hanzlíková 1976; Nikolić et al. 2001; Tomlinson and Irving 1977). One strategy to explore the functional significance of this remodeling has been to compare fine motor skills in young and old adults. Accordingly, it was shown that the ability of old adults to maintain a steady, submaximal force is impaired, especially at low forces (Galganski et al. 1993) and the deficit appears to be associated with elevated levels of discharge variability (Laidlaw et al. 2000). Studies that examined the variability in motor unit discharge rate, however, yielded mixed findings; some found greater discharge variability for old adults (Kornatz et al. 2005; Laidlaw et al. 2000; Tracy et al. 2005), whereas others found similar discharge variability for young and old adults (Semmler et al. 2000; Vaillancourt et al. 2003). Furthermore, only in some experiments was reduced force steadiness associated with elevated levels of discharge variability (Kornatz et al. 2005; Laidlaw et al. 2000; Tracy et al. 2005).

One of the difficulties in motor unit studies is that the activity of only a few motor units can be recorded in each experiment. As a consequence, the implicit assumption in these studies is that the behavior of a few motor units adequately represents that of the entire motor unit population. The mixed results on the contribution of discharge variability to force steadiness thus might be caused by differences in the motor units that were used to represent the activity of the population. To assess the association between discharge variability and force steadiness, Moritz et al. (2005) imported experimental observations on the discharge of single motor units into a computational model of motor unit recruitment and rate coding (Fuglevand et al. 1993). The results indicated that discharge variability changes across the activation range of a motor unit and that when this characteristic was specified in the model there was a strong association between discharge variability and the steadiness of muscle force.

The combined approach of measuring and then incorporating physiologically accurate behavior for individual motor units in a computer model of the entire motor unit pool is the most effective mean of evaluating the influence of motor unit discharge characteristics on the output of the motor unit pool (Jones 2005; Moritz et al. 2005). Because Moritz et al. (2005) measured motor unit activity only in young adults, the question remained as to whether or not discharge variability contributes to the difference in force steadiness between young and old adults. The purpose of this study was to examine the influence of motor unit discharge variability on the force fluctuations in a hand muscle of old adults. This study has been published (Barry et al. 2007).

Methods

The experimental protocols replicated the previous study with young adults (Moritz et al. 2005). In two separate experimental protocols, single motor unit discharge and fluctuations in force during steady contractions were measured over a wide range of forces. The discharge times of thirty-four motor units were recorded from twenty subjects (10 men; 77.1 ± 6.9 yr) and fluctuations in force were quantified in a separate experiment from twenty-two subjects (12 men; 78.5 ± 7.3 yr). All subjects were right handed, as verified by the Edinburgh Handedness Inventory (Oldfield 1971) and free from cardiovascular and neurological disorders. The ethics committee at the University of Colorado at Boulder approved the experimental protocol.

Force measurement

Subjects were seated with the left shoulder abducted by 0.79 rad and the forearm was restrained in a neutral posture and rested on a metal table. The elbow joint and forearm were immobilized with a vacuum pillow (Tumble Forms, Trenton, Ontario, Canada) and Velcro^{TM} straps. The left hand was supported with the palm vertical and the third through fifth digits were flexed slightly at the metacarpophalangeal joints and restrained in a brace (Figure 1). The thumb was extended and held in the same vertical plane as the palm using a separate brace. The angle between the index finger and the thumb was approximately 1.1 rad. The index finger was extended and secured to a hinged splint using Velcro^{TM} straps to maintain both interphalangeal joints extended and to constrain finger excursion to the abduction-adduction plane. The isometric abduction force exerted by the index finger was measured aligning a force transducer with the proximal interphalangeal joint. Low (0.049 V/N) and high (0.472 V/N) sensitivity transducers (Model 13, Sensotec, Columbus, OH) were used to enable the measurement of forces from <2% up to 100% of maximum with a sufficiently high signal-to-noise ratio. The force signals were digitized with a Power 1401 (CED, Cambridge, UK) at 200 samples•s^{-1} during the collection of motor unit discharge times and at 1,000 samples•s^{-1} during the experiments examining force fluctuations. Visual feedback of index finger abduction force was provided on a 17-in computer monitor located at eye level in front of the subjects at a distance of 1.6 m.

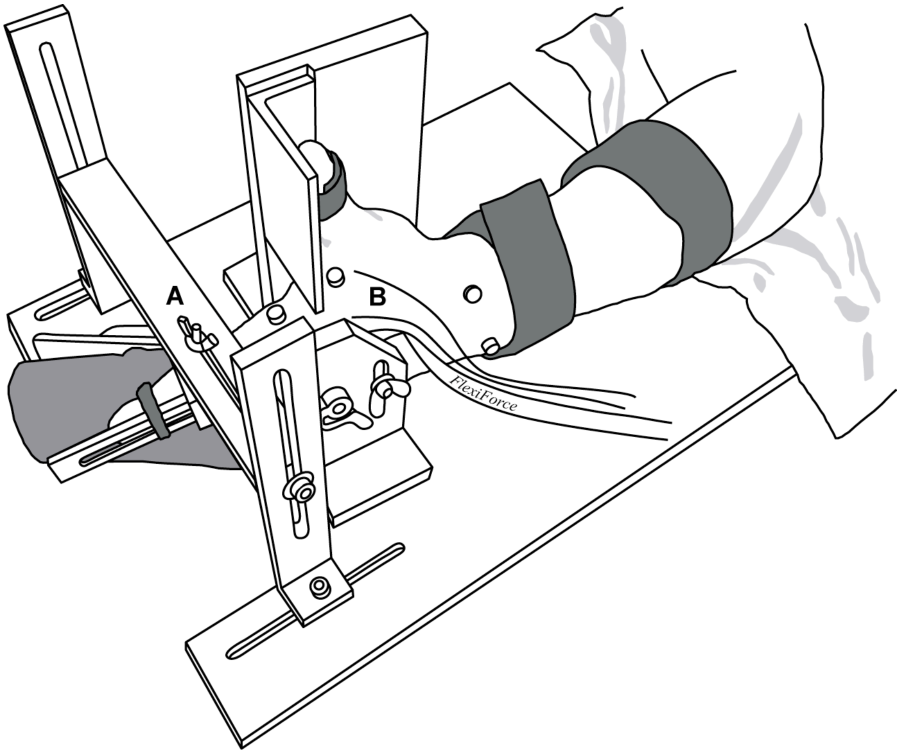

Figure 1. The left hand was placed in a custom device that isolated index finger abduction. A force transducer was placed above the knuckle (*A*) and fine-wire recordings were made of FDI motor unit activity and antagonist muscle activity (*B*).

EMG measurement

Single motor unit action potentials were recorded from the first dorsal interosseus muscle using stainless-steel wires (50 μm in diameter; California Fine Wire Company, Grover Beach, CA) that were glued together and inserted into the muscle using a 27-gauge, 1.125-cm long hypodermic needle. The needle was removed, leaving only the wires in the muscle for the duration of the experiment. Three wires were included in each electrode to allow for alternate bipolar configurations. The fine-wire EMG signal was amplified X5,000 or 10,000, and band-pass filtered between 300 Hz and 8.5 kHz (S-series, Coulbourn Instruments, Allentown, PA). The motor unit signal was sampled at 20k samples•s^{-1} with a Power 1401 (CED) and stored on a computer. Single motor unit potentials were visualized during the experiment using an oscilloscope and a dual-window discriminator (S-series, Coulbourn Instruments). A reference electrode was placed over the styloid process of the ipsilateral ulna (silver-silver chloride, 4-mm diameter).

EMG recordings of the antagonist muscle, the second palmar interosseus, were concurrently made using a second fine-wire electrode to monitor the degree of agonist-antagonist coactivation during the single motor unit experiments. The electrode comprised two stainless-steel wires (50 μm in diameter; California Fine Wire Company, Grover Beach, CA) that were inserted through the dorsum of the hand into the belly of the muscle using a 30-gauge, 2.25-cm hypodermic needle. The needle was removed, leaving only the wires in the muscle for the duration of the experiment. The fine-wire EMG signal was amplified X1,000 or 5,000, and band-pass filtered between 13 Hz and 1 kHz (S-series, Coulbourn Instruments, Allentown, PA). The intramuscular signal was sampled at 2k samples•s^{-1} with a Power 1401 (CED) and stored on a computer. A reference electrode was placed over the styloid process of the radius (silver-silver chloride, 4-mm diameter). Selective recordings from the second palmar interosseus were assumed when there was negligible EMG activity during a strong contraction of the thumb adductors (adductor pollicis) a weak contraction of the third digit abductors (second dorsal interosseus).

Protocol

At the start of both experimental protocols, subjects performed several trials of the MVC task to determine the maximal force capacity of the first dorsal interosseus muscle. Subjects were given strong verbal encouragement as they increased force from rest to maximum over a 3-s period and then held the maximal force for 1-2 s. Visual feedback of the abduction force exerted by the index finger was provided on the computer monitor and the subjects' hand was closely monitored by one of the experimenters to ensure that the task was performed correctly. MVC force was defined as the peak value from three or four trials that was within 5% of the peak force for another trial. A maximal index finger adductor task was also performed in order to normalize antagonist EMG amplitudes.

Motor unit experiment

Single motor unit action potentials were recorded during brief isometric contractions performed with the first dorsal interosseus muscle as subjects performed three tasks: *1*) a graded minimal-rate task with audio feedback of discharge rate, *2*) a ramp contraction in which force was increased gradually and continuously, and *3*) a serious of discrete isometric target matching tasks.

The experiment began by the subject gradually increasing contraction intensity as the investigators observed the fine-wire EMG signal for the appearance of a candidate motor unit. The subject was provided with visual feedback of index finger force and audio feedback of the discharge times for the motor unit being tracked. Once a motor unit had been identified, the subject was instructed to increase the force gradually until the motor unit became active and then to reduce the force slowly to identify the minimal rate at which the motor unit could discharge action potentials regularly. This task will be referred to as the graded minimal-rate task and was repeated three times. Each trial lasted between 5 to 20 s.

Subjects next performed two to three ramp contractions using twice the force associated with the graded minimal-rate contractions as the target force. The motor unit recruitment threshold force was characterized as the force at which the motor unit began to discharge action potentials repetitively. Each ramp lasted between 3 to 6 s for the up and down phases of the contraction (6 to 12 s total). Subsequently, an additional ramp contraction was performed to determine the peak force at which the discharge of the motor unit could be discriminated.

The peak force defined an upper limit for 10 target forces that encompassed the range of forces over which the motor unit could be discriminated. The actual number of target forces varied across motor units. Successive target forces were presented on the computer monitor in front of the subjects and they were instructed to produce enough index finger abduction force to match the target without exceeding it. Subjects were required to maintain the target force for 2 to 10 s, with briefer durations for high target forces. Subjects were given at least 30 s of recovery time between trials. Force targets were presented in ascending order, except for the targets that were adjusted in small increments (<1% MVC force) around the recruitment threshold to identify the discrete force target associated with the minimal discharge rate. This minimal discharge rate was referred to as the discrete minimal discharge rate. Following the completion of the discrete target matching tasks, MVCs were collected to ensure the observed discharge rates were minimally influenced by muscle fatigue.

Force steadiness task

Twenty-two subjects performed isometric contractions with the first dorsal interosseus muscle to match eight difference target forces. Each subject practiced the task at moderate intensity before beginning the series. The target forces (2, 5, 15, 30, 50, 70, 85, and 90% MVC force) were presented in a random order and the subject performed a brief isometric contraction to match each target force twice. The task involved increasing index finger abduction force from rest to the target force in ~1 s and exerting a steady force for 6 s. Visual feedback of the target force and the index finger force was provided for the first 3 s. The entire force trace was shown to the subject after completing the trial. The gain of the force display was adjusted so that the target-force line was always at the same position on the monitor relative to zero force. The 3 s of non-visual feedback from each trial were included in the analysis. A minimal rest interval of 30 s was provided between each trial, with considerably longer rest periods after high-force contractions.

Motor unit model

A model of motor unit recruitment and rate coding, originally developed by Fuglevand et al. (1993) and later modified by Moritz et al. (2005), was used to simulate the isometric force produced by a pool of motor units with characteristics resembling the first dorsal interosseus muscle. The model, which was implemented in MATLAB (v. 7.2, The MathWorks Inc., Natick, MA), has been described in detail previously (Fuglevand et al. 1993; Moritz et al. 2005; Taylor et al. 2003; Taylor et al. 2002).

The model comprised a pool of motor units with systematic variation in recruitment threshold, minimal, and maximal discharge rates, twitch force, and twitch contraction times. The simulated pool had 180 motor units (Jenny and Inukai 1983) and a recruitment range to an upper limit of 60% MVC. Variability in interspike intervals was included in the model by independently adjusting the timing of each discharge according to a normal distribution, with a specified coefficient of variation about the mean discharge rate. Motor unit recruitment and discharge rate were controlled by an excitation function (*E*) that acted on the entire motor unit pool. Motor unit #1 was the first to be recruited, had the smallest twitch force [1 arbitrary unit (au)], and the longest twitch time (90 ms). Motor unit #180 was the last to be recruited, had the largest twitch force (100 au), and the briefest contraction time (30 ms). Each motor unit generated a twitch force in response to a single discharge and a tetanic force when the activation involved multiple discharges. The amplitude of each tetanus was defined by a gain function that depended on discharge rate. The simulated muscle force was taken as the sum of all the active motor unit forces. Muscle force at each level of excitation was normalized to the force produced when all motor units were recruited and discharging at maximal rates (analogous to the MVC).

In early versions of the motor unit model (Fuglevand et al. 1993; Taylor et al. 2003; Taylor et al. 2002; Yao et al. 2000), the minimal discharge rate was set at 8 pulses per second (pps) for all motor units, and the maximal discharge rate decreased from 35 pps for the first recruited unit to 25 pps for the last recruited unit. In the Moritz et al. (2005) version of the model, minimal discharge rate was increased linearly with recruitment threshold force from 7.6 to 17.9 pps and maximal discharge rate was also increased linearly with recruitment threshold force from 17.6 to 34.8 pps. Both of these changes to the model were based on physiological data collected from young adults (Moritz et al., 2005). In addition, the coefficient of variation for interspike interval was modified from a constant value of 20% to a variability of roughly 30% at recruitment that rapidly declined to 10% as the discharge rate increased. The minimal and maximal discharge rates and the coefficient of variation for interspike interval were adjusted in terms of the excitation drive delivered to the pool of motor units in the model. The coefficient of variation for interspike interval declined exponentially for each motor unit as force increased above recruitment threshold force.

The current version of the model included interspike interval distributions that were skewed rather than being symmetrically distributed about the mean (Calvin and Stevens 1968; Enoka et al. 1989; Matthews 1996). Interspike interval distributions were generated such that they were mostly skewed for newly recruited motor units when the coefficient of variation for interspike interval was high and became progressively less skewed as the discharge rate increased above recruitment levels, until the interspike interval distribution became entirely normal.

A final version of the model was developed with parameters adjusted to simulate the motor unit pool of an old adult (Table 1). The number of motor units was reduced to reflect the loss of motor units that occurs with advancing age (Brown et al. 1988; Doherty and Brown 1993; Larsson and Ansved 1995; Tomlinson and Irving 1977). The range of twitch torques was reduced, the average twitch torque was increased (Doherty and Brown 1997; Galganski et al. 1993; Kadhiresan et al. 1996; Larsson and Ansved 1995; McComas 1995; Spiegel et al. 1996), and twitch contraction times were increased (Andersen 2003; Doherty and Brown 1997; Kadhiresan et al. 1996; Klitgaard et al. 1990; Larsson et al. 1979; Lexell 1995; Lexell et al.

1988; Sugiura and Kanda 2004). Maximal discharge rates were also lowered (Kamen et al. 1995). All simulations were run 20 times so that the variability assigned to each parameter could influence the variability in the simulated forces.

Table 1. *Model parameters used to simulate the motor unit pools of young and old adults.*

Model Parameter	Young	Old
Motor unit number (n)	180	100
Recruitment range (% MVC)	60	60
Average twitch force (au)	21.8	26.2
Twitch force range (au)	1 – 100	3.5 – 113
Twitch force sum (au)	3919	2624
Average contraction time (ms)	54.4	63.9
Range of contraction times (ms)	90 – 30	101 – 38
Minimal discharge rate (pps)	7.6 – 16.4	7.7 – 16.2
Maximal discharge rate (pps)	16.7 – 31.1	14.4 – 24.0
Peak force (au)	28392	21366

au: arbitrary units, pps: pulses per second

Data analysis

Single motor unit action potentials were discriminated off-line using the template-matching features of Spike2 (v. 5.02, CED) and were verified by visual inspection. Interspike intervals >250 ms (<4 pps; *n* = 89, 0.23% of discharges) or <10 ms (>100 pps; *n* = 15, 0.04% of discharges) were excluded from the calculations of discharge rate. Long interspike intervals (<4 pps) likely arose from the brief cessation of motor unit discharge, whereas very short intervals (>100 pps) exceed the rates normally observed during these types of contractions for human motor units (Bigland-Ritchie and Lippold 1954; De Luca et al. 1982; Kanosue et al. 1979; Tanji and Kato 1973) and likely resulted from discrimination error or double discharges. To determine the region over which to calculate the mean and coefficient of variation for discharge rate during each contraction, the force plateau was identified as beginning when the force was within 90% of the target force and ending 1 s before the force dropped to <90% of the target force. The force and discharge rate measurements were made over the intervening interval.

The recruitment threshold of each motor unit was determined by moving a 0.5-s window forward in time in 1-ms steps during the ramp task until the coefficient of variation for interspike intervals separating the potentials within the window was <50%. The force corresponding to the first discharge in this window was taken as the recruitment threshold of the motor unit.

The minimal discharge rate of each motor unit was measured in two tasks: *1*) a graded decrease in force until a minimal rate was achieved and *2*) discrete increments in target force around the recruitment threshold of the motor unit. The minimal discharge rate during the graded test corresponded to the lowest rate measured during a 2-s interval when the coefficient of variation for interspike interval was <50%. The minimal rate during the discrete test was identified by moving a 2-s window forward in 1-ms steps and noting the rate when both the coefficient of variation for interspike interval was <50% and at least one discharge occurred both before and after the 2-s window.

Different types of distributions were fit to the interspike interval histograms for each motor unit for each discrete target force performed by the young and old adults. Interspike interval histograms were constructed with a 1-ms bin width for a period of 1 to 250 ms. Normal, lognormal, and gamma distributions were fit to each histogram by maximum-likelihood estimation (Statistics Toolbox, MATLAB). The goodness-of-fit of the different distributions was evaluated by calculating the root-mean-square error (RMSE) between the fitted distributed and the interspike interval count for each bin. The RMSE was calculated for the entire interspike interval histogram and also tail regions of the histogram lying outside of ± 1 SD from the mean. Frequencies were collated for the distribution type that best fit each individual interspike interval histogram.

Force steadiness task

The abduction force exerted by the index finger at each target force during the steady contractions was quantified for a 1-s period commencing 500 ms after visual feedback of the force was removed. The 1-s epoch was linearly detrended before the assessment of the coefficient of variation for force to minimize the contribution of gradual drifts away from the target force. The mean force was calculated over this 1-s region for the two trials at each target level. For some trials, it was necessary to select a slightly later or earlier time window if the mean force was not sufficiently close to the target. The coefficient of variation for force for the trial with the mean force closer to the target force was used in the analysis.

Motor unit model

Simulations were run for eight target forces with 10- and 5-s contractions at each target force. Force data were measured during the middle 1 s of the 5-s simulated contraction and discharge rate statistics were extracted for the middle 8 s of the 10-s simulated contraction.

Statistical analysis

Repeated-measures ANOVA and paired samples *t*-tests were used to compare the recruitment forces and discharge rates obtained during the ramp, graded, and discrete tasks. The relations between recruitment threshold and the minimal and peak discharge rates observed during the brief contractions at different target forces were characterized with linear regression analyses. Independent samples *t*-tests were used to compare the young and old adults. For some variables, ANCOVA assessed age-related differences to verify that detected differences in discharge rate variables did not arise from sampling differences. Lilliefors test evaluated the goodness-of-fit of a normal distribution to the interspike interval histograms for each target force of each motor unit. A two-factor, repeated-measures ANOVA compared the simulated and experimental measures of the coefficient of variation for force (between-subject factor) at each of the eight target forces (repeated-measures factor). Post hoc analyses with paired samples *t*-tests were used to identify the target forces that differed when interactions were identified by ANOVA. Regressions were also performed on the average coefficient of variation for force at each target level as a means of quantifying the similarity between force output from the model and experimental data. All statistical procedures were performed with SPSS (v. 14.0) and SigmaPlot (v. 8.02, SPSS, Chicago, IL). Alpha was set at 0.05 and all reported values are mean ± standard deviation or 95% confidence intervals (CIs).

Results

Recordings were made from 34 motor units at 9 ± 3 target forces during contractions that lasted 11.3 ± 2.8 s. The target forces ranged from recruitment threshold to an average of 17.9% maximal voluntary contraction (MVC) force above recruitment (4.6 – 42.0% MVC). There were 117 ± 33 (range 68 – 198) discharges recorded at each force target over an average duration of 8.6 ± 2.4 s. EMG recordings during the experiments for 21 of the 34 motor units indicated that the activity of the antagonist muscle, second palmar interosseus, was minimal and consistent across the different target forces. The data were compared with that for 38 motor units from young adults from a previous study (Moritz et al. 2005; Table 2). MVC force was significantly greater for the young adults (35.1 ± 8.6 N) compared with the old adults (27.8 ± 8.0 N, $P < 0.005$).

Table 2. *Characteristics of the motor unit data for the old adults.*

MU #	Min. Force (%MVC)	Peak Force (%MVC)	Force Range (%MVC)	Min. DR (pps)	Peak DR (pps)	DR Range (pps)	CV for ISI at recruitment	Min CV of ISI	SD-Mean ISI slope	Proportion of ISI histograms NOT normally distributed
1	0.0	14.2	14.2	12.9	19.1	6.2	19.9	9.1	0.335	0.25
2	0.0	14.8	14.8	12.8	19.4	6.6	23.3	14.0	0.366	0.50
3	0.2	11.1	10.9	7.6	15.4	7.9	33.5	13.0	0.397	0.31
4	0.7	28.1	27.4	8.6	16.7	8.1	31.6	11.8	0.505	0.43
5	0.9	6.6	5.7	12.9	16.7	3.8	13.8	9.3	0.315	0.00
6	1.1	14.8	13.7	8.6	15.0	6.3	24.3	10.8	0.530	0.50
7	1.4	6.8	5.4	8.1	16.3	8.2	31.6	18.3	0.459	0.33
8	1.7	7.0	5.3	10.5	14.0	3.4	34.9	10.9	1.057	0.71
9	2.0	9.2	7.2	11.4	17.4	6.0	32.8	15.4	0.471	0.55
10	2.0	6.6	4.6	12.2	16.0	3.9	17.0	11.7	0.330	0.33
11	2.3	12.7	10.4	6.2	14.8	8.6	19.5	12.0	0.251	0.14
12	2.3	43.6	41.3	6.5	16.8	10.3	22.3	11.2	0.243	0.50
13	2.5	9.5	7.0	8.8	11.6	2.7	20.8	10.0	0.414	0.45
14	3.0	11.7	8.6	13.3	16.1	2.7	23.5	11.0	0.622	0.00
15	3.7	15.1	11.4	9.1	15.2	6.2	17.5	9.9	0.393	0.50
16	4.4	12.5	8.1	6.2	9.9	3.6	21.8	8.9	0.388	0.29
17	5.1	16.0	10.8	10.7	13.3	2.6	39.2	18.7	0.720	0.90
18	5.3	28.3	23.0	7.9	22.2	14.3	31.0	16.4	0.285	0.20
19	5.8	47.8	42.0	7.9	20.5	12.7	23.9	10.0	0.331	0.56
20	6.3	17.9	11.6	9.7	15.0	5.3	32.7	13.6	0.486	0.50
21	6.7	21.6	14.9	8.1	16.8	8.7	31.3	11.6	0.493	0.50
22	7.2	20.0	12.8	8.4	15.9	7.5	24.5	16.6	0.404	0.33
23	12.9	28.6	15.7	11.6	14.5	3.0	8.8	8.4	0.030	0.00
24	13.5	36.5	23.0	8.7	16.1	7.4	16.2	12.7	0.242	0.44
25	15.3	27.2	11.9	8.8	13.1	4.3	29.9	11.7	0.439	1.00
26	24.0	53.2	29.2	13.9	22.4	8.5	21.2	15.4	0.312	0.40
27	24.1	63.0	38.9	8.9	25.0	16.1	36.6	17.5	0.580	0.33
28	26.2	54.4	28.2	11.4	22.9	11.5	44.6	21.0	0.730	0.83
29	26.3	46.2	19.9	15.2	26.4	11.2	20.2	14.6	0.262	0.13
30	30.4	53.7	23.3	17.5	27.9	10.4	19.7	15.3	0.182	0.50
31	31.6	68.7	37.2	18.2	24.2	6.0	24.8	17.1	0.445	0.38
32	40.0	73.7	33.7	10.8	20.0	9.3	32.3	12.4	0.518	0.56
33	44.0	66.0	22.1	11.0	16.5	5.5	23.9	12.6	0.439	0.29
34	53.3	67.6	14.2	15.4	18.5	3.0	16.2	14.8	0.163	0.40
Mean	**12.0**	**29.8**	**17.9**	**10.6**	**17.7**	**7.1**	**25.4**	**13.2**	**0.42**	**0.41**
SD	14.4	21.7	11.1	3.1	4.2	3.4	7.9	3.1	0.19	0.23
Young adult values										
Mean	**16.4**	**38.4**	**22.0**	**10.3**	**22.4**	**12.1**	**27.1**	**14.2**	**0.38**	**0.39**
SD	16.5	25.9	15.8	3.5	6.9	5.6	8.1	3.6	0.11	0.23
t-test P	*0.231*	*0.134*	*0.202*	*0.773*	***0.001***	***< 0.001***	*0.389*	*0.210*	*0.319*	*0.719*

Mean values and standard deviation for all units. The data comprises the ranges for force and discharge rate (DR) observed for each motor unit during the discrete task and indices of discharge variability. Min. Force: recruitment threshold force during the discrete task; Peak Force: highest discrete target force at which the motor unit's activity was recorded; Force Range: difference between peak force and min. force; Min. DR: lowest mean discharge rate for a discrete force target; Peak DR:

Recruitment threshold

Recruitment threshold forces (Figure 2*A*) measured during the discrete and graded minimal-rate tasks were similar to those for the ramp task (task main effect: $P = 0.358$). The discharge rates (Figure 2*B*) recorded at recruitment did not differ for the ramp and discrete minimal tasks, but were significantly lower for the graded minimal rate task (task main effect: $P < 0.001$).

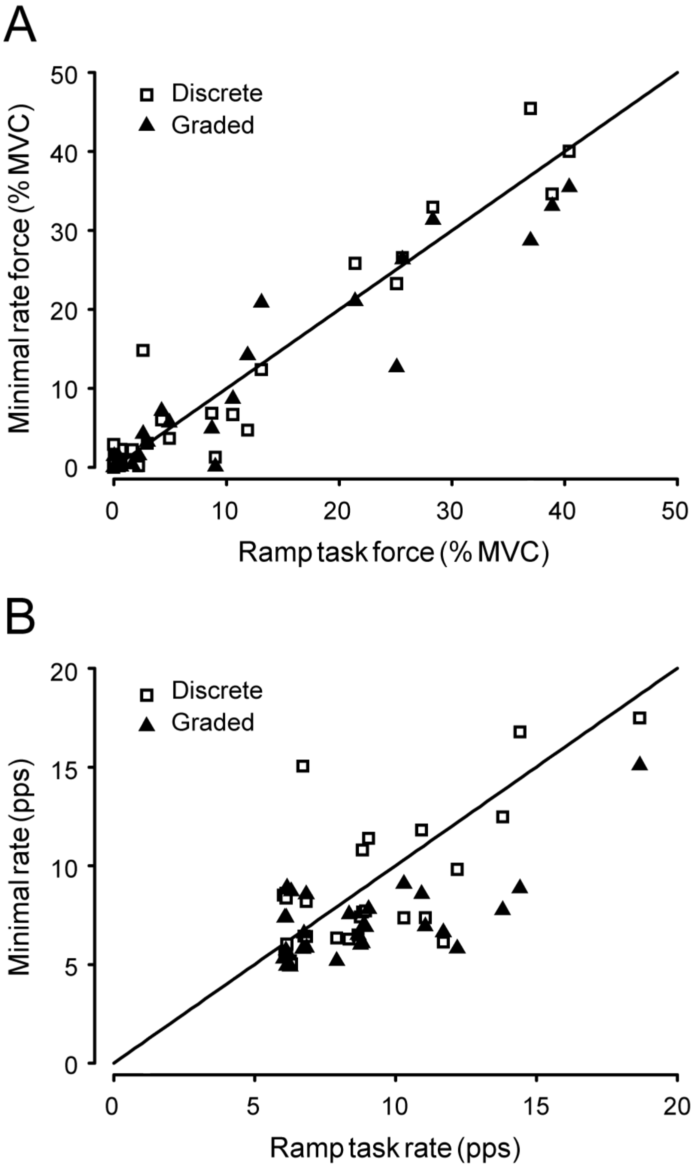

Figure 2. Relations between the forces and discharge rates for 28 motor units during the ramp task, graded minimal discharge task, and the discrete minimal rate task. *A*, force measured during the three tasks. Clustering of the data about the line of identity indicates similar relations for graded ($P = 0.358$) and discrete ($P = 0.425$) minimal rate tasks compared with the ramp task. *B*, minimal discharge rates observed during the three tasks. Minimal discharge rate during the discrete minimal rate task did not differ from the line of identity with the ramp task ($P = 0.653$), whereas discharge rates were lower for the graded minimal rate task ($P < 0.001$).

Discharge rate

Motor unit discharge rate increased with index finger abduction force for all units (Figure 3*A*). Minimal discharge rates were similar for young and old adults ($P = 0.773$), whereas peak discharge rates were lower for old adults ($P = 0.001$). Consequently, the range of rate coding for each motor unit of the old adults (7.1 ± 3.4 pps) was less than that observed for the young adults (12.1 ± 5.6 pps, $P < 0.001$; Figure 3*B*). Although the force range across which motor units were tracked did not differ significantly between young and old adults ($P = 0.202$), there was a trend for the mean force range to be smaller for the old adults (Table 2) and for the average peak force over which the motor units of young and old adults were tracked to be less ($P = 0.134$). The significant reduction in rate coding for old adults persisted when ANCOVA was used with either force range ($P < 0.001$) or peak force ($P = 0.002$) as covariates. Although it appears from the two data sets in Figure 3*B* that motor unit recruitment may also be compressed for old adults, the recruitment threshold force for the young and old adult motor unit samples did not differ significantly ($P = 0.231$).

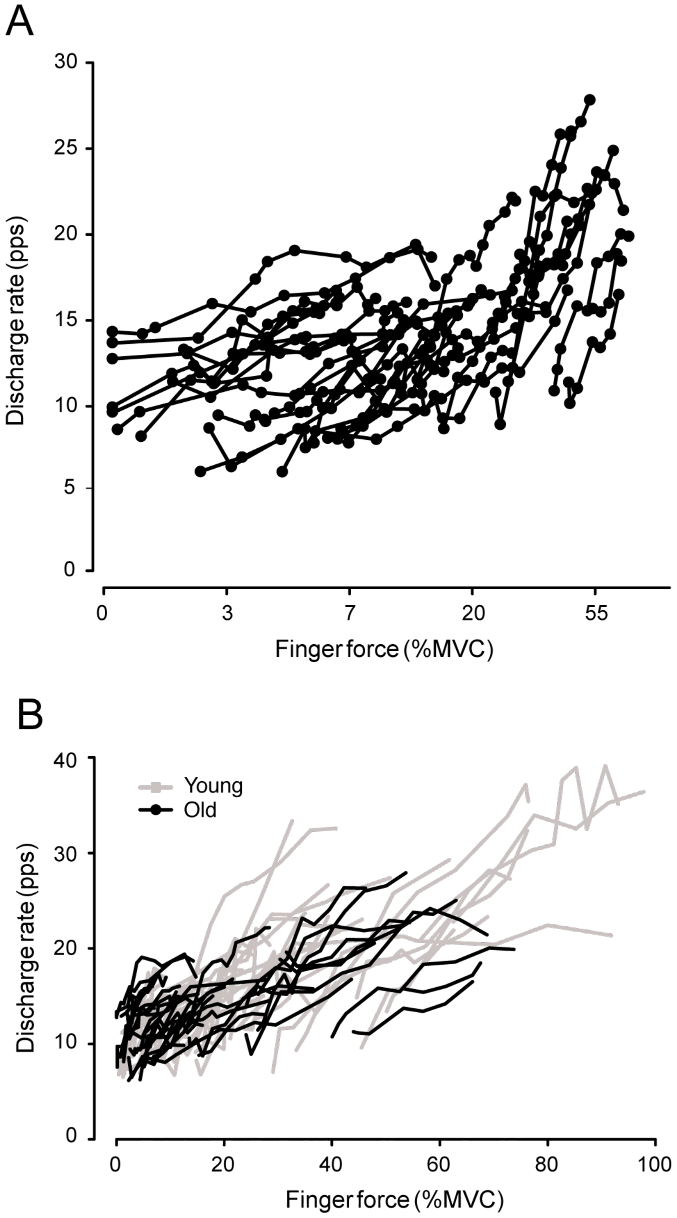

Figure 3. Association between discharge rate and index finger force for 34 motor units during the discrete task. *A*, motor unit sample from old adults with each data point corresponding to the mean ± SD of the discharge rate during an isometric contraction. A line connects the data points for each single motor unit and data are plotted on a log-based scale. *B*, 34 motor units from the old adults overlaid on the previously reported 38 motor units for young adults (light gray).

