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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Ann Louise Revill entitled The Role of Synaptic and Non-Synaptic Mechanisms Underlying Motor Neuron Control and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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ABSTRACT

While motor neuron activity has been studied for many decades, the relative contribution of synaptic and non-synaptic mechanisms underlying this activity during natural behaviors is not well understood. Thus, the goal of this dissertation was to further understand the role of non-synaptic properties of motor neurons during voluntary activity. In particular, I considered three non-synaptic properties: persistent inward currents (PICs) that boost synaptic inputs, spike-threshold accommodation that affects recruitment threshold as excitation rates of rise slow, and spike-frequency adaptation that leads to a decrease in firing rate despite constant excitation levels. Computer simulations were employed to understand the potential effect that these properties could have on firing rate behavior. In particular, the focus was on paired motor unit recordings where a lower threshold motor unit’s firing rate served as a proxy for synaptic drive, and differences in firing rate ($\Delta F$) were compared at a higher threshold unit’s recruitment and derecruitment. While $\Delta F$ has been used by others to estimate PIC activation, the simulation results indicated that each of these non-synaptic mechanisms could lead to positive $\Delta F$. Furthermore, by varying contraction speed and duration it seemed possible to determine which property contributes to $\Delta F$ in vivo. The results from human experiments indicated that adaptation is most likely the predominant contributor to $\Delta F$ during natural behaviors. Additionally, positive $\Delta F$ was even observed in the genioglossus muscle of the tongue, where the role of PICs has been debated. These results suggested that $\Delta F$ may not the best method to detect PICs during natural
behaviors. As such, I also considered whether there might be another metric to infer PIC activation during natural behaviors. Motor unit firing rates tend to plateau, or saturate, despite continued force increase, and one hypothesis is that PICs contribute to this behavior. Indeed, motor unit firing rate saturation was diminished by the addition of inhibition, which should have limited PIC activation. Therefore, this final study provided possible evidence for PIC activation during natural behaviors. Overall, this dissertation highlights that non-synaptic properties of motor neurons are activated during natural behaviors and that they contribute significantly to firing rate output.
I. INTRODUCTION

Motor neurons, the final common pathway from the central nervous system, have been well studied. Nevertheless, while much is known about how motor neurons integrate synaptic inputs to generate firing rate output, the contribution of non-synaptic properties in shaping firing rate output during natural behaviors is not yet well understood. The focus of this dissertation is on the role non-synaptic properties play during voluntary activity.

Non-synaptic motor neuron properties have been studied in reduced preparations. The results have highlighted some intriguing properties of motor neurons. For example, persistent inward currents (PICs) provide a relatively fixed level of excitatory current that sums with synaptic inputs. PICs might affect how motor neurons integrate information, how motor neuron firing is modulated, or how motor neurons are recruited and derecruited. Additionally, motor neuron firing threshold appears not to be fixed, but instead may vary based on the rate of rise of excitation through a process called spike-threshold accommodation. And finally, motor neuron firing rates can decay over time, despite a steady level of excitatory drive, this is due to spike-frequency adaptation. All of these properties may lead to non-linear changes in how motor neurons respond to synaptic inputs. Thus I may also refer to these properties collectively as non-linear properties. The literature review in Chapter II considers in more detail information about motor unit physiology (a motor unit is the motor neuron and unique muscle fibers it
innervates), including what is presently known about the role of these non-linear properties on firing rate output.

A next logical step would be to determine under which conditions the above-mentioned properties are activated, for example, during normal behavior, only during situations of high arousal, or maybe after fatigue has set in. However, since it is not presently possible to measure these properties directly, indirect methods must be employed. Much of my dissertation concerns an interesting observation originally developed as a method to detect PIC activation during natural behavior. When firing rate of a lower threshold motor unit is compared at recruitment and derecruitment of a higher threshold motor unit is recruited, there is often a difference in firing rate (ΔF), which has been attributed to PIC activation (Gorassini et al. 2002a; Kiehn and Eken 1997). However, it seemed feasible that other non-linear motor neuron properties could also contribute to this behavior. Therefore, the first objective of this dissertation was to ascertain whether other intrinsic, non-linear motor neuron properties could contribute to ΔF. Chapter III presents the results from simulation experiments addressing this particular topic. Furthermore, since these three non-linear properties are differentially affected by excitation rate of rise as well as total contraction duration, Chapter III also developed hypotheses that could be evaluated in vivo to determine the primary contributor to ΔF. The experimental results testing the hypotheses developed through the simulations are presented in Chapter IV. Ultimately, the results from this study suggest that ΔF may be predominantly related to spike-frequency adaptation.
It is also possible that the relative effects of these non-linear properties may be different depending on the motor unit pool in question. For example, PICs are often hypothesized to be important for postural control (e.g. Heckman et al. 2008), but no role has yet been proposed for fine motor control, or for the muscles of the tongue that are responsible for many complicated tasks. Chapter V explores how the $\Delta F$ measurement varies across motor unit populations, including a postural muscle (tibialis anterior), a hand muscle used in fine motor control (extensor digitorum) and a tongue muscle (genioglossus).

Ultimately, the results from these studies were that the $\Delta F$ measurement may not be the best means to ascertain whether PICs have been activated during voluntary activity. However, when PICs are naturally engaged remains an important question and the last portion of my dissertation focused on another peculiar motor unit behavior that might be explained by PIC activation. Briefly, during a voluntary contraction, motor unit firing rates tend to increase rapidly, but then firing rates plateau (or saturate) at relatively modest rates, despite continued increases in force. One hypothesis is that this firing rate profile may be due to changes in PIC activation. Since PICs are susceptible to inhibition, in Chapter VI I tested whether firing rate saturation was affected when inhibition was added to a motor unit pool. The results indicated that firing rate saturation was less pronounced during contractions with added inhibition, providing support for the idea that PIC activation might underlie this phenomenon.
Motor neurons are perhaps the most studied of all neuronal populations. This is due to many features that have made them particularly amenable to investigation. Spinal motor neurons are relatively large and accessible within the spinal cord. Furthermore, the consequences of motor neuron activity are reflected concretely in muscle force that is easily measured. The first extracellular neural recordings in the 1920s in mammals involved recording the output of motor neurons (Adrian and Bronk 1929) and recordings have continued since. Despite this relatively long history of motor neuron research, little is understood as to how a variety of non-linear properties shape motor neuron output during natural behaviors. This is the focus of this dissertation.

1. Overview of motor unit organization

   i. Introduction to motor neurons

   There are two main types of motor neurons: alpha motor neurons that innervate the fibers that constitute skeletal muscle (extrafusal fibers) and gamma motor neurons that innervate the delicate muscle fibers (intrafusal fibers) that reside within fusiform-shaped sensory organs called muscle spindles. A third, but more rare, type of motor neuron called beta motor neurons also exists that innervates both intrafusal and extrafusal
muscle fibers (Burke 1981; Henneman and Mendell 1981). The focus of this dissertation is on alpha motor neurons. I will refer to them as motor neurons for simplicity.

Motor neurons are multipolar neurons with an extensive dendritic network that makes up more than 95% of the cell volume (Chen and Wolpaw 1994; Cullheim et al. 1987). A myelinated axon extends from the soma and projects to the periphery, where it branches multiple times to innervate a subset of muscle fibers within a muscle. While motor neurons vary in size, the dendritic area and axon diameter scale with the soma size (Kernell 1966). Finally, the post-synaptic currents generated by dendritic and somatic synaptic inputs converge at the initial segment of the axon, which possesses a high density of sodium channels and is thought to be the site where action potentials are generated (Kernell 2006).