Minimal discharge rates ranged from 6.2 to 18.2 pps and peak discharge rates varied from 9.9 to 27.9 pps. Regression analyses revealed that both minimal ($r^2 = 0.28$, $P < 0.002$) and peak ($r^2 = 0.26$, $P < 0.003$) discharge rates increased as a function of recruitment threshold (Figure 4).

Similar relations were observed with young adults for the minimal ($r^2 = 0.55$, $P < 0.001$) and peak discharge rates ($r^2 = 0.51$, $P < 0.001$), but the strength of these relations was notably weaker in old adults.

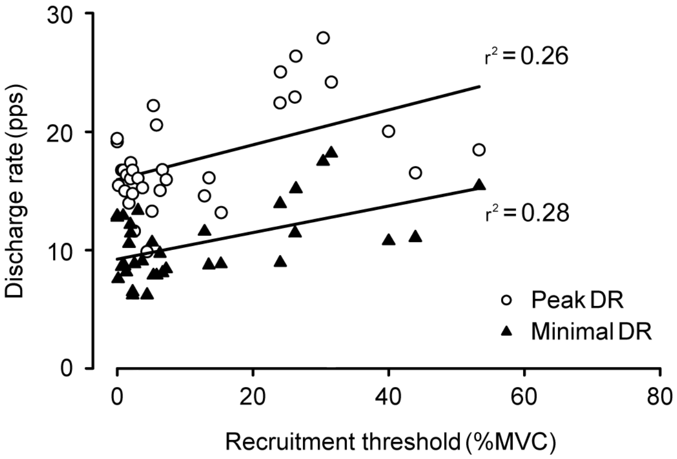

Figure 4. Minimal and peak discharge rates as measured during the discrete task (discharge rate calculated over the entire train) for 34 motor units as a function of recruitment threshold. Peak discharge rate represents the greatest rate that could be discriminated reliably, which may not correspond to the maximal discharge rate. Both minimal ($r^2 = 0.28$; $y = 0.11x + 9.2$) and peak discharge rate ($r^2 = 0.26$; $y = 0.15x + 15.9$) increased significantly with recruitment threshold ($P < 0.003$).

Discharge variability

Variability in motor unit discharge was similar for the young and old adults. The coefficient of variation for the interspike interval (relative variability) was similar at recruitment (young: 27.1%, old: 25.4%, $P = 0.389$) and declined to almost the same level with an increase in discharge rate (young: 14.2%, old: 13.2%, $P = 0.210$). The rate of change in discharge variability was also similar for young and old adults; the slope of the linear relation between SD of interspike intervals and mean interspike interval (Figure 5*A*) was 0.38 for young adults and

0.42 for old adults (*P* = 0.319). The SD of the interspike interval (Figure 5*B*), which is an index of the absolute variability in discharge rate, was similar for the young and old adults at recruitment (young: 27.9 ± 10.9 ms, old: 25.1 ± 10.3 ms, *P* = 0.255) and reached a similar minimal value (young: 7.5 ± 1.9 ms, old: 8.1 ± 1.9 ms, *P* = 0.240). Relative variability in discharge times, measured as either the coefficient of variation for interspike interval or the coefficient of variation for discharge rate, declined progressively with a decrease in mean interspike interval (Figures 5*C*, *E*, and *G*).

Discharge rate variability was calculated in units of pulses per second (pps; Stein et al. 2005). The SD of discharge rate was high at recruitment, declined to a minimum as the discharge rate increased, but subsequently increased with further elevations in discharge rate (Figures 5*D* and *F*). Similar SDs of discharge rate were observed for the young (0.88 ± 0.28 pps) and old adults (0.83 ± 0.26 pps) at recruitment (*P* = 0.255). The minima reached by the young and old adults, however, differed (young: 0.61 ± 0.21 pps, old: 0.52 ± 0.16 pps, *P* = 0.054) and the SD of discharge rate differed significantly between groups at peak force (young: 0.84 ± 0.32 pps, old: 0.65 ± 0.20 pps, *P* < 0.005). Peak discharge rates were greater for the young adults (young: 22.4 ± 6.9 pps, old: 17.7 ± 4.2 pps). The coefficient of variation for discharge rate was similar for the young and old adults at recruitment (young: 8.7 ± 2.8%, old: 7.9 ± 2.8%, *P* = 0.265) and declined to almost the same level at peak force (young: 3.8 ± 0.8%, old: 3.7 ± 0.9%, *P* = 0.802; Figures 5*E*, and *G*).

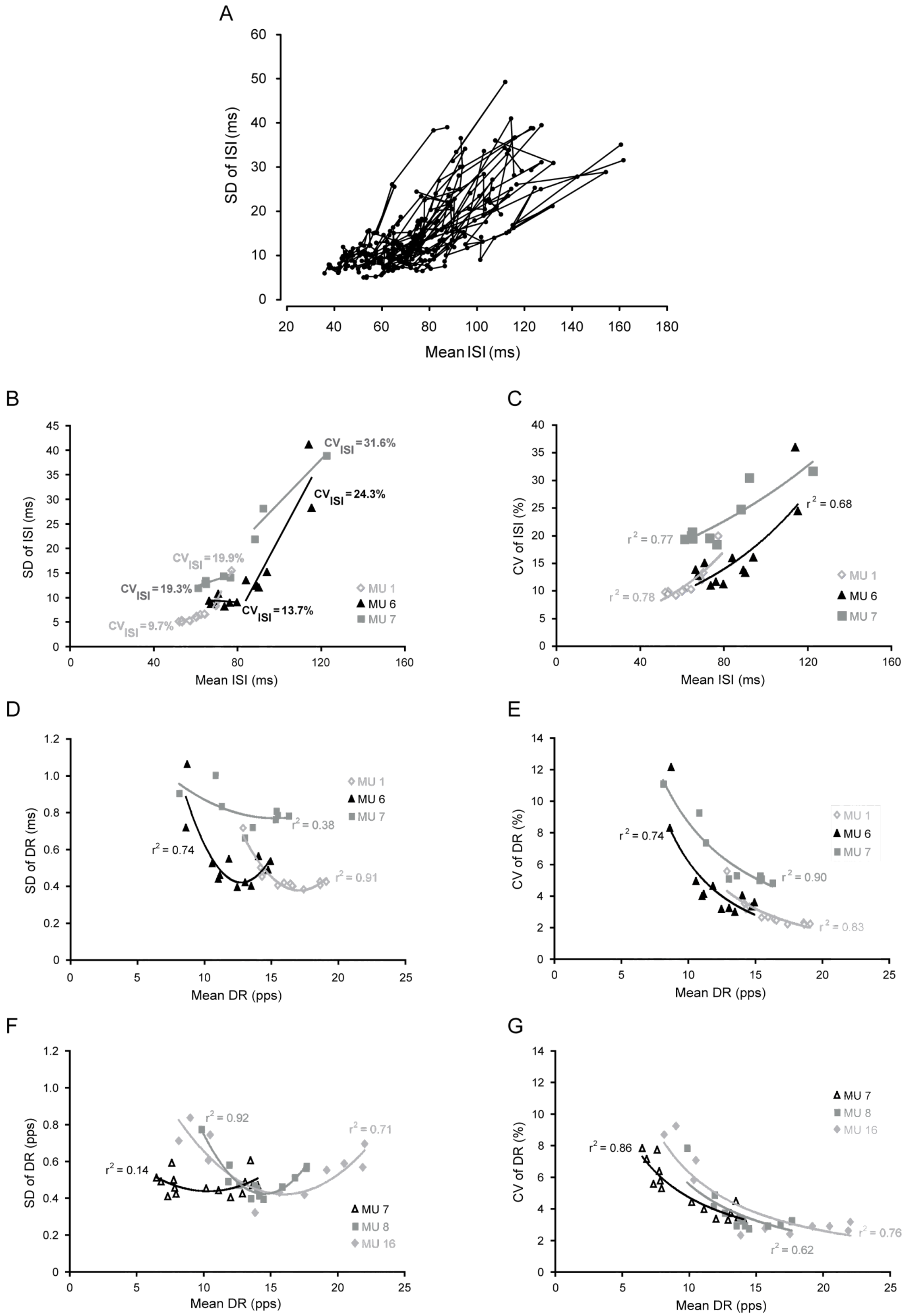
A
SD of ISI (ms)
Mean ISI (ms)
B
SD of ISI (ms)
Mean ISI (ms)
CV_{ISI} = 31.6%
CV_{ISI} = 24.3%
CV_{ISI} = 19.9%
CV_{ISI} = 19.3%
CV_{ISI} = 13.7%
CV_{ISI} = 9.7%
MU 1
MU 6
MU 7
C
CV of ISI (%)
Mean ISI (ms)
r^2 = 0.77
r^2 = 0.78
r^2 = 0.68
MU 1
MU 6
MU 7
D
SD of DR (ms)
Mean DR (pps)
r^2 = 0.74
r^2 = 0.38
r^2 = 0.91
MU 1
MU 6
MU 7
E
CV of DR (%)
Mean DR (pps)
r^2 = 0.74
r^2 = 0.90
r^2 = 0.83
MU 1
MU 6
MU 7
F
SD of DR (pps)
Mean DR (pps)
r^2 = 0.14
r^2 = 0.92
r^2 = 0.71
MU 7
MU 8
MU 16
G
CV of DR (%)
Mean DR (pps)
r^2 = 0.86
r^2 = 0.62
r^2 = 0.76
MU 7
MU 8
MU 16

Figure 5. Variability of motor unit discharge times across the discrete target forces. *A*, SD of the interspike interval (ISI) relative to the mean ISI for 34 motor units from old adults. *B*-*G*, representative data are shown for 3 motor units (1, 6, and 7 from Table 2) from the old adults (*B*, *C*, *D*, and *E*) and 3 motor units (7, 8, and 16 from Table 1 in Moritz et al. 2005) from the young adults (*F* and *G*). *B*, coefficient of variation (CV) for the ISI for 3 motor units; the data for each motor unit are indicated with 2 lines to denote the initial rapid decline in the SD of ISI from the high value at recruitment to the subsequent plateau. *C*, progressive decline in the CV for ISI as mean ISI declines for the old adults. Note the considerably higher CV for the ISIs for each motor unit at high mean ISIs (low discharge rates) compared with the CV values at the lowest mean ISI values (peak discharge rate). *D*-*G*, direct measures of variability in discharge rate (DR). SD and CV of discharge rate were calculated with equations reported by Stein et al. (2005); $V = \sigma^2 T/\mu^3$, where V is the variance in motor unit discharge rate, μ is the mean ISI, σ is the SD of the ISI, and T is the period over which the discharge rate is quantified (i.e., 1 s). In contrast to the monotonic decline in CV with an increase in mean discharge rate (*E* and *G*), the SD of discharge rate first decreased and then increased as the units discharged more rapidly (*D* and *F*).

The shape of the interspike interval distributions for every discrete force target was also examined. At low discharge rates, the interspike interval distributions were skewed with a greater proportion of extra-long interspike intervals, but became normally distributed as discharge rate increased. Sample data for two concurrently recorded motor units that display the changing shape of the histograms are shown in Figure 6. Of the 375 histograms for young adults and 322 for old adults, the proportion of there that differed from a normal distribution was similar for young (0.41) and old adults (0.39, $P = 0.719$). Normal, lognormal, and gamma distributions were fit to each histogram and the goodness-of-fit was quantified by calculating the root-mean-square error for each histogram bin. The best fit for most histograms was a lognormal distribution and there was a remarkable similarity in these data for the young and old groups. This is evident in the proportion of the interspike interval histograms from all motor unit and target forces that were best fit by lognormal (young: 0.62 ± 0.21, old: 0.66 ± 0.23), normal (young: 0.22 ± 0.18, old: 0.21 ± 0.17), or gamma distributions (young: 0.16 ± 0.14, old: 0.13 ± 0.14).

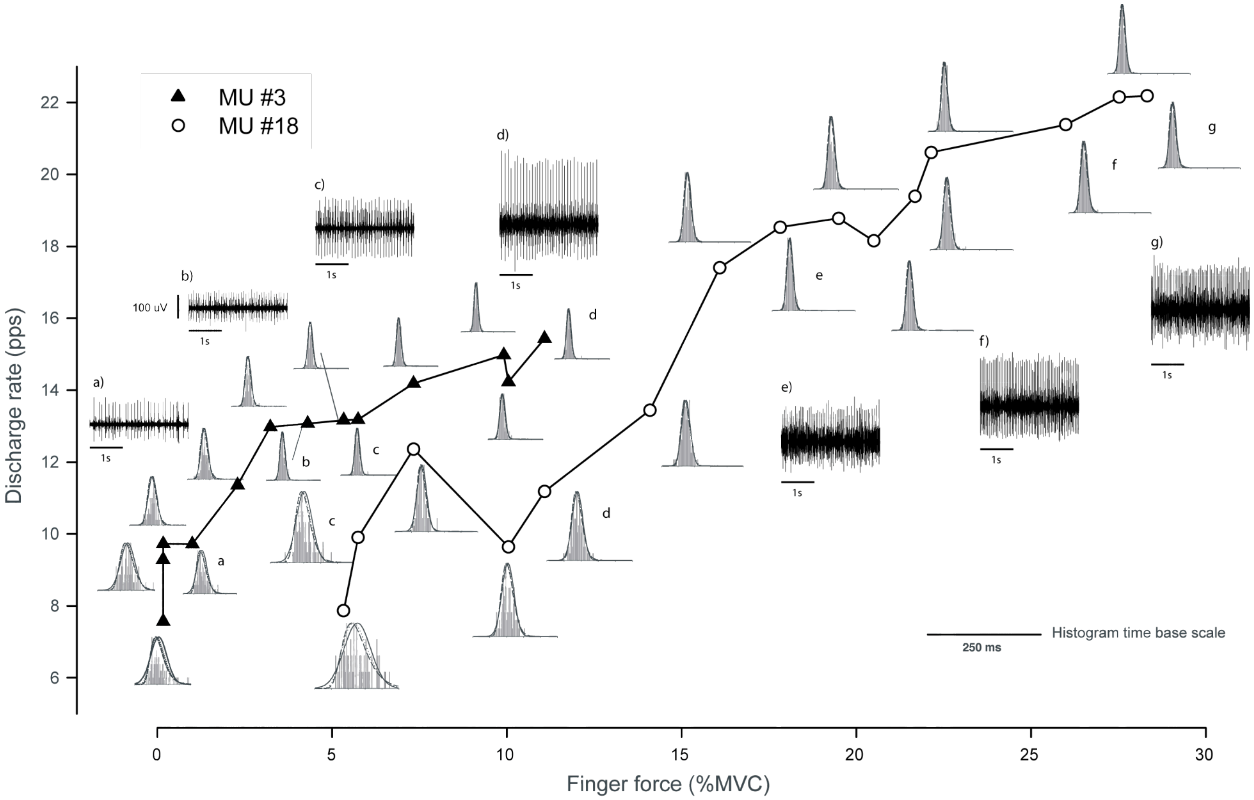

Figure 6. Change in discharge rate relative to the abduction force exerted by the index finger for motor units 3 and 18 in Table 2. The figure shows the corresponding ISI histogram for each target force: 13 histograms for motor unit 3 and 15 histograms for motor unit 18. Each ISI histogram is displayed with the fitted distributions (dotted lines: lognormal distribution; solid lines: Gaussian distribution; dashed lines: gamma distribution). Segments of the single motor unit recordings are shown for 7 of the discrete force targets, with letters denoting the corresponding ISI histogram (sample trains of action potentials labeled *c* and *d* are referenced beside histograms for both motor units because activity from the 2 units was recorded concurrently). These simultaneously recorded motor units display high discharge rate variability near recruitment, which is indicated by the spread in the ISI histograms, although this declined as discharge rate increased. At low levels of activation, the ISI histograms were skewed to the *right* as the result of a grater incidence of longer ISIs. Skewed histograms were better fit by a lognormal distribution than by a normal (Gaussian) distribution. As discharge rate increased, the distributions became more normal, although with more kurtosis than a standard normal distribution.

Force variability and simulations

When the minimal and peak discharge rates and the variability in discharge rate were specified in terms of the motor unit pool excitation parameter *E*, the outputs of the model were similar to those found by Moritz et al. (2005; Figures 7*A* and *B*). Similarly, the use of lognormal and normal distributions for the interspike intervals rather than only a normal distribution did not alter the variability in the simulated interspike intervals (Figures 7*C* and *F*). The only difference was that the combined distribution (normal + lognormal) produced longer interspike intervals at low discharge rates. This addition had only a minor impact on the simulated force steadiness, but it did typically elevate the coefficient of variation for force for the 2% MVC simulation, bringing it closer to the experimental data than previous versions of the model. Discharge variability had a substantial influence on the match between simulated and experimental measures of force steadiness (Figures 7*G* and *H*). The match between the experimental and simulated data was greater with a changing coefficient of variation for interspike interval (r^2 = 0.93) compared with a constant coefficient of variation for interspike interval of 20% (r^2 = 0.69).

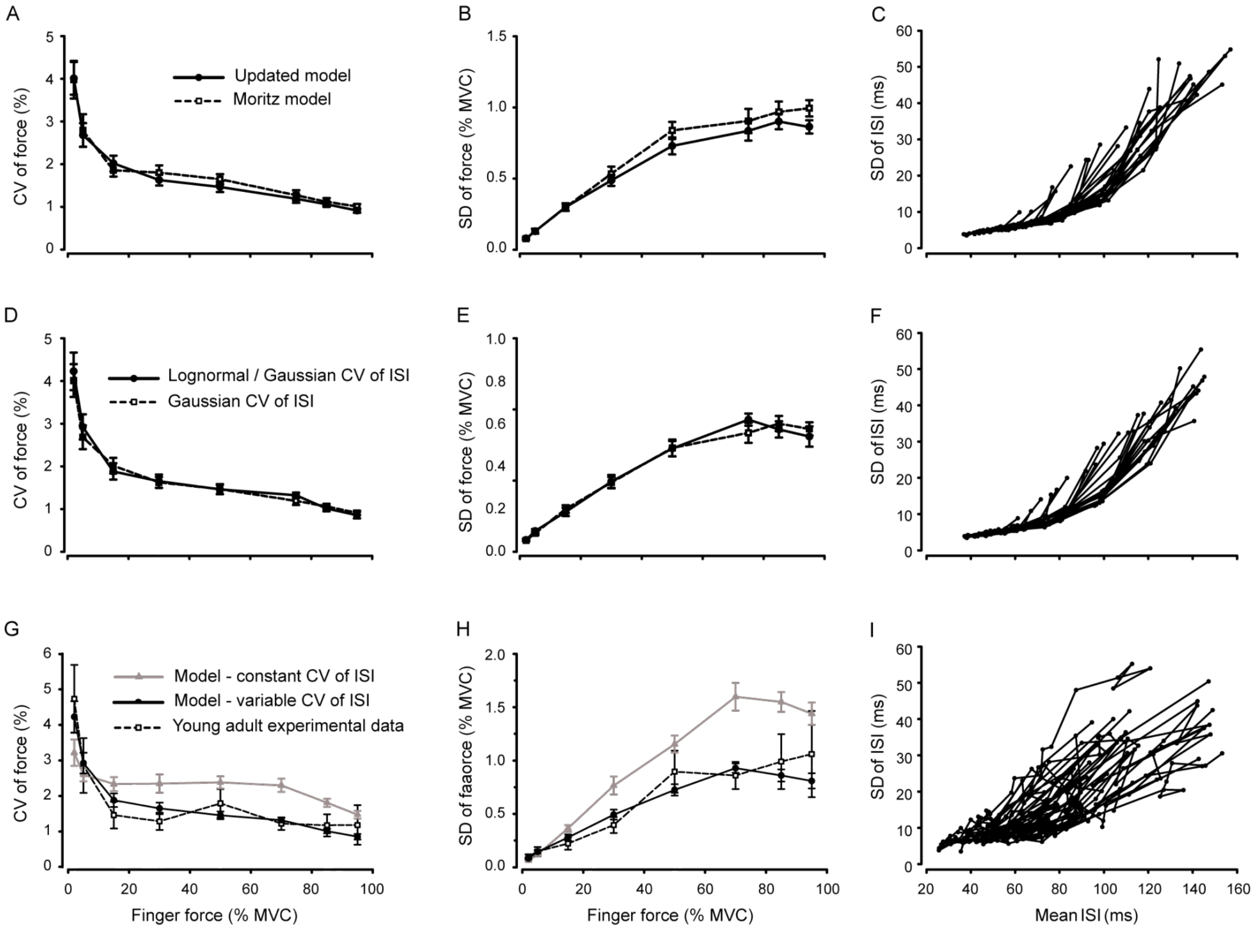

Figure 7. Comparison of simulated and measured forces at 8 different target forces (2, 5, 15, 30, 50, 70, 85, and 95% MVC force). *A* and *B*: relative (CV) and absolute (SD) variability of simulated force for the previous (Moritz et al. 2005) and current versions of the model. Normal distributions for the ISIs were used in both models. *C*: simulated SDs of the ISI relative to mean ISI for 29 motor units from the model with normal distributions. *D* and *E*: influence of ISI distributions on the simulated SD and CV for force. There was no difference in the simulated force between the normal (Gaussian) and combined (normal + lognormal) distributions. *F*: SD of the ISI relative to mean ISI for 31 motor units derived with the simulation that included the combined distributions. *G* and *H*: measured and simulated force variability (CV and SD). Simulated data were obtained with models that used constant and variable values for the CV for ISI with combined (normal + lognormal) distributions. Measured forces are for 22 young adults. Output of the variable CV model did not differ for either CV (main effect: $P = 0.717$) or SD (main effect: $P = 0.31$) of force. *I*: SD of the ISIs relative to mean ISI for 38 motor units from the previous sample of young adults. These data do not cluster as cleanly as the model data in *E* and *F* because the experimental data include measurement noise and were collected from 18 subjects whose motor unit pools naturally differ. Error bars in *A*, *B*, *D*, *E*, *G*, and *H* are 95% CIs.

Force-steadiness data were collected from a group of 22 young and 22 old adults (Figure 8*A* and *B*) who differed in the abduction force exerted by the index finer during a maximal voluntary contraction with the first dorsal interosseus muscle (young: 31.6 ± 8.5 N, old: 27.0 ± 6.8 N, $P < 0.05$). A significant main effect arising from age was observed for the coefficient of variation for force ($P < 0.05$) measured at eight different target forces (2, 5, 15, 30, 50, 70, 85, and 95% MVC). The greatest difference between the young and old adults was at the lowest force (2% MVC, $P < 0.05$). An index of absolute variability, the SD of force, did not quite reach statistical significance ($P = 0.074$). The model was adjusted to approximate the properties of the motor unit pool for old adults and the simulated measures of force were compared with the experimental measurements. Force-steadiness data from the old adults was closely matched ($r^2 = 0.92$ for coefficient of variation for force) by the simulated data (Figure 8*C*). Comparisons of force-steadiness data from simulations of the motor units pools of young and old adults (Figure 8*E* and *F*) showed subtle, yet significant (main effect: $P < 0.001$ for coefficient of variation and SD of force), differences that were reasonably consistent with the differences between the experimental measurements for young and old adults (Figure 8*A* and *B*).

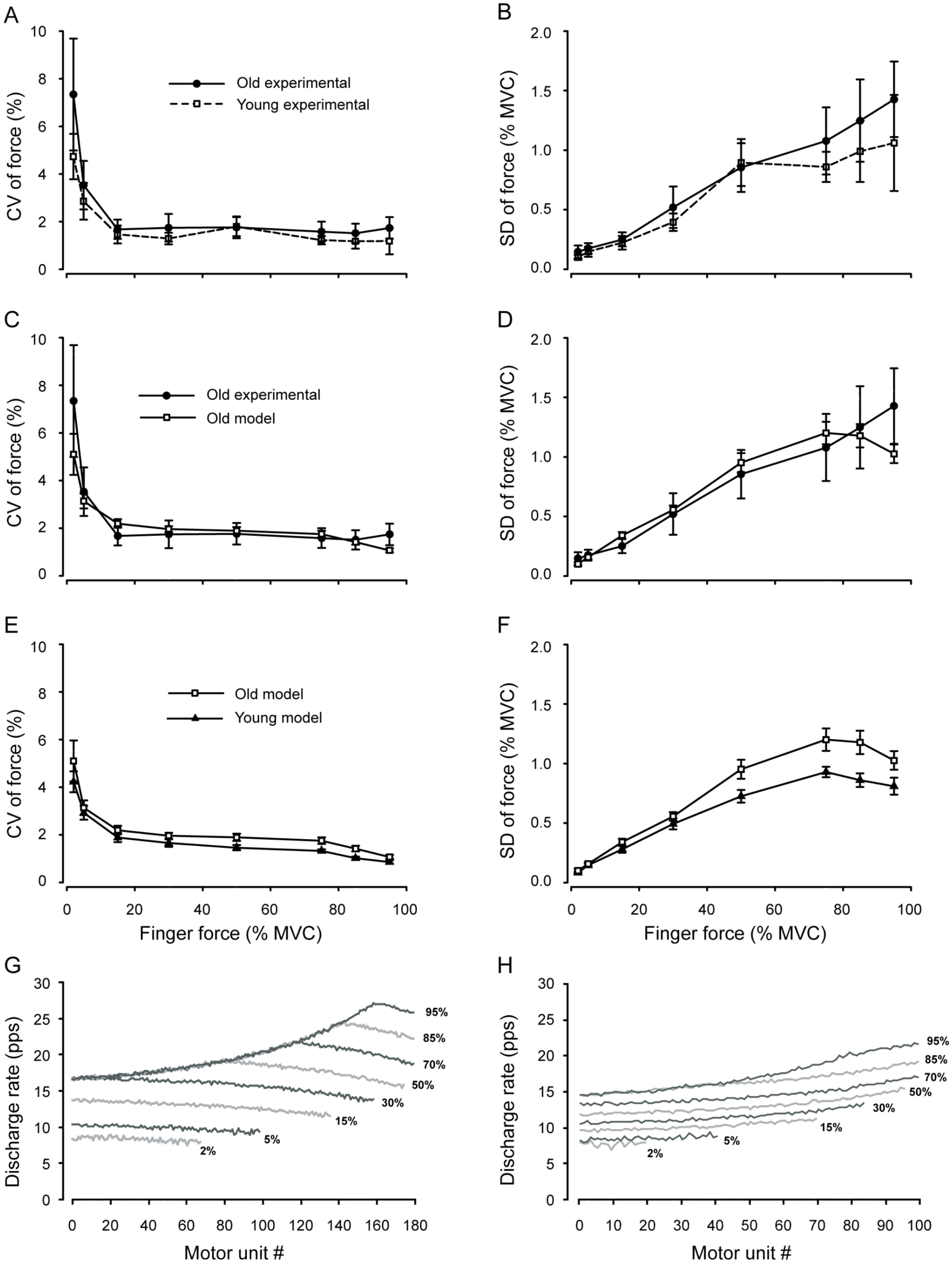
A
CV of force (%)
Old experimental
Young experimental
B
SD of force (% MVC)
C
CV of force (%)
Old experimental
Old model
D
SD of force (% MVC)
E
CV of force (%)
Old model
Young model
Finger force (% MVC)
F
SD of force (% MVC)
Finger force (% MVC)
G
Discharge rate (pps)
95%
85%
70%
50%
30%
15%
5%
2%
Motor unit #
H
Discharge rate (pps)
95%
85%
70%
50%
30%
15%
5%
2%
Motor unit #

Figure 8. Comparison of measured and simulated values for force steadiness and the variation in discharge rate across the population of motor units. *A* and *B*, experimental measures of relative (CV) and absolute force (SD) steadiness for 22 old and 22 young adults. A significant main effect of age was found for the CV of force, with multiple *t*-tests identifying significantly greater ($P < 0.05$) relative variability of force at the 2% MVC target for the old adults. *C* and *D*, comparison of old experimental data with model data simulating the motor unit pool of old adults. There were no significant main effects for contrasts of the model and experimental data (CV: $P = 0.26$, SD: $P = 0.201$), indicating that the model provides a good approximation of the force exerted for old adults. Separate comparisons at each target force with multiple *t*-tests, however, did reveal significant differences between the measured and simulated values for CV and SD of force at the 15 and 95% MVC target forces. *E* and *F*, comparison of simulated data for young and old adults. There was a significant main effect for both the CV and SD of force ($P < 0.001$), with significant differences between 6 of the 8 forces for both the CV and SD of force. *G* and *H*, simulated mean discharge rate for each motor unit activated at the 8 different target forces for young (*G*) and old adult (*H*) versions of the model. Different pattern of mean discharge rate for each active motor unit was not specified explicitly, but rather emerged from the specification of reduced rate coding, fewer motor units, and altered twitch torques for old adults. On the basis of experimental data, the variability in motor unit discharge rate for each individual motor unit was the same for the 2 models. Rate of increase in discharge rate for all active motor units was slower for old adults, which resulted in differences in discharge rate variability across the pool. Average coefficient of variation of the ISI for all the active motor units was significantly greater for old adults during the simulated contractions at 2% (old: 27.4%, young: 25.6%; $P < 0.001$), 5% (old: 20.2%, young: 16.7%; $P < 0.001$), and 15% MVC force (old: 11.7%, young 10.9%; $P < 0.05$), but average variability was the same for the remaining forces ($P > 0.212$). Error bars in *A*-*F* are 95% CIs.

Discussion

The discharge characteristics of motor units with a wide range of recruitment threshold were recorded in a hand muscle of old adults as the muscle performed isometric contractions at many target forces. The experimental measurements indicated that the old adults exhibited a compressed range of rate coding compared with young adults, but there was no difference between the two groups in discharge variability. When these data were combined with other known changes that accompany aging, a computational model of motor unit recruitment and rate coding was able to achieve a close match between measured and simulated values for force steadiness across the operating range of the muscle for the old adults.

Recruitment threshold and discharge rate

Motor unit recruitment thresholds were assessed with three different tasks: a ramp increase in force, discrete constant-force targets, and a graded minimal-discharge task with audio feedback of motor unit discharge. Consistent with young adults (Moritz et al. 2005) and a previous comparison of young and old adults (Spiegel et al. 1996), motor unit recruitment thresholds were characterized equally well with all tasks. The discharge rates at recruitment, however, were significantly lower for the graded minimal-discharge task than for the ramp task or discrete-force targets. A similar association was observed for the young adults. Late adaptation in motor unit discharge rate could explain the observation of lower discharge rates during the longer-duration graded minimal-discharge task compared with the briefer ramp and discrete tasks (Kernell 1965a; Kernell and Monster 1982b; Sawczuk et al. 1995). Presumably, the lower discharge rate during the graded minimal-discharge task is not accompanied by lower force because of the delayed mechanical response of muscle to reductions in discharge rate (Macefield et al. 1996).

Both minimal and peak discharge rates increased with recruitment threshold, but the strength of these relations were notably weaker for old adults compared with young adults. The decline in these relations for the old adults is likely a consequence of the motor unit remodeling that occurs with aging, perhaps arising from the change in innervation number that disrupts the associations between recruitment threshold, contractile speed of the innervated muscle fibers, and discharge rate. Erim et al. (1999) found a similar effect of aging on the relation between minimal discharge rate and recruitment threshold force.

Although Erim et al. (1999) also found that the relation between peak discharge rate and recruitment is disturbed in old adults, the associations differed from those observed in the current study. The observation that peak discharge rate increases with recruitment threshold contrasts with results from other studies on hand muscles found low threshold motor units achieving higher discharge rates than later recruited motor units (De Luca et al. 1982; Tanji and Kato 1973). The data in the current study are limited by technical constraints that prevented the recording of motor unit discharge up to maximal force. Other studies that assessed maximal discharge rates with ramp contractions to a set force level (De Luca et al. 1982; Duchateau and Hainaut 1990) may have underestimated the range of rate coding for high threshold motor units if the force at the peak of these ramps was not sufficient to elicit maximal discharge rates for later recruited motor units. Regardless of the methodological issues that limit the measurement of maximal discharge rates, the observation of reduced rate coding in old adults is consistent with other studies (Erim et al. 1999; Kamen et al. 1995; Knight and Kamen 2007; Patten et al. 2001). Furthermore, it has been reported that the difference in discharge rates of consecutively recruited motor units is less for old adults (Erim et al. 1999).

Discharge variability

The absence of an age-related difference in discharge variability is consistent with some previous investigations (Semmler et al. 2000; Vaillancourt et al. 2003), but not others (Laidlaw et al. 2000; Tracy et al. 2005). Variability in motor unit discharge times was previously attributed to synaptic noise and its interaction with the time course of the post-spike afterhyperpolarization (AHP) of a motor neuron (Calvin and Stevens 1968; Matthews 1999; 1996). From the limited number of motor neuron investigations with cells from old animals, there is no evidence of age-related changes in the intrinsic motor neuron properties that control the AHP and influence the susceptibility to activation by synaptic noise (Engelhardt et al. 1989;

Morales et al. 1987). Therefore it is not surprising that no age-related difference in the variability of motor unit discharge times was observed with the various measures that were used.

Rather, the similarity in discharge variability for the young and old adults provides converging evidence to support the previous description of the pattern of discharge variability in motor units across their activation range (Moritz et al. 2005). The results indicate that relative variability is high at recruitment and it declines as discharge rate increases, whereas absolute variability, measured as the SD of the number of motor unit discharge occurring in any 1-s period, increase with an increase in discharge rate (Jones et al. 2002; Stein et al. 2005). However, there is an initial rapid decline in absolute variability at low discharge rates just above recruitment threshold, which may explain why elevated levels of motor unit discharge variability were found for old adults in some studies (Kornatz et al. 2005; Laidlaw et al. 2000; Tracy et al. 2005) but not others (Christie and Kamen 2009a; Semmler et al. 2000; Vaillancourt et al. 2003; Welsh et al. 2007). Accordingly, the variability in discharge times for a motor unit should not be determined from a measurement at a single force.

Updating the Fuglevand model

The skew that is apparent in interspike interval histograms at low levels of excitation was described previously (Calvin and Stevens 1968; Enoka et al. 1989; Matthews 1996; Poliakov et al. 1995). The variable shape of the interspike interval distribution was included in the model because of the possibility that variability in force might be exacerbated by a greater frequency of longer duration interspike intervals (Duchateau et al. 2006). The skewed feature of interspike interval histograms was implemented in the model by drawing random interspike intervals from a combination of normal and lognormal distributions. Inclusion of a lognormal distribution provided the long tail observed in interspike interval histograms. The predicted effect of more variable motor unit forces was apparent in the model output. For the range of twitch durations and twitch forces in the simulated motor unit pool, however, this postulated contributor to force variability had only a minor effect.

There is recent evidence that the incidence of brief interspike intervals (≤10 ms), referred to as double discharges, may be reduced in old adults (Christie and Kamen 2006a). The analysis in the current study excluded any double discharges, but this amounted to only 0.04% of interspike intervals recorded during the discrete constant-force contractions. Although twice as many double discharges were recorded for the young adults, the extremely low incidence is indicative of the irrelevance of this motor unit behavior to steady-force contractions examined in this experiment. Accordingly, double discharges were not included in the computer model.

Force variability and old adults

The experimental protocols used in the present study for the assessment of force steadiness involved brief contractions performed in the absence of visual feedback. Under these conditions, the difference between young and old adults in both relative and absolute indices of force steadiness was relatively minor, with a clear difference only at the lowest force level (Galganski et al. 1993). Had longer duration contractions been examined (Laidlaw et al. 2000), variable force targets been presented (Vaillancourt and Newell 2003), or visual feedback provided (Sosnoff and Newell 2006a; 2005), there may have been more substantial differences in force steadiness. By observing only a short-duration, constant-force contraction without visual feedback, the intent was to assess steadiness under conditions of a relatively constant central drive to the motor neuron pool. This permitted the investigation of the contribution of alterations in the motor unit pool to age-related declines in force steadiness.

The close match between experimental and simulated data for the old adults confirms that the key features of the motor unit pool of old adults that contribute to the decline in force steadiness with old age have been identified. It is notable that there was a difference in the output of the young- and old-adult versions of the model, despite the two models including the same degree of discharge variability for each individual motor unit. The old-adult model had reduced rate coding, fewer motor units, and different distribution of twitch forces, which resulted in lower discharge rates at a given force. As a consequence, there were higher levels of discharge variability across the motor unit pool of old adults at low forces, which likely contributed to some of the difference in force steadiness between the young and old adults. Nonetheless, the slightly reduced force steadiness for the old-adult model was apparent across the entire operating range, despite similar levels of variability in discharge times. Although previous studies suggested that a reduction in the number of motor units and an increase in motor unit twitch forces does not reduce force steadiness (Enoka et al. 2003; Keen et al. 1994), these effects may emerge only when distributed across the entire population with realistic patterns of motor unit discharge variability. Furthermore, the measured and simulated differences in strength between young and old adults (Sosnoff and Newell 2006b) were associated with relatively minor differences in force steadiness.

The relatively similar force-steadiness profiles for the experimental and model data for both the young and old adults indicate that age-related changes in the input to the motor neuron pool may be responsible for the more substantial decrements in steadiness found for old adults (Vaillancourt et al. 2003; Vaillancourt and Newell 2003). For example, changes in spinal reflex pathways (Earles et al. 2001; Kido et al. 2004), a reduction in the number of corticospinal fibers (Eisen et al. 1996), or potential alterations in monoaminergic drive to the motor neuron pool (Christou et al. 2004) may influence the ability of old adults to provide a constant input to the motor neuron pool.

The discharge variability hypothesis

The finding of similar discharge variability for young and old adults may seem to contradict earlier reports from our laboratory (Kornatz et al. 2005; Laidlaw et al. 2000; Tracy et al. 2005). Indeed, one motivation for conducting the current study was the conflicting evidence from other experiments, such as those by Semmler et al. (2000) and Vaillancourt et al. (2003), which found no difference in discharge variability between young and old adults. The primary reason for the apparent disparity is the rapid change in discharge variability as the activation of a motor unit increases (Moritz et al. 2005). Importantly, the study by Moritz et al. (2005) established the potent influence of discharge variability on force steadiness. These findings encouraged our continued investigation of discharge variability in old adults, but indicated that it could be assessed accurately only by measuring the magnitude of discharge variability within the same motor unit over a range of contraction intensities. The current study demonstrates that discharge variability also exerts a potent influence on force steadiness in old adults, but found that this mechanism contributes minimally to the difference in force steadiness between young and old adults.

In summary, old adults exhibited reduced rate coding of motor unit discharge compared with that of young adults, but the two groups had similar levels of variability in motor unit discharge times. In agreement with a previous study (Moritz et al. 2005), discharge variability exerted a critical influence on the steadiness of force, especially at low forces. The inclusion of these characteristics of motor unit recruitment and rate coding provided an accurate simulation of the force produced by old adults.

Chapter III

Discharge characteristics of motor units during long duration contractions performed by young and old adults

Abstract

The modulation of motor unit discharge rate has been studied in a range of tasks, but rarely in contractions that last >5 min. Although reduced preparations allow for long-duration recordings of motor neuron activity in animals, it is unclear if the results from intracellular current injection *in vitro* generalize to motor neuron activation arising from synaptic input *in vivo*. The purpose of the second dissertation study was to determine how long motor unit activity could be sustained during a voluntary contraction by humans. The discharge times of single motor units were recorded from the first dorsal interosseus muscle in young (27.8 ± 8.1 yr) and old adults (80.5 ± 6.7 yr) as they exerted a low abduction force with the index finger. The task was to maintain the discharge of an isolated single motor unit for as long as possible. Subjects received auditory feedback of the discharge times and were asked to keep the rate relatively constant for the duration of the task. The task was terminated when the motor unit stopped discharging action potentials despite the ability of the subject to sustain the abduction force. Twenty-four single motor units were recorded in young adults, but due to technical difficulties only five were recorded in old adults. The mean task duration was 20.6 ± 17.9 min (range: 1.2 – 65.2 min) for young and 8.2 ± 10.2 min (range: 0.9 – 25.8 min) for old adults. When analyzed across discharge duration, mean discharge rate (10.8 ± 1.9 pulses•s^{-1}) and mean abduction force (6.1 ± 3.1% maximum) did not change (discharge rate: $P = 0.119$, abduction force: $P = 0.235$), however relative variability (coefficient of variation) of the interspike intervals during the initial 30 s of the task was 21.1 ± 6.5% and this increased across the task to 34.2 ± 10.6% during the final 30 s ($P < 0.001$). Discharge characteristics changed to the same extent with time and did not differ between young and old adults. All motor units could be recruited within 60 s of recovery. The results indicate that subjects were able to sustain a relatively

constant discharge rate based on audio feedback, the cessation of the discharge was preceded by an increase in discharge variability, and the duration was less for the few motor units recorded in old adults. The findings indicated that the maximal duration of human motor unit activity could far exceed the previously reported durations for motor neurons that were activated by intracellular current injection *in vitro*.

Introduction

When a person is asked to exert a constant force during a voluntary contraction, the discharge rate of motor units that were active from the beginning of the contraction typically declines due to adjustments in synaptic input and the intrinsic properties of the motor neuron (Bigland-Ritchie et al. 1983; Carpentier et al. 2001; De Luca et al. 1996; Enoka et al. 1989; Garland et al. 1994; Mottram et al. 2005; Riley et al. 2008a). One approach to estimate the relative significance of these adjustments is to determine how long a motor unit can continue to discharge action potentials despite changes in the intrinsic properties of its motor neuron. Previous studies do not address this issue as they involved constant-force, voluntary contractions that lasted <5 min (Christie and Kamen 2009a; Conwit et al. 2000; De Luca et al. 1996; Garland et al. 1994; Kamo and Morimoto 2001; Mottram et al. 2005; Person and Kudina 1972; van Groeningen et al. 2000). Furthermore, the longest duration of sustained motor neuron discharge in response to injected current is 5 min for a hypoglossal motor neuron in a brain stem slice preparation (Sawczuk et al. 1995) and 4 min in an anesthetized cat spinal motor neuron (Kernell and Monster 1982a). An alternative approach is to determine how long a motor unit can continue to discharge action potentials at a constant rate during a sustained voluntary contraction, which will mean that the excitatory synaptic input must increase to overcome the progressive adjustments in the intrinsic motor neuron properties.

Given age-associated changes in both the synaptic input that is delivered to motor neurons (Boxer et al. 1988; Eisen et al. 1996; Oliviero et al. 2006) and in the intrinsic properties of the motor neurons (Engelhardt et al. 1989; Morales et al. 1987; Piotrkiewicz et al. 2007; Rossini et al. 1992), it is likely that the discharge characteristics of motor units active for long durations differ for old adults when they perform such tasks. The afterhyperpolarization period the of action potential, for example, is known to influence motor neuron discharge rates (Eccles and Lundberg 1958; Kernell 1965b) and several animal (Cameron et al. 1991; Engelhardt et al. 1989; Kalmar et al. 2009) and human (Christie and Kamen 2009b; Piotrkiewicz et al. 2007) studies indicate that this period is prolonged and corresponds with reduced discharge rates in old humans (Christie and Kamen 2009b). Additionally, there are age-related reductions in the amount of synaptic input received by motor neurons from many pathways (Boxer et al. 1988; Earles et al. 2001; Eisen et al. 1996; Johnson et al. 1993; Kido et al. 2004; Oliviero et al. 2006). Reductions in the number of synaptic inputs from these various pathways may represent differences in the strength and the relative balance of excitatory and inhibitory inputs received by the motor unit pools of old adults. Evidence suggests, for example, that changes in the balance between excitatory and inhibitory inputs can influence the discharge characteristics of neurons (Abbott and Chance 2005; Berg et al. 2007; Stein 2010), which may contribute to age-associated differences in the discharge characteristics of motor units during long-duration contractions.

The purpose of the study was to determine how long motor unit activity could be sustained during a voluntary contraction performed by humans. Of secondary interest was the influence of age on this capability. These data have been presented in abstract form (Pascoe et al. 2006; Pascoe et al. 2009).

Methods

Twenty healthy young (27.8 ± 8.1 yrs; range, 18 – 57 yrs; 17 men) and five old adults (80.5 ± 6.7 yrs; range, 71 – 89 yrs; all men) who were free from cardiovascular and neurological disorders volunteered for the study and participated in one to three experimental sessions. All subjects were right handed, as verified by the laterality quotient (young: 0.79 ± 0.12, old: 0.84 ±

0.17) of the Edinburgh Handedness Inventory (Oldfield 1971). Written informed consent was obtained from all the participants and the experimental procedures were approved by the Institutional Review Board at the University of Colorado (Protocol #0908.6) and were in accordance with the *Declaration of Helsinki*.

Experimental setup measurement

Subjects were seated in a modified dental chair in a shielded experimental room. The left shoulder was abducted 0.79 rad, and the forearm restrained in a neutral position with the ulnar surface resting on a platform. The elbow joint and forearm were supported with a vacuum pillow (Tumble Forms, Trenton, Ontario, Canada) and secured with Velcro^{TM} straps. The left hand was supported with the palm vertical, the third through fifth digits flexed slightly at the metacarpophalangeal joints, and restrained in a brace. The index finger was secured to a hinged splint to maintain both interphalangeal joints extended and to constrain finger movement to the abduction-adduction plane. To maximize the contribution of the first dorsal interosseus muscle to the abduction force, the index finger was flexed at the metacarpophalangeal joint ~0.1 rad. The thumb was extended vertically and held with a separate brace in the same plane as the palm of the hand at an angle of ~1.1 rad to the index finger (Figure 9). The right forearm and hand rested on a platform at the right side of the dental chair.

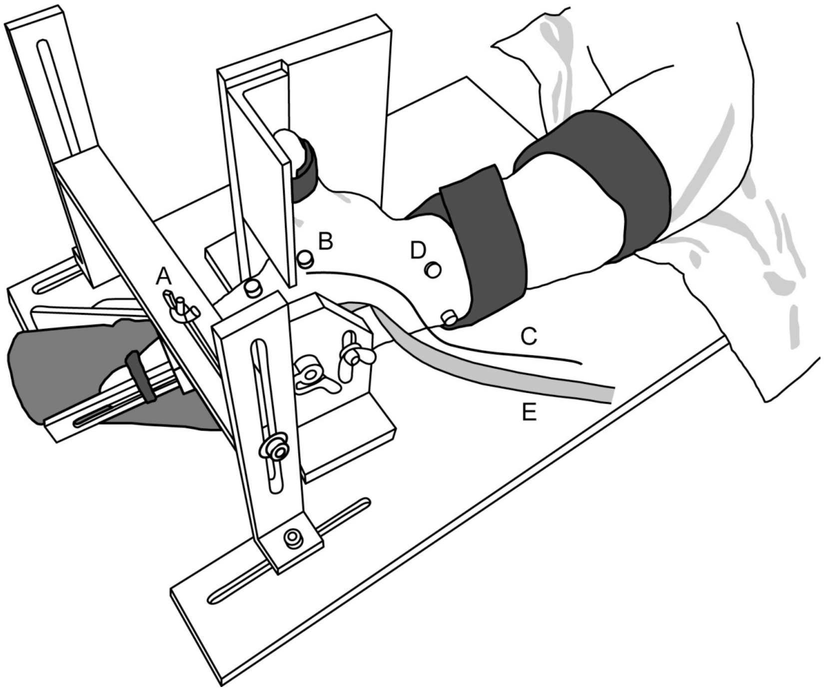

Figure 9. The experimental setup consisted of a custom-fabricated device that limited index finger movement to the abduction-adduction plane. Index finger abduction force was measured with a force transducer (*A*) aligned with the radial border of the proximal interphalangeal joint. Surface EMG from the first dorsal interosseus (FDI) muscle was obtained with 4-mm-diameter electrodes (*B*). A fine-wire electrode (three 50 µm-diameter insulated stainless steel wires) was inserted into the FDI to record discharge times (*C*). Reference electrodes were placed on the styloid processes of the radius and ulna (*D*). Index finger flexion was monitored using a FlexiForce transducer placed at the back of the hinge of index finger splint (*E*).

Force measurement

The abduction force exerted by the index finger was measured with a force transducer (Model 13, Sensotec, Columbus, Ohio) that was aligned with the proximal interphalangeal joint. Abduction force was digitized with a Power 1401 (Cambridge Electronic Design, Cambridge, UK) at 200 samples•s^{-1} and stored on a computer. Visual feedback of the abduction force exerted by the index finger was provided on a 17-in computer monitor located at eye level in front of the subjects at a distance of 1.4 m.

The presence of flexion force by the index finger about the metacarpophalangeal joint was detected with a force sensor (FlexiForce A201, Tekscan, Boston, MA) positioned at the rear of the hinged index finger splint. Flexion force was monitored throughout the experiment and subjects were required to keep this force minimal. Flexion force was digitized with a Power 1401 (CED) at 200 samples•s^{-1} and stored on a computer.

Electromyographic recordings

Single motor unit potentials were recorded from the first dorsal interosseus muscle using Formvar-insulated, stainless steel wires (diameter: 50 μm, California Fine Wire, Grover Beach, CA) that were glued together at the recording tip and inserted into the muscle belly using a 27-gauge hypodermic needle. The needle was inserted into the muscle to a depth of 10-20 mm and then withdrawn, leaving the wires within the belly of the muscle. Each electrode consisted of three wires to permit alternative bipolar configurations. Single motor unit recording quality was optimized by using different pairs of recording wires or by making slight adjustments in the depth of the electrode. A reference surface electrode for the single motor unit recordings was placed over the styloid process of the radius or ipsilateral ulna (silver-silver chloride, 4-mm diameter). The EMG signal was amplified X5,000 and band-pass filtered between 300 Hz and 8.5 kHz (S-series, Coulbourn Instruments, Allentown, PA). The motor unit signal was sampled at 20k samples•s^{-1} with a Power 1401 (CED) and stored on a computer. Single motor unit potentials were identified on-line using a dual-window discriminator (Coulbourn). Discharge times were presented to the subject as audible events through a stereo speaker.

The surface EMG of the first dorsal interosseus muscle was recorded using a bipolar configuration of circular electrodes (silver-silver chloride, 4-mm diameter) using a belly-tendon configuration. A reference surface electrode was placed over the styloid process of the ipsilateral radius or ulna (silver-silver chloride, 4-mm diameter). The EMG signal was amplified X1,000 and band-pass filtered between 13 Hz and 1 kHz (S-series, Coulbourn), sampled at 2k samples•s^{-1} with a Power 1401 (CED) and stored on a computer.

Protocol

At the beginning of the experiment subjects performed several trials of a maximal voluntary contraction (MVC) task to obtain maximal force and surface EMG values for use in normalizing submaximal contractions. Subjects were provided with verbal encouragement as they increased abduction force from baseline to maximum over a 3-s period and then held this maximal level for a further 1-3 s. Visual feedback of abduction force exerted by the index finger was provided on the computer monitor, and the gain of the force was altered between trials to remove subject bias from previous trials. The subject's hand was closely observed by one of the experimenters to ensure other upper extremity muscles did not aid in the task. The peak force value from the three or four trials was taken as the MVC force, provided it was within 10% of the peak value for the previous trial. Subjects were provided with 60 s of rest between each MVC trial.

Subjects were asked to produce a low abduction force with the index finger and to gradually increase contraction intensity as the investigators observed the intramuscular EMG signal for the appearance of a candidate motor unit. The subject was provided with visual feedback of index finger force and audio feedback of the discharge times for the motor unit being tracked. A candidate motor unit was selected for further study if the subject was able to modulate the audible discharge rate using subtle changes in contraction intensity, and its amplitude remained relatively constant despite the subtle changes in contraction intensity. Great care was taken to optimize motor unit recordings to ensure accurate discrimination of discharge times for all tasks. The approximate force associated with repetitive discharge was noted.

The recruitment threshold of the motor unit was characterized as the force at which the unit began to discharge action potentials repetitively during a ramp increase in index finger force. The target force for the ramp contractions was set at twice the approximate force at which repetitive discharge of the candidate unit was observed. The ramp contractions comprised a ~10 s linear increase in force up to the target followed by a ~10 s linear decrease in contraction intensity back to baseline. Visual feedback was provided during the ramp contractions, and no audio feedback was given. Subjects were asked to perform four ramp contraction trials and rested for 30 s between trials.

Subjects were then instructed to slowly increase force until the candidate unit began to discharge action potentials and to then keep the discharge rate relatively constant based exclusively on the audio feedback. When discharge rate changed or became variable, subjects were given verbal guidance to keep the contraction intensity similar to the force exerted at the start of the long contraction. Subjects were also informed that their perceived effort might increase as the contraction progressed. When the motor unit stopped discharging action potentials, subjects were encouraged to maintain the force for a further 60 s before relaxing. Within 15 s of task termination, subjects performed another maximal voluntary contraction. Thirty seconds later, subjects performed another ramp contraction to assess recovery of the motor unit and to verify that discharge cessation was not due to electrode movement.

Data analysis

The recruitment threshold of each unit was determined with a Spike2 script that was based on an algorithm developed by Moritz et al. (2005). The algorithm identified the recruitment threshold by moving a 500-ms window forward in time in 1-ms steps until the coefficient of variation for the discriminated discharges within the window was <50%. The force corresponding to the first discharge within that window was taken as the recruitment threshold of the unit (Figure 10). The same method was used to define the derecruitment threshold force of the motor unit. The discharge rate and the coefficient of variation for interspike interval were determined for a 500-ms window at recruitment and derecruitment.

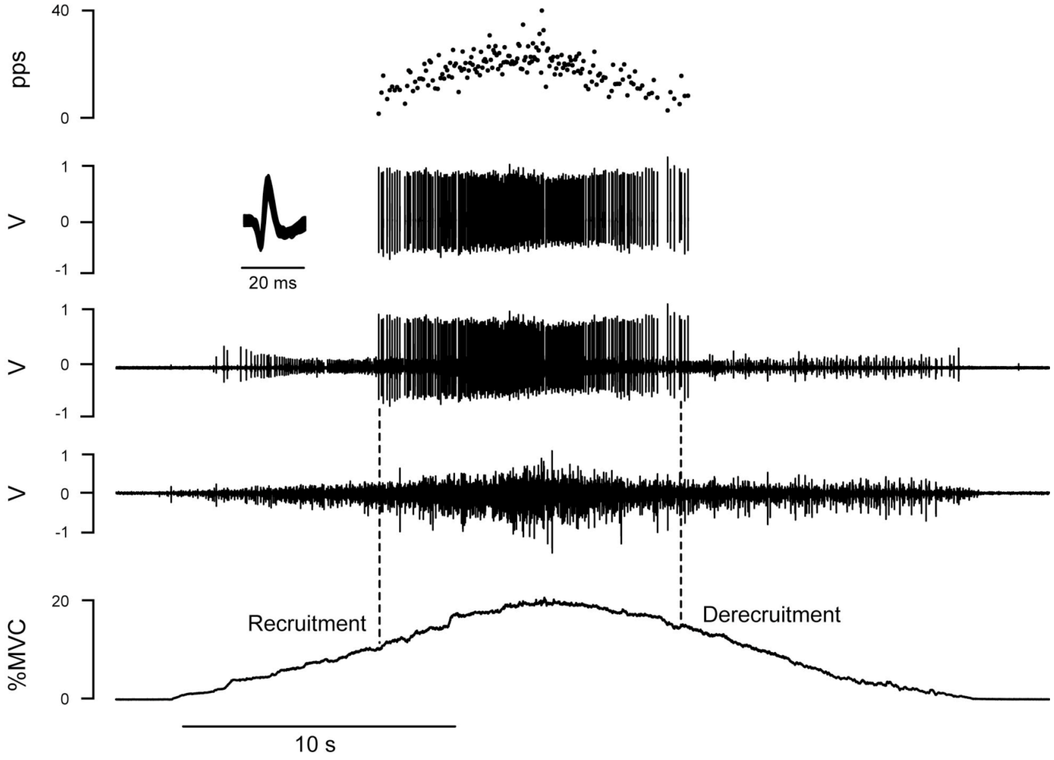

Figure 10. Representative data from a ramp contraction depicting, from top to bottom, the instantaneous discharge rate of the motor unit (pulses per second; pps), discriminated action potentials with waveform overlay (Volts; V), interference electromyogram (EMG) from the fire-wire electrode, surface EMG from the FDI muscle, and index finger abduction force normalized to maximal voluntary contraction (MVC). The motor unit had a recruitment threshold force of 10.6% MVC and a derecruitment threshold force of 13.2% MVC.

Discrimination of single motor unit action potentials was performed off-line by visual inspection of each potential and by using the template-matching features of the Spike2 software (v. 5.12, CED). Interspike intervals >250 ms (<4 pps) or <20 ms (>50 pps) were excluded from the calculations of discharge rate (*n* = 2,504, 1.2% of discharges). Long interspike intervals (>250 ms) were likely due to cessation of motor unit discharge, whereas short intervals (<20 ms) exceed the rates normally observed during sustained isometric contractions of human motor units in hand muscles (Barry et al. 2007; Bigland-Ritchie and Lippold 1954; De Luca et al. 1982; Kanosue et al. 1979; Moritz et al. 2005; Tanji and Kato 1973) and were likely due to discrimination error or double discharges. Once discriminated, custom code was used to assign the discharge times into five epochs defined by a 30-s window placed at the beginning, 25, 50, 75%, and end of the contraction duration. Within each epoch, discharge times were converted into interspike intervals and the mean and coefficient of variation were calculated. Additionally, interspike interval histograms were constructed for each epoch and skewness and kurtosis values were calculated.

Fluctuations in index finger abduction force were quantified as the coefficient of variation (standard deviation / mean X 100). The surface EMG for first dorsal interosseus was quantified using the root mean square (RMS) amplitude and expressed as a percentage of the maximal root mean square (% Max) value. The maximal RMS value was calculated over a 500-ms window that was centered about the peak force of the MVC trial with the highest peak force. To assess any changes in surface EMG, RMS values were calculated across a 30-s window at six points, including four equally spaced (20, 40, 60, 80%) between the beginning and end of the contraction.

Statistical analysis

Paired samples *t*-tests were used to investigate differences in force between the maximal voluntary contractions performed before and immediately after the long-duration contraction. Additional *t*-tests were used to examine differences in force, mean discharge rate, and coefficient of variation for interspike interval between recruitment and derecruitment during ramp contractions. Multiple two-factor ANOVAs with repeated measures on time and a between subjects factor of age were used to detect changes in mean discharge rate, coefficient of variation for interspike interval, mean force, coefficient of variation for force, histogram skewness and kurtosis, and normalized EMG RMS amplitude across discharge duration and between young and old groups. When ANOVAs yielded significant main effects, post hoc analysis using the Bonferroni adjustment for multiple comparisons was performed to locate differences within the tasks.

The interspike interval histograms were constructed in MATLAB (v. 7.3, The MathWorks, Inc., Natick, MA) using a bin width of 1 ms and included all interspike intervals between 1 ms (1,000 pps) and 250 ms (4 pps). Normal, lognormal, and gamma functions were fit to each histogram using maximum-likelihood estimation (Statistics Toolbox, MATLAB). The goodness-of-fit for all three functions was evaluated by calculating the root mean square error (RMSE) between the fitted function and the interspike interval count for each bin across the entire histogram. Skewness and kurtosis values were also calculated; skewness describes the degree of symmetry and kurtosis indicates the breadth of a distribution. The normal distribution has a skewness value of zero and a kurtosis of 3. Therefore, a sample interspike interval distribution with skewness of 1.2 and kurtosis of 3.5 is described as being skewed to the right due to a greater proportion of extra long interspike intervals (positively skewed) and to be more narrow than the normal distribution due to values clustering about a central value (leptokurtic).

An alpha level of $P < 0.05$ was used to identify significant differences and statistical analyses were performed using SPSS (v. 16.0, Chicago, IL, USA). Data are presented in the text as mean ± standard deviation and in figures as mean ± standard error of the mean.

Results

The results comprise recordings for 24 motor units for young (success rate: 31%) and 5 for old adults (success rate: 16%) from first dorsal interosseus when subjects maintained the discharge of an isolated single motor unit for as long as possible. An example of a long duration contraction for a young adult is shown in Figure 11. The subject increased index finger abduction force to 6.9% MVC during the first 30 s of the contraction and the motor unit discharged action potentials at a rate of 9.9 pps, with a coefficient of variation for interspike interval of 22.9% (Figure 11*A*). The motor unit stopped discharging action potentials after 5.2 min of discharge, at which time the mean discharge rate was 7.3 pps and the coefficient of variation for interspike interval had increased to 29.3%, despite no change in abduction force (8.1% MVC) from the start of the task (Figure 11*B*). MVC force for all tasks, however, was significantly less (30.0 ± 6.8%) at the end of the protocol (27.5 ± 8.8 N) than the beginning (38.7 ± 9.0 N, $P < 0.001$), which indicates that the task involved substantial fatigue.

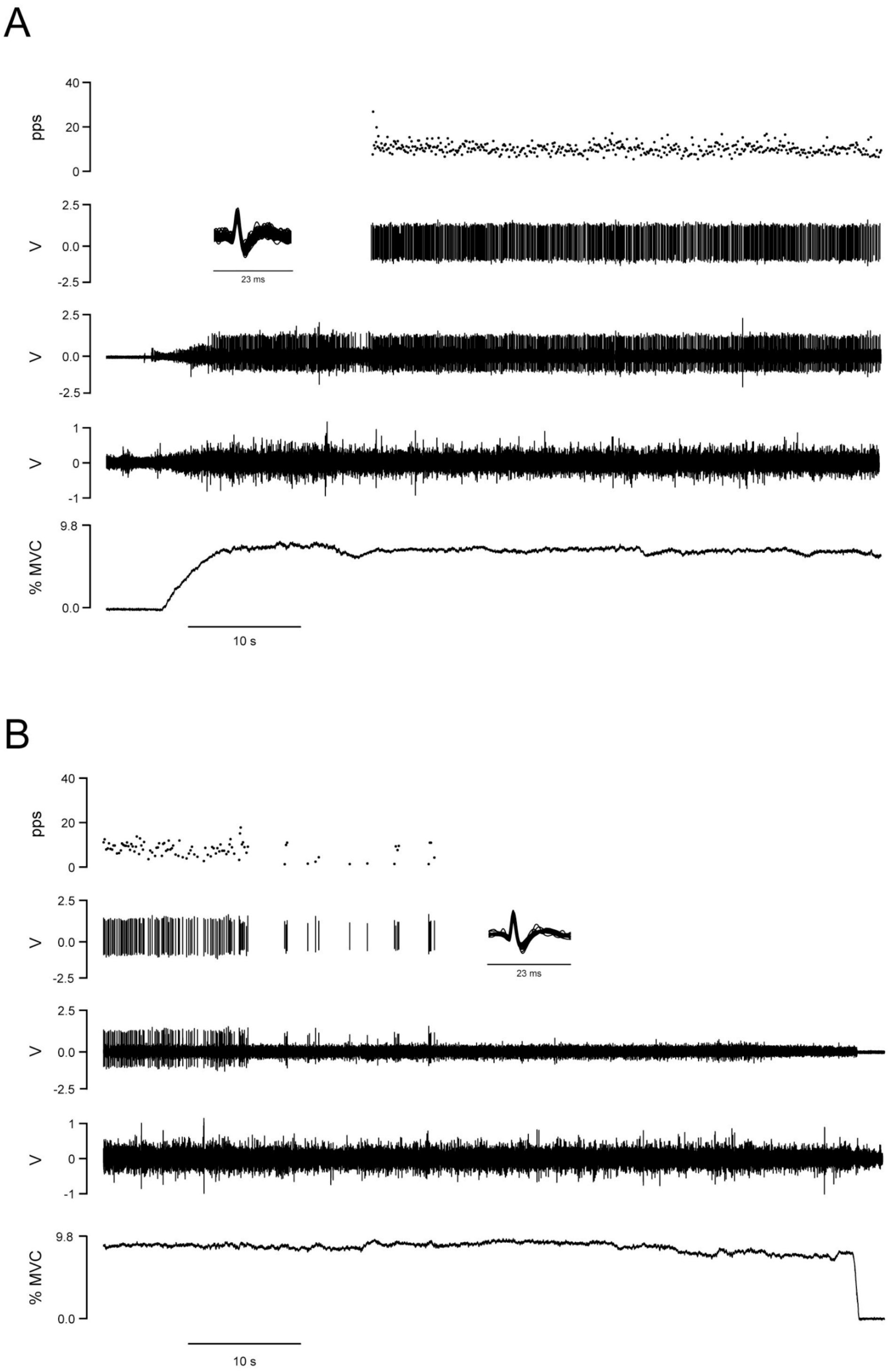

Figure 11. Representative data from the beginning (*A*) and end (*B*) of a 5.2 min contraction depicting, from top to bottom, the instantaneous discharge rate of the motor unit (pulses per second; pps), discriminated action potentials with waveform overlay (Volts; V), interference electromyogram (EMG) from the fire-wire electrode, surface EMG from the FDI muscle, and index finger abduction force normalized to maximal voluntary contraction (MVC).

Recruitment threshold

The average recruitment threshold force for motor units in young adults was 3.3 ± 3.7% MVC (range: 0.01 – 13.3% MVC) and for old adults it was 7.3 ± 9.7% MVC (range: 0.01 – 23.8% MVC) for old adults. Identifiable derecruitment threshold forces were obtained for 26 of the 29 motor units examined. The derecruitment threshold forces (4.7 ± 3.2% MVC) were greater than the recruitment threshold forces ($P = 0.18$). The discharge rate at recruitment (9.0 ± 2.3 pps) was significantly greater than that at derecruitment (7.8 ± 1.2 pps, $P = 0.023$). The coefficient of variation for interspike interval at recruitment (23.1 ± 6.7%) was similar to that at derecruitment (22.2 ± 6.8%, $P = 0.655$).

Long-duration contractions

Twenty-four motor units for young and five for old adults were tracked as subjects performed long duration contractions (Figure 12). Contractions were held for an average of 20.6 ± 17.9 min (range: 1.2 - 65.2 min) for young and 8.2 ± 10.2 min (range: 0.9 - 25.8 min) for old adults at a mean abduction force of 6.1 ± 3.1% MVC, which did not change over time (time main effect: $P = 0.235$, Figure 13*A*). A significant main effect for age ($P = 0.039$) indicated that mean abduction force was on average less for young (5.4 ± 3.1) than old adults (9.6 ± 8.1% MVC). Fluctuations in index finger abduction force were relatively high (20.7 ± 19.1%), as subjects were not required to keep index finger abduction force steady during the contraction, but this did not change across time (time main effect: $P = 0.69$, Figure 13*B*) and did not differ between age groups ($P = 0.978$).