Within the spinal cord, motor neurons innervating axial, or postural, muscles tend to reside in the medial portion of the ventral horn, whereas motor neurons innervating limb muscles tend to reside in the lateral portion of the ventral horn. All of the spinal motor neurons for a particular muscle tend to cluster together in a long rostral-caudal column extending over a few vertebral segments (Burke et al. 1977). Cranial motor neurons, on the other hand, originate from distinct nuclei in the brainstem (e.g. cranial nucleus XII, Uemura et al. 1979). They innervate ocular muscles, facial muscles and muscles of the upper airway including the tongue.
ii. Introduction to motor units

Each motor neuron in adult mammals innervates a unique set of muscle fibers; the neuron and its associated muscle fibers are referred to as a motor unit (Liddell and Sherrington 1925). Furthermore, the muscle fibers of a motor unit have been referred to as a muscle unit (Kernell 2006). Additionally, all of the motor units of a particular muscle are referred to as a motor unit pool. The number of motor units of a muscle varies based on muscle function as well as body size, and other factors such as the age of the animal (reviewed in Burke 1981). In humans, small intrinsic hand muscles may have a couple hundred motor units whereas large locomotor muscles may have several hundred to thousands of motor units (Buchthal and Schmalbruch 1980; Enoka 1995). Similar muscles in rats or cats will have fewer motor units (Buchthal and Schmalbruch 1980). The number of muscle fibers of a particular motor unit, the innervation number, also varies within a motor pool and also between muscle types (see II.1.v.d Motor unit innervation number). The smallest motor units supplying ocular muscles innervate only a few muscle fibers whereas the largest motor unit in a lower-limb muscle might have several thousand muscle fibers (Buchthal and Schmalbruch 1980; Burke 1981).

iii. Muscle fiber properties

Muscle fibers are complex structures made up of repeating elements called sarcomeres, which are the units that contain the contractile elements of muscle.
Sarcomeres are made of thin filaments comprised of actin, thick filaments made of myosin, as well as many other proteins. Myosin molecules are comprised of two myosin heavy chains (MHC), two essential myosin light chains (MLCs) and two regulatory MLCs. MHCs have myofibrillar adenosine triphosphatase (mATPase) activity, which is responsible for the break down of ATP to provide energy required for muscle contraction. Muscle fibers of different types have varying levels of mATPase activity, which was exploited originally to differentiate fiber types. By pre-incubating fibers in solutions of varying pH, and following with a standard histochemical process to determine mATPase activity, three different fiber types were observed in limb muscle fibers: type I, IIA, and IIB (Brooke and Kaiser 1970). Subsequent work has identified a fourth type of fiber, type IIX fibers, found in rodents (Lind and Kernell 1991) and probably also humans (see Kernell 2006).

Subsequently, it has been demonstrated that there are at least eight different MHC isoforms. Most skeletal muscles contain four, “conventional”, isoforms: MHC1 (which can be divided into MHC1α, MHC1β), MHC-IIa, MHC-IIb and MHC-IIx (reviewed in Reggiani et al. 2000). While some cranial muscles express non-conventional MHC isoforms (e.g. Kjellgren et al. 2003), tongue muscles contain a similar profile of MHC expression to skeletal muscles (Rahnert et al. 2010). MHC characteristics appear to be the main determinant of muscle fiber contraction characteristics, although MLC isoforms can contribute subtle effects (reviewed in Reggiani et al. 2000). For example, muscle fibers with MHC-I myosin contract more slowly than MHC-2 myosin isoforms (see Table 1, Reggiani et al. 2000). Finally, for muscles with complex functions such as
many head and neck muscles, more than one MHC isoform may be present in muscle fibers (such as extraocular muscles, Kjellgren et al. 2003).

Muscle fiber types also have varied metabolic profiles. That is, type I fibers nearly exclusively obtain energy oxidatively, whereas type IIB (or type IIX, depending on species) fibers utilize almost entirely anaerobic metabolism. Type IIA fibers have an intermediate profile, using both aerobic and anaerobic metabolism (Burke 1981). As such, type I fibers have higher concentrations of enzymes used for aerobic metabolism, type IIA have moderate concentrations of these enzymes, and type IIB have low concentrations (Nemeth et al. 1986). By contrast, type IIB fibers have high concentrations of enzymes used in glycosis, and type I fibers have a low concentration of these enzymes (Nemeth et al. 1986). Additionally, type II fibers also have the largest glycogen stores, and typically are surrounded by a less dense capillary network than type I fibers that need a greater oxygen supply for aerobic metabolism (Burke 1981). Finally, there may be some anatomical differences between fiber types. In general, cross-sectional area increases from type I muscle fibers to type IIa and type IIb (Bodine et al. 1987; Totosy de Zepetnek et al. 1992b), although there are exceptions such as human female vastus lateralis type I fibers have a larger diameter than type II fibers (Simoneau and Bouchard 1989).
v. Motor unit physiology

All of the muscle fibers of a given motor unit are of the same type (Burke et al. 1973; Nemeth et al. 1986), although these muscle fibers are broadly distributed over a wide area of the muscle and may have a density of only 2-9 fibers/100 fibers (Burke and Tsairis 1973; Kanda and Hashizume 1992). Furthermore, the physiological properties of motor units generally correspond to the histochemical properties of muscle fibers (Burke et al. 1973). As such, when motor units were classified into different categories, it was found that type I muscle fibers were associated with slow twitch, fatigue resistant (S) motor units, type IIA muscle fibers were usually part of fast twitch, fatigue resistant (FR) motor units and type IIB muscle fibers were part of fast twitch, fatiguable (FF) motor units (Burke et al. 1973). In some cases, a fourth motor unit category, fast intermediate fatigability, has occasionally been reported that may be similar to FF motor units (e.g. Emonet-Denand et al. 1988; Totosy de Zepetnek et al. 1992b). For simplicity, I will only consider the three most common motor unit types: S, FR, and FF. Finally, despite broad categorizations, muscle fiber types do not always match motor unit physiological profiles (Totosy de Zepetnek et al. 1992b), and motor unit properties exist along a continuum (rather than in discrete clusters), which makes categorization potentially problematic (Bawa et al. 1984).

Each motor unit will have particular contraction characteristics. Generally the assessed properties include: force generated to a single impulse (twitch force) or maximal force due to prolonged stimulation (tetanic force), twitch contraction time, as well as
fatigability (Burke et al. 1973). Finally, the exact range of values for each of these properties will vary with the motor unit pool in question.

a. Motor unit twitch contraction time

Muscle fiber contractile speed has been used to distinguish motor unit populations. Burke (1973) originally used repetitive stimulation of motor units, which led to tension production that either showed “sag”, that is force decreased from an initially higher value, or no sag. Those units that sagged were classed as F motor units, whereas the units that didn’t sag were S motor units. F motor units had faster contraction times than S motor units. Furthermore, contraction times have been measured for some motor unit populations. For heterogeneous muscles, such as gastrocnemius or deep lumbrical muscles of the cat, there is a range of twitch contraction times of approximately 4-5 fold (Burke et al. 1973; Kernell et al. 1975). Additionally, the absolute values of twitch contraction times will vary between motor unit populations. For example, twitch contraction times for extraocular motor units will be quite short, on the order of ~6-12 ms (Lennerstrand 1974), whereas contraction times range from <30 ms to more than 100 ms in cat gastrocnemius motor units (Burke et al. 1973). While twitch contraction times are continuously distributed across a motor unit population, Burke (1973) found that fatigue resistant motor units tended to have the slowest twitch contraction speed and the fatigable motor units had the fastest twitch contraction speed. However, the relationship between twitch contraction time and fatigability is not clearly present in other motor unit
populations. For example, there is often no relationship between twitch contraction time and fatigability, in human motor units (Fuglevand et al. 1999; Thomas et al. 1991b; Thomas et al. 1990).

b. Motor unit fatigue

Early work on motor unit classification carried out by Burke and colleagues used susceptibility to fatigue as the primary distinguishing characteristic of motor unit populations (Burke et al. 1973). Fatigue was classified based on a fatigue index developed by Burke (1973), which allowed motor units to be separated into categories. The fatigue index is the relationship between the force produced after 2 minutes of stimulation compared to the amount of force initially generated by the motor unit. A fatigue index of 1 corresponded to a sustained force output, while a fatigue index of 0 indicated the motor unit did not produce any force after 2 minutes of stimulation. Using this measure, Burke observed that cat medial gastrocnemius motor units tended to cluster into three groups: S motor units with a fatigue index greater than 0.75, FR motor units with a fatigue index between 0.25 and 0.75, while FF motor units with a fatigue index less than 0.25.