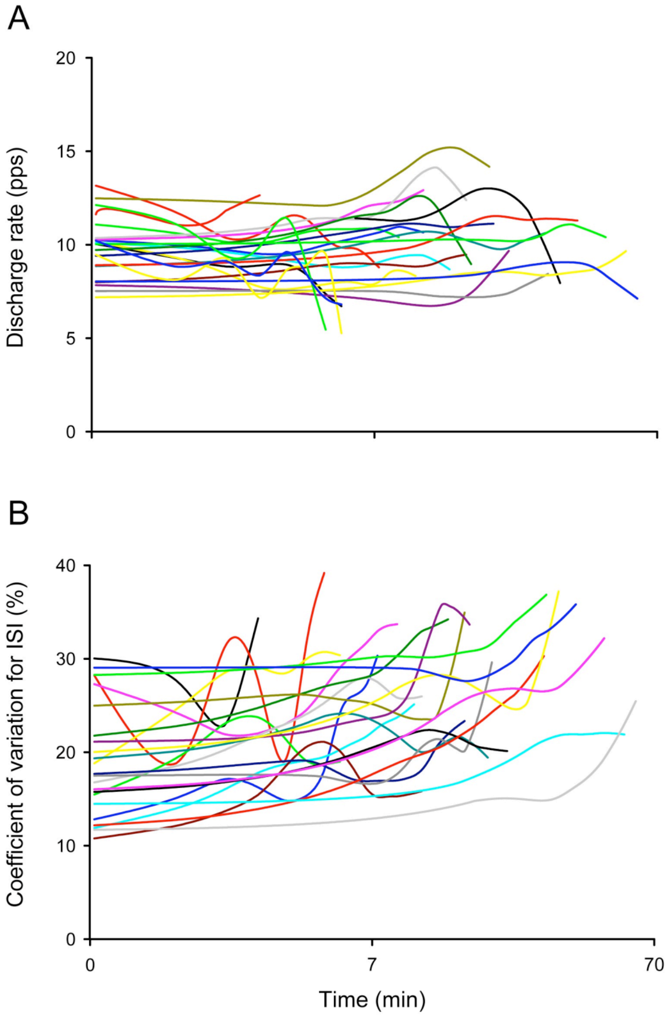

Figure 12. Mean discharge rate (*A*) and coefficient of variation for interspike interval (ISI; *B*) for 21 motor units from young and 3 from older adults plotted across task duration. Data are presented as 3rd-order regressions and plotted on a log-based scale. Data for 3 motor units from young and 2 from old subjects were too brief (<2 min) to be plotted and are not depicted.

Motor unit discharge was tracked throughout the sustained contractions and the trains of action potentials contained an average of 11,957 ± 9,887 interspike intervals for young and 4,852 ± 4,798 for old adults. Mean discharge rate was relatively low (10.8 ± 1.9 pps) and remained unchanged with time for both age groups (time X age interaction: *P* = 0.180, Figure 13*C*). The coefficient of variation for interspike interval during the initial 30 s of the tasks was 21.1 ± 6.5%

and increased across tasks to 34.2 ± 10.6% during the final 30 s (time main effect: $P < 0.001$) for both young and old adults (time X age interaction: $P = 0.984$). Post hoc analysis revealed that coefficient of variation for interspike interval for the final 30 s was elevated compared with the initial three time points ($P \leq 0.009$, Figure 13*D*). There was no change in EMG amplitude for first dorsal interosseus muscle during the contraction for either young or old adults (14.1 ± 2.2% maximum, $P = 0.815$, Figure 13*E*). All motor units were recruited after a period of recovery (30 – 60 s), which verified that the electrode had not become displaced.

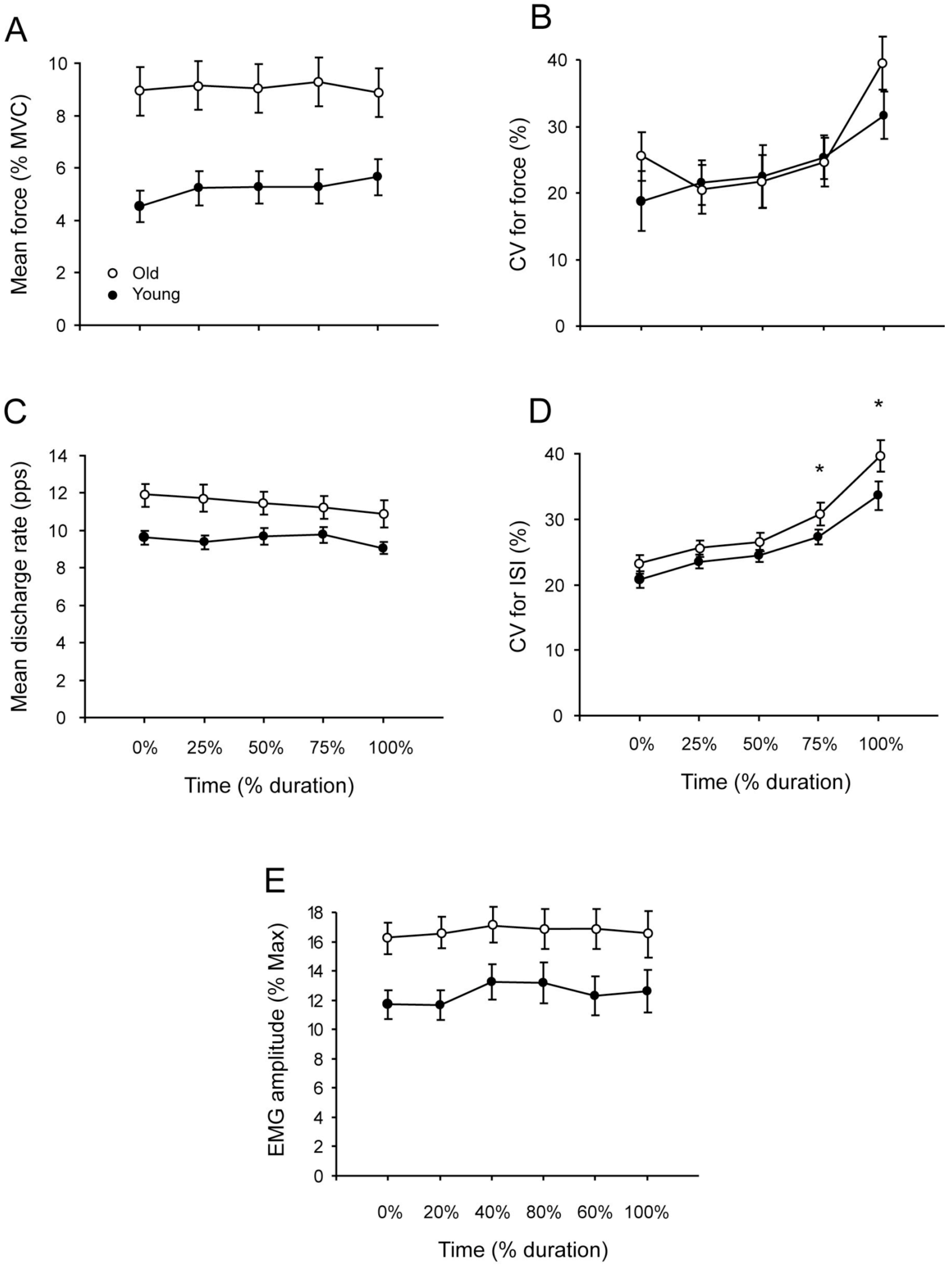

Figure 13. Task and discharge characteristics for the long-duration contractions for young (●) and old (○) adults. Mean index finger abduction force (*A*, maximal voluntary contraction; MVC), coefficient for variation (CV) for force (*B*), and mean discharge rate (*C*) did not change with time for either young or old adults. The coefficient for variation for interspike interval (CV for ISI) increased with time, but to the same extent for young and old adults (*D*). The normalized amplitude of the surface EMG did not change with time for either young or old adults (*E*). $*P < 0.05$ compared with the previous time points.

Interspike interval histograms constructed at each of five 30-s intervals contained on average 277 ± 83 interspike intervals (Figure 14*A*). Histograms were positively skewed (1.2 ± 0.6) and this value remained constant (time main effect: *P* = 0.857) for both young and old adults (time X age interaction: *P* = 0.887, Figure 14*B*) throughout the tasks. However, kurtosis values were leptokurtic (3.2 ± 2.9) and remained unchanged with time (*P* = 0.09) to the same extent for young and old adults (time X age interaction: *P* = 0.887) from initial values of 3.2 ± 2.9. A majority of interspike interval histograms were best fit by a lognormal distribution (89%, Table 3).

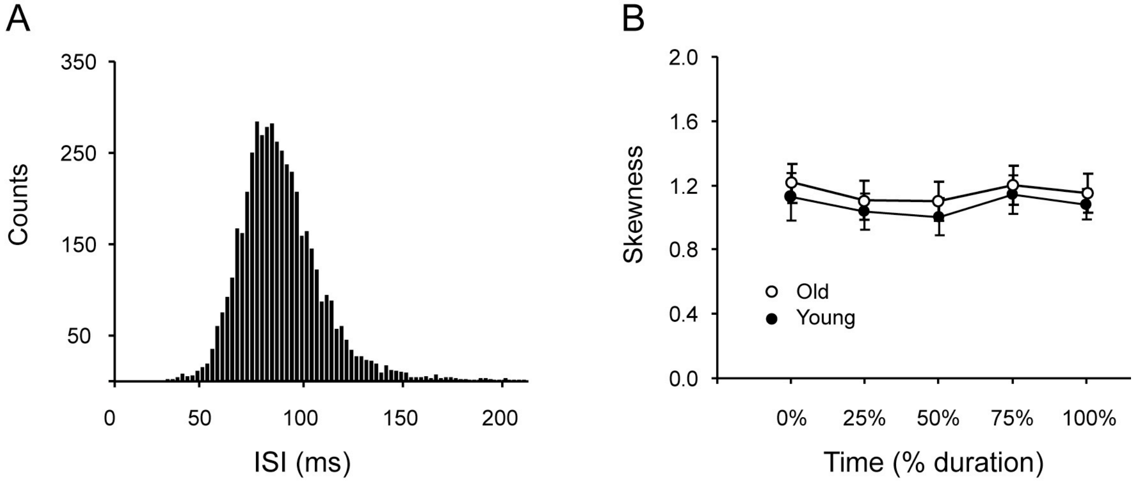

Figure 14. *A*, a sample interspike interval histogram with a mean interspike interval (ISI) of 84.3 ms, skewness of 1.2 and a kurtosis of 3.9. *B*, skewness values remained positive and did change with time for young (●) and old (○) adults.

Table 3. The totals (frequencies) for distribution functions that best fit each histogram.

Percent of duration	No. interspike Intervals	Lognormal	Gamma	Normal
Beginning	307 ± 65	24 (0.83)	3 (0.13)	2 (0.04)
25%	297 ± 68	27 (0.96)	1 (0.00)	1 (0.04)
50%	305 ± 87	25 (0.88)	2 (0.08)	2 (0.04)
75%	301 ± 94	26 (0.92)	2 (0.04)	1 (0.04)
End	176 ± 99	27 (0.92)	1 (0.04)	1 (0.04)
Total	1,380 ± 312	129 (0.89)	9 (0.06)	7 (0.05)

Discussion

This study was the first to record the discharge of single motor units during a continuous contraction until the motor unit stopped discharging action potentials. Subjects sustained a low abduction force with the index finger and kept the motor unit discharging action potentials at a constant rate for as long as possible. These recordings comprise a previously unexplored range of durations for human motor unit recordings during a continuously sustained isometric contraction.

Discharge characteristics during ramp contractions

The recruitment threshold forces were lower than the derecruitment threshold forces for ramp contractions performed before the long-duration contractions. Although a lower derecruitment threshold force has been observed in some studies (Baudry et al. 2009; Denier van der Gon et al. 1985; Garland et al. 1994; Pascoe et al. 2010; Romaiguère et al. 1993; Suzuki et al. 1990), many have also found a lower recruitment threshold force (De Luca et al. 1982; Freund et al. 1975; Jesunathadas et al. 2010; Milner-Brown et al. 1973; Patten and Kamen 2000; Spiegel et al. 1996). With the exception of (Patten and Kamen 2000), the majority of studies found lower recruitment thresholds were performed in the first dorsal interosseus muscle, including the present study. Given that studies with the opposite finding examined contractions in joints that resulted from the activation of several muscles, the number of muscles contributing to torque about the joint could influence the relation between recruitment and derecruitment threshold force. Motor units may stop discharging action potentials at higher forces than those when they became active due to the fact that there is a delay between motor unit discharge and the development of force in the muscle (De Luca et al. 1982). As ramp contractions involved a gradual linear change in force, differences in threshold forces are accompanied by differences in discharge rate to achieve comparable changes in force. This was the case in the current study, with greater discharge rate at recruitment (9.0 ± 2.3 pps) than at derecruitment (7.8 ± 1.2 pps, $P = 0.023$).

Increased discharge variability during sustained contractions

In line with previous findings, variability in discharge times increased during the prolonged isometric contractions (Carpentier et al. 2001; Mottram et al. 2005; Nordstrom and Miles 1991). In these previous studies, however, force rather than discharge rate was controlled. Motor unit discharge variability has been attributed to synaptic noise and its interaction with the time course of the afterhyperpolarization phase (AHP; Calvin and Stevens 1968; Matthews 1999; 1996). It was also observed in all recordings that discharge variability peaked prior to the cessation of discharge. As the contractile apparatus experiences a decrease in force capacity during a sustained contraction, the central nervous system compensates by increasing the descending drive to the motor neuron pool that recruits additional motor units (Löscher et al. 1996b). This results in the progressive increase in the number of synaptic inputs onto a motor neuron thereby increasing synaptic noise and augmenting the variability in motor neuron discharge times (Berg et al. 2007).

A change in discharge variability could also be a consequence of time-dependent alterations in the intrinsic properties of the motor neurons. For example, Kernell and Monster (1982) observed that the discharge rate of motor neurons that innervated the cat medial gastrocnemius muscle slowed progressively despite the injection of a constant current. The gradual prolongation of the time between successive action potentials was attributed to a lengthening of the AHP. In the current study, however, mean discharge rate did not change over the course of the contraction for young or old participants, which suggests that the AHP duration did not increase. Furthermore, there was no change in the distributions of interspike intervals across the tasks, which were best described by a lognormal function throughout the contraction (Table 3). Therefore, the observed increase in discharge variability during the contraction could be due to changes in either the synaptic inputs received by the motor neuron or in intrinsic properties other than the afterhyperpolarization. Further study is required to determine which of these two factors may play a bigger role in the progressive increase in discharge variability during a sustained contraction.

Comparisons with intracellular depolarization

When tracked using audio feedback of discharge times, human motor units discharged for longer periods on average than motor neurons in experimental animals that were driven by intracellular current (Kernell and Monster 1982a; Sawczuk et al. 1995). The recording of 65.2 min of continuous motor unit discharge in the current study appears to be the longest duration to be reported in the literature, although many studies have examined motor unit discharge over several minutes during intermittent contractions (Adam and De Luca 2003; Bigland-Ritchie et al. 1986b; Carpentier et al. 2001; Christova and Kossev 1998; Dorfman et al. 1990; Enoka et al. 1989; Seyffarth 1940). Whereas most studies that report motor unit discharge characteristics over prolonged period of time require subjects to keep contraction intensity constant (Christie and Kamen 2009a; Conwit et al. 2000; De Luca et al. 1996; Garland et al. 1994; Kamo and Morimoto 2001; Person and Kudina 1972; van Groeningen et al. 2000), the task in the present study was to keep discharge timing constant. Additionally, the cessation of motor unit discharge has been reported during maximal (Peters and Fuglevand 1999), but not sustained submaximal contractions. Although several studies have presented subjects with audio feedback of motor unit discharge times for several minutes (Bawa and Murnaghan 2009; Bawa et al. 2006; Manning et al. 2010), these studies focused on motor unit rotation and did not follow individual motor units to cessation.

The upper limit of motor neuron discharge duration in animal preparations has been investigated in a few studies that recorded discharge times in response to current injection into motor neurons. In one experiment, rat hypoglossal motor neurons were driven using a sustained injected current and one motor neuron discharged repetitively for 5 min, but the trial was ended before the motor neuron stopped discharging action potentials (Sawczuk et al. 1995). In another study, motor neurons of the cat medial gastrocnemius were stimulated to discharge action potentials repetitively in response to a constant injected current and the maximal duration observed was 4 min, during which time mean discharge rate decreased and the response was referred to as spike frequency adaptation (Kernell and Monster 1982b). Furthermore, no study has examined long-duration current injection in the motor neurons of old animals.

Recordings from old adults

Although a reasonable number of young adult motor units could be tracked in the current protocol, a notably lower success rate (16%) resulted in only five successful recordings in old adults. Sessions failed due to the contamination of the fine-wire EMG signal by the recruitment of additional motor units as the task progressed, making it impossible to provide subjects with accurate audio feedback of discharge times from the primary motor unit. It is speculated that aging is accompanied by a reduction in motor neuron number (Campbell et al. 1973; Kawamura et al. 1977; Tomlinson and Irving 1977) and motor neuron excitability (Engelhardt et al. 1989; Morales et al. 1987; Roos et al. 1997) that may alter the distribution of recruitment threshold forces within the motor unit pool. If these changes resulted in a compression of the recruitment threshold forces in old adults, the contamination of the intramuscular signal by the recruitment of another motor unit of similar threshold was more likely. However, the difference between motor unit recruitment threshold forces does not appear to change with age (Jesunathadas et al. 2010). When a muscle fiber loses its neural innervation, it will either be re-innervated from a collateral sprout of a surviving motor unit or it will perish (Kanda and Hashizume 1989) and several studies indicate that innervation number increases with advancing age (de Koning et al. 1988; Masakado et al. 1994; Stålberg et al. 1989; Stålberg and Fawcett 1982). Furthermore, there is an increase in motor unit territories with advancing age (Bodine et al. 1988). With fewer, larger motor units that span a larger cross section of the muscle of old adults, there was an increased probability of the fine wires detecting the activity from a nearby motor unit. Furthermore, it has been shown that the morphology of motor unit waveforms becomes more complex as a consequence of the denervation-reinnervation process (Daube and Rubin 2009; Erim et al. 1999; Kamen and De Luca 1989) and as a result, it was difficult to selectively trigger from one phase of the action potential.

Therefore, it seems reasonable to speculate that the age-related remodeling of the motor unit territories for old adults created a less than optimal condition for recording motor unit activity during a long contraction. Clearly additional experiments are required to address the question of how the age-related remodeling of the motor unit pool influences the maximal duration a motor unit can discharge. Perhaps surface electrode arrays could be used to record concurrent activity of multiple motor units during long sustained contractions (Farina and Merletti 2004) and motor unit decomposition techniques used to identify the discharge times of multiple motor units (McGill et al. 2005).

In summary, the findings of the current study indicated that the maximal duration of human motor unit activity could far exceed the previously reported durations for motor neurons that were activated by intracellular current injection *in vitro*. Despite the absence of change in discharge rate for young adults, discharge variability exhibited a marked increase. The duration of motor unit activity in old adults was briefer, yet discharge variability increased to a similar extent as for young adults. However, a lower success rate for old adults leaves the question of age-related changes in motor unit activity unresolved.

Chapter IV

Discharge characteristics of biceps brachii motor units at recruitment when old adults sustained an isometric contraction

Abstract

The purpose of this study was to compare the discharge characteristics of motor units recruited during an isometric contraction that was sustained with the elbow flexor muscles by old adults at target forces that were less than the recruitment threshold force of each isolated motor unit. The discharge times of 27 single motor units were recorded from the biceps brachii in 11 old adults (78.8 ± 5.9 yrs). The target force was set at either a relatively small (6.62 ± 3.7% maximum) or large (11.4 ± 4.5% maximum) difference below the recruitment threshold force and the contraction was sustained until the motor unit was recruited and discharged action potentials for ~60 s. The time to recruitment was longer for the large target-force difference ($P = 0.001$). At recruitment, the motor units discharged repetitively for both target-force differences, which contrasts with data from young adults when motor units discharged intermittently at recruitment for the large difference between recruitment threshold force and target force (Riley et al. *J Physiol* 586: 2183, 2008). The coefficient of variation for the first five interspike intervals increased from the small (18.7 ± 7.9%) to large difference (35.0 ± 10.2%, $P = 0.008$) for the young adults, but did not differ for the two target force differences for the old adults (26.3 ± 14.7% to 24.0 ± 13.1%, $P = 0.610$). When analyzed across the discharge duration, the average coefficient of variation for interspike interval decreased similarly for the two target-force differences ($P = 0.618$) in old adults. These findings contrast with those of young adults and indicate that the integration of synaptic input during sustained contractions differs between young and old adults.

Introduction

When a muscle contraction is sustained at a force that is less than the upper limit of motor unit recruitment, not all motor units are activated at the onset of the contraction. As the contraction progresses, the force exerted by motor units active from the beginning of the contraction decreases due to a decline in motor unit discharge rate (Bigland-Ritchie et al. 1986a; Carpentier et al. 2001; Enoka et al. 1989; Garland et al. 1994; Gatev et al. 1986; Mottram et al. 2005; Person and Kudina 1972; Rudroff et al. 2010) and a decrease in the force-generating capacity of the muscle fibers (Allen et al. 2008; Westerblad et al. 2002). To compensate for the reduction in motor unit force, the net muscle force is maintained by a gradual increase in the descending drive that recruits previously inactive motor units (Löscher et al. 1996a). Once activated, the newly recruited motor units discharge action potentials either repetitively or intermittently (Bawa et al. 2006; Carpentier et al. 2001; Fallentin et al. 1993; Garland et al. 1994; Maton and Gamet 1989; Miller et al. 1996) and the previously active motor unit may either continue to discharge action potentials or cease to discharge action potentials for the remainder of the contraction (Person 1974) or until the motor unit has recovered sufficiently to resume activity (Bawa et al. 2006).

Riley et al. (2008a) found that the discharge characteristics of motor units at recruitment could differ substantially depending on the difference between the recruitment threshold of the motor unit and the target force for the sustained contraction. When the target force was ~5% of maximum less than the recruitment threshold of an isolated motor unit in biceps brachii, the discharge was less variable than when the target force was ~10% of maximum below its recruitment threshold. As recordings obtained from the same motor unit in a second set of data ($n = 12$) were consistent with the larger set of data made from separate motor units ($n = 53$), the difference in discharge characteristics for the two tasks was attributed to differences in the integration of synaptic input received by the motor neuron (Riley et al. 2008a). Given age-associated changes in both the synaptic input that is delivered to motor neurons (Boxer et al. 1988; Eisen et al. 1996; Oliviero et al. 2006) and in the intrinsic properties of the motor neurons (Engelhardt et al. 1989; Morales et al. 1987; Piotrkiewicz et al. 2007; Rossini et al. 1992), it seems likely that the discharge characteristics of motor units at recruitment will differ for old adults when they perform such tasks. The purpose of the study was to compare the discharge characteristics of motor units recruited during an isometric contraction that was sustained with the elbow flexor muscles by old adults at target forces that were less than the recruitment threshold force of each isolated motor unit. Some of these data have been presented in abstract form (Holmes et al. 2010; Pascoe et al. 2008).

Methods

Eleven healthy adults (78.8 ± 5.9 yrs; range, 71 – 88 yrs; 8 men) who were free from cardiovascular and neurological disorders volunteered for the study and participated in one to six experimental sessions. Informed consent was obtained from all the participants and the experimental procedures were approved by the Institutional Review Board at the University of Colorado (Protocol #0109.11) and were in accordance with the *Declaration of Helsinki*.

Experimental setup

Subjects were seated upright in an adjustable chair in a shielded experimental room with the left upper arm vertical and slightly abducted from the trunk. The elbow was flexed to 1.57 rad and secured in a padded brace. The forearm was oriented in a neutral position midway between supination and pronation. The hand and forearm were secured with a modified wrist-hand orthosis (Orthomerica, Newport Beach, CA, USA). The force exerted by the elbow flexor muscles was measured in the upward direction at the wrist with a force-moment sensor (900-N range, 182 N/V; JR-3, Woodland, CA, USA). The orthosis was attached to the transducer at the level of the wrist (Figure 15*A*). Visual feedback of elbow flexion force was provided on a 17-in computer monitor located at eye level ~1.2 m in front of the subject. Force was digitized with a Power 1401 (Cambridge Electronic Design; CED, Cambridge, UK) at 200 samples•s^{-1} and stored on a computer.

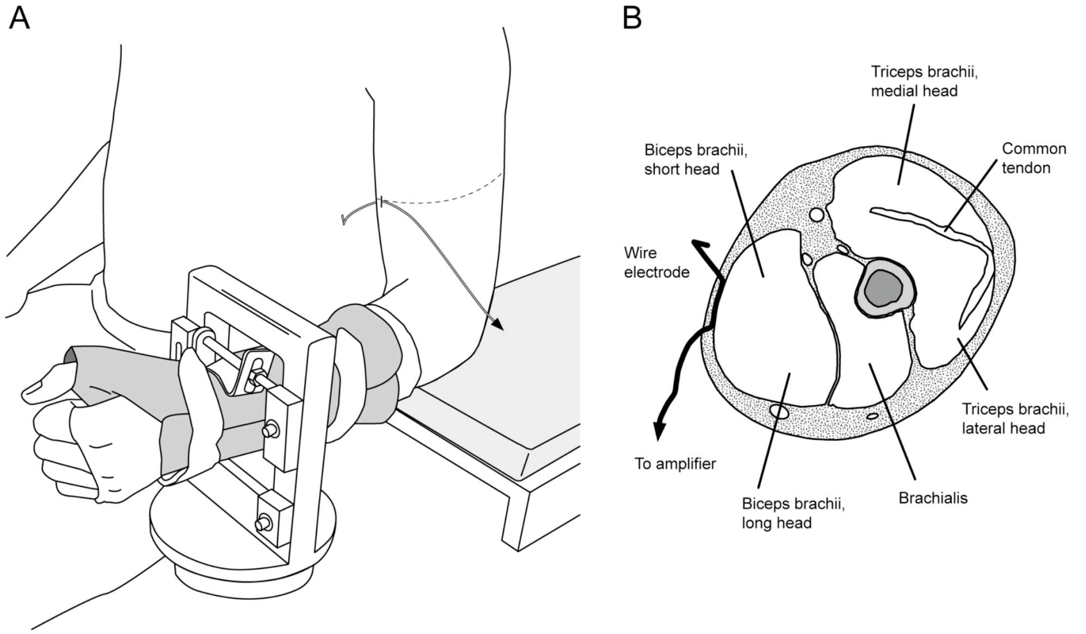

Figure 15. *A*, position of the left arm in the experimental setup. Subjects were seated with the arm slightly abducted from the body and with the elbow resting on a padded platform. Vertical force was measured as the elbow flexor muscles contracted and the left wrist pulled up against a force transducer. A subcutaneous wire electrode was placed over the short head of the biceps brachii muscle. The broken line denotes the level of a transverse section, inset *B*, through the upper arm to show the location of the electrode as it passes from the medial to the lateral aspect of the arm, overlaying the muscle fascia of the biceps brachii, and continues to the amplifier.

Electromyographic recordings

Single motor unit recordings were obtained from the short head of biceps brachii using a branched bipolar electrode (stainless steel, diameter: 50-µm, Formvar insulated, California Fine Wire Company, Grover Beach, CA, USA; Enoka et al. 1988; Gydikov et al. 1986b; Mottram et al. 2005). The electrode comprised two insulated fine wires that were glued together with three ~1 mm regions of the insulation removed; two regions ~1 mm on one wire, separated by 3 mm, and a single exposed ~1 mm region on the other wire positioned in between the two regions of the other wire. The lateral margin of the short head of biceps brachii and the intermarginal septum of the biceps brachii were identified and a 25-gauge, 3.81 cm disposable hypodermic needle was used to insert the wires subcutaneously across the muscle belly of the short head of biceps brachii without penetrating the muscle fascia and approximately perpendicular to the orientation of the muscle fibers (Figure 15*B*). The needle was removed prior to recording motor unit activity. The location of the recording sites was adjusted by pulling on the exposed ends of the electrode and its position was optimized to provide the greatest signal-to-noise ratio during 10-s ramps to a target force of 50% maximal voluntary contraction (MVC) force. A reference electrode was placed on the lateral epicondyle of the humerus. Single motor unit recordings were amplified X5,000 and band-pass filtered between 300 Hz and 8.5 kHz (S-series, Coulbourn Instruments, Allentown, PA, USA). The motor unit signal was sampled at 20k samples•s^{-1} with a Power 1401 (CED) and stored on a computer (Spike2, v. 5.20, CED).

Surface electromyographic (EMG) recordings were made using a bipolar configuration of circular silver-silver chloride electrodes (8-mm diameter) placed on one side of the innervation zone for the short and long heads of biceps brachii and the lateral head of the triceps brachii (interelectrode distance of ~20 mm). Reference electrodes were placed over the acromion process of the ipsilateral scapula. Smaller electrodes (4-mm diameter) were placed over the brachioradialis muscle. The surface EMG signals were amplified X1,000, band-pass filtered between 13 Hz and 1 kHz (S-series, Coulbourn Instruments), sampled at 2k samples•s^{-1}, and stored on a computer (Spike2, v. 5.20, CED).

Protocol

The experimental protocol replicated the previous study with young adults (Riley et al. 2008a). The following six tasks were performed with the elbow flexor muscles of the left arm of the volunteers in each experimental session: *1*) assessment of the MVC force; *2*) identification of a single motor unit; *3*) measurement of the recruitment and derecruitment thresholds of the motor unit; *4*) performance of the sustained isometric contractions at 5-10% below the recruitment threshold of the motor unit; *5*) evaluation of the recruitment and derecruitment thresholds of the motor unit immediately after the final sustained contraction; and *6*) completion of another MVC with the elbow flexor muscles. Each experiment lasted about two hours.

The experimental session began with a minimum of two MVCs in the flexion direction and at least one in the extension direction. The MVC task involved increasing the force from zero to maximum over 3 s and then holding the maximum for a further 1-2 s. To minimize fatigue, subjects rested for 90-120 s between trials. If the peak MVC force for the two elbow flexor trials were within 5% of each other, the larger of the two values was recorded as the maximum and used as a reference value for the recruitment threshold force of the motor unit. Otherwise, additional trials were performed until the 5% criterion was met. Efforts that the subject did not regard as maximal were rejected, and the visual gain of the force feedback was varied across trials to minimize the subjects' awareness of differences in performance.

Motor units were identified in the subcutaneous EMG recording by asking the subjects to slowly increase elbow flexor force from rest to 50% MVC force in ~10 s and then gradually relax during the subsequent 10 s to produce a triangular force profile. Subjects were instructed to change force at a constant rate. Once a potential unit was identified, subjects performed three ramp contractions that were separated by 60 s of rest. The recruitment threshold was estimated during the experiment by noting the force after the third interspike interval, and averaging the values from the ramp contractions.

The target force for the sustained contraction was set at either 5 or 10% MVC force below the recruitment threshold force of the motor unit, which were denoted as small and large differences between the two forces, respectively. The absolute force difference was scaled to the recruitment threshold force of the motor unit. A line was set at the target force on the monitor and the subject was asked to increase the force up to the line gradually (~15 s) and to maintain the target force as steadily as possible until instructed by the investigator to relax. The gain of the display was adjusted to keep both the target and zero force lines consistent across subjects. Subjects observed the two force traces during the task. The time to recruitment was defined as the time from target acquisition to the first action potential discharged by the isolated motor unit. The task was terminated when the motor unit became active and discharged action potentials repetitively for ~60 s. After a brief rest period (~180 s), the contraction at the other target force was performed. The order in which the two target forces (small and large differences) were performed was counterbalanced across subjects. The presence of the same motor unit after each sustained contraction was later verified offline by comparing waveform overdraws created with Spike2 software.

In four separate sessions, subjects maintained target forces at larger differences below recruitment threshold force (range: 15.2 – 27.2% MVC). Furthermore, when a subject was willing and the experimental conditions remained viable, the position of the subcutaneous electrode was adjusted to obtain recordings from a second motor unit, which was possible in four experimental sessions.

Data analysis

Template matching with Spike2 software was performed offline to discriminate individual motor unit action potentials. The accuracy of the discrimination was verified by close visual inspection of each discriminated spike and by reviewing the interspike intervals.

Interspike intervals >250 ms (<4 pulses per second; pps; n = 42, 0.10% of discharges) or <20 ms (>50 pps; n = 6, 0.01% of discharges) were excluded from the calculations of discharge rate. Long interspike intervals likely arose from the brief cessation of motor-unit discharge, whereas short intervals exceed the rates normally observed during these types of contractions for human motor units (Bigland-Ritchie and Lippold 1954; De Luca et al. 1982; Kanosue et al. 1979; Tanji and Kato 1973) and likely resulted from discrimination error or a double discharge.

Recruitment threshold force was determined with an algorithm that involved advancing a 500-ms window in 1-ms steps across the discharge times of the motor unit until the coefficient of variation for interspike interval in the window was <50% (Moritz et al. 2005). The force corresponding to the time of the first discharge in the window was taken as the recruitment threshold force. The same method was used to define the derecruitment threshold force of the motor unit. The discharge rate and the coefficient of variation for interspike interval were determined for a 500-ms window at recruitment and derecruitment. A linear trend was fit through the ascending and descending force trajectories to calculate the rate of force development and relaxation, respectively.