While it is clear that motor unit populations demonstrate a range of fatigue susceptibilities, the distinctions may not be as clear as originally observed. For example, in some motor unit populations, only a subset of F motor units sag (Bakels and Kernell 1993; Totosy de Zepetnek et al. 1992b). Furthermore, sag appears to be completely
absent in human motor units, as demonstrated in thenar motor units (Thomas et al. 1991b) and in toe extensors (Macefield et al. 1996). According to conventional criteria, this would imply that human muscles possess only S motor units, yet many human motor units are fatigable. Additionally, when calculating the fatigue index for human motor units, most motor units are classified as having intermediate fatigability (i.e. fatigue index between 0.25 and 0.75) and very few units, if any, are highly fatigable (i.e. fatigue index < 0.25) (Fuglevand et al. 1999; Thomas et al. 1991b). Some other nonhuman motor unit populations also display predominantly intermediate fatigability (e.g. Bakels and Kernell 1993; Totosy de Zepetnek et al. 1992b).

c. Motor unit tension characteristics

A motor unit population typically has a large range in force from the weakest to the strongest units. In nonhuman animal experiments, this is often measured as the tetanic (or maximum) tension the unit can achieve. There may be ~100 fold difference between the weakest and strongest motor units in a muscle, like in the hindlimb muscles of the cat (Burke et al. 1973; Emonet-Denand et al. 1988) or rat tibialis anterior (Totosy de Zepetnek et al. 1992b). There is as much as a ~350 fold range of tetanic tension in rat gastrocnemius muscle (Kanda and Hashizume 1992). Additionally, a motor unit population has a skewed distribution of force, such that most motor units produce low levels of force (Wuerker et al. 1965). Finally, while tetanic tensions tend to exist along a continuum (e.g. Burke et al. 1973; Kanda and Hashizume 1992), when motor units are
divided into groups according to fatigability, motor unit tetanic tension is smallest in S
motor units, intermediate in FR motor units and greatest in FF motor units (e.g. Kanda
and Hashizume 1992).

Motor unit strength has also been measured in human subjects, however, for
technical reasons, twitch tension rather than tetanic force is usually reported. Similar to
many nonhuman animal experiments, a ~100 fold range in twitch tensions has been
reported (Milner-Brown et al. 1973b; Monster and Chan 1977). Additionally, the
distribution of motor unit strength is skewed such that most motor units produce low
levels of force and relatively few units produce large forces (Milner-Brown et al. 1973b;
Monster and Chan 1977). Therefore, human and other animal motor unit populations
appear to be similar regarding the organization of motor unit tension production.

d. Motor unit innervation number

There are three motor unit properties that can contribute to the force produced by
a motor unit: muscle fiber cross-sectional area, muscle fiber specific tension, and the
number of muscle fibers innervated by a motor neuron, or “innervation number” (Burke
and Tsairis 1973). Branches arising from motor axons allow each motor neuron to
contact many muscle fibers. In fact, larger motor neurons branch more than smaller
motor neurons (Eccles and Sherrington 1930), which mean that larger motor neurons tend
to innervate a greater number of muscle fibers. It turns out that the innervation number is
probably the most influential motor unit property in determining the maximum force a
unit can produce (Bodine et al. 1987; Kanda and Hashizume 1992; Totosy de Zepetnek et al. 1992a). Furthermore, the range of innervation numbers of a motor unit pool is the primary determinant of the force capacity range of that muscle (Enoka and Fuglevand 2001).

Innervation number ranges have been estimated in a few experimental animal muscles by using the glycogen depletion technique (reviewed in Burke 1981). By stimulating a single motor unit for a sufficient length of time, the muscle fibers of that motor unit will consume all of the stored glycogen. This may require stimulation for >2 hours for S motor units, but only a few minutes for FF motor units (Totosy de Zepetnek et al. 1992a). Subsequent histochemical staining causes glycogen-depleted muscle fibers to stain white, while the surrounding muscle fibers appear in varying shades of gray. The number of white muscle fibers in cross-section can then be counted to determine the innervation number of that motor unit (Burke 1981). While there are some caveats to this method, including incomplete depletion of glycogen in some fibers (particularly in S motor units) and spurious glycogen depletion of muscle fibers associated with other motor units, this is the most accurate means of calculating innervation numbers currently available (Burke et al. 1973; Enoka and Fuglevand 2001).

The results from glycogen depletion studies indicate that innervation numbers have a two- to nine-fold range in values for cat and rat (Bodine et al. 1987; Kanda and Hashizume 1992; Totosy de Zepetnek et al. 1992a). These ranges, however, may underestimate the actual range of innervation numbers, since these studies only sampled a small number of units. Further to this point, based on calculations of innervation
numbers for a human hand muscle, Enoka and Fuglevand (2001) suggested that this muscle could have an ~80-fold range in innervation numbers. However, actual innervation number ranges for any animal species are presently unknown.

The use of the glycogen depletion technique is limited to nonhuman animals, but average innervation numbers have been estimated in humans as well as in other animals. The average innervation number can be estimated for a particular muscle by counting the number of muscle fibers in cross section and estimating the number of motor neurons innervating that muscle. The results from these studies suggest that human muscles have larger average innervation numbers than for analogous nonhuman animals (see summary in Tables 1 and 2, Enoka 1995).

v. Motor neuron properties

As well as variations in muscle fiber types, motor neuron properties vary systematically in motor unit populations. In particular, motor neurons vary in size, ranging from ~40 to 75 µm in soma diameter in cat lumbar motor neurons (Burke et al. 1977), but are smaller in rodent motor neurons (Chen and Wolpaw 1994). Some motor neuron properties are directly related to motor neuron size, including: motor neuron input resistance, rheobase current (which is the amount of excitatory current needed to elicit an action potential), and axonal conduction velocity. Additionally, some motor neuron properties are not explicitly related to motor neuron size but do show systematic variation in a motor unit population, such as the afterhyperpolarization (AHP).
a. Input resistance

Motor neuron input resistance provides information about cell size, and is also related to motor neuron excitability. Input resistance is a function of specific membrane resistance as well as the surface area of a motor neuron. While there is a strong correlation between input resistance and cell surface area (Barrett and Crill 1974), the specific membrane resistance appears to be greater in small than large motor neurons (Kernell and Zwaagstra 1981). Therefore, ranges in input resistance will tend to slightly overestimate actual motor neuron sizes. For cat spinal motor neurons, input resistance may be as high as 3 MΩ and as low as 0.3 MΩ (Fleshman et al. 1981a; Zengel et al. 1985), i.e. about a 10-fold range. Average motor neuron input resistance is higher in rats (Bakels and Kernell 1993) and mice (Meehan et al. 2010), likely due to smaller motor neuron sizes. The distribution of input resistances is skewed, such that most motor neurons have a relatively larger input resistance (Gustafsson and Pinter 1984). Between motor unit categories, average input resistance is highest in S motor units, followed by FR and then FF motor units (Fleshman et al. 1981a; Zengel et al. 1985).

Recalling Ohm’s law ($\Delta V = IR$), a change in membrane voltage depends on the amount of excitatory current as well as the input resistance of a neuron. Therefore, motor neurons with high input resistances need less current to reach the same level of depolarization as motor neurons with low input resistances. In other words, S motor units are more excitable than FR and FF motor units, while FR motor units are more excitable than FF motor units. Indeed, when comparing motor neuron input resistance to
recruitment threshold, those units with the highest input resistance are recruited first (Gustafsson and Pinter 1984) (see also II.2.i Motor unit recruitment).

b. Rheobase

Rheobase tends to correlate inversely with input resistance (Fleshman et al. 1981b; Gustafsson and Pinter 1984). That is, motor neurons with the highest input resistance will have the lowest rheobase. In cat spinal motor neurons, rheobase values may have close to a 30-fold range, from ~2 nA to ~30 nA (Gustafsson and Pinter 1984). This means that the reported rheobase range is typically greater than the input resistance range (Gustafsson and Pinter 1984), suggesting that input resistance is not the only determinant of rheobase. There is some evidence that voltage threshold is lower for smaller (i.e. higher input resistance) motor neurons (Gustafsson and Pinter 1984), which could explain why there is a greater range of rheobase compared to input resistance values. Finally, like input resistance, most motor neurons in a pool tend to have relatively smaller rheobase values (Gustafsson and Pinter 1984).

c. Axon conduction velocity

Axonal conduction velocity also varies across motor unit pools. A range of velocity values of ~60 to ~120 m/s were reported for cat hindlimb motor neurons (Kernell and Zwaagstra 1981; Zengel et al. 1985), and slower conduction velocities were
reported in the rat, ranging from ~30 to ~85 m/s (Bakels and Kernell 1993). Conduction velocity increases with axonal diameter (Arbuthnott et al. 1980; Rushton 1951), in part because the axial resistance decreases with increased axonal diameter (Rushton 1951). Thus, larger motor neurons, which have larger diameter axons (Kernell 1966), conduct action potentials more quickly than smaller motor neurons in cat hindlimb motor unit populations (Kernell and Zwaagstra 1981). This relationship, however, may be less clear in rat motor neurons (Bakels and Kernell 1993). Furthermore, when motor units were categorized, S motor units had a slower average conduction velocity than F motor units (Bakels and Kernell 1993; Zengel et al. 1985), although there is a considerable range of conduction velocity values for each motor unit class (Zengel et al. 1985).