As the duration of the contraction differed across individuals, each contraction after recruitment was divided into five epochs of equal duration. The discharge rates during the sustained contractions were averaged for each 20% interval and the coefficient of variation for interspike interval was calculated from the first five interspike intervals in each 20% interval. Force fluctuations were calculated as the normalized measure of variability, the coefficient of variation (standard deviation/mean force X 100). Surface EMG values are presented as the root-mean-square (RMS) amplitude of the signal normalized to the RMS for a 500-ms epoch centered about the peak force during the MVC. Coactivation ratios were quantified as the quotient of the averaged, rectified and normalized EMG values of the elbow extensors (lateral head of triceps brachii) relative to that for the pooled average of the elbow flexors (brachioradialis, short and long head of biceps brachii).

Statistical analysis

Paired samples *t*-tests were used to compare MVC force before and after the end of the protocol; recruitment threshold force and derecruitment threshold force to one another and also to assess the change after the end of the protocol; discharge rate and the coefficient of variation for interspike interval at recruitment and derecruitment threshold; the duration of the two sustained contractions; and the rate of force development and relaxation during the ramp contractions. Independent samples *t*-test were used to compare recruitment threshold forces between old adults of this study and the young adults of Riley et al. (2008a). The mean discharge rate and coefficient of variation for interspike interval in each 20% epoch of discharge duration were compared with repeated-measures two-way ANOVAs (target-force x time).

The target force, difference between target force and recruitment threshold force, and the time to recruitment of the motor unit were compared with a subset of 12 motor units from young adults (Riley et al. 2008a) with repeated-measures two-way ANOVAs (age x target-force). In addition, the mean discharge rate and coefficient of variation for the first five interspike intervals obtained at recruitment during the sustained contractions were compared using a two-way ANOVAs (age x target-force) with repeated-measures on the target-force factor. Post-hoc analysis with paired samples *t*-tests were used when appropriate to compare the mean discharge rate between each 20% epochs of discharge duration. The coefficient of variation for force, average RMS amplitude of the surface EMG, and coactivation ratios were compared for the first and last third of discharge duration between young and old subjects using multivariate three-way ANOVAs (age X target-force X time). An alpha level of $P < 0.05$ was used to identify significant differences. All statistical analyses were performed using SPSS (v. 16.0, Chicago, IL, USA). Data are presented in the text as mean ± standard deviation and in figures as mean ± standard error of the mean.

Results

The results comprise recordings for 27 motor units from the short head of biceps brachii when 11 old adults sustained an isometric contraction with the elbow flexor muscles at a target force that was less than the recruitment threshold of the isolated motor unit. The discharge of each motor unit was recorded during two contractions that differed in the magnitude of the difference between the target force and the recruitment threshold force. An example of the sustained contractions at two different target forces below recruitment threshold force (34.2% MVC) from the same motor unit is shown in Figure 16. The time from the start of the contraction to the recruitment of this motor unit was 7.3 s when the target was set at 8.7% MVC force below recruitment threshold force (Figure 16*A*) and 118 s when the target force was set at 13.8% MVC force below recruitment threshold (Figure 16*B*). MVC force for the old subjects did not change from the beginning (158 ± 64 N) to the end of the protocol (154 ± 53 N, $P = 0.489$), which indicates that the two tasks involved minimal fatigue. In the four experiments in which more than one motor unit recording could be obtained, the decline in MVC force from the beginning to the end of the first experiment was 3.12%, which indicated that recording of the second motor unit began with minimal fatigue.

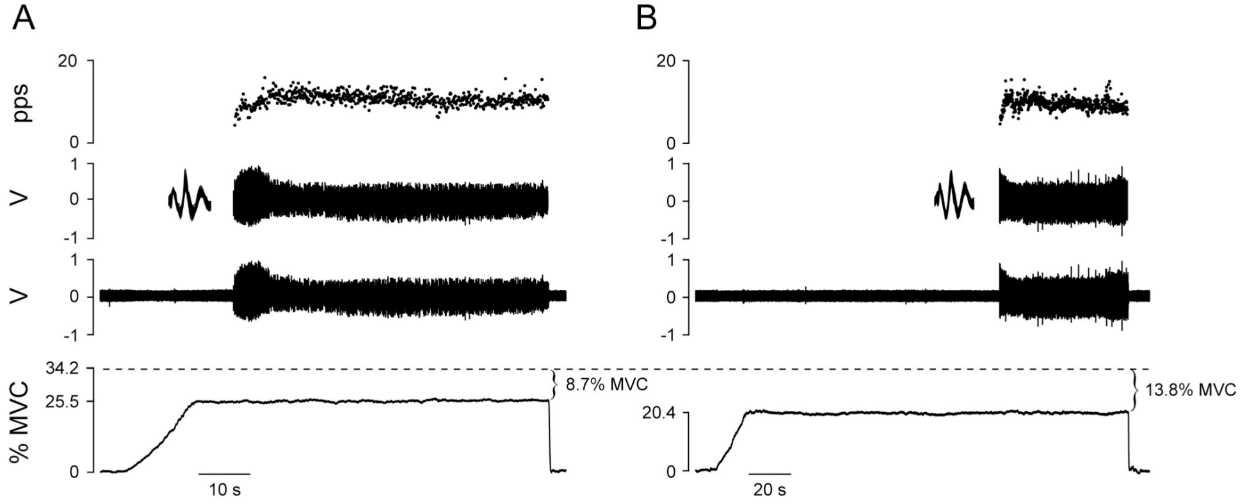

Figure 16. *A*, representative data from a sustained contraction depicting, from top to bottom, the instantaneous discharge rate of the motor unit (pulses per second; pps), discriminated action potentials with waveform overlay, interference electromyogram from the wire electrode, and the vertical force exerted against the force transducer by the wrist. The motor unit had a recruitment threshold force of 34.2% MVC. The time to recruitment was 7.3 s for a target set at 8.7% MVC force below recruitment threshold force. *B*, time to recruitment for the same motor unit increased to 118 s when the target was set at 13.8% MVC force below recruitment threshold force.

Recruitment threshold

The average recruitment threshold force for the motor units from the old adults was 25.4 ± 10.2% MVC (range: 9.47 – 48.3% MVC) and this was unchanged (23.8 ± 10.1% MVC) at the end of the protocol ($P = 0.08$). Identifiable derecruitment threshold forces were obtained for 15 of the 27 motor units examined. The derecruitment threshold forces (16.6 ± 9.8% MVC) were less than the recruitment threshold forces for the set of ramp contractions that preceded (27.7 ± 11.4% MVC, $P < 0.001$) the sustained contractions. The rate of force development (5.00 ± 2.72% MVC•s^{-1}) was not significantly different from the rate of force relaxation (6.92 ± 9.53% MVC•s^{-1}) during the ramp contractions ($P = 0.290$). The discharge rate at recruitment (10.0 ± 2.5 pps) was significantly greater than that at derecruitment (8.0 ± 2.7 pps, $P = 0.001$). The coefficient of variation for the interspike intervals at recruitment (19.3 ± 6.8%) was significantly less than that at derecruitment (25.6 ± 7.2%, *P* = 0.026). The recruitment thresholds were lower for old adults (25.4 ± 10.2% MVC) when compared with both the group of 53 units (31.1 ± 12.3% MVC, *P* = 0.043) and the separate subset of 12 units (32.8 ± 9.6% MVC, *P* = 0.04) from the young adults in Riley et al. (2008a).

Sustained contractions

The duration over which discharges were recorded did not differ for the two differences in target force (64.0 ± 10.8 s, *P* = 0.340). Also, the motor units discharged repetitively at recruitment for both target-force differences (Figure 17*A*), which contrasts with a previous observation for young adults when motor units discharged intermittently at recruitment with a large difference between recruitment threshold force and target force (Figure 17*B*).

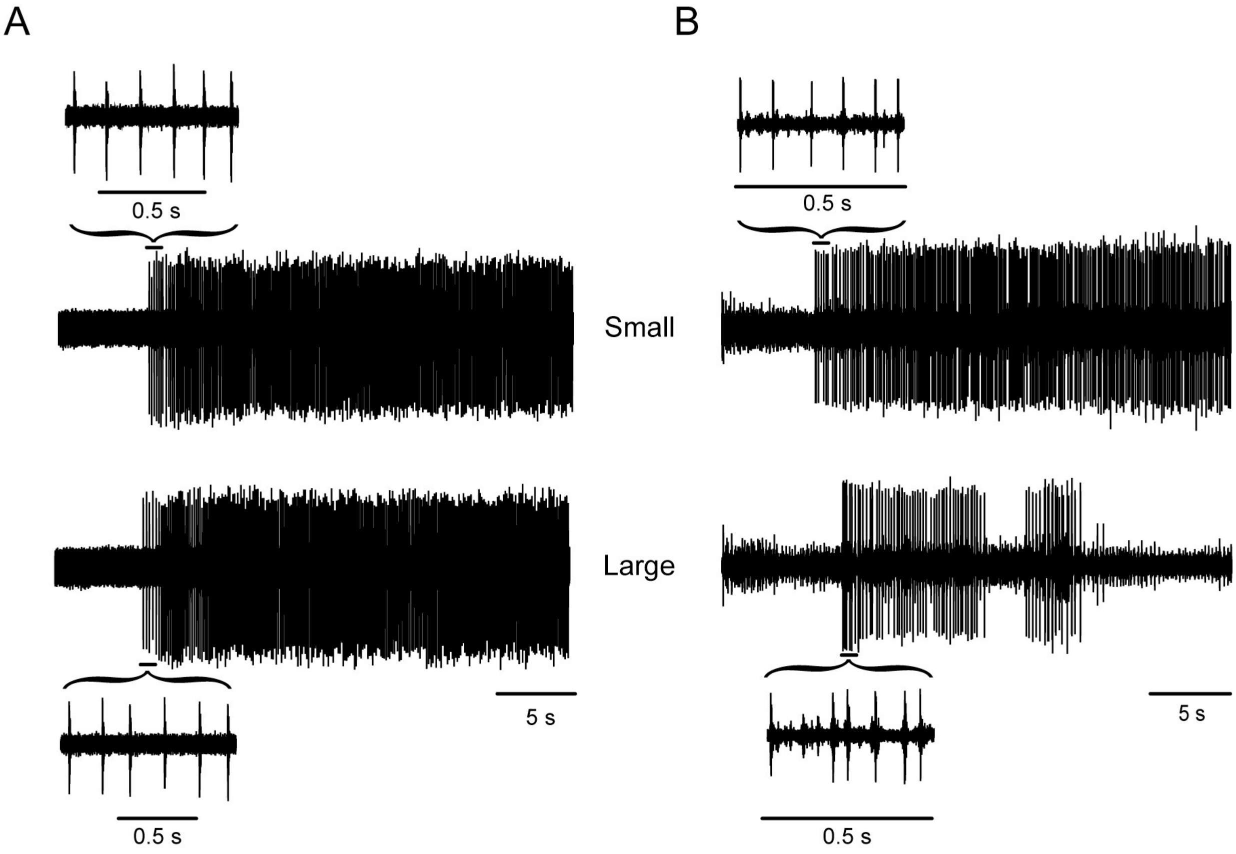

Figure 17. *A*, representative trains of action potentials at the time of recruitment for a motor unit in biceps brachii of an old adult for both the small and large difference between target force and recruitment threshold force. *B*, trains of action potentials for a motor unit in the biceps brachii of a young adult. The variability of the first five interspike intervals was increased in the young adult for the large difference between the target force and recruitment threshold force, but not in the old adult.

The target forces were greater for young adults (age main effect: $P = 0.004$) for both small and large differences in target force (27.1 ± 10.3 and 22.3 ± 10.1% MVC force) compared with old adults (18.4 ± 7.9 and 13.5 ± 7.0% MVC force, Figure 18*A*). The difference between target force and recruitment threshold force was less for the small difference in both age groups (age X target-force interaction: $P = 0.923$), and increased from 6.62 ± 3.7 to 11.4 ± 4.5% MVC force for the large difference (target-force main effect: $P < 0.001$). The small and large target-force differences for the old adults were 7.01 ± 4.2 and 11.9 ± 5.2% MVC, respectively. The time to recruitment was longer for the large difference (target-force main effect: $P = 0.001$, Figure 18*B*), especially for the old adults (age main effect: $P = 0.015$). There was a significant interaction (age X target-force interaction: $P = 0.004$) as the time to recruitment increased from 22.5 ± 21.7 s to 37.1 ± 50.4 s for the young adults compared with 17.9 ± 27.0 s to 160 ± 145 s for the old adults.

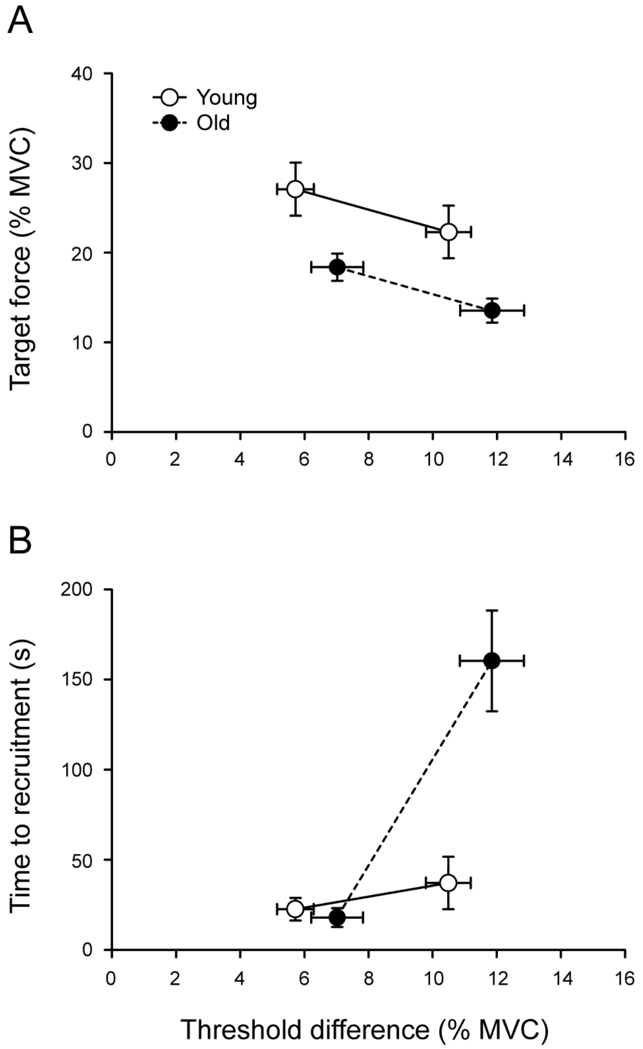

Figure 18. Task characteristics for the 27 motor units recorded from old adults (●) and the 12 motor units from young adults (○). Young data are from Riley et al. (2008). *A*, target force (% MVC) was greater for young adults than for old adults ($P = 0.004$) for both the small and large difference between target force and recruitment threshold force. The target force was greater for the small difference for both ages ($P = 0.923$), and decreased for the large difference ($P < 0.001$). *B*, the time to recruitment (s) increased for the large difference between target force and recruitment threshold force ($P = 0.001$), but more so for the old adults ($P = 0.004$).

The mean discharge rate at recruitment was greater in younger adults (age main effect: $P < 0.001$, Figure 19*A*) and did not differ for the two target-force differences for either the young (small difference 13.2 ± 3.5, large difference 13.9 ± 4.3 pps) or old adults (small difference 8.3 ± 2.5, large difference 8.4 ± 3.3 pps). The coefficient of variation for the first five interspike intervals did not differ between young and old adults (age main effect: $P = 0.610$, Figure 19*B*); however, a main effect for target-force difference ($P = 0.008$) and a significant interaction ($P < 0.001$) indicated that the coefficient of variation increased for the young adults (small difference 18.7 ± 7.9%, large difference 35.0 ± 10.2%) but did not change for the old adults (small difference 26.3 ± 14.7%, large difference 24.0 ± 13.1%).

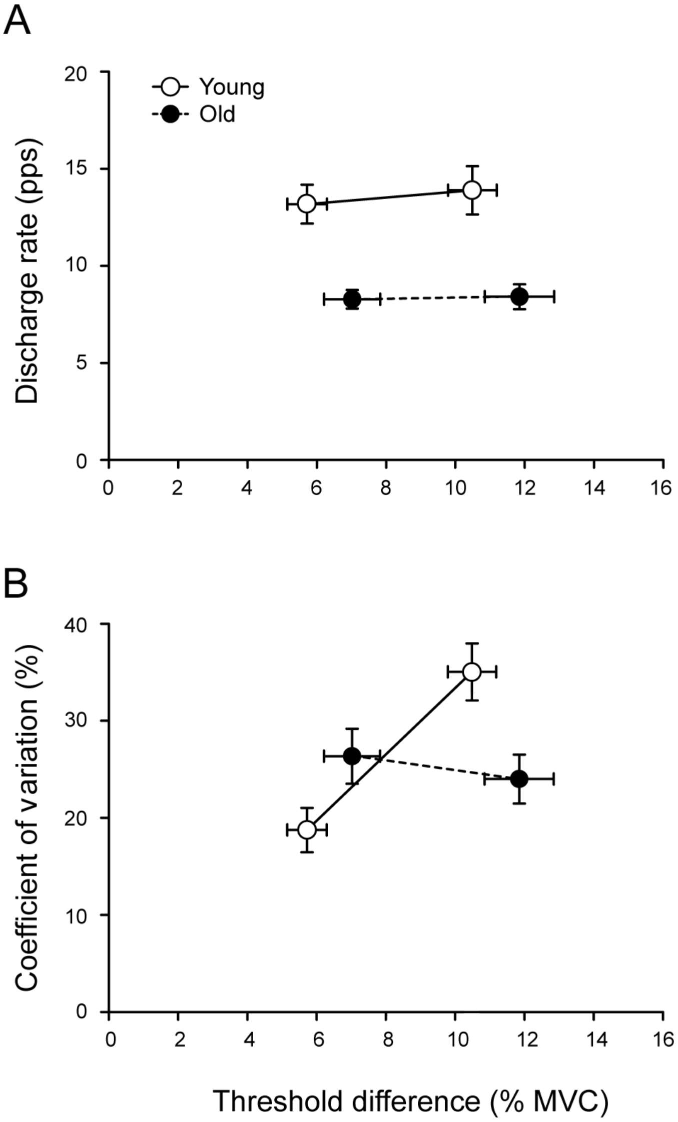

Figure 19. Discharge characteristics at recruitment for the 27 motor units recorded from old adults (●) and the 12 motor units from young adults (○). Young data are from Riley et al. (2008). *A*, mean discharge rate (pps) for the first five interspike intervals was greater for the young adults (age main effect: $P < 0.001$) and did not differ between tasks for both the young and old adults (target force main effect: $P = 0.458$). *B*, the coefficient of variation (%) for the first five interspike intervals did not differ between young and old adults (age main effect: $P = 0.610$); however, the coefficient of variation increased with the difference between target force and recruitment threshold force for the young, but not for the old adults (age X target force interaction: $P = 0.001$).

Mean discharge rate for the old adults was similar at all 20% epochs (target force main effect: $P = 0.179$); discharge duration was 63.2 ± 27.9 s for the young adults and 64.0 ± 10.8 s for the old adults. When the data were collapsed across target-force differences, a paired comparison indicated there was no difference between the first 20% (11.8 ± 3.1 pps) and the last

20% (11.0 ± 2.0 pps) epoch of discharge duration ($P = 0.091$, Figure 20*A*). The average coefficient of variation for interspike interval also decreased similarly for the two target-force differences (time X target force interaction: $P = 0.618$, Figure 20*C*), decreasing from 25.2 ± 13.8% at the start of the contractions to 10.8 ± 5.6% at the end of the contractions (time main effect: $P < 0.001$). These findings contrast with those for young adults (Riley et al. 2008a) when the mean discharge rate (Figure 20*B*) and coefficient of variation for interspike interval (Figure 20*D*) changed in opposite directions for the two target-force differences.

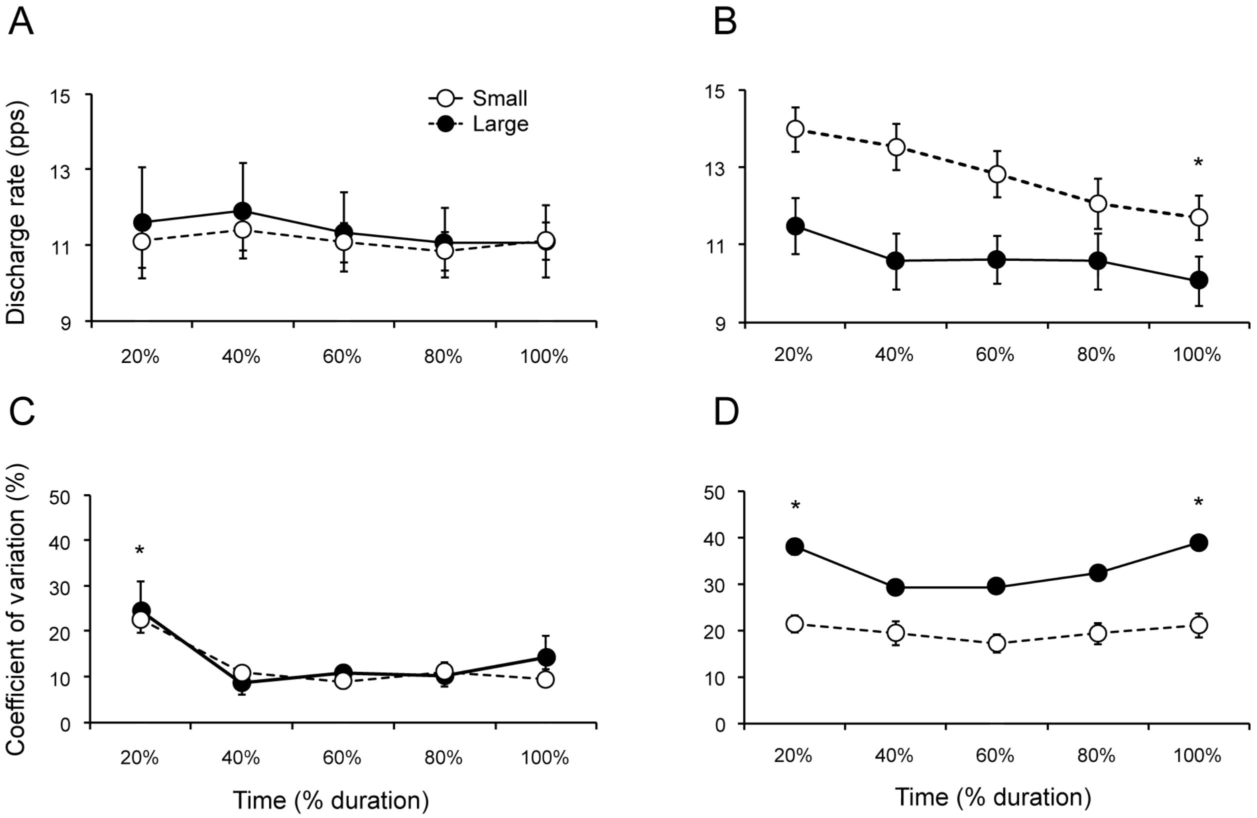

Figure 20. Discharge characteristics for the motor units during the sustained contractions for young (left column) and old (right column) adults. Young data are from Riley et al. (2008). *A*, mean discharge rate (pps) for the 27 units of old adults for the small (○) and large (●) difference between target force and recruitment threshold force (% MVC) for each 20% of discharge duration. Discharge rate did not change from the first to the last 20% of discharge duration ($P = 0.091$). B, coefficient of variation for first five interspike intervals was greater during the first 20% of discharge duration for old adults (*time main effect: $P < 0.001$) and decreased to the same extent for both differences in target force ($P = 0.618$). *C*, mean discharge rate for young adults decreased (*time main effect: $P = 0.04$) for the small (○) difference in target force, but not the large difference (●). D, the average coefficient of variation for the first five interspike intervals for the young adults was less for the small difference in target force (target-force difference main effect: $P < 0.001$) and it was elevated for the first and last 20% of discharge duration (*time main effect) compared with the middle time points for the large difference in target force.

The coefficient of variation for force did not differ between young and old adults (age main effect: *P* = 0.328), but the value was greater for the large difference (target force main effect: *P* = 0.002) and increased over time (time main effect: *P* < 0.001), from 1.6 ± 1.0% in the first third to 2.0 ± 0.9% in the final third of discharge duration. Surface EMG amplitude for all elbow flexors pooled together did not differ between young and old adults (age main effect: *P* = 0.161, Figure 21), but the amplitude was greater for the large target-force difference during the first third of the contraction (target-force main effect: *P* = 0.013) and increased significantly over time (time main effect: *P* = 0.004), from 15.5 ± 9.7 to 20.8 ± 13.0% maximum. Surface EMG amplitude for the antagonist (triceps brachii) was minimal, but as significantly less for young adults, (5.9 ± 5.0%; age main effect: *P* < 0.001) compared with old adults (8.9 ± 6.1%), but did not change over time (age X time interaction: *P* = 0.680, Figure 7). The coactivation ratios did not differ between young (0.60 ± 1.7) and old adults (0.60 ± 0.4; age main effect: *P* = 0.795), between target-force differences (target-force main effect: *P* = 0.124), or from the beginning to the end of the task (time main effect: *P* = 0.749).

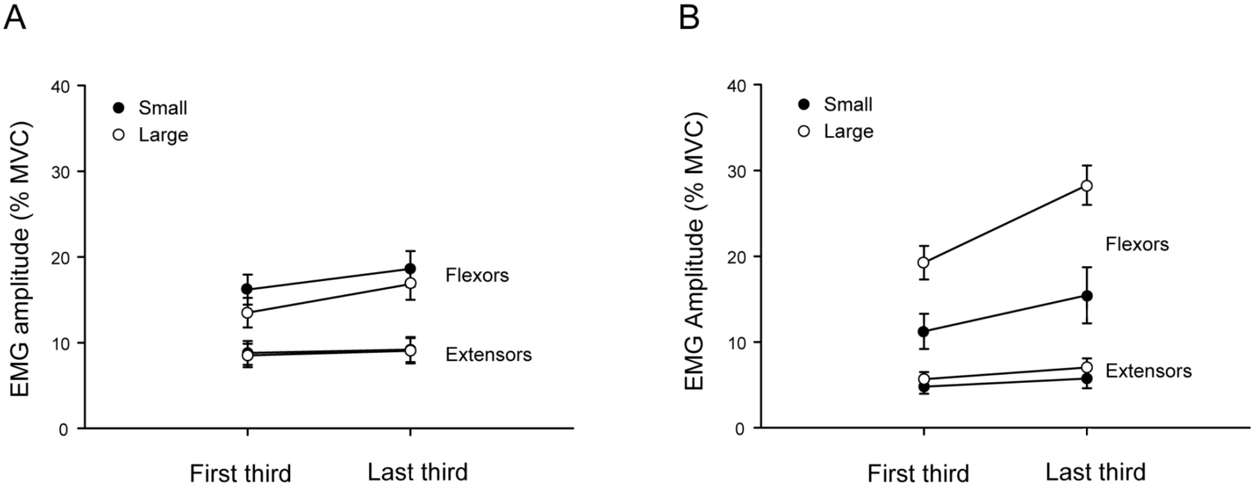

Figure 21. Changes in amplitude of the surface electromyogram (EMG) recorded from the elbow flexors and extensor (triceps brachii) muscles from the first to the last third of contraction duration, normalized to maximum EMG (% MVC) for young (*A*) and old adults (*B*). Young data are from Riley et al. (2008). Surface EMG amplitude for all elbow flexors did not differ between young and old adults (age main effect: $P = 0.161$). Agonist EMG amplitude for the old adults (*A*) did not change from the first one-third (14.8 ± 7.6% maximum) to the last one-third (17.7 ± 8.5% maximum) of the contraction (total duration: 154.8 ± 122.6 s). Agonist EMG amplitude for the young adults (*B*) was greater for the large target-force difference (○) during the first one-third of the contraction (target-force main effect: $P = 0.013$) and increased significantly over time (time main effect: $P = 0.004$), from 15.9 ± 11.0 to 23.0 ± 15.1% maximum. Surface EMG amplitude for triceps brachii was significantly greater for old adults, (8.89 ± 6.11%; age main effect: $P < 0.001$) compared with young adults (5.89 ± 5.01%), and did not change over time (time main effect: $P = 0.330$) for either age group (age X time interaction: $P = 0.680$).

When the experiment involved a target force that was much greater than 10% below recruitment threshold force (range: 15.2 – 27.3% MVC), the four motor units from four different subjects all discharged repetitively for 60 s following recruitment (Figure 22*A-D*). The time to recruitment for the four motor units was 2287, 1005, 1677, and 323 s. The mean discharge rate and coefficient of variation for interspike interval for the first five intervals at recruitment for the four motor units were 14.2, 13.0, 14.7, and 15.9 pps, and 10.9, 48.3, 37.4, and 42.4%, respectively.

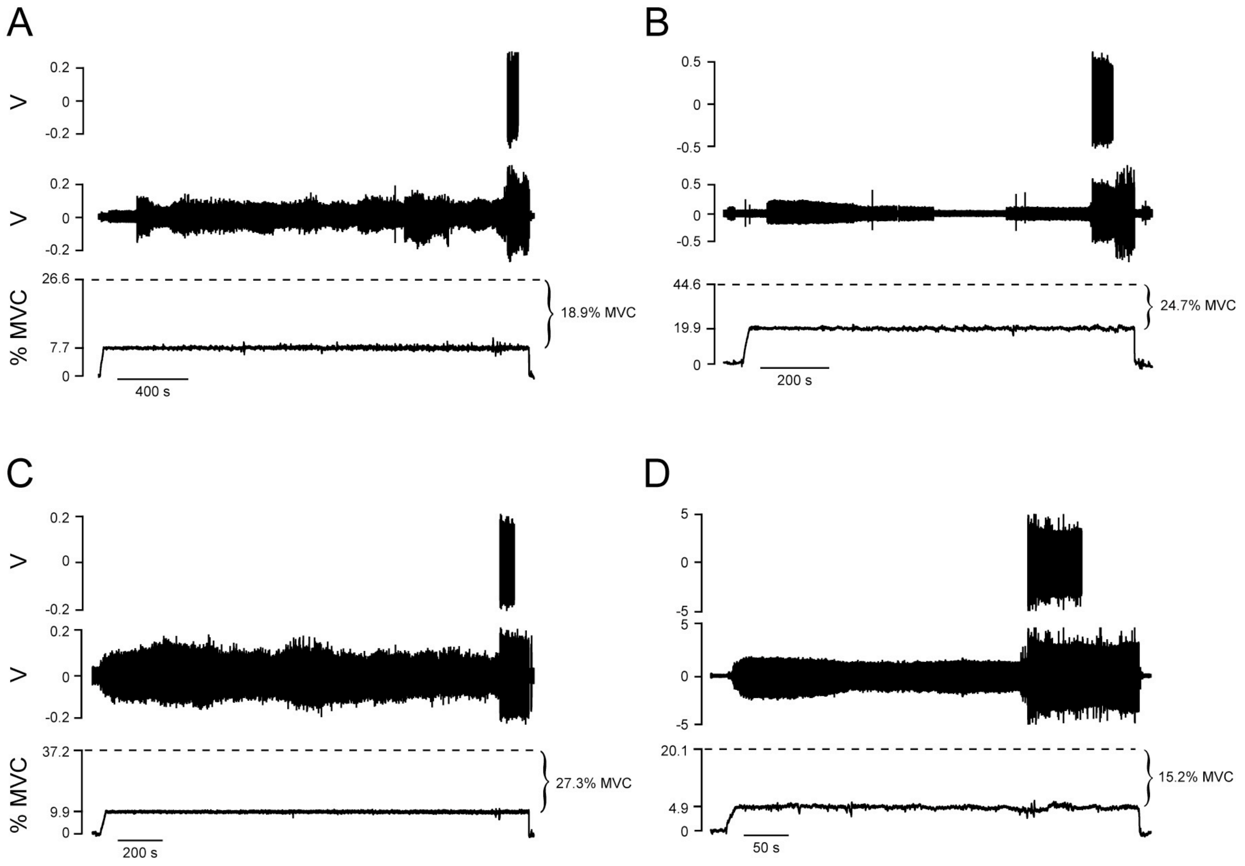

Figure 22. Data from the four sustained contractions held at target forces much lower than recruitment threshold force. Each panel depicts the discriminated action potentials, interference electromyogram (EMG), and the vertical force produced by the elbow flexors during these contractions. Despite the substantial difference between the target force and the recruitment threshold force for panels *A-D* (18.9, 24.7, 27.3, and 15.2% MVC, respectively), the unit discharged repetitively once recruited.

Discussion

The purpose of the study was to compare the discharge characteristics of motor units recruited during an isometric contraction that was sustained with the elbow flexor muscles by old adults at target forces that were less than the recruitment threshold force of each isolated motor unit. The main finding of this study was that discharge characteristics of motor units at recruitment in old adults were not influenced by the relative target force, which contrasts with the results observed in young adults (Riley et al. 2008a). The results suggest that the integration of synaptic input by motor neurons during sustained contractions differs for old adults compared with young adults.