Conduction velocity is one of a few motor neuron properties that can presently be measured in human subjects. Much like the available data from nonhuman animals, the lowest threshold human motor units (which probably also have the smallest motor neurons) have the slowest conduction velocity and the highest threshold motor units (which probably have the largest motor neurons) have the fastest conduction velocities (Dengler et al. 1988; Freund et al. 1975). Dengler and colleagues (1988) reported a range of conduction velocities in the human thenar muscle of 40 to 62 m/s with a median value of ~52 m/s. Thus, based on the available data, human motor units appear to have conduction velocities similar to rat motor neurons, but slower than cat motor neurons.
d. Afterhyperpolarization

Finally, motor neuron afterhyperpolarization (AHP) is a period of depressed excitability that occurs just post-action potential, and may help set the minimum firing rate (Kernell 1965c). The AHP also varies with other motor unit properties. The predominant AHP conductance in motor neurons is mediated through a calcium-activated potassium current (Sah 1996; Viana et al. 1993b). Pharmacological studies indicate that calcium influx for the AHP is mediated through the voltage dependent high-voltage activated N- and P-type calcium channels (Viana et al. 1993b), which then activates the small conductance calcium-dependent potassium channel (Sah 1996). AHP duration ranges continuously from ~30 ms to > 270 ms in cat medial gastrocnemius motor units (Zengel et al. 1985). Furthermore, on average, S motor units have longer duration AHPs (~160 ms in cat medial gastrocnemius, ~75 ms in rat medial gastrocnemius) and larger amplitude than F motor units (AHP duration ~ 65 ms in cat medial gastrocnemius, ~54 ms in rat medial gastrocnemius) (rat data from Bakels and Kernell 1993; cat data from Zengel et al. 1985). Systematic variations in AHP amplitude may be explained by similar variations in input resistance (Bakels and Kernell 1993), while variations in AHP duration were speculated to be due to differences in channel density and/or channel properties responsible for the AHP (Zengel et al. 1985).

In human motor unit studies, two techniques have been developed to estimate AHP duration: the interval death rate analysis (Matthews 1996; 1999) and a method that uses changes in interspike interval variability (Piotrkiewicz 1999). While each of these
methods cannot measure exact AHP duration, they appear to provide a rough estimate of AHP duration, as tested in cat motor neurons (Powers and Binder 2000a). Using the interval death rate analysis, average AHP is ~140 ms in duration in human tibialis anterior (Macdonell et al. 2008) and first dorsal interosseus (Gossen et al. 2003). Thus, from the available data, AHP duration may be longer in humans than in other experimental animals. Furthermore, this may account for lower firing rates at recruitment in humans (~8 Hz) compared to rats and cats.

Overall, there is a systematic variation in the muscle fiber and motor neuron properties of a particular motor pool. In general, the weakest motor units are comprised of few muscle fibers and are resistant to fatigue. The neurons that innervate these units have a relatively slow conduction velocity, have high input resistance and low rheobase current, thus making them the most excitable neurons in the pool. The strongest motor units have a large number of muscle fibers that tend to be highly fatigable. The neurons that innervate these muscle units have a faster conduction velocity, have lower input resistance and higher rheobase current, resulting in them being less excitable.

vi. Sources and distribution of synaptic inputs

Motor neurons receive synaptic input from a variety of sources, which are outlined below. Most synapses in mammalian motor systems are chemical, although gap junctions may play a role in rhythmic behavior, particularly during development (reviewed in Kiehn et al. 2000). It has been estimated that a motor neuron receives
50,000-140,000 synaptic contacts (Ornung et al. 1998), however some of these contacts are likely from the same pre-synaptic source (Burke and Glenn 1996). The predominant motor neuron excitatory neurotransmitter is glutamate whereas the main source of inhibition to motor neurons is from GABA (gamma-aminobutyric acid) or glycine (reviewed in Kernell 2006; Rekling et al. 2000). Together boutons containing these two neurotransmitters make up approximately 95% of the synaptic contacts onto motor neurons (Ornung et al. 1998). The complement of synapses on motor neurons is comprised of serotonin (5HT), noradrenaline, dopamine and other peptidergic neurotransmitters (reviewed in Rekling et al. 2000). Additionally, in spinal motor neurons, there are almost four times as many inhibitory as excitatory contacts in proximal dendrites, although this ratio approaches one-to-one on the distal dendritic tree (Ornung et al. 1998).

a. Descending inputs to spinal motor neurons

Spinal motor neurons receive input from descending tracts originating from the cerebrum and brain stem as well as from reflex pathways arising in the periphery. These sources of input may be mediated through monosynaptic connections to motor neurons or may be relayed through interneurons or propriospinal neurons (reviewed in Baldissera et al. 1981; Rekling et al. 2000).

Descending inputs to spinal motor neurons can be divided into two broad categories: the medial and lateral pathways. The medial pathway is primarily comprised
of the vestibulospinal tract that originates from Deiter’s nucleus (Petras 1967), and the reticulospinal tract, which arises from areas in the reticular formation (Peterson et al. 1978). These uncrossed pathways make synaptic connections primarily in the medial aspect of the ventral horn, thus mainly influencing axial muscles (Kuypers et al. 1962). Indeed, in studies when the medial pathway was lesioned, monkeys were unable to maintain posture, but with appropriate support they could make fine movements to retrieve morsels of food (Lawrence and Kuypers 1968b). It is now known that the vestibulospinal tract provides inputs important for maintaining balance, and activity in this pathway is responsible for many vestibulospinal reflexes (Wilson and Peterson 1978). Most of these inputs from vestibulospinal pathways are not monosynaptic, but some direct connections exist, for example monosynaptic excitatory post-synaptic potentials (EPSPs) have been observed to hindlimb extensor motor neurons in cats (Grillner et al. 1970; Wilson and Yoshida 1969) and neck extensor muscles in cats (Wilson and Yoshida 1969). These direct connections to motor neuron pools used for balance and posture emphasize the role of the vestibulospinal pathway for these functions. The reticulospinal tract, on the other hand, includes inputs important for initiating and modulating locomotion (Mori 1987) and also includes sources of neuromodulatory inputs to spinal motor neurons (see II.1.vii.e Neuromodulatory input to motor unit pools). Furthermore, recent evidence suggests that reticulospinal neurons make monosynaptic connections with hand muscles in primates, similar to the corticospinal tract (Riddle et al. 2009). Thus the role of the reticulospinal tract may in fact be more extensive than presented here.
The lateral pathway is comprised of the rubrospinal tract, which originates from the red nucleus in the midbrain (Keifer and Houk 1994), and the corticospinal tract, which originates primarily, but not exclusively, from the motor cortex (Kuypers 1960). Neurons from the lateral pathway synapse predominantly contralaterally in the lateral portion of the ventral horn (Kuypers 1960) where motor neurons supplying distal muscles reside. Additionally, when the lateral pathway was lesioned in monkeys, the animals could balance and locomote but had difficulty with fine motor control, especially of the fingers (Lawrence and Kuypers 1968a). Further support for the role in fine motor control comes from the observation that each of these pathways has more potent connections to muscles supplied by distal motor neurons (e.g. red nucleus projections in rat Kuchler et al. 2002; corticospinal projections reviewed in Lemon and Griffiths 2005). In particular, the corticospinal tract has direct excitatory monosynaptic connections to motor neurons in some primate species, including humans (Lemon 2008). Furthermore, measurements of dexterity correspond to the strength of corticomotoneuronal EPSPs; humans have the greatest dexterity and also the most potent corticomotoneuronal inputs (Lemon 2008).