Discharge characteristics of motor units in old adults

Motor unit recruitment thresholds extended across a wide range of forces (9.5 – 48.3% MVC). Although not statistically significant ($P = 0.08$), the recruitment threshold forces tended to decrease after the sustained contractions. As recruitment threshold forces typically decrease following a fatiguing contraction (Adam and De Luca 2003; Baudry et al. 2009; Carpentier et al. 2001; Enoka et al. 1989; Garland et al. 1994; Suzuki et al. 1990), the protocol used in the current study involved minimal amounts of muscle fatigue. In addition, there was no difference ($P = 0.489$) in MVC forces before (158 ± 64 N) and after (154 ± 53 N) the 60-s sustained contractions, consistent with the two tasks involving minimal muscle fatigue.

The derecruitment threshold forces were lower than the recruitment threshold forces for ramp contractions performed before and after the sustained contractions. Although a lower derecruitment threshold force has been observed in some studies (Baudry et al. 2009; Denier van der Gon et al. 1985; Garland et al. 1994; Romaiguère et al. 1993; Suzuki et al. 1990), many have also found a lower recruitment threshold force (De Luca et al. 1982; Freund et al. 1975; Jesunathadas et al. 2010; Milner-Brown et al. 1973; Patten and Kamen 2000). As both ramp contractions involved a gradual linear change in force, differences in threshold forces must be accompanied by differences in discharge rate to achieve comparable changes in force. Accordingly, discharge rate at derecruitment was lower in the current study as it has been found in other studies (Christova and Kossev 1998; De Luca et al. 1982; Denier van der Gon et al. 1985; Gorassini et al. 2002; Oya et al. 2009; Romaiguère et al. 1993). As lower discharge rates are usually associated with higher discharge variability (Barry et al. 2007; Moritz et al. 2005), the coefficient of variation for interspike interval in the current study was greater at derecruitment compared with recruitment.

A decrease in mean discharge rate during a sustained contraction is well documented in young adults (Christova and Kossev 1998; Gantchev et al. 1986; Garland et al. 1994; Gatev et al. 1986; Person and Kudina 1972). Relatively few studies have recorded motor unit discharge rate during sustained contractions performed by old adults and have either indicated no reduction in discharge rate for both young and old adults during a sustained submaximal contraction (Christie and Kamen 2009a), or similar decline in discharge rate for young and old adults during intermittent contractions (Rubinstein and Kamen 2005). In agreement with previous findings, the mean discharge rate in the current study did not change from the first 20% to the last 20% epoch of the ~60-s contraction ($P = 0.091$).

However, there was a marked decrease in discharge variability in the current study from 25.2% during the first 20% of the contraction to 10.8% for the remainder of the contraction. This result is consistent with previous findings that at low levels of relative synaptic input (e.g., motor unit recruitment) the motor neuron experiences fluctuations in membrane potential resulting in variable discharge times (Berg et al. 2007; Berg et al. 2008; Calvin and Stevens 1968; Matthews 1999; 1996; Stein et al. 2005). Previous studies have found a rapid reduction in discharge variability with an increase in contraction intensity (Barry et al. 2007; Baudry and Enoka 2009; Moritz et al. 2005), presumably due to a decline in synaptic noise at higher levels of synaptic input (Matthews 1999; 1996), which would occur progressively during sustained submaximal contractions (Löscher et al. 1996a).

Age-related differences in motor unit discharge

The most striking difference between the current results and those obtained from young adults (Riley et al. 2008a) is the influence of the two target forces on the discharge characteristics of motor units in biceps brachii at recruitment. Old adults exhibited the same discharge characteristics for the two conditions, whereas the motor units recorded in young adults discharged repetitively for the small difference and intermittently for the large difference. Indirect evidence for an age-associated difference in discharge characteristics at recruitment during sustained contractions was suggested previously by the marked depression of bursts of activity in the surface EMG recordings from the elbow flexor muscles in old adults compared with young during fatiguing contractions that were sustained 20% MVC force (Hunter et al. 2005a).

The current study found that the biceps brachii motor units of old adults had lower mean discharge rates for the first five interspike intervals (~8.4 pps) when compared with young adults (~13.6 pps) at recruitment (Figure 19*A*, $P = 0.001$). Similarly, others have described lower mean discharge rates for old adults (Barry et al. 2007; Connelly et al. 1999; Dalton et al. 2010; Kamen and Knight 2004; Kamen et al. 1995; Knight and Kamen 2007; Nelson et al. 1984; Patten et al. 2001), which can be attributed to such age-related changes as prolongation of the afterhyperpolarization phase (Piotrkiewicz et al. 2007; Rossini et al. 1992) and motor unit twitch contraction times (Bellemare et al. 1983; Connelly et al. 1999; Roos et al. 1999). The current study found that the discharge variability of biceps brachii motor units did not differ between young and old adults. The opposite was seen in a recent study of biceps brachii motor units, reporting that the coefficient of variation for the interspike interval was lower in young (5 ± 3%) compared with old men (11 ± 7%) when they produced low intensity (~10% MVC) elbow flexion forces (Harwood et al. 2010). However, these differences are likely explained by the use of target forces *above* recruitment threshold force selected by Harwood et al. (2010) and the use of target forces *below* recruitment threshold in the current study. Unlike young adults, the coefficient of variation for the first five interspike intervals did not vary across the two target forces for old adults (Figure 19*B*), and this may indicate less synaptic noise at the time of recruitment for the old adults (Matthews 1999).

The discharge characteristics at recruitment depend on how the intrinsic properties of the motor neurons integrate synaptic input (Kernell 1965a; Schwindt and Calvin 1972). Studies have shown age-related reductions in the amount of synaptic input received by motor neurons from corticomotor (Eisen et al. 1996; Oliviero et al. 2006), brainstem (Johnson et al. 1993), and spinal reflex (Boxer et al. 1988; Earles et al. 2001; Kido et al. 2004) pathways. Reductions in the number of synaptic inputs from these various pathways may represent differences in the strength and the relative balance of excitatory and inhibitory inputs received by the motor unit pools of old adults. Evidence suggests, for example, that changes in the balance between excitatory and inhibitory inputs can influence the discharge characteristics of neurons (Abbott and Chance 2005; Berg et al. 2007; Stein 2010), which may explain the age-associated difference in the discharge characteristics of motor units observed in the current study.

However, the intrinsic properties of motor neurons also differ between young and old experimental animals. For example, motor neurons from old animals exhibit greater input resistance (Chase et al. 1985; Kalmar et al. 2009; Morales et al. 1987), reduced rheobase current (Kalmar et al. 2009; Morales et al. 1987), and either longer (Cameron et al. 1991; Kalmar et al. 2009), or unchanged (Engelhardt et al. 1989; Morales et al. 1987) afterhyperpolarization duration. Such parameters can only be estimated with indirect approaches in humans, such as the interval death rate analysis of single motor unit interspike intervals (Matthews 1996) or evaluation of the relation between the mean and standard deviation of interspike intervals within a train of single motor unit action potentials (Piotrkiewicz et al. 2007). The interval death rate analysis technique has been validated in animal experiments (Powers and Binder 2000) and used in several studies of young adults (MacDonell et al. 2008; MacDonell et al. 2010; MacDonell et al. 2007). When this technique was used to compare young and old adults, it was shown that old adults have an longer afterhyperpolarization duration (Christie and Kamen 2009b). Additionally, an indirect estimate suggests that afterhyperpolarization duration is prolonged in old adults (Piotrkiewicz et al. 2007). If such age-associated adaptations can be confirmed, then the intrinsic properties of motor neurons could also contribute to the difference in discharge characteristics of motor units in young and old humans.

The observation by Riley et al. (2008a) that motor units in young adults discharged action potentials intermittently when there was a large difference between the target force and recruitment threshold force (Figure 17*B*) and that mean discharge rate declined over time (Figure 20*B*) were interpreted to indicate the absence of a significant role for persistent inward currents during this task. In contrast, motor units in old adults discharged repetitively at recruitment for both target forces and there was no reduction in mean discharge rate during the 60-s contractions, which is consistent with a significant contribution by persistent inward currents to the net excitation of the motor neurons. As the proportion of motor neurons that demonstrate persistent inward currents may increase with age (Bae et al. 2008; Kalmar et al. 2009), the different discharge characteristics of the two groups of participants may reflect an age-associated change in the contribution of persistent inward currents during such tasks. Nonetheless, the putative persistent inward currents were not substantial enough to influence the relative values for recruitment and derecruitment thresholds across the two age groups. Additionally, the relative influence of persistent outward currents (Hamm et al. 2010; Turkin et al. 2010) may change with age.

In summary, the discharge characteristics of motor units in the biceps brachii of old adults were not influenced by the difference between target force and recruitment threshold force of a motor unit. This result contrasts with previous observations in young adults when the discharge characteristics varied with the force difference (Riley et al. 2008a). In addition, the observed lower mean discharge rates for the old adults that did not vary with time across the contraction. Taken together, these results suggest that the integration of synaptic input during sustained contractions changes with age. The lack of modulation exhibited by old adults likely reflects a reduced flexibility in the output from the spinal cord as a consequence of aging.

Chapter V

Motor unit activity at recruitment when young and old adults support compliant loads

Abstract

Two variables known to influence the integration of synaptic inputs, and thereby modulate the discharge characteristics of motor units, are the age of the individual and the compliance of the load supported by a limb. The purpose of the study was to compare the discharge characteristics of motor units when recruited during contractions that required young and old adults to maintain a constant elbow angle while supporting a load that was less than the recruitment threshold force of each isolated motor unit. The discharge times of single motor units were recorded from the biceps brachii in 16 young adults (28 ± 4 yr) and 14 old adults (75 ± 4 yr). Two sustained contractions were performed with the elbow flexors using target forces set at small (11.7 ± 4.4) or large (17.8 ± 6.5% maximum) differences below the recruitment threshold force of an isolated motor unit. The task required subjects to maintain elbow angle at 1.57 rad until the isolated motor unit was recruited and discharged action potentials for ~120 s. Twenty motor units were recorded for each group of subjects. The average recruitment threshold force did not differ between groups (young: 25.9 ± 12.6%; old: 30.0 ± 10.9% maximum, $P = 0.07$). Young adults had a greater number of motor units that discharged action potentials intermittently at recruitment than old adults. The time to recruitment for both young and old was longer for the large difference (187 ± 227 s vs. 23 ± 46 s, $P < 0.001$). Discharge rate was similar for the two target-force differences in old adults (small: 9.13 ± 2.9, large: 9.3 ± 4.8 pps), but was less for the large difference in young adults (small: 10.9 ± 3.3, large: 8.6 ± 2.5 pps, $P = 0.04$). The coefficient of variation for interspike interval was similar between target-force differences for young and old (small: 24.2 ± 11.8%, large: 25.8 ± 10.5%, $P = 0.43$) and tended to be less in old (22.4 ± 9.4%) compared with young adults (27.6 ± 12.3%, age group main effect: $P = 0.07$). Discharge rate did not differ between small and large differences in either young or old for either

load condition (*P* = 0.06). Similar to previous experiments involving pulling up against a rigid restraint, old adults did not exhibit modulation in discharge rate or discharge variability at recruitment when the force below recruitment threshold varied, whereas young adults modulated discharge rate in the present study and discharge variability in a previous study involving a rigid restraint. These findings suggest that the integration of synaptic input differs between young and old adults while supporting a compliant load with the elbow flexor muscles.

Introduction

As the decrease in skeletal muscle mass does not fully account for the reduced movement capabilities of old adults (Clark and Manini 2008; Delbono 2003; Roos et al. 1997; Vandervoort 2002), adaptations in the activation of muscle by the central nervous system have been implicated in the decline in motor performance (Aagaard et al. 2010; Doherty 2003; Doherty et al. 1993). At the level of the basic functional unit of the neuromuscular system, old adults exhibit reduced instantaneous discharge rates for motor units in tibialis anterior during ballistic contractions with dorsiflexor muscles (Klass et al. 2008), lower peak discharge rates during maximal voluntary contractions with the first dorsal interosseus and tibialis anterior muscles (Christie and Kamen 2009b; Kamen et al. 1995; Knight and Kamen 2008), a lower incidence of double discharges for motor units in tibialis anterior during ramp isometric contractions with dorsiflexor muscles (Christie and Kamen 2009b), compressed discharge rate modulation in first dorsal interosseus (Barry et al. 2007), and less modulation of discharge rate in first dorsal interosseus when tracking sinusoidal waveforms with the index finger (Knight and Kamen 2007).

The adaptations experienced by old adults involve both intrinsic motor neuron properties (Christie and Kamen 2009b; Piotrkiewicz et al. 2007) and the magnitude and distribution of synaptic inputs (Earles et al. 2001; Eisen et al. 1996; Erim et al. 1999; Kido et al. 2004; Oliviero et al. 2006; Patten and Kamen 2000), which together influence the transformation of synaptic inputs into trains of motor unit action potentials. It has recently been shown that the discharge characteristics of the same motor unit in the biceps brachii that was recruited during a sustained contraction was influenced by the relative target force in young adults (Riley et al. 2008a), but not in old adults (Pascoe et al. 2010). In both studies, a motor unit was isolated in the biceps brachii and subjects maintained an isometric contraction at one of two target forces below the recruitment threshold force for the motor unit. Once the motor unit was recruited, those in old adults discharged action potentials repetitively at both target forces, whereas the motor units of young adults discharged repetitively when the difference between target force and recruitment threshold was small, and intermittently when the difference was large. Additionally, the mean and variability of the discharge times for the first five interspike intervals at recruitment did not differ between the two target-force conditions for old adults, whereas it did for young adults. These differences in motor unit discharge characteristics suggested that synaptic input received by the motor neuron pool, either ionotropic, neuromodulatory, or both, differed across conditions for young but not old adults.

In contrast to the preceding observations that were all based on experiments in which participants exerted a force against a rigid restraint, almost nothing is known about the influence of age on motor unit activity during the more ecologically valid task of controlling compliant loads. It is known, however, that load compliance influences reflex responsiveness (Akazawa et al. 1983; Baudry et al. 2008; Maluf et al. 2007; Perreault et al. 2008), and that motor unit recruitment threshold force, rate of recruitment, mean discharge rate, and discharge variability are all influenced by load compliance (Baudry et al. 2009; Mottram et al. 2005; Tax et al. 1990; Tax et al. 1989; Theeuwen et al. 1994). Given that reflex responses (Corden and Lippold 1996; Earles et al. 2001; Mynark and Koceja 2001; Wolfarth et al. 1997) and motor unit properties (Barry et al. 2007; Erim et al. 1999; Galganski et al. 1993; Kamen et al. 1995; Semmler et al. 2000; Vaillancourt et al. 2003) change with advancing age, the purpose of the current study was to compare the discharge characteristics of motor units when recruited during contractions that required young and old adults to maintain a constant elbow angle while supporting a compliant load that was less than the recruitment threshold force of each isolated motor unit.

Methods

Sixteen young (28.0 ± 3.8 yrs; range, 23 – 37 yrs; 13 men) and 14 old adults (75.1 ± 3.9 yrs; range, 66 – 81 yrs; 12 men) who were free from cardiovascular and neurological disorders volunteered for the study and participated in one to three experimental sessions. Informed consent was obtained from all the participants and the experimental procedures were approved by the Institutional Review Board at the University of Colorado (Protocol #0110.23) and were in accordance with the *Declaration of Helsinki*.

Experimental setup

Subjects were seated upright in an adjustable chair with the left upper arm vertical and slightly abducted from the trunk. The elbow was flexed to 1.57 rad and secured in a padded brace. The forearm was oriented in a neutral position midway between supination and pronation. The hand and forearm were secured with a modified wrist-hand orthosis (Orthomerica, Newport Beach, CA, USA). The force exerted by the elbow flexor muscles was measured as subjects pulled upward at the wrist against a low-compliance force transducer (900-N range, 182 N/V; JR-3, Woodland, CA, USA). The orthosis was attached to the transducer at the level of the wrist (Figure 23*A*). Visual feedback of elbow flexion force was provided on a 17-in computer monitor located at eye level ~1.2 m in front of the subject. Force was digitized with a Power 1401 (Cambridge Electronic Design; CED, Cambridge, UK) at 200 samples•s^{-1} and stored on a computer.

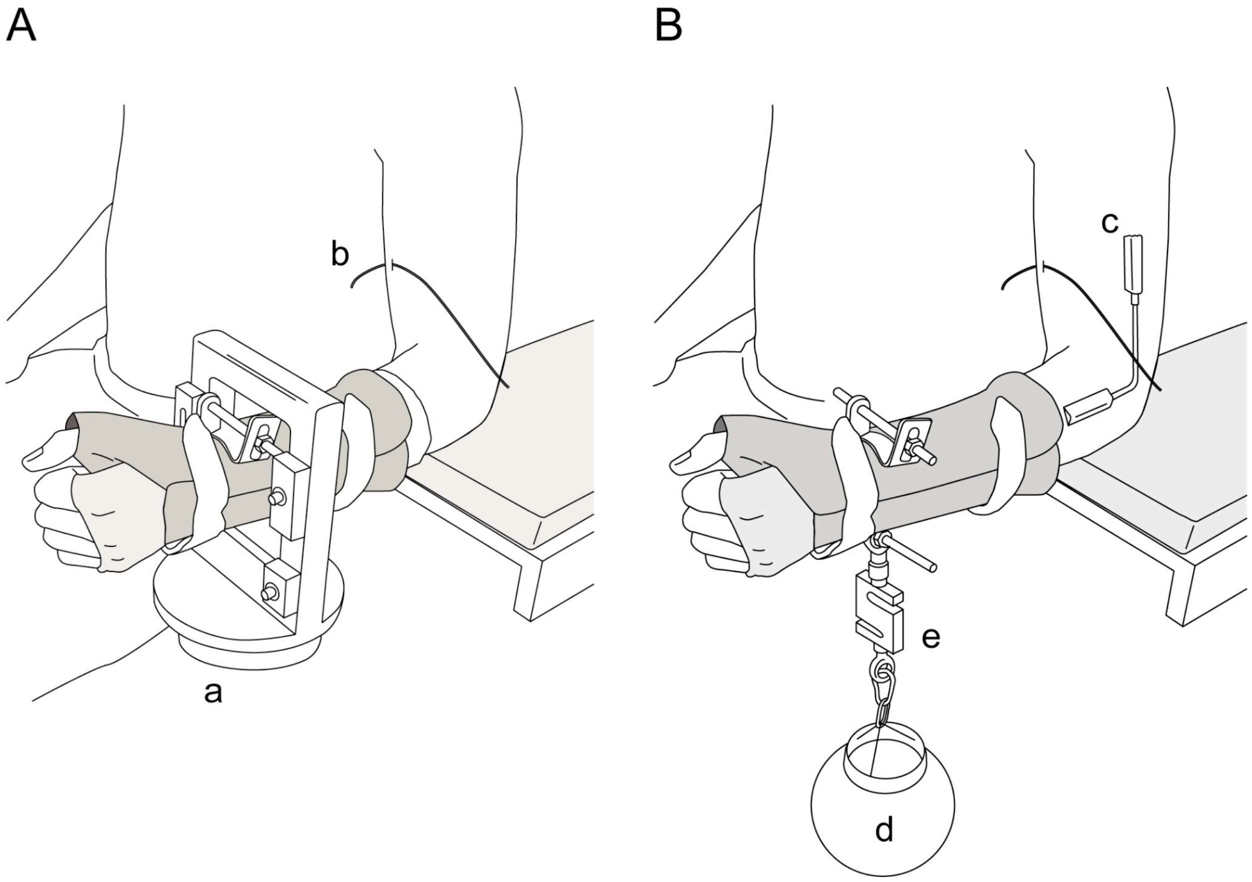

Figure 23. *A*, position of the arm for tasks performed against a rigid restraint. Subjects were seated with the arm slightly abducted from the body with the elbow resting in a padded support. Vertical force was measured as the elbow flexor muscles contracted and the left wrist pulled up against a force transducer (a). A subcutaneous wire electrode (b) was placed over the short head of the biceps brachii muscle. *B*, position of the arm for tasks supporting a compliant load. An electrogoniometer (c) was placed on the lateral aspect of the elbow to provide subjects with visual feedback of elbow angle. The task required subjects to maintain a constant elbow angle of 1.57 rad while a load (d) was hung from the wrist. A strain gauge force transducer (e) was placed in series with the load to determine the start of the task and to measure fluctuations in the limb during the task.

Participants were required to keep the elbow joint at a constant angle (position task) while supporting a mass that comprised lead shot placed in a plastic spherical container. The mass was placed in series with a strain gauge force transducer (SBO-200, Transducer Techniques, Temecula, CA, USA) and attached to the orthosis (Figure 23*B*). The weight of the load and transducer was set equal to a target force that depended on the recruitment threshold of the motor unit. The force transducer measured the force fluctuations during the protocol. Elbow joint angle was measured using an electrogoniometer (SG110 and K800, Biometrics, Cwmfelinfach, Gwent, UK) attached to the lateral aspect of the elbow joint. The output from the electrogoniometer was displayed on the monitor in front of the subject and stored on a computer. The force and elbow angle signals were digitized with a Power 1401 (CED) at 200 samples•s^{-1} and stored on a computer.

Electromyographic recordings

Single motor unit recordings were obtained from the short head of biceps brachii using branched bipolar electrodes (Figure 23*A*, stainless steel, 50-µm diameter, Formvar insulated; California Fine Wire Company, Grover Beach, CA, USA; Enoka et al. 1988; Gydikov et al. 1986a; Mottram et al. 2005). The electrode comprised two insulated fine wires that were glued together with three ~1 mm regions of the insulation removed; two regions ~1 mm on one wire, separated by 3 mm, and a single exposed ~1 mm region on the other wire positioned in between the two regions of the other wire. The lateral margin of the short head of biceps brachii and the intermarginal septum of the two heads were identified and a disposable hypodermic needle (25-guage, 3.91 cm) was used to insert the wires subcutaneously across the muscle belly of the short head of biceps brachii without penetrating the muscle fascia and approximately perpendicular to the orientation of the muscle fibers. The needle was removed prior to recording motor unit activity. The recording sites were adjusted by pulling on the exposed ends of the electrode and its position was optimized to provide the greatest signal-to-noise ratio during 10-s ramps to a target force of 60% maximal voluntary contraction (MVC) force. Once recordings began, the electrode was not moved again until the experiment was completed. A reference electrode was placed on the lateral epicondyle of the ipsilateral humerus. Single motor unit recordings were amplified (X5,000) and band-pass filtered (0.3-8.5 kHz; S-series, Coulbourn Instruments, Allentown, PA, USA). The motor unit signal was sampled at 20k samples•s^{-1} with a Power 1401 (CED) and stored on a computer (Spike2, v. 5.20, CED).

Surface electromyographic (EMG) recordings were made using a bipolar configuration of circular silver-silver chloride electrodes (8-mm diameter) placed on one side of the innervation zone for the short and long heads of biceps brachii and the lateral head of the triceps brachii (interelectrode distance of ~20 mm). Reference electrodes were placed over the acromion process of the ipsilateral scapula. Smaller electrodes (4-mm diameter) were placed over the brachioradialis muscle. The surface EMG signals were amplified (X1,000), band-pass filtered (13 Hz - 1 kHz; S-series, Coulbourn Instruments), sampled at 5k samples•s^{-1}, and stored on a computer (Spike2, v. 5.20, CED).

Protocol

The experimental protocol replicated the previous studies with young (Riley et al. 2008a) and old adults (Pascoe et al. 2010), but involved a compliant load hung from the wrist. The following six tasks were performed with the elbow flexor muscles of the left arm of the volunteers in each experimental session: *1*) assessment of the MVC force; *2*) identification of a single motor unit; *3*) measurement of the recruitment and derecruitment thresholds of the motor unit; *4*) performance of sustained contractions while supporting a mass that was less than the recruitment threshold of the motor unit; *5*) evaluation of the recruitment and derecruitment thresholds of the motor unit immediately after the final sustained contraction; and *6*) completion of a final MVC with the elbow flexor muscles. Each experiment lasted about two hours.

The experimental session began with a minimum of two MVCs in the direction of elbow flexion and at least one in the direction of elbow extension. The MVC task involved increasing the force from zero to maximum over 3 s and then holding the maximum for a further 1-2 s. To minimize fatigue, subjects rested for 90-120 s between trials. When the peak MVC force for the two elbow flexor trials were within 5% of each other, the larger of the two values was recorded as the maximum and used as a reference value for the recruitment threshold force of the motor unit. Otherwise, additional trials were performed until the 5% criterion was met. Efforts that the subject did not regard as maximal were rejected, and the visual gain of the force feedback was varied across trials to minimize the subjects' awareness of differences in performance.

Motor units were identified in the subcutaneous EMG recording by asking the subjects to slowly increase elbow flexion force from rest to 60% MVC force at a constant rate taking ~10 s and then gradually relax during the subsequent 10 s to produce a triangular force profile. Once a potential unit was identified, subjects performed three ramp contractions separated by 60 to 90 s of rest. The recruitment threshold was estimated during the experiment by noting the force at the end of the third interspike interval, and averaging the values from four ramp contractions. Ramp contractions were performed until the coefficient of variation for recruitment threshold force across four ramp contractions was ≤10%. Prior to removing the wrist-orthosis from the JR-3 device, an electrogoniometer (SG110, Biometrics) was attached to the elbow using adhesive tape and a reference angle of 1.57 rad was established.

Subsequently, the subject was instructed to relax as the investigator flexed the arm to the reference angle of 1.57 rad and a second investigator attached the load to the orthosis. To avoid transient recruitment of the motor unit, the load was transferred to the subject slowly (~5 s). The subject was instructed to maintain the target elbow angle of 1.57 rad as steadily as possible by matching the signal from the electrogoniometer to a target line on the monitor until instructed to relax by the investigator. The task was terminated when the motor unit became active and discharged action potentials continuously for ~120 s. After 15 min of rest (Mottram et al. 2005), the contraction with the other load was performed. The presence of the same motor unit after each sustained contraction was later verified offline by comparing waveform overdraws created with Spike2 software (CED).

Although the goal of the study was to compare discharge characteristics of motor units at recruitment using loads similar to those in previous studies on young (Riley et al. 2008a) and old adults (Pascoe et al. 2010), pilot experiments indicate that it was difficult to apply a load that was <10% MVC force below the recruitment threshold of the motor unit without it being recruited during the loading phase. Therefore, the loads used in the current study represent greater differences between recruitment threshold force and target force compared with the previous studies. Similar to the previous chapter, small and large will be used to refer to the relative difference between target force and recruitment threshold force for an isolated motor unit.

To compare the discharge characteristics of the same motor unit between load compliances, three young (22.8 yrs, 3 women) and four old adults (74.1 yrs, 2 women) performed one sustained contraction requiring force control and one requiring position control in the same experiment. The order in which the tasks were performed was counterbalanced across subjects.

Data analysis

Template matching with Spike2 software was performed offline to discriminate individual motor unit action potentials. The accuracy of the discrimination was verified by close visual inspection of each discriminated spike and by reviewing the interspike intervals. Interspike intervals >250 ms (<4 pulses per second; pps; *n* = 762, 3.6% of discharges) or <20 ms (>50 pps; *n* = 11, 0.02% of discharges) were excluded from the calculations of discharge rate. Long interspike intervals likely arose from the brief cessation of motor-unit discharge, whereas short intervals exceed the rates normally observed during these types of contractions for human motor units (Bigland-Ritchie and Lippold 1954; De Luca et al. 1982; Kanosue et al. 1979; Tanji and Kato 1973) and likely resulted from discrimination error or a double discharge (Garland and Griffin 1999; Nordstrom et al. 1992).

Recruitment threshold force was determined with an algorithm that advanced a 500-ms window in 1-ms steps across the discharge times of the motor unit until the coefficient of variation for interspike interval in the window was <50% (Moritz et al. 2005). The force corresponding to the time of the first discharge in the window was taken as the recruitment threshold force. The same method was used to define the derecruitment threshold force of the motor unit. Discharge rate and the coefficient of variation for interspike interval were determined for the 500-ms window at recruitment and derecruitment. The rate of force development and relaxation were taken from the slope of a linear trend that was fit through the ascending and descending force trajectories, respectively.

Each train of action potentials was divided into five epochs of equal duration. The discharge rates were averaged across the entire 20% interval and the coefficient of variation for interspike interval (standard deviation/mean interspike interval X 100) was calculated from the first five interspike intervals in each 20% interval. Fluctuations in the signals from the electrogoniometer (elbow angle) and strain gauge transducer were also quantified as the coefficient of variation. The time to recruitment was defined as the time from complete load support to the first action potential discharged by the isolated motor unit. Surface EMG values are presented as the root-mean-square (RMS) amplitude of the signal normalized to the RMS for a 500-ms epoch centered about the peak force during the MVC. Coactivation ratios were quantified as the quotient of the averaged, rectified and normalized EMG values for the elbow extensor (lateral head of triceps brachii) relative to the pooled average of the elbow flexors (brachioradialis, short and long head of biceps brachii).

Statistical analysis

Independent samples *t*-tests and repeated-measures ANOVAs were used to compare the MVC forces between young and old adults and to assess changes with time. Multiple three-factor, repeated-measures ANOVAs assessed force, rate of force development, discharge rate, and coefficient of variation for interspike interval between young and old adults during ramp contractions (between-subject factor) to compare recruitment and derecruitment and before and after the sustained contractions (within-subjects factor). Multiple repeated-measures, two-way ANOVAs were used to compare the target-force difference, time to motor unit recruitment, duration of motor unit discharge, contraction duration, load, equivalent target force, and mean discharge rate and coefficient of variation for the first five interspike intervals between the young and old adults (between-subjects factor) and the two target forces (within-subjects factor). A χ^2 analysis was used to determine whether significant differences were present in observed discharge patterns (repetitive vs. intermittent) between young and old adults.

In addition, the dependent variables were compared with sustained tasks involving low-compliance loads from previous studies on young (Riley et al. 2008a) and old adults (Pascoe et al. 2010) using repeated-measures, three-way ANOVAs (between-subjects factors: age and load compliance; within-subjects factor: target-force difference). Repeated-measures, three-way ANOVA were used to compare the discharge rate and coefficient of variation for interspike interval between the young and old adults (between-subjects factor) for the two target forces and across 20% epochs of discharge duration (within-subjects factors). Post-hoc analyses with paired samples *t*-tests were used when appropriate to compare the mean discharge rate and coefficient of variation for interspike interval between each 20% epoch of discharge duration. Repeated-measures, three-way ANOVA were also used to compare the amplitude of the surface EMG amplitude and coefficient of variation for force in the strain gauge transducer between the young and old adults (between-subjects factor) for the two target-force difference tasks, from the first to last third of the contraction duration (within-subjects factors). An alpha level of $P < 0.05$ was used to identify significant differences. All statistical analyses were performed using SPSS (v. 16.0, Chicago, IL, USA). Data are presented in the text as mean ± standard deviation and in figures as mean ± standard error of the mean.

Results

The results comprise recordings from 20 motor units in the short head of biceps brachii from each age group while supporting a mass with the elbow flexor muscles that was less than the recruitment threshold force of the isolated motor unit. The discharge of each motor unit was recorded during two contractions that differed in the magnitude of the difference between the weight of the load hung from the wrist and the recruitment threshold force of the isolated motor unit. MVC force for the elbow flexor and extensor muscles at the beginning of the protocol was greater for the young (280 ± 91 N, 170 ± 76 N, respectively) than the young adults (200 ± 67 N, 116 ± 53 N, $P < 0.012$). MVC force declined (young: 9 ± 21%, old: 7 ± 23%) significantly from the beginning to the end of the protocol (young: 251 ± 89 N, old: 185 ± 70 N, time main effect: *P* = 0.003) and to a similar extent between young and old adults (age X time interaction: *P* = 0.295).