b. Peripheral and spinal inputs to spinal motor neurons

As well as inputs from the cerebrum and brainstem, motor neurons receive synaptic input from peripheral and local interneurons. The best-studied input to spinal motor neurons is the monosynaptic muscle spindle Ia input to spinal motor neurons (Henneman and Mendell 1981). Anatomical, as well as electrophysiological, evidence suggests that a
single Ia afferent diverges extensively to contact nearly every homonymous motor neuron of the pool (Fleshman et al. 1981a; Mendell and Henneman 1971). This monosynaptic excitatory input has been used in many studies as a controlled way to examine the effects of synaptic input on motor neuron behavior (e.g. Homma et al. 1970; Hultborn et al. 2003; Powers et al. 2008). Additionally, Ia afferents branch to contact Ia inhibitory interneurons supplying antagonist muscles. Through this disynaptic pathway, referred to as reciprocal inhibition, antagonist motor neurons are inhibited (Eccles and Lundberg 1958).

Motor neuron axons branch to create axons collaterals that synapse onto Renshaw cells, which are inhibitory interneurons (Renshaw 1941). Renshaw cells synapse onto homonymous motor neurons to form a disynaptic inhibitory reflex pathway referred to as recurrent inhibition (Eccles et al. 1961). Additionally, Renshaw cells are more common in proximal compared to distal motor neurons, at least from what is known from studies in cats and humans (reviewed in Illert and Kummel 1999). While Renshaw cell circuits have been well characterized, their functional role is still not entirely clear (Kernell 2006). Other common peripheral inputs derive indirectly from group II and III muscle afferents, joint afferents and cutaneous afferents (all of the former are sometimes grouped as flexor reflex afferents), and Golgi tendon organs. Ib afferents that arise from Golgi tendon organs excite Ib inhibitory interneurons that inhibit homonymous motor neurons, sometimes referred to as autogenic inhibition (reviewed in Baldissera et al. 1981).

While some common peripheral pathways involve specific spinal cord interneurons, many of the descending pathways also synapse onto these, and other, interneurons
(reviewed in Baldissera et al. 1981; Hongo et al. 1969; Hultborn 2001). Thus, while these peripheral input pathways can be activated in isolation, and sometimes evoke reflexes, they should be considered in a larger context of converging activity from many sources onto these interneurons during natural behaviors. For example, cutaneous and muscle spindle inputs help to shape the locomotor pattern (reviewed by Zehr and Stein 1999). Finally, presynaptic inhibition likely also modulates the afferent input to interneuron and motor neuron targets (reviewed in Rudomin 1999).

c. Inputs to brainstem motor neurons

Brainstem motor nuclei also receive a diverse array of input, which accounts for the varied tasks of these motor unit pools. For example, activity in the hypoglossal motor nucleus (which innervates the tongue) is associated with a wide variety of behaviors including breathing, chewing, swallowing, speaking, as well as volitional movement. The preBötzinger complex provides respiratory drive to respiratory motor neurons by way of the parahypoglossal region and the nucleus tractus solitaries (NTS) (Tan et al. 2010), which then projects to the hypoglossal motor nucleus (Chamberlin et al. 2007; Dobbins and Feldman 1995). The ventral respiratory group (which projects to phrenic, intercostal, and abdominal motor neurons) receives input caudal to the preBötzinger complex (Tan et al. 2010). For swallowing behavior, the hypoglossal motor nucleus receives relatively specific input from the central subnucleus of the solitary tract, which contains the swallowing pattern generator (Amri and Car 1988). Furthermore, in order to
coordinate jaw and tongue movements, premotor neurons to the hypoglossal nucleus receive input from masseter (jaw closer muscle) afferents (Luo et al. 2006). Other afferent input to hypoglossal, facial and trigeminal nuclei is predominantly routed through the NTS (Rekling et al. 2000). Finally, the extent of direct cortical projections for volitional movement is species dependent, such that primates most closely related to humans have more direct projections than other primates (Jurgens and Alipour 2002). However, cortical projections may also relay through other brainstem neurons (Jurgens and Alipour 2002; Schmitt and Gacek 1986).

d. Relative contribution of synaptic sources in a motor pool

Activation and control of a motor unit pool will depend on the organization of synaptic inputs to that pool. For example, all motor units in a pool could receive similar levels of synaptic input from a particular source, or they could receive an asymmetrical distribution. Heckman and colleagues addressed this topic by developing a method to determine the magnitude of a synaptic input as measured at the soma, called “effective synaptic current” (Binder et al. 1996; 2002; Heckman and Binder 1988). Effective synaptic current is both a function of the time integral of the postsynaptic potential and the inverse of motor neuron input resistance (Heckman and Binder 1988). Effective synaptic current is a useful concept when considering inputs to motor units since the magnitude of synaptic current that reaches the soma is particularly relevant for spike
generation. Therefore, a larger effective current will have a greater impact on the probability of spiking.

Figure II.1. Variations of effective synaptic currents. The six different inputs illustrated here are: pyramidal tract (PT, also known as the corticospinal tract), rubrospinal tract (via stimulation of the red nucleus, RN), vestibulospinal tract (via stimulation of Deiter’s nucleus, DN), monosynaptic Ia excitatory input, disynaptic Ia inhibitory input and finally inhibition from the Renshaw cell (RC). Data from cat spinal cord motor neurons of varying input resistances. From (Binder et al. 2002).

Effective synaptic currents were measured from six different sources of input that impinge, either monosynaptically or oligosynaptically, on cat medial gastrocnemius motor units (while the animal was under barbituate anesthesia) (reviewed in Binder et al. 1996; 2002). Ia excitatory effective synaptic current was larger in higher input resistance motor compared to motor neurons of low input resistance (Fig. II.1) (Heckman and
Binder 1988) and therefore should have a greater effect on membrane potential changes in high input resistance motor neurons. Indeed, as a consequence of Ohm’s law, the difference in input resistance from the smallest to largest motor neurons would further magnify this effect. Reciprocal Ia inhibition (Heckman and Binder 1991a) and recurrent inhibition mediated by Renshaw cells (Lindsay and Binder 1991) were of relatively uniform strength across motor neurons of different input resistances (Fig. II.1). Inputs from the ipsilateral vestibulospinal tract (by stimulating Deiter’s nucleus) were excitatory to medial gastrocnemius motor neurons, but had a larger effective synaptic current onto lower input resistance neurons than higher input resistance motor neurons (Westcott et al. 1995) (Fig. II.1). Finally, when neurons from the contralateral corticospinal (Binder et al. 1998) and rubrospinal tract (Powers et al. 1993) were stimulated, predominantly excitatory effects were observed to large motor neurons and inhibitory effects predominated to the smallest motor neurons (Fig. II.1). This last result may seem contradictory, since neurons in these pathways are excitatory (see II.1.vii.a Inputs to spinal motor neurons). However, in the cat, corticospinal and rubrospinal neurons do not make monosynaptic connections with lumbar motor neurons, and thus their effects are mediated through interneurons, some of which are inhibitory. From these results, Binder and colleagues (Binder et al. 1998) conclude that corticospinal or rubrospinal input could lead to disruptions in normal recruitment order (however, see 2.i.b Recruitment reversals).
e. Neuromodulatory input to motor unit pools

Many inputs to motor neurons are mediated through ionotropic receptors, that is, they act by directly opening a neurotransmitter gated ion channel. However, there are also some inputs to motor neurons that act primarily through metabotropic receptors. Typically these metabotropic receptors are G-protein coupled receptors that start a signaling cascade to subsequently facilitate or inhibit other ionic currents in the neuron. The present data suggests that serotonin (5HT) and noradrenaline are the predominant neuromodulators in motor neurons, although dopamine, glutamate, and acetylcholine also have effects on motor neurons (reviewed in Rekling et al. 2000). 5HT is the most well studied neuromodulatory system relevant for motor control.