Once recruited, motor units discharged action potentials with one of two patterns: repetitive or intermittent (Figure 24). The time from the start of the task to the first action potential for the motor unit that discharged intermittently was 23.6 s when the target force was 15.7% MVC force below recruitment threshold force (46.8% MVC, Figure 24*A*) and 103 s when it was 28.3% MVC force below recruitment threshold (Figure 24*B*). The time from the start of the task to the first action potential for the motor unit that discharged repetitively was 1.23 s when the target force was 11.4% MVC force below recruitment threshold force (20.1% MVC, Figure 24*C*) and 205 s when it was 15.4% MVC force below recruitment threshold (Figure 24*D*)

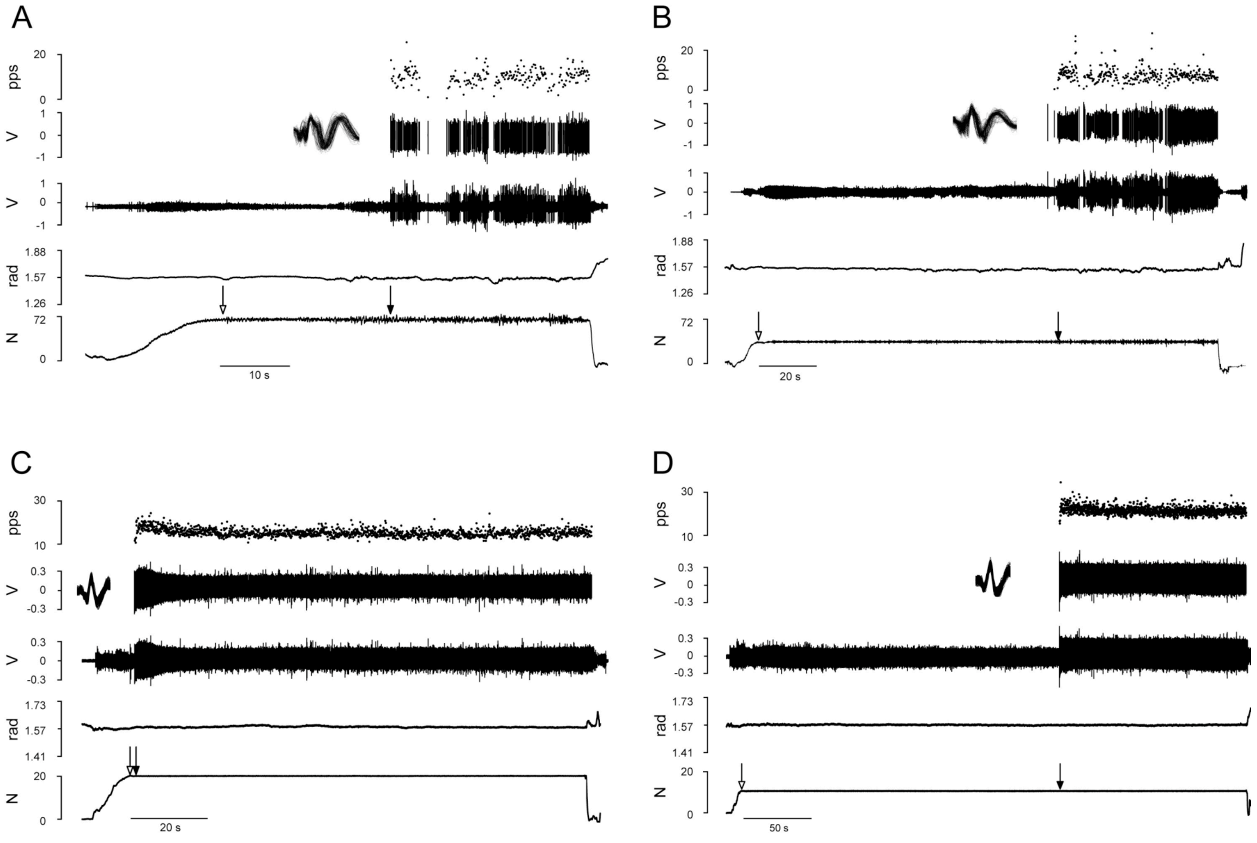

Figure 24. Representative data from sustained contractions depicting, from top to bottom, the instantaneous discharge rate of the motor unit (pulses per second; pps), discriminated action potentials with waveform overlay, interference electromyogram (EMG) from the wire electrode, elbow angle from the electrogoniometer, and the force from the strain gauge transducer in series with the load. Data from an old adult that demonstrated intermittent motor unit activity during small (*A*), and large (*B*) target-force differences. The motor unit had a recruitment threshold force of 46.8% MVC. The time to recruitment was 23.6 s for a load equivalent to 15.7% MVC force below recruitment threshold force and 103 s for a load equivalent to 28.3% MVC force below recruitment threshold force. Data from an old that demonstrated repetitive activity during small (*C*), and large (*D*) target-force differences. The motor unit had a recruitment threshold force of 20.1% MVC. The time to recruitment was 1.23 s for a load equivalent to 11.4% MVC force below recruitment threshold force and 205 s for a load equivalent to 15.4% MVC force below recruitment threshold force. Open arrows indicate the start of the task; filled arrows indicate the onset of motor unit activity.

Recruitment threshold ramp tasks

The average coefficient of variation for the four recruitment threshold forces during the ramp contractions was similar for young and old adults (6.1 ± 3.3%, $P = 0.45$). There was a trend for faster rates of change in force during the relaxation phases of the ramp tasks for both young (*P* = 0.084) and old adults (*P* = 0.056). Average recruitment threshold force did not differ between young and old adults (age main effect: *P* = 0.06, Table 4), but was significantly reduced (10 ± 10%) when measured after the sustained contractions (time main effect: *P* = 0.001). Derecruitment thresholds were identified for 37 of the 40 motor units examined (18 young, 19 old, Table 4). Average derecruitment threshold forces were less than recruitment threshold forces (*P* < 0.001), in both young and old adults (threshold force X age interaction: *P* = 0.120, Table 4). Discharge rates at recruitment were significantly greater than those at derecruitment (*P* < 0.001) and were 20% lower for old adults (age main effect: *P* = 0.001, Table 4). Coefficient of variation for the interspike intervals at recruitment was significantly less than that at derecruitment (*P* = 0.017), but overall did not differ between young and old adults (age main effect: *P* = 0.095, Table 4).

Table 4. *Ramp task characteristics.*

	Rate of change in force (% MVC•s^{-1})		Threshold force (% MVC)		Discharge rate (pps)		CV for ISI (%)	
	Development	Relaxation	Recruitment	Derecruitment	Recruitment	Derecruitment	Recruitment	Derecruitment
Young	5.0 ± 1.4	5.5 ± 1.9	30.0 ± 10.9	22.9 ± 11.7†	13.6 ± 2.8*	9.57 ± 1.8†*	22.0 ± 4.6	24.7 ± 3.9†
Old	5.6 ± 1.7	6.2 ± 2.4	25.9 ± 12.6	14.8 ± 8.19†	11.0 ± 3.4	8.64 ± 2.0†	20.3 ± 4.6	22.6 ± 5.8†

*$P \leq 0.013$ compared with old adults; †$P \leq 0.004$ compared with recruitment. Discharge rate and coefficient of variation for interspike interval (CV for ISI) calculated from first five interspike intervals at recruitment. MVC, maximal voluntary contraction; pps, pulses per second.

Sustained contractions while supporting a mass

The two target forces (16.5 ± 8.0 and 10.4 ± 6.3% MVC, $P < 0.001$) did not differ between young and old adults (age main effect: $P = 0.340$, Figure 25*A*). Due to lower elbow flexor MVC values, the absolute loads supported were heavier for young adults (small: 4.9 ± 2.1 kg, large: 3.2 ± 1.5 kg) compared with old adults (small: 3.1 ± 2.0 kg, large: 1.8 ± 1.5 kg, age main effect: $P = 0.009$). The difference between the recruitment threshold force and target force for the small and large differences was 11.7 ± 4.4 and 17.8 ± 6.5% MVC ($P < 0.001$), respectively, and did not differ between young and old adults (age main effect: $P = 0.297$). The time to recruitment was longer for the large target-force difference (187 ± 227 s) compared with the small target-force difference (23 ± 46 s, $P < 0.001$), but did not differ between young and old adults (age main effect: $P = 0.586$, Figure 25*B*).

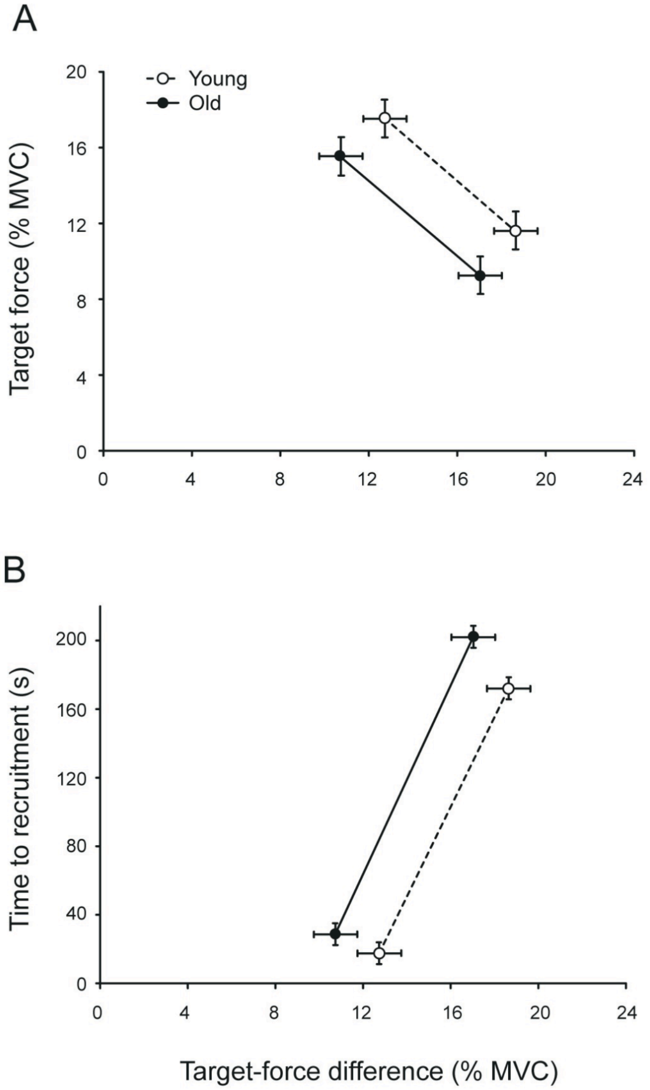

Figure 25. Task characteristics for the 20 motor units recorded from old (●) and young adults (○). *A*, target force (% MVC) was greater for the small compared with the large difference between target force and recruitment threshold force ($P < 0.001$) and to the same extent for both age groups ($P = 0.34$). *B*, the time to recruitment (s) was greater for the large difference between target force and recruitment threshold force ($P < 0.001$) and to the same extent for both age groups ($P = 0.59$).

Young adults had a greater proportion ($\chi^2 = 18.34$, $df = 1$, $P < 0.01$) of motor units that discharged action potentials intermittently at recruitment compared with old adults (Figure 26*A*). This contrasts with the consistent repetitive pattern observed when old adults performed a similar protocol with the wrist fixed in a rigid restraint (Figure 26*B*). The mean discharge rate at recruitment tended to be higher for the small difference (10.0 ± 3.2 pps) compared with the large difference (8.9 ± 3.8 pps) between recruitment threshold force and target force, but did not reach statistical significance (force-difference main effect: *P* = 0.063). However, a significant age X force-difference interaction (*P* = 0.036, Table 5) suggested that only the young adults exhibited a difference in discharge rate between the small and large target-force differences, whereas old adults did not (Table 5, Figure 27*A*). The coefficient of variation for the first five interspike intervals did not differ between the small (24.2 ± 11.8%) and large target-force differences (25.8 ± 10.5%, force-difference main effect: *P* = 0.426). Additionally, the coefficient of variation for interspike interval was generally greater for young adults compared with old adults (Table 5) but this did not reach statistical significance (age main effect: *P* = 0.071, Figure 27*B*).

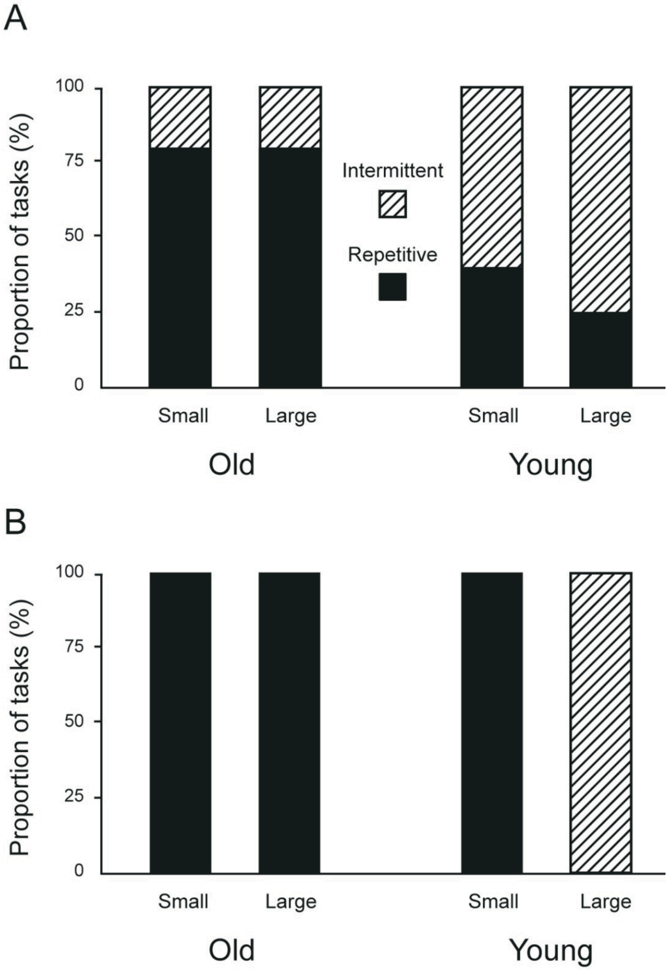

Figure 26. *A*, a greater proportion of tasks demonstrated intermittent motor-unit activity in young compared with old adults when the task was to support a compliant load hung from the wrist ($P < 0.001$). *B*, a summary of previous findings in young and old adults when the task was to produce a constant force against a rigid force transducer. Young adults demonstrated repetitive motor unit activity only when there was a large target-force difference (Riley et al. 2008a), whereas old adults only demonstrated repetitive activity, regardless of the magnitude of the target-force difference (Pascoe et al. 2010).

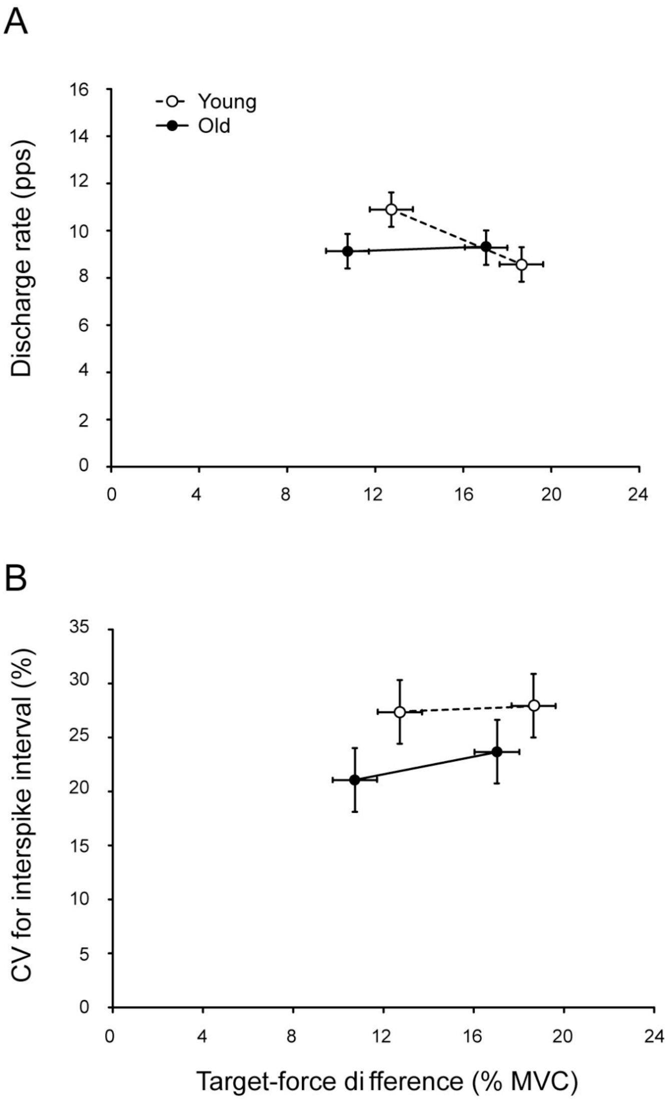

Figure 27. Discharge characteristics at recruitment for the 20 motor units recorded from old (●) and young adults (○). *A*, mean discharge rate (pps) for the first five interspike intervals was lower for the large compared with the small difference between target force and recruitment threshold force in young adults only (age X force-difference interaction: $P = 0.036$). *B*, the coefficient of variation (%) for the first five interspike intervals did not differ between young and old adults ($P = 0.07$) or between the small and large differences between target force and recruitment threshold force ($P = 0.43$).

Table 5. *Task and motor unit discharge characteristics for both force and position tasks, in young and old adults.*

	Young		**Old**	
	Force	Position	Force	Position
Recruitment threshold force (% MVC)				
	32.8 ± 9.7	30.0 ± 10.9	25.4 ± 16.6	26.1 ± 12.4
Target force (% MVC)				
Small	27.1 ± 10	17.5 ± 6.7	18.4 ± 7.9	15.5 ± 9.3
Large	22.3 ± 10*	11.6 ± 5.1*	13.5 ± 7.0*	9.24 ± 7.2*
Force below recruitment threshold (% MVC)				
Small	5.72 ± 2.0	12.7 ± 4.4	7.05 ± 4.2	10.7 ± 4.2
Large	10.5 ± 2.4*	18.4 ± 6.2*	11.9 ± 5.2*	17.0 ± 6.9*
Time to onset (s)				
Small	22.5 ± 22	17.4 ± 28	17.9 ± 27	28.5 ± 59
Large	37.1 ± 50	172 ± 187*	160 ± 145*	202 ± 265*
Task duration (s)				
Small	-	138 ± 21	84.4 ± 29	185 ± 219
Large	-	295 ± 195*	223 ± 147*	325 ± 266*
Mean discharge rate (pps)				
Small	13.2 ± 3.5	10.9 ± 3.3	8.3 ± 2.5	9.1 ± 2.9
Large	13.9 ± 4.3	8.6 ± 2.5*	8.4 ± 3.3	9.3 ± 4.8
Coefficient of variation for ISI (%)				
Small	18.7 ± 7.9	27.3 ± 13	26.3 ± 15	21.0 ± 9.8
Large	35.0 ± 10*	27.9 ± 12	24.0 ± 13	23.7 ± 9.1

*$P < 0.007$ compared with small target force for the same age and load compliance. Data for young and old adults force tasks taken from Riley et al. 2008a and Pascoe et al. 2010. MVC, maximal voluntary contraction; pps, pulses per second.

Discharge characteristics during the sustained contractions

Motor unit discharge times were recorded for 116 ± 21 s following recruitment and discharge characteristics were calculated across five equal-duration epochs for each motor unit. Overall, the mean discharge rates did not differ between young (10.2 ± 2.5 pps) and old adults (10.8 ± 2.1 pps) for the sustained contractions (age main effect: $P = 0.650$). Mean discharge rate decreased during the sustained contractions (time main effect: $P < 0.001$), but to the same extent for young and old adults (time X age interaction: $P = 0.405$). Mean discharge rate was greater for the small target-force difference (force-difference main effect: $P = 0.009$). However, a significant force-difference X age interaction ($P = 0.026$) suggests that old adults did not exhibit a difference in discharge rate between the small (10.8 ± 1.5) and large target-force differences (10.7 ± 2.5 pps, Figure 28*A*), whereas young adults did (small: 11.0 ± 2.2, large: 9.3 ± 2.6 pps, Figure 28*B*). Additionally, a non-significant force-difference X age X time interaction ($P = 0.335$) indicates that the decrease in discharge rate for the two force differences occurred to the same extent in both young and old adults.

The average coefficient of variation for interspike interval was greater for young (23.5 ± 11.0%) than old adults (16.0 ± 6.7%) for the sustained contractions (age main effect: $P = 0.020$). The coefficient of variation for interspike interval decreased during the sustained contractions (time main effect: $P < 0.001$), but to the same extent for young and old adults (time X age interaction: $P = 0.217$). The coefficient of variation for interspike interval was lower for the large target-force difference (force-difference main effect: $P = 0.006$). However, a significant force-difference X age interaction ($P = 0.039$) suggests that old adults did not exhibit a difference in discharge variability between the small (15.7 ± 6.9%) and large target-force differences (16.4

± 6.6%, Figure 28*C*), whereas young adults did (small: 22.8 ± 10.2, large: 24.4 ± 12.1%, Figure 28*D*).

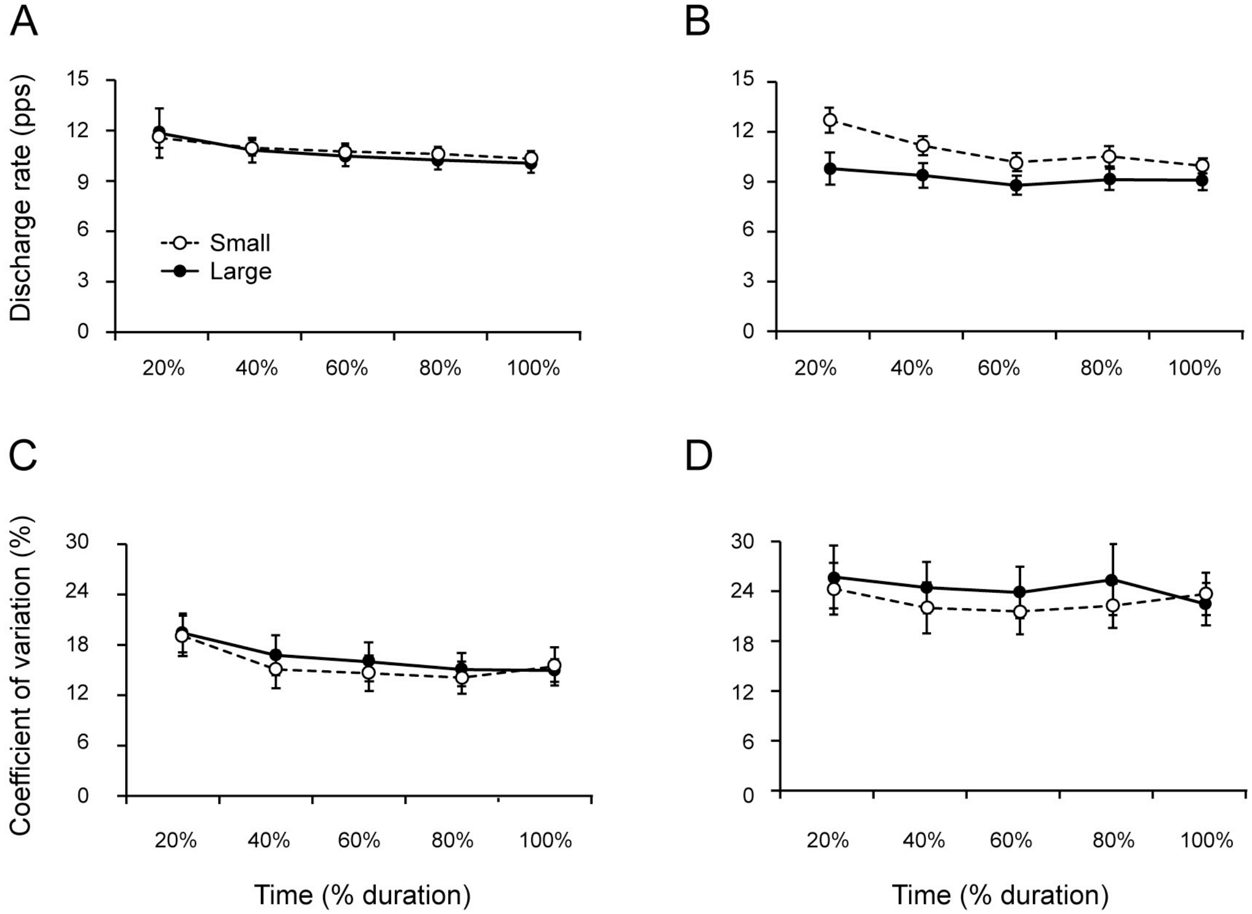

Figure 28. Discharge characteristics for motor units during the sustained contraction for old (left column) and young adults (right column). *A*, mean discharge rate (pps) for the 20 motor units of old adults for the small (○) and large (●) difference between target force and recruitment threshold force (% MVC) for each 20% epoch of discharge duration. Discharge rate did not change from the first to the last 20% epoch of discharge duration (time X force-difference X age group interaction: $P = 0.335$). However, discharge rate was greater for the small target-force difference compared with the large target-force difference in the young adults (*C*, force-difference X age group interaction: $P = 0.026$). *B*, coefficient of variation for the first five interspike intervals (CV for ISI) did not change from the first to the last 20% epoch of discharge duration (time X force-difference X age group interaction: $P = 0.830$), however, CV for ISI was greater for the large target-force difference compared with the small target-force difference in the young adults (*D*, force-difference X age group interaction: $P = 0.039$). Additionally, the CV for ISI values was lower in old adults ($P = 0.020$).

Surface electromyography and limb fluctuations

The normalized amplitude of the surface EMG was greater for all muscles during the small target-force difference (force-difference main effect: $P < 0.001$) and increased from the first to the last one-third of the contraction duration for the elbow flexor muscles ($P \leq 0.004$), but did not change for the elbow extensor muscle (triceps brachii: $P = 0.087$). Only the long head of biceps brachii had significant force-difference X age ($P = 0.02$) and time X age interactions ($P = 0.045$), indicating that the EMG of old adults increased to a greater extent with time (first one-third: 15.6 ± 11.7, last one-third: 21.9 ± 19.1% maximum EMG) and was elevated during the small target-force difference (small target-force difference: 20.9 ± 16.1, large target-force difference: 16.6 ± 14.7% maximum EMG). Additionally, the triceps brachii was the only muscle to demonstrate a significant main effect for age ($P = 0.037$), indicating that the EMG amplitude was less in the young (4.3 ± 3.0%) compared with old adults (9.9 ± 10.5 maximum EMG). Although the coactivation ratio was generally less for young (35.1 ± 22.9%) compared with old adults (52.7 ± 27.9%), this did not reach statistical significance (age main effect: $P = 0.063$). However, coactivation decreased from the first (43.6 ± 27.0%) to the last one-third (40.6 ± 25.2%) of the contraction duration (time main effect: $P = 0.004$) and was less for small (38.9 ± 24.3%) compared with large target-force difference (45.4 ± 27.8%, force-difference main effect: $P < 0.001$).

Fluctuations in the force applied to the load, as measured with the strain gauge transducer (Figure 23*B*), were lower for the small difference (1.4 ± 1.0%) compared with the large target-force difference (2.5 ± 2.4%, force-difference main effect: $P < 0.001$). However, there were no differences between young and old adults (age main effect: $P = 0.731$) and the fluctuations did not change with time (time main effect: $P = 0.168$).

Comparisons of load compliance and age

The data from old adults in the present study performing tasks requiring position control (old and young; *n* = 20 each) were compared with data obtained from previous studies using the identical protocol in young (Riley et al. 2008a, *n* = 12) and old adults (Pascoe et al. 2010, *n* = 27), but with a task that required force control (Table 6). There was a significant main effect for target-force difference ($P < 0.001$) for both load conditions (force and position control). The target forces varied to a similar extent between the young and old adults and between the two loads (age X load compliance interaction: $P = 0.073$). As a consequence, the target-force differences varied to the same extent for both age groups and load compliances (age X load compliance interaction: $P = 0.164$). The duration of the sustained contractions was briefer when the task involved a small difference (120 ± 119 s) compared with a large difference (242 ± 207 s, force-difference main effect: $P < 0.001$).

The time to recruitment was briefer for small target-force differences (21.2 ± 37.0 s) compared with larger target-force differences (155 ± 189 s, force-difference main effect: $P < 0.001$) and similarly between young and old adults (age X load compliance interaction: $P = 0.396$). Additionally, motor units were recruited sooner when the limb pulled against a rigid restraint (70.9 ± 80.6 s), compared with supporting the mass (105 ± 136 s, load compliance main effect: $P = 0.049$). A significant main effect for load compliance ($P < 0.001$) indicated that the target-force differences were greater for contractions supporting the mass and likely accounted for the longer time to recruitment for position control tasks. The mean discharge rates for the first five interspike intervals were higher for young (11.2 ± 3.8 pps) than old adults (8.7 ± 3.4 pps, age main effect: $P = 0.035$) and differed for position control (9.5 ± 3.5 pps) compared with force control (9.9 ± 4.0 pps, load compliance main effect: $P = 0.035$). However, a significant age X load compliance interaction (*P* = 0.001) indicated that old adults did not exhibit a difference between force (8.4 ± 2.9 pps) and position (8.8 ± 3.9 pps) control, whereas young adults did (force tasks: 13.5 ± 3.9, position tasks: 9.7 ± 2.9 pps). The coefficient of variation for the interspike interval demonstrated a significant main effect for the force-difference (*P* = 0.009), but a significant age X force-difference interaction (*P* = 0.01) suggests that old adults did not exhibit a difference between small (24.1 ± 13.0%) and large target-force differences (23.9 ± 11.4%), whereas young adults did (small: 24.1 ± 12.0, large: 30.6 ± 11.4%). Additionally, a significant force-difference X age X load compliance interaction (*P* = 0.002) suggests that discharge variability did not change with target-force difference for either force or position control for old adults. In contrast, young adults exhibited a change in discharge variability during force, but not position control (Table 6).

Direct comparison between force and position tasks in the same motor unit

Four motor units from young (recruitment threshold force range: 12.1 – 64.2% MVC) and four from old adults (14.6 – 38.5% MVC) were tested under both load conditions (Table 6). The decrease in the target force below recruitment threshold force ranged from 6.6 – 27.2% MVC for young adults, which resulted in target forces that ranged from 5.5 – 37.0% MVC. The time to the first action potential did not differ consistently between the two load conditions, but the discharge pattern was typically intermittent during force control (3/4) and mixed during position control (2/4). Mean discharge rate was greater during force control (3/4 units) than position control, and the coefficient of variation for interspike interval was greater during position control (3/4 units).

The decrease in the target force below recruitment threshold force ranged from 7.6 – 17.0% MVC for the old adults, which resulted in target forces that ranged from 7.0 – 21.5% MVC. The time to the first action potential was always greater in force control (21.1 – 27.8 s) than position control (0.13 – 1.31 s). Most motor units discharged repetitively when recruited (force control: 4/4, position control 3/4) and mean discharge rate for the first five interspike intervals was usually elevated during force control (3/4 units). All motor units had a greater coefficient of variation for interspike interval during force control (24.4 – 48.9%) than position control (11.8 – 22.1%).

Table 6. *Discharge characteristics for the four motor units in young and old adults recruited during force and position control tasks.*

	Recruitment Threshold	Target-Force Difference		Pattern	Target	Time to Onset	Discharge Rate	Coefficient of Variation
Young								
1	12.1	6.6	F	Repet	5.5	869	9.3	6.0
			P	Repet		1.7	6.6	20.3
2	27.2	12.6	F	Interm	14.6	52.6	7.3	21.6
			P	Repet		0.2	12.5	20.3
3	43.9	19.1	F	Interm	24.8	27.8	23.5	23.2
			P	Interm		156	19.6	23.5
4	64.2	27.2	F	Interm	37.0	54.7	15.1	45.9
			P	Interm		68.8	10.0	48.7
Old								
1	14.6	7.6	F	Repet	7.0	27.8	8.4	24.4
			P	Repet		0.2	7.7	13.7
2	19.3	9.4	F	Repet	9.9	21.1	7.9	27.4
			P	Repet		0.1	7.5	11.8
3	36.8	16.4	F	Repet	20.4	24.5	13.7	40.3
			P	Interm		0.2	15.1	22.1
4	38.5	17.0	F	Repet	21.5	23.7	15.5	48.9
			P	Repet		1.3	11.3	18.0

F, Force task; P, position task; Repet, repetitive; Interm, intermittent. Recruitment threshold, target-force difference, and target presented as % maximum voluntary contraction (MVC), time to onset presented as seconds (s), discharge rate presented as pulses per second (pps), and coefficient of variation presented for the interspike interval (%).

Discussion

The purpose of the study was to compare the discharge characteristics of motor units when recruited during contractions that required young and old adults to maintain a constant elbow angle while supporting a compliant load that was less than the recruitment threshold force of each isolated motor unit. The main finding of this study was that discharge characteristics of motor units at recruitment did not differ for either force difference in old adults, whereas mean discharge rate was influenced between force differences for young adults. Discharge rate changed across discharge duration for both young and old adults, which contrasts with findings from studies when the force was exerted against a rigid restraint (Pascoe et al. 2010; Riley et al. 2008a). These results extend previous work, to indicate that age-related differences in the integration of synaptic input occur when the limb supports a compliant load.

Discharge characteristics during ramp contractions

Motor unit discharge characteristics were calculated at the time of recruitment and derecruitment as subjects performed ramp contractions that required an increase in elbow flexion force from rest to 60% MVC and a subsequent decrease in force back down to resting levels. The coefficient of variation for of recruitment threshold force for each motor unit (6.1 ± 3.3%) was relatively low compared with previous reports in the wrist extensor muscles (7.2 – 117%; Romaiguère et al. 1989), which indicated a reliable estimate of motor unit recruitment threshold force. Additionally, the rate of force development exhibited a trend for slightly faster rates of change in force ($P \geq 0.056$) during the relaxation phase of the ramp contractions for both young and old adults.

Motor unit recruitment thresholds extended across a wide range of forces in both young (14.0 – 52.5% MVC) and old adults (7.5 – 47.0% MVC) and were well below the upper limit of recruitment for biceps brachii (88% MVC; Kukulka and Clamann 1981). Recruitment threshold force was significantly lower (*P* = 0.001) when reassessed immediately following the sustained contractions, indicating the contractions in this study were fatiguing (Adam and De Luca 2003; Baudry et al. 2009; Carpentier et al. 2001; Enoka et al. 1989; Garland et al. 1994; Suzuki et al. 1990). This result contrasts with a previous study involving sustained contractions against a rigid restraint which found no significant change between the two thresholds (Pascoe et al. 2010). It has been suggested that position control involves a more rapid increase in the neural to the motor unit pool (Hunter et al. 2002), resulting in more rapid recruitment of the motor unit pool to accommodate the faster decline in discharge rate (Mottram et al. 2005). Indeed, fatiguing contractions that involve position control are accompanied by more rapid declines in motor unit recruitment thresholds than comparable contractions requiring force control (Baudry et al. 2009). This seems to explain why reductions were observed when subjects performed position control in the current study, but not when performing contractions that required force control (Pascoe et al. 2010).