Serotonergic boutons comprise 1-2% (~1500 boutons) of the inputs to motor neurons (Alvarez et al. 1998). 5HT inputs to the spinal cord derive almost exclusively from the raphe nucleus in the brainstem. The rostral portion of the raphe projects to the forebrain, whereas the caudal portion of the raphe projects to the brain stem and spinal cord, and in particular the nuclei raphe obscurus and pallidus provide serotonin directly to spinal motor neuron pools (Jacobs et al. 2002). Additionally, the raphe pallidus, obscurus and magnus also project to trigeminal, hypoglossal and facial nuclei (Li et al. 1993). Synaptic boutons containing 5HT are in direct opposition to spinal motor neurons (Alvarez et al. 1998) as well as cranial motor neurons (Dobbins and Feldman 1994). However, 5HT staining is most prevalent in the medial aspects of the ventral horn, suggesting that 5HT has more effects on gross motor behaviors rather than fine control.
(Jacobs et al. 2002). 5HT has generally excitatory effects on motor neurons by activating $I_h$ (this cationic current acts at hyperpolarized membrane potentials and depolarizes the membrane potential) (Larkman and Kelly 1997), reducing potassium conductances (Larkman and Kelly 1998), reducing the magnitude and duration of the AHP (White and Fung 1989), and uncovering an L-type Ca current, which underlies persistent inward currents (PICs) (Hounsgaard and Kiehn 1993) (see also 3.i Persistent Inward Currents). While these serotonergic neurons have a basal level of activity on the order of 5-6 spikes/s in quiet wakefulness, they show an increase in activity coincident with the onset of a motor task (Veasey et al. 1995). At the cessation of the motor task, the activity levels of the neuron drop back to baseline levels (Veasey et al. 1995). Therefore, 5HT levels in the ventral horn probably increase during voluntary activity and generally acts to facilitate motor neuron activity (however see Gerin and Privat 1998).

The locus ceruleus in the brainstem provides the primary source of noradrenaline to motor neurons (Lyons and Grzanna 1988). Activity of this brainstem region is positively correlated with the waking state of the animal (Berridge 2008; Hobson et al. 1983) as well as its stress level (reviewed in Jacobs 1986). Noradrenergic neurons directly oppose motor neurons, although at least some of their inputs are extrasynaptic and thus actions of noradrenaline may be more diffuse (Card et al. 1986). Noradrenergic terminals are more prevalent in the lateral ventral horn, where distal limb motor neurons reside, and also more prevalent in the ventral aspects of the hypoglossal motor nucleus, where tongue protruder muscles are located (Rekling et al. 2000). Functionally, noradrenaline increases motor neuron excitability by blocking barium-sensitive
potassium leak currents and promoting a barium-insensitive sodium current, decreasing AHP amplitude in some hypoglossal motor neurons (Parkis et al. 1995) and also promotes PICs (Conway et al. 1988).

Overall, motor neurons receive a wide variety of inputs that may vary both within a motor unit pool as well as between motor unit pools. Specifically how all of these inputs are coordinated to generate complex motor output is still not well understood. However, results from simulation experiments (e.g. Fuglevand et al. 1993), as well as results during natural behavior demonstrating commonly modulated motor unit activity (De Luca and Erim 1994; De Luca et al. 1982), suggest that all motor neurons in a pool may receive similar levels of net synaptic drive. In the next few sections, I will consider how motor neuron activity is coordinated to control force output.

2. Organization of motor neuron output

Motor neurons are activated to generate appropriate muscle force for a task. In one of the first observations of motor unit activity, Adrian and Brock (1929) pointed out that there are two ways to modulate force output: varying the number of active motor units, “recruitment”, or varying the rate of spiking of active motor units, “rate coding”. These authors felt that rate coding was the more important mechanism in varying the total force from a muscle. While the contribution of each mechanism may still not be entirely clear, it now appears that most motor pools use both strategies. However, recruitment or rate coding may be emphasized differently in motor pools. In some muscles, almost all
motor units are recruited by ~50% maximum force (Kukulka and Clamann 1981; Moritz et al. 2005) with rate coding used to increase force output beyond this point. In other muscles, however, recruitment appears to be ongoing until nearly maximum force is attained (Kukulka and Clamann 1981; De Luca et al. 1982; Oya et al. 2009). And in some instances, it appears that motor pools might vary the strategy used for different tasks. For example, in the tongue, increases in isometric force appear to be primarily mediated by recruitment whereas displacement tasks appear to be dominated by rate coding (Pittman and Bailey).

i. Recruitment

The observation that motor units are recruited in a fixed sequence dates to at least the 1930s (Denny-Brown and Pennybacker 1938). Subsequently, the mechanism underlying orderly recruitment was elucidated to be motor neuron size, referred to as the “size principle” (Henneman 1957; Henneman et al. 1965b; Henneman and Olson 1965). That is, the smallest motor neurons have the highest input resistance, which means that they are more susceptible to discharge than larger motor neurons. Henneman and colleagues demonstrated that recruitment order is relatively invariant across pairs of motor units excited by a variety of reflex pathways (Henneman et al. 1965a) or various descending pathways (Somjen et al. 1965). The recruitment order of pairs of motor units was compared and, approximately 90% of the time, the smaller unit (as assessed by extracellular spike height) was recruited first across a variety of different stimuli.
However, certain motor unit size properties correlate more strongly with recruitment order than others. As summarized by Binder and colleagues (1996), those properties include: twitch tension (Milner-Brown et al. 1973b; Olson et al. 1968), tetanic force (Mizote 1982; Zajac and Faden 1985), axonal conduction velocity (Bawa et al. 1984), as well as the amplitude of the extracellular axonal action potential (measured from ventral root filaments) (Henneman et al. 1965b). Thus, the weakest motor units with the most slowly conducting axons are usually the first to be activated (however see Zajac and Faden 1985).

Inhibition also appears to act in an orderly manner on motor neurons (Henneman et al. 1965a). The largest motor neurons are silenced with the smallest amount of inhibition whereas the smallest motor neuron requires the strongest inhibition, i.e. inhibition is directly correlated with motor neuron size (Henneman et al. 1965a). This result, while robust, does not seem to be in accordance with the size principle (which would have predicted that inhibition is inversely correlated with motor neuron size). Furthermore, inhibitory post-synaptic potential amplitude is inversely correlated to cell size (Eccles et al. 1961), which is as would be predicted based on the size principle. To account for this discrepancy, Henneman suggested that changes in conductance, which determine the inhibitory current, might be independent of cell size (Henneman et al. 1965a).
a. Recruitment during natural behaviors

Recruitment order has additionally been investigated during voluntary activity in human subjects. In these experiments, recruitment threshold is measured in terms of total muscle force, which is related to synaptic drive (synaptic drive cannot presently be evaluated directly in human subjects). Additionally, twitch force is typically used as the metric for motor unit size. Overall, results from a variety of muscles consistently demonstrate that there is a positive relationship between twitch tension and motor unit recruitment threshold (Monster and Chan 1977; Milner-Brown et al. 1973b; Thomas et al. 1987; Stephens and Usherwood 1977). Thus the size principle is also valid during natural behaviors.