The derecruitment threshold forces were lower than the recruitment threshold forces for ramp contractions performed before and after the sustained contractions. Although a lower derecruitment threshold force has been observed in some studies (Baudry et al. 2009; Denier van der Gon et al. 1985; Garland et al. 1994; Pascoe et al. 2010; Romaiguère et al. 1993; Suzuki et al. 1990), many have also found a lower recruitment threshold force (De Luca et al. 1982; Freund et al. 1975; Jesunathadas et al. 2010; Milner-Brown et al. 1973; Patten and Kamen 2000). As ramp contractions involved gradual changes in force, differences in threshold forces must have been accompanied by differences in discharge rate to achieve comparable rates of change in force. Accordingly, discharge rate at derecruitment was lower in the current study as it has been reported in other studies (Christova and Kossev 1998; De Luca et al. 1982; Denier van der Gon et al. 1985; Gorassini et al. 2002; Oya et al. 2009; Pascoe et al. 2010; Romaiguère et al. 1993). As lower discharge rates are usually associated with higher discharge variability (Barry et al. 2007; Baudry et al. 2009; Moritz et al. 2005; Pascoe et al. 2010), the coefficient of variation for interspike interval in the current study was greater at derecruitment compared with recruitment.

Influence of target-force difference on discharge characteristics when supporting a compliant load

Just as in previous studies using the same experimental protocol, each isolated motor unit was exposed to two target forces of varying magnitude below its recruitment threshold force (Pascoe et al. 2010; Riley et al. 2008a). As expected, setting the target force further away from recruitment threshold force resulted in a longer time from the start of the task to the first action potential ($P < 0.001$). Unlike previous studies (Riley et al. 2008a), however, the current study found no association between the target-force difference and whether the motor unit discharged repetitively or intermittently upon recruitment (Figure 25*B*). The divergent behavior in the current study cannot be attributed to recruitment threshold force or the age of the subject as both patterns were seen across the range of motor unit recruitment thresholds and in both age groups (Figure 25*A*). Furthermore, differences in the steadiness of the contraction were not responsible for the two discharge patterns, as the overall levels of force fluctuations did not differ between young and old adults ($P = 0.731$), despite differences in the proportion of motor units responding with intermittent discharge ($P < 0.01$). Additionally, no correlation was found between the coefficient of variation for force produced by the elbow flexor muscles and the number of trains of action potentials for intermittent patterns of activity in motor units of young adults (Riley et al. 2008a). Furthermore, Rudroff et al. (2010) found values for the coefficient of variation for force (~3%) similar to those observed in the current study. Additionally, there were no differences in the coefficient of variation for force between pulling up against a rigid restraint and supporting a compliant load with the elbow flexor muscles. Taken together, fluctuations in force were similar between tasks requiring force and position control and do not explain the greater proportion of intermittent activity for young and old adults of this study compared with that shown in force control tasks of previous studies.

Rather, the lower incidence of intermittent activity exhibited by old adults was likely a consequence of the reduced capacity to modulate discharge rate. At the time of recruitment, mean discharge rate was modulated between the two target forces only for young adults, such that discharge rate was greater for the small (10.9 ± 3.3 pps) compared with the large (8.6 ± 2.5 pps) target-force difference. This difference in discharge rate for young adults between target forces was also seen throughout the five equal duration epochs, such that discharge rates were again greater for small (11.0 ± 2.2 pps) than the large (9.3 ± 2.6 pps, Figure 28*B*) target-force difference ($P = 0.026$). However, the lack of a difference in discharge rate between the tasks for the old adults may indicate a reduced capacity of the motor neurons of old adults to modulate motor unit discharge rate between the two target-force differences. Previous reports of reduced discharge rate modulation include a smaller difference between minimal and peak motor unit discharge rates for a small hand muscle across multiple isometric target forces (Barry et al. 2007) and during a sinusoidal matching task (Knight and Kamen 2007). The results from this study support a reduction in discharge rate modulation by old adults. This decrease in discharge rate is likely mediated by presynaptic inhibition or disfacilitation of the Ia afferent rather than by direct inhibitory inputs received by the motor neuron (Duchateau et al. 2002; Duchateau and Hainaut 1993) or by late spike-frequency adaptation (Kernell and Monster 1982a; b). A decrease in discharge rate with time has also been noted in biceps brachii motor units in previous studies involving sustained contractions involving compliant loads (Baudry et al. 2009; Mottram et al. 2005; Rudroff et al. 2010).

Although the coefficient of variation for interspike interval did not vary between the two target-force differences for either age group at the time of recruitment (Figure 27*B*), the change with time indicated that there was an overall difference across the five epochs between small and large target-force differences in young, but not old adults (*P* = 0.039, Figure 28*CD)*. This may be related to a greater amount of discharge variability for young (23.5 ± 11.0%) compared with old adults (16.0 ± 6.7%, *P* = 0.020). When studied systematically across multiple target forces, the coefficient of variation for interspike interval is highest at forces close to the motor unit recruitment threshold force and decreases with increases in target force (Moritz et al. 2005) and this relation is similar between young and old adults (Barry et al. 2007). This relation indicates that as the descending drive to the motor neuron increases there is a reduction in discharge variability. The different amount of discharge variability between small and large target-force differences seen in young adults in the current experiment suggests that there are either different amounts of synaptic input received by the motor neuron or that the synaptic input is integrated differently compared with that of old adults.

Influence of load compliance on motor unit discharge characteristics

Most of the sustained contractions performed by old adults in the current study resulted in the repetitive discharge of action potentials at recruitment, whereas young adults primarily discharged action potentials intermittently (Figure 25*A*). This age-related difference in motor unit discharge pattern at recruitment is similar to the result of previous studies that showed old adults responded with only repetitive discharge (Pascoe et al. 2010), whereas young adults with both repetitive and intermittent patterns (Riley et al. 2008a; Figure 25*B*). The presence of both discharge patterns across both target-force differences and both age groups suggests that tasks involving position control involve an interaction between the synaptic input received by the motor neuron and its intrinsic properties. For example, it is likely that there are differences in the synaptic input received by the motor neuron pool between the position tasks of the current study and the force tasks of recent studies (Pascoe et al. 2010; Riley et al. 2008a). These differences likely arise from a heightened sensitivity of stretch reflex pathways (Maluf and Enoka 2005) and the associated differences in presynaptic Ia inhibition (Baudry and Enoka 2009) that have been observed in tasks involving compliant loads. In addition to adjustments in stretch reflex sensitivity in the spinal cord, it known that activity of supraspinal pathways is augmented with differences in load compliance (Shemmell et al. 2010). Therefore, the combination of adjustments in both spinal and supraspinal pathways likely provides the motor neuron pool with relatively different amounts of synaptic input, accounting for differences in discharge pattern between tasks involving force or position control.

It has been shown that there is a high correspondence between intermittent motor unit discharge and the rate of bursts in the surface electromyogram for biceps brachii (Riley et al. 2008b). Indirect evidence supporting a tendency towards repetitive motor unit discharge in old adults has been suggested previously by the decreased rate of bursts of activity in the surface EMG recordings from the elbow flexor muscles during force (0.5 ± 1.0 bursts•min^{-1}) compared with position tasks (0.8 ± 1.3 bursts•min^{-1}) during fatiguing contractions that were sustained at 20% MVC force (Hunter et al. 2005b).

In summary, the discharge characteristics of motor units in the biceps brachii of old adults were not influenced by the difference between the mass supported by the arm and recruitment threshold force of a motor unit. This result extends previous observations on old adults when discharge characteristics were not influenced when the task involved pulling up against a rigid restraint (Pascoe et al. 2010). Intermittent motor unit activity was observed in some tasks for old adults while supporting a mass, whereas only repetitive activity was observed when the contraction required force control. Furthermore, mean discharge rate declined with time during position control in contrast to the absence of a change during force. The lack of differences in discharge rate between the target-force differences for old adults suggests that the capacity to modulate discharge rate is reduced with advancing age.

Chapter VI

Conclusions

This dissertation comprised four studies that examined the discharge characteristics of human motor units in young and old adults as they performed voluntary contractions. The findings of these studies indicate that old adults have a reduced ability to modulate discharge rate, which contributes to a decline in motor system performance with advancing age.

The purpose of the first study was to examine the influence of motor unit discharge variability on the force fluctuations in a hand muscle of old adults. The discharge of single motor units in a hand muscle and the fluctuations in force during steady contractions were measured across a range of index finger abduction forces in old adults. Although minimal discharge rates were similar between motor units of young and old adults, maximal rates were significantly reduced for old adults. As a result, old adults exhibited reduced rate coding compared with young adults. The two groups had similar levels of variability in motor unit discharge and when incorporated into a computer model of an aged motor unit pool resulted in an accurate simulation of the forces produced by old adults.

The purpose of the second study was to determine how long motor unit activity could be sustained during a voluntary contraction in humans. The discharge of single motor units in a hand muscle of young and old adults was measured as subjects maintained a constant discharge rate. The duration of motor unit discharge was longer for young compared with old adults; however, technical difficulties limited the number of motor units recorded from old adults. Despite the absence of change in discharge rate for young adults, discharge variability exhibited a marked increase. The findings indicated that the maximal duration of human motor unit activity could far exceed the previously reported durations for motor neurons that were activated by intracellular current injection *in vitro*.

The purpose of the third study was to compare the discharge characteristics of motor units recruited during an isometric contraction that was sustained with the elbow flexor muscles by old adults at target forces that were less than the recruitment threshold force of each isolated motor unit. The pattern of activity as well as discharge rate and discharge variability were quantified and compared with data for young adults that performed similar tasks. In contrast to motor units in young adults that discharged action potentials either repetitively or intermittently depending on the magnitude of the target-force difference, the motor units of old adults discharged action potentials repetitively for both target-force differences. Additionally, mean discharge rate and discharge variability did not differ between target forces for old adults, whereas discharge rates were greater for small target-force differences and discharge variability was greater for small target-force differences. These results indicate that the rate modulation capabilities of the motor unit pool for biceps brachii are reduced in old adults.

The purpose of the fourth study was to compare the discharge characteristics of motor units when recruited during contractions that required young and old adults to maintain a constant elbow angle while supporting a compliant load that was less than the recruitment threshold force of each isolated motor unit. The pattern of activity as well as discharge rate and discharge variability were quantified and compared between age groups as well as with data for young and old adults that performed similar tasks while exerting force against a rigid restraint. Although there were a few contractions at which motor units discharged action potentials intermittently upon recruitment for old adults, a majority of tasks resulted in the repetitive discharge of action potentials and a significantly greater proportion of tasks in young adults exhibited intermittent discharge. Similar to the third study, mean discharge rate and discharge variability did not differ between tasks involving loads of different magnitude, whereas mean discharge rate showed differences between target forces sustained by young adults. The results indicated that the discharge of motor units in old adults was not modulated by an increase in load compliance.

In conclusion, the ability of old adults to modulate discharge rate is less than that for young adults. This decline is attributable to numerous changes in the synaptic input and intrinsic properties of motor neurons with advancing age. As motor performance is related to the ability to control muscle force, and muscle force is controlled by modulating motor unit activity, it is suggested that the adaptations in motor unit activation observed in these studies underlies a reduction in motor performance with advancing age.

Chapter VII

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Chapter VIII

Appendix

Long Duration Contractions

Principal Investigator Michael Pascoe

PARTICIPANT INFORMED CONSENT FORM

October 29, 2009

Please read the following material that explains this research study. Signing this form will indicate that you have been informed about the study and that you want to participate. We want you to understand what you are being asked to do and what risks and benefits—if any—are associated with the study. This should help you decide whether or not you want to participate in the study.

You are being asked to take part in a research project conducted by Michael A. Pascoe, a Ph.D. candidate in the University of Colorado at Boulder's Department of Integrative Physiology, 354 UCB, Boulder, CO 80309-0354. This project is being done under the direction of Professor Roger M. Enoka, Department of Integrative Physiology, 354 UCB, Boulder, CO 80309-0354. Mr. Pascoe can be reached at 303-492-4975. Professor Enoka can be reached at 303-492-7232.

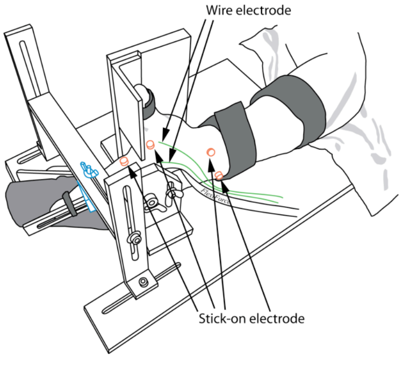
Project Description:

This research study will examine the electrical recordings of muscle activity with probes placed into the muscle as young and old adults perform a long contraction. You are being asked to participate in this study because you are a healthy adult between the ages of 18-35 or ≥ 70 years. Participation in this study is entirely your choice.

Procedures:

Following the completion of this consent form, you will be asked to complete two brief questionnaires. The first simply asks you questions on which hand you prefer to use during various activities of daily living. The second contains questions regarding your cardiovascular health that will inform Mr. Pascoe of your overall health. In addition, we ask that you provide us with information about your ethnicity (e.g., Asian, African American, White, etc.) because we are required to report this to our funding agency (the National Institutes of Health). If you agree to take part in this study, you will be asked to perform a number of muscle contractions with a hand muscle. The project involves one experimental session. Upon completion of the experiment, you will be invited to return for up to two additional sessions (a total of three sessions). Each session will take place on a separate day with at least 2-3 days between sessions.

Approximately 40 young (18-35 years) and 40 older (≥ 70 years) adults will visit the Neurophysiology of Movement Laboratory (Carlson Gymnasium 202G) to perform sustained, submaximal muscle contractions with a hand muscle.



Each experimental session will last approximately two hours and will involve several muscle contractions during which you will increase force gradually and one sustained muscle contraction. You will receive specific instructions on how to perform the muscle contractions and you will practice the tasks before testing begins. In the experimental set up, your left forearm and hand will be placed in an experimental apparatus and secured with Velcro straps (see Figure on left). Your goal in one task will be to increase and then decrease your index finger force gradually (i.e., the contraction will get harder and then easier) by pushing your index finger against a rigid bar. As you perform the task, we will record the force produced by your index finger and the electrical activity from two hand muscles. The electrical activity of one muscle located between your index finger and thumb on the back of your hand will be recorded with pairs of stick-on electrodes placed on the skin surface over the belly and with three thin wires (an electrode), each thinner than a human hair, inserted into the muscle belly. In older adults only, another electrode will be inserted into a second muscle located on the back of your hand between your index and middle fingers.

The wires will be inserted together with a small hypodermic needle so that the electrode is close to the active muscle fibers. There may be from two to three insertions in each experiment. The electrode may be moved slightly while in your muscle to improve the quality of the recording. All wire electrodes and needles will be sterilized and each electrode will be used with only one subject. Subjects on blood thinners, specifically Coumadin (Warfarin) and Lovenox (Enoxaparin), will be excluded from the study because of an increased risk of bleeding, but subjects on baby aspirin can still participate.

Risks and Discomforts:
There are some potential risks if you take part in this study. First, the insertion of the fine wires to record the electrical activity in the hand muscles will cause some discomfort and may cause you to feel lightheaded or faint. In about 1 in 10 cases, there may be a small amount of bleeding that produces a bruise. The risk of infection or significant blood loss is 1 in 1,000. Second, the attachment of electrodes to your skin with tape sometimes causes a minor irritation, but this will disappear in a day or so. Please let us know during the experiment if you experience any discomfort so that we can make adjustments.

Benefits:
There are no direct benefits to you from taking part in this study. The information we obtain will help us learn about the function of the nerve cells during muscle contractions.

Source of Funding:
Funding for this study is being provided by the National Institutes of Health, which is a federal agency that requires data be collected in a form that permits for differences between men and women and races or ethnic groups to be analyzed.

Cost to Participant:
There is no cost to you for participating in this study.

Subject Payment:
You will be paid $10/hr for your participation in this study with a maximum of $60 (2 hrs x 3 sessions x $10/hr = $60 max). In addition, parking will be provided at no charge for the duration of your testing sessions. If you choose to withdraw before the conclusion of the study, you will receive $10 for each completed hour of testing.

Injury and Compensation:
If you feel that you may have been harmed while participating in this study, you should inform Michael Pascoe at 303-492-4975 immediately. The cost for any treatment will be billed to you or your medical or hospital insurance. The University of Colorado at Boulder has no funds set aside for the payment of health care expenses for this study. If you should find the need to make an injury claim, Colorado State Law allows for claims to be made within 180 days of the discovery of injury (Art. 24-10-109).

Ending Your Participation:
You have the right to withdraw your consent or stop participating at any time. You have the right to refuse to answer any question(s) or refuse to participate in any procedure for any reason. Refusing to participate in this study will not result in any penalty or loss of benefits to which you are otherwise entitled.

If you are enrolled in more than one research study, please let the principal investigators of the studies know. The investigators reserve the right to withdraw you from the study for reasons that they deem necessary for subject safety, or if you fail to comply with study procedures.

Confidentiality:
We will make every effort to maintain the privacy of your data. Prior to filing, your data will be coded using your initials. The records will be locked in a filing cabinet and stored for a minimum of 10 years in the Neurophysiology of Movement Laboratory (Carlson Gymnasium, Room 202G) with only the study title and date the study was performed. The records will then be shredded and recycled. Only Mr. Pascoe and his associates will know your identity (filed separately from the coded data).

Other than the research team, only regulatory agencies such as the Office of Human Research Protections, the University of Colorado Institutional Review Board, the Food and Drug Administration, and the National Institutes of Health may see your individual data as part of routine audits.

Invitation for Questions:
If you have any questions regarding your participation in this research, you should ask the investigator before signing this form. If you have questions regarding your rights as a participant, any concerns regarding this project or any dissatisfaction with any aspect of this study, you may report them -- confidentially, if you wish -- to the Executive Secretary, Institutional Review Board, 563 UCB, ARCE Room A15, University of Colorado at Boulder, Boulder, CO 80309, (303) 735-3702.

Authorization:
I have read this paper about the study or it was read to me. I know the possible risks and benefits. I know that being in this study is voluntary. I choose to be in this study. I know that I can withdraw at any time. I have received, on the date signed, a copy of this document containing four pages.

Name of Participant (printed) ____

Signature of Participant ____________________________ Date _______________
(Also initial all previous pages of the consent form.)

Are you interested in participating in other studies in our laboratory?

__ Yes, you may contact me for future studies in your laboratory.

__ No, I do not want to be contacted for future studies in your laboratory.

For IRB Use Only

This consent form is approved for use from ________________ through ________________.

________________________________ Panel Coordinator, Institutional Review Board
(Signature)

Motor unit recruitment in older adults
Principal Investigator Michael Pascoe

PARTICIPANT INFORMED CONSENT FORM
February 20, 2009

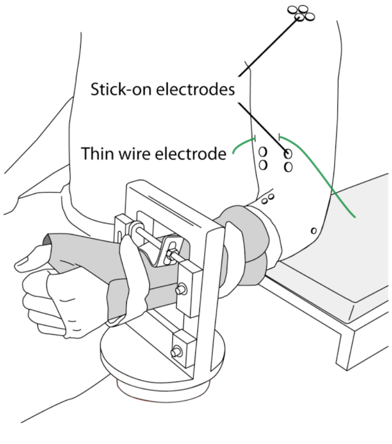
Please read the following material that explains this research study. Signing this form will indicate that you have been informed about the study and that you want to participate. We want you to understand what you are being asked to do and what risks and benefits—if any—are associated with the study. This should help you decide whether or not you want to participate in the study.

You are being asked to take part in a research project conducted by Michael A. Pascoe, a Ph.D. candidate in the University of Colorado at Boulder's Department of Integrative Physiology, 354 UCB, Boulder, CO 80309-0354. This project is being done under the direction of Professor Roger M. Enoka, Department of Integrative Physiology, 354 UCB, Boulder, CO 80309-0354. Mr. Pascoe can be reached at (303) 492-4975. Professor Enoka can be reached at (303) 492-7232.

Project Description:
This research study will examine the electrical recordings of muscle activity with probes placed under the skin and on top of the muscle as older adults perform a voluntary contraction. You are being asked to participate in this study because you are a healthy adult ≥ 70 years old. Participation in this study is entirely your choice.

Procedures:
If you agree to take part in this study, you will be asked to perform a number of muscle contractions with the upper arm muscles. The project involves one experimental session. Upon completion of the experiment, you will be invited to return for up to two additional sessions (a total of three sessions). Each session will take place on a separate day with at least seven days between sessions.

Approximately 30 older (≥ 70 years) adults will visit the Neurophysiology of Movement Laboratory (Carlson Gymnasium 202G) to perform sustained, submaximal muscle contractions with their upper arm muscles.



Each experimental session will last approximately three hours. You will be asked to perform several muscle contractions: a maximal contraction, multiple submaximal contractions in which you will increase force gradually, and two sustained submaximal contractions. You will receive specific instructions on how to perform the muscle contractions and you will practice the tasks before testing begins. In the experimental set up, your left forearm and hand will be placed in a plastic removable brace (see Figure on left) that will be held in place by an instrument to record force (force transducer). The brace easily slides over the hand and can be adjusted to secure the forearm into position. Your goal in one task will be to increase and then decrease the strength of the muscle contraction gradually (i.e., the contraction will get harder and then easier) by pulling up at your wrist. Your goal in the sustained task will be to reach a target force level on a computer screen in front of you by pulling up against the force transducer. As you perform the task, the electrical activity from the arm muscles will be recorded with pairs of stick-on electrodes placed on the skin surface over the belly of each muscle and with two thin wires (an electrode), each thinner than a human hair, inserted under the skin over the biceps muscle.

The wires will be inserted together with a small hypodermic needle so that the electrode is close to the active muscle fibers. There will only be one electrode insertion per session. The electrode may be moved slightly while under the skin and on top of your muscle to improve the quality of the recording. All wire electrodes and needles will be sterilized and each electrode will be used with only one subject. Subjects on blood thinners, such as Coumadin (Warfarin) and Lovenox (Enoxaparin), will be excluded from the study because of an increased risk of bleeding, but subjects on baby aspirin can still participate.

Risks and Discomforts:
There are some potential risks if you take part in this study. First, the insertion of the thin wires to record the electrical activity in the biceps muscle will cause some discomfort and may cause you to feel lightheaded or faint. In about 1 in 10 cases, there may be a small amount of bleeding that produces a bruise. The risk of infection or significant blood loss is 1 in 1,000. Second, the attachment of electrodes to your skin with tape sometimes causes a minor irritation, but this will disappear in a day or so. Please let us know during the experiment if you experience any discomfort so that we can make adjustments.

Benefits:
There are no direct benefits to you from taking part in this study. The information you contribute will help us learn about the function of the nerve cells during muscle contractions.

Source of Funding:
Funding for this study is being provided by the National Institutes of Health, which is a federal agency that requires data be collected in a form that permits for differences between men and women and races or ethnic groups to be analyzed.

Cost to Participant:
There is no cost to you for participating in this study.

Subject Payment:
You will be paid $10/hr for your participation in this study with a maximum of $90 (e.g., 3 hrs x 3 sessions x $10/hr = $90 max). In addition, parking will be provided at no charge for the duration of your testing sessions. If you choose to withdraw before the conclusion of the study, you will receive $10 for each completed hour of testing.

Injury and Compensation:
If you feel that you may have been harmed while participating in this study, you should inform Michael Pascoe at (303) 492-4975 immediately. The cost for any treatment will be billed to you or your medical or hospital insurance. The University of Colorado at Boulder has no funds set aside for the payment of health care expenses for this study. If you should find the need to make an injury claim, Colorado State Law allows for claims to be made within 180 days of the discovery of injury (Art. 24-10-109).

Initials:

Ending Your Participation:
You have the right to withdraw your consent or stop participating at any time.
You have the right to refuse to answer any question(s) or refuse to participate in any procedure for any reason. Refusing to participate in this study will not result in any penalty or loss of benefits to which you are otherwise entitled.

If you are enrolled in more than one research study, please let the principal investigators of the studies know. The investigators reserve the right to withdraw you from the study for reasons that they deem necessary for subject safety, or if you fail I comply with study procedures.

Confidentiality:
We will make every effort to maintain the privacy of your data. Prior to filing, your data will be coded using your initials. The records will be locked in a filing cabinet and stored for a maximum of 10 years in the Neurophysiology of Movement Laboratory (Carlson Gymnasium, Room 202G) with only the study title and date the study was performed. The records will then be shredded and recycled. Only Mr. Pascoe and his associates will know your identity (filed separately from the coded data).

Other than the research team, only regulatory agencies such as the Office of Human Research Protections, the University of Colorado Human Research Committee, the Food and Drug Administration, and the National Institutes of Health may see your individual data as part of routine audits.

Invitation for Questions:
If you have any questions regarding your participation in this research, you should ask the investigator before signing this form. If you have questions regarding your rights as a participant, any concerns regarding this project or any dissatisfaction with any aspect of this study, you may report them -- confidentially, if you wish -- to the Executive Secretary, Human Research Committee, 26 UCB, Regent Administrative Center 308, University of Colorado at Boulder, Boulder, CO 80309-0026, (303) 735-3702.

Authorization:
I have read this paper about the study or it was read to me. I know the possible risks and benefits. I know that being in this study is voluntary. I choose to be in this study. I know that I can withdraw at any time. I have received, on the date signed, a copy of this document containing four pages.

Initials:

Name of Participant (printed) ____

Signature of Participant __________________________ Date ______________
(Also initial all previous pages of the consent form.)

Are you interested in participating in other studies in our laboratory?

__ Yes, you may contact me for future studies in your laboratory.

__ No, I do not want to be contacted for future studies in your laboratory.

For HRC Use Only

This consent form is approved for use from _______________ through _______________.

______________________________ Panel Coordinator, Human Research Committee
(Signature)

Motor unit recruitment in young and older adults while supporting a weight with the arm

Principal Investigator Michael Pascoe

PARTICIPANT INFORMED CONSENT FORM
January 15, 2010

Please read the following material that explains this research study. Signing this form will indicate that you have been informed about the study and that you want to participate. We want you to understand what you are being asked to do and what risks and benefits—if any—are associated with the study. This should help you decide whether or not you want to participate in the study.

You are being asked to take part in a research project conducted by Michael A. Pascoe, a Ph.D. candidate in the University of Colorado at Boulder's Department of Integrative Physiology, 354 UCB, Boulder, CO 80309-0354. This project is being done under the direction of Professor Roger M. Enoka, Department of Integrative Physiology, 354 UCB, Boulder, CO 80309-0354. Mr. Pascoe can be reached at (303) 492-4975. Professor Enoka can be reached at (303) 492-7232.

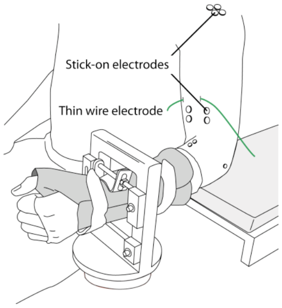
Project Description:

The motor unit comprises a nerve cell in the spinal cord and all of the muscle fibers it innervates. The few hundred motor units that innervate each muscle are known as the motor unit pool. When a subject performs a muscle contraction, the force exerted by the muscle depends on the number of motor units in the pool that are activated and the rates at which they discharge action potentials. Recordings of motor unit activity from young and old adults during a sustained contraction provide information about the activity of the motor unit pool during voluntary contractions and, therefore, inform investigators about the function of the nervous system.

This research study will examine the electrical recordings of motor units with wire electrodes placed under the skin and on top of the muscle as you perform a voluntary contraction. You are being asked to participate in this study because you are a healthy adult between the ages of 18-35 years or 70-85 years. Participation in this study is entirely your choice.

Procedures:

If you agree to take part in this study, you will be asked to perform a number of muscle contractions with the upper arm muscles. The project involves one experimental session. Upon completion of the experiment, you will be invited to return for up to two additional sessions (a total of three sessions). Each session will take place on a separate day with at least seven days between sessions.



Approximately 30 young (18-35 years) and 30 older (70-85 years) adults will visit the Neurophysiology of Movement Laboratory (Carlson Gymnasium 202G) to perform sustained, submaximal muscle contractions with their upper arm muscles.

Each experimental session will last approximately three hours. You will be asked to perform several muscle contractions. For one type of contraction you will be asked to increase your force from rest to maximum taking three seconds. Once you reach your maximum, you will be asked to maintain this for a further two seconds. For another type of contraction you will increase force gradually from rest up to an intensity that is 60% of your maximum. In the final type of contractions, you will sustain a force that is approximately equal to 25% of your maximum. You will receive specific instructions on how to perform the muscle contractions and you will practice the tasks before testing begins. In the experimental set up, your left forearm and hand will be placed in a plastic removable brace (see Figure on left) that will be held in place by an instrument to record force (force transducer). The brace easily slides over the hand and can be adjusted to secure the forearm into position. Your goal in one task will be to increase and then decrease the strength of the muscle contraction gradually (i.e., the contraction will get harder and then easier) by pulling up at your wrist. Your goal in the sustained task will be to maintain a target elbow angle on a computer screen in front of you while supporting a bag of weights hung under your wrist. The duration of these sustained tasks will range from one to 10 minutes. As you perform the task, the electrical activity from the arm muscles will be recorded with pairs of stick-on electrodes placed on the skin surface over the belly of each muscle and with two thin wires (an electrode), each thinner than a human hair, inserted under the skin over the biceps muscle.

The wires will be inserted together with a small hypodermic needle through a fold of skin, entering through one side of the fold and emerging from the opposite side, so that the electrode is close to the active muscle fibers. There will only be one electrode insertion per session. The electrode may be moved slightly while under the skin and on top of your muscle to improve the quality of the recording. All wire electrodes and needles will be sterilized and each electrode will be used with only one subject. Subjects on blood thinners, such as Coumadin (Warfarin) and Lovenox (Enoxaparin), will be excluded from the study because of an increased risk of bleeding, but subjects on baby aspirin can still participate.

Risks and Discomforts:

There are some potential risks if you take part in this study. First, the insertion of the thin wires to record the electrical activity in the biceps muscle will cause some discomfort and, in about 1 in 1,000 cases, may cause you to feel lightheaded or faint. In about 1 in 10 cases, there may be a small amount of bleeding that produces a bruise. The risk of infection or significant blood loss is 1 in 1,000. Second, the attachment of electrodes to your skin with tape sometimes causes a minor irritation, but this will disappear in a day or so. Please let us know during the experiment if you experience any discomfort so that we can make adjustments. Third, you may experience muscle strain / soreness as a result of the maximal contractions. Fourth, the collection of your personal information may put you at risk for a breach of confidentiality.

Benefits:

There are no direct benefits to you from taking part in this study. The information you contribute will help us learn about the function of the nerve cells during muscle contractions.

Source of Funding:
Funding for this study is being provided by the National Institutes of Health, which is a federal agency that requires data be collected in a form that permits for differences between men and women and races or ethnic groups to be analyzed.

Cost to Participant:
There is no cost to you for participating in this study.

Subject Payment:
You will be paid $10/hr for your participation in this study with a maximum of $90 (e.g., 3 hrs x 3 sessions x $10/hr = $90 max). In addition, parking will be provided at no charge for the duration of your testing sessions. If you choose to withdraw before the conclusion of the study, you will receive $10 for each completed hour of testing.

Injury and Compensation:
If you feel that you may have been harmed while participating in this study, you should inform Michael Pascoe at (303) 492-4975 immediately. The cost for any treatment will be billed to you or your medical or hospital insurance. The University of Colorado at Boulder has no funds set aside for the payment of health care expenses for this study. If you should find the need to make an injury claim, Colorado State Law allows for claims to be made within 180 days of the discovery of injury (Art. 24-10-109).

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If you are enrolled in more than one research study, please let the principal investigators of the studies know. The investigators reserve the right to withdraw you from the study for reasons that they deem necessary for subject safety, or if you fail to comply with study procedures.

Confidentiality:
We will make every effort to maintain the privacy of your data. Prior to filing, your data will be coded using your initials. The records will be locked in a filing cabinet and stored for at least 10 years and a maximum of 25 years in the Neurophysiology of Movement Laboratory (Carlson Gymnasium, Room 202G) with only the study title and date the study was performed. The records will then be shredded and recycled. Only Mr. Pascoe and his associates will know your identity (the key code will be filed separately from the coded data) and the only the coded data will be stored on laboratory computers.

Other than the research team, only regulatory agencies such as the Office of Human Research Protections, the University of Colorado Institutional Review Board, the Food and Drug Administration, and the National Institutes of Health may see your individual data as part of routine audits.

Invitation for Questions:
If you have any questions regarding your participation in this research, you should ask the investigator before signing this form. If you have questions regarding your rights as a participant, any concerns regarding this project or any dissatisfaction with any aspect of this study, you may report them -- confidentially, if you wish -- to the Executive Secretary, Institutional Review Board, 563 UCB, ARCE, Room A15, University of Colorado, Boulder, CO 80309-0026, (303) 735-3702.

Authorization:
I have read this paper about the study or it was read to me. I know the possible risks and benefits. I know that being in this study is voluntary. I choose to be in this study. I know that I can withdraw at any time. I have received, on the date signed, a copy of this document containing four pages.

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Signature of Participant ___________________________ Date _______________
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______________________________ Panel Coordinator, Human Research
(Signature) Committee