The effect of contraction speed on recruitment threshold has been investigated in human subjects during volitional activation. Büdingen and Freund (1976) showed that as contraction speed increased, the recruitment threshold of every motor unit decreased. This has the effect of compressing the range over which motor units are recruited. Indeed, recruitment order appears to remain the same even during so-called “ballistic” (very rapid) contractions (Desmedt and Godaux 1977; 1978).

b. Recruitment reversals

Furthermore, there are few examples of recruitment reversals in either animal experiments or human experiments. Some of the reversals in animal experiments may be
attributed to experimental error, or to comparisons between motor units of similar threshold (Henneman and Mendell 1981). However, recruitment reversals could also occur if motor neurons received an asymmetrical distribution of synaptic inputs from different input pathways. For example, cutaneous afferent input from the dorsum of the foot, which is innervated by the sural nerve, tends to inhibit lower threshold hindlimb motor neurons and excite higher threshold motor neurons (Kanda et al. 1977). Indeed, lower threshold medial gastrocnemius motor units activated by tendon vibration were silenced during skin pinch (thus stimulating the sural nerve), and higher threshold motor units were also recruited during the skin pinch (Kanda et al. 1977). Therefore, sural nerve stimulation has the ability to disrupt the normal order of recruitment. However, most attempts to demonstrate disruptions in recruitment order have had limited success, and recruitment order appears to be almost entirely invariant (Binder et al. 1996).

c. Derecruitment

As well as the level of excitation required for recruiting motor units, some research has also investigated the level of excitation at motor unit derecruitment. Henneman and colleagues showed that motor units are silenced in the reverse order that they are recruited, i.e. the largest motor units are the last to be recruited and the first to be derecruited (Henneman et al. 1965b). Considered at a simplistic level, motor neurons cross their spiking threshold when an excitatory current activates a critical number of sodium channels through positive feedback, thus generating the large sodium current that
is the upstroke of the action potential (Hodgkin and Huxley 1952a). All other things being equal, once the excitatory current drops below the level necessary to activate this critical number of sodium channels, the motor neuron should derecruit. Thus, theoretically, recruitment and derecruitment thresholds should be highly similar. In both animal and human experiments, however, this is often not the case. In some animal experiments, the level of injected current at derecruitment is greater than the injected current at recruitment (e.g. Turkin et al. 2010) and in other cases, the converse is seen (e.g. Bennett et al. 2001b). Similarly, in humans, some results demonstrate greater derecruitment compared to recruitment threshold (Fuglevand et al. 2006; Milner-Brown et al. 1973a; De Luca et al. 1982), but other studies show smaller derecruitment compared to recruitment thresholds (Gorassini et al. 2002a; Person and Kudina 1972; Romaiguere et al. 1993; 1989).

One possible explanation for these observations is that non-synaptic motor neuron properties led to changes in the apparent recruitment or derecruitment threshold (see 3. Non-linear motor neuron properties, as well as Chapters III, IV, and V). Additionally, when recruitment and derecruitment are measured in terms of force level, there are mechanical effects that may contribute to a higher derecruitment compared to recruitment threshold. Particularly, there is a delay between a motor unit spike and the development of force associated with those spikes (De Luca et al. 1982; Fuglevand et al. 2006; Milner-Brown et al. 1973a). Thus, the force at motor unit recruitment is only the result of activity of all previously active motor units whereas force at derecruitment would include
previously active motor units plus force that hasn’t yet dissipated from derecruited motor units (including the unit being investigated) (Fuglevand et al. 2006).

ii. Rate coding

Motor unit spiking behavior is often characterized in the following ways. Motor units have a minimum firing rate, whereby excitation below the level associated with the minimum rate leads to no repetitive spiking. Motor units also have a maximum firing rate; excitation above the level necessary to elicit this firing rate will probably lead to depolarization block (and thus no further spiking) (e.g. Meehan et al. 2010). Finally, the relationship between excitatory current and firing rate frequency, as well as the relationship between motor unit force and stimulation frequency, can be considered.

a. Minimum firing rate

Motor neurons fire repetitively at their minimum firing rate when the excitatory current exceeds the rhythmic firing threshold (see point a in lower panel of Fig. II.2), which is approximately 1.5 times the rheobase (Kernell 1965b). The minimum firing rate is often closely related to the length of the AHP. Motor units with the longest AHP duration (which are usually the first recruited) tend to have the lowest minimum firing rate, while motor units with the shortest AHP (and usually the last to be recruited) typically have the highest minimum firing rate (Kernell 1965b). In fact, the AHP may
help to set the minimum firing rate (Kernell 1965b). By definition, the AHP hyperpolarizes the membrane below the spiking threshold; thus, if a steady level of just-suprathreshold current is applied, a new spike cannot occur until the cessation of the AHP.

During voluntary activity in human subjects, the relationship between minimum firing rate and recruitment threshold is less clear. For example, some results suggest that minimum firing rates of voluntarily activated human motor units are relatively fixed, independent of recruitment threshold (Monster and Chan 1977) (Fig. II.3) whereas other results suggest that minimum firing rate varies with recruitment threshold (Barry et al. 2007; Moritz et al. 2005). However, when the AHP was estimated in human subjects, motor units with the longest estimated AHP duration also tended to have the lowest minimum firing rate (Macdonell et al. 2008). That being said, in this study, the interspike interval associated with the minimum firing rate was longer than the estimated AHP duration, suggesting that other factors also influence the minimum firing rate (Macdonell et al. 2008).
b. Current-frequency relationship and maximum firing rate

Systematic study of rate coding was originally conducted in lumbar motor neurons of deeply anesthetized animals (Granit et al. 1963; Kernell 1965b). Once motor neurons started spiking regularly, their firing rate increased linearly with increased excitatory current (Granit et al. 1963; Kernell 1965b; Schwindt and Crill 1982) (Fig. II.2).
II.2). In some motor neurons, the slope of the relationship between excitatory current and frequency changed as the level of excitatory input increased. Traditionally, there was an initial lower slope region, called the primary range, followed by a second, higher slope region observed in some motor neurons, called the secondary range (Kernell 1965a). More recently, a subprimary region has also been described in rodent motor neurons. In mice, the subprimary region is a region of higher gain characterized by irregular spiking behavior and subthreshold oscillations in membrane potential (Manuel et al. 2009; Iglesias et al. 2011), whereas the subprimary firing rate is less variable in rats (Hamm et al. 2010). As well as for current injection, this linear relationship between current and firing rate appears to hold for motor neuron activation with some synaptic inputs (Binder et al. 1999; Granit et al. 1966; Kernell 1969; Powers and Binder 2000b). Finally, while the minimum firing rate is relatively easy to obtain, the maximum firing rate during current injection has been less well studied, however rates in excess of 100 imp/s have been reported (Granit et al. 1963; Schwindt and Crill 1982) (Fig. II.2).

During voluntary activity in human subjects, it is possible to examine the relationship between motor unit firing rate and total muscle force (because force should be closely related to net excitatory synaptic current). There have been relatively fewer studies examining human motor unit behavior at higher force levels due to the difficulty of recording single motor units during this level of activity in the motor unit pool. While some aspects of motor unit behavior are similar to the results from reduced preparations, other aspects from these studies differ from those in experimental animals. For example, motor unit firing rates initially tend to increase linearly with force (e.g. Monster and Chan
1977) (Fig. II.3). However, a secondary range characterized by a higher current-frequency slope is not typically seen, and instead firing rates tend to plateau at higher force levels (see 2.ii.d Firing rate saturation section, also Fig. II.3). Finally, when motor unit activity was followed to high force levels, peak firing rates were much lower than 100 imp/s, often between 20 and 40 Hz (De Luca et al. 1982; Gydikov and Kosarov 1974; Monster and Chan 1977; Oya et al. 2009; Tanji and Kato 1973) (Fig. II.3).

Figure II.3. Motor unit recruitment in a human hand muscle. Each dot and line represent an additionally recruited motor unit. From (Monster and Chan 1977)

c. Motor unit force-frequency relationship

One possible explanation for the discrepancies observed between feline motor neuron activity during current injection and natural human motor unit behavior may be related to the force (tension)-frequency relationship of individual motor units. By
stimulating a single motor unit at varying rates, the resultant motor unit force can be measured. At low stimulation rates, there is little twitch summation, but as stimulation rates increase, force increases linearly with stimulation rate and then finally force production plateaus. Therefore, there is a sigmoidal relationship between stimulation rate and tension (e.g. Kernell et al. 1983). The force-frequency relationship tends to depend on the twitch contraction time of the motor unit, as observed in feline motor units (Botterman et al.; Kernell et al. 1983), as well as in human motor units (Fuglevand et al. 1999; McNulty et al. 2000; Thomas et al. 1991a). Furthermore, the stimulation rate required for half-maximal force was used to provide information about how quickly motor units produce maximum force. Thus, for many human motor units, a stimulation rate of ~9-15 imp/s (Fuglevand et al. 1999; Macefield and Johansson 1996; McNulty et al. 2000; Thomas et al. 1991a) was required for half-maximum force, whereas higher stimulation rates of ~20-60 imp/s (Botterman et al. 1986; Kernell et al. 1983) were needed to reach half-maximum force in feline motor units. Finally, human motor units may reach maximum force at stimulation rates of ~30-80 imp/s (Fuglevand et al. 1999; Macefield and Johansson 1996; McNulty et al. 2000; Thomas et al. 1991a). Feline motor units, on the other hand, may require stimulation rates of 100 imp/s or greater to achieve maximum force (Botterman et al. 1986; Kernell et al. 1983). Overall, the data presented above suggest that human motor units appear to be optimized to function at lower firing rates compared to cat motor units.
d. Firing rate saturation

As mentioned above, human motor unit firing rates initially show rate modulation with force but then firing rates reach a plateau at relatively modest levels, that is firing rates tend to saturate (Clamann 1970; Milner-Brown et al. 1973a; Monster and Chan 1977; Person and Kudina 1972; Pittman and Bailey 2009) (Fig. II.3). This peculiar motor unit behavior deviates from observations in most experimental animal preparations. Furthermore, little research has attempted to understand the mechanisms that might underlie firing rate saturation.

Figure II.4. Firing rates of motor neurons in the presence of neuromodulators. Neuromodulators were used to activate PICs. Firing rates look similar to those presented in Fig. II.3. From (Hornby et al. 2002a)

Experiments conducted by Hornby and colleagues, however may provide insight into this phenomenon (Hornby et al. 2002b; a). The firing rate profile of turtle motor
neurons was compared in the presence and absence of neuromodulators. Without the addition of neuromodulators, motor neurons responded linearly to current injection (Hornby et al. 2002a). However, when neuromodulators, like serotonin or muscarine were added, firing rates saturated at higher levels of excitatory current (Hornby et al. 2002a) (Fig. II.4). Furthermore, this behavior was more prevalent in motor neurons that generated PICs (Hornby et al. 2002a). In all motor neurons, however, neuromodulators promoted higher firing rates at the same level of injected current (Hornby et al. 2002a). Finally, a role for PICs in firing rate saturation has also been suggested by other authors (Heckman et al. 2008; Lee et al. 2003; Taylor and Enoka 2004).

The above discussion highlights one example of how non-synaptic mechanisms may alter the relationship between synaptic input and firing rate output. Human motor unit activity does not usually mimic the firing rate activity of motor neurons during typical current injection experiments, suggesting that there may be additional non-synaptic mechanisms that contribute to motor unit spiking during natural behaviors. For the work in this dissertation, I have considered possible effects that the following three properties might have on the firing rate input-output relationship: PICs, spike threshold accommodation, and spike frequency adaptation. The subsequent sections provide information about these non-linear properties.
3. Non-linear motor neuron properties

i. Persistent inward currents

Persistent inward currents (PICs), discovered approximately thirty years ago (Schwindt and Crill 1977), are a voltage-dependent, as well as neuromodulation dependent, intrinsic source of excitation to motor neurons. PICs have been demonstrated across different motor neuron populations and different species, for example, cat spinal motor neurons (Bennett et al. 1998; Hultborn et al. 2003), rat spinal motor neurons (Hamm et al. 2010) and sacral motor neurons (Bennett et al. 2001b), mouse spinal motor neurons (Meehan et al. 2010; Carlin et al. 2000b) and turtle motor neurons (Hornby et al. 2002a; Perrier and Delgado-Lezama 2005; Perrier and Cotel 2008). Their functional role in behavior is debated, but PICs could be useful for postural control (Hounsgaard et al. 1988a), or more generally for boosting synaptic inputs (Hultborn et al. 2003; Prather et al. 2001). PICs may also underlie the muscle spasticity that often occurs in a spinal cord injured person (Murray et al. 2010).

a. Properties of PICs

There are likely multiple currents that sum together to create the net observed PIC. The predominant current underlying PIC behavior is mediated through L-type calcium channels (Cav 1.3) (Carlin et al. 2000b). It also appears that the channels
underlying calcium PICs have an activation threshold that roughly corresponds to the membrane depolarization associated with recruitment of the motor neuron (Bennett et al. 1998). However, the L-type calcium channel appears to play little role in juvenile hypoglossal motor neurons while most of the calcium PIC is carried via N- and P-type calcium channels (Powers and Binder 2003). There is also a sodium PIC, which tends to activate below spiking threshold for a motor neuron, and in fact has been suggested to be integral to the ability for a motor neuron to generate spikes at all (Harvey et al. 2006a). This sodium PIC, however, is not particularly persistent and tends to inactivate in a few seconds (Lee and Heckman 1999b; Li and Bennett 2003; Harvey et al. 2006a; Harvey et al. 2006b). In some cases a non-specific cation current may also contribute to the PIC (Zhang et al. 1995; Rekling and Feldman 1997; Perrier and Hounsgaard 1999). Finally, as well as inward currents, there is recent evidence for an ongoing persistent outward current (Li and Bennett 2007; Hamm et al. 2010), which might be a potassium current (Li and Bennett 2007) or related to an ongoing adaptive process (Hamm et al. 2010). A long-lasting outward current would diminish the net inward current (Li and Bennett 2007).
The role of neuromodulation in promoting PICs has been best studied with regard to serotonin (5HT). 5HT acts to facilitate PICs by blocking certain potassium conductances and may also potentiate L-type calcium currents (Hounsgaard and Kiehn 1993; Booth et al. 1997; Perrier and Hounsgaard 2000; 2003) (Fig. II.5). As well as serotonergic pathways, noradrenaline can promote PICs (Conway et al. 1988). Furthermore, there are likely local neuromodulators in the spinal cord that may activate muscarinic acetylcholine receptors and metabotropic glutamate receptors (demonstrated in turtle motor neurons Svirskis and Hounsgaard 1998; Hornby et al. 2002a) to promote PICs (Heckman et al. 2003).

As mentioned, PICs tend to be activated around motor neuron recruitment threshold (Bennett et al. 1998), but may require a several hundred milliseconds to be fully expressed (Bennett et al. 2001b; Bennett et al. 2001a; Li et al. 2004a; Li and Bennett 2003). PICs may then remain at a relatively fixed magnitude for many seconds (Lee and
Heckman 1998a), at least in some motor neurons. It is less clear, however, how PICs inactivate. Some motor neurons, often higher threshold motor neurons, express PICs that are rather unstable and naturally decay in a few seconds (Lee and Heckman 1998a). In addition, PICs are known to be particularly sensitive to inhibition (Bui et al. 2008a; b; Hounsgaard and Kiehn 1989; Kuo et al. 2003) (Fig. II.5). Inhibitory inputs are often located near PIC channels (Bui et al. 2008b), and activation of inhibitory reflex pathways may minimize PIC magnitude (Kuo et al. 2003). The effects of possibly inhibiting PICs are investigated in Chapter VI.

b. Prevalence of PICs

While PICs have been demonstrated in many motor neuron populations, there is variability in their expression. For example, motor neuron behavior consistent with PICs is more readily elicited in extensor motor neurons compared to flexor motor neurons (Cotel et al. 2009; Hounsgaard et al. 1988a). Also, it appears that PICs are more prevalent, more persistent, and larger in magnitude in lower threshold motor neurons than in high threshold motor neurons (Hamm et al. 2010; Lee and Heckman 1998a). PIC magnitude (Hultborn et al. 2003) and duration (Hounsgaard and Kiehn 1989) may be affected by the level of excitation.
c. The role of PICs during natural behaviors

While there is ample evidence that motor neurons have the ability to generate PICs, it has been frustratingly difficult to demonstrate whether or not PICs are active during voluntary behavior. The difficulty lies in the fact that it is presently impossible to measure synaptic drive in awake, behaving animals, and, as such it is not possible to assess whether PICs have also been activated. Nonetheless, some behaviors have been associated with PICs. For example, self-sustained firing, whereby a motor unit continues to generate action potentials even though the subject has been instructed to relax completely, could be explained by ongoing PICs (Gorassini et al. 1998; Gorassini et al. 2004; Mottram et al. 2009; Walton et al. 2002).

Perhaps the most widely used measurement proposed to detect PICs to date is a method originally developed by Gorassini and others (2002a) (Fig. II.6).

This method uses the firing rate of a lower threshold, “control”, motor unit as an indicator of changes in synaptic drive to the motor pool. The relative level of synaptic drive, as measured by the control

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**Figure II.6.** Schematic to illustrate the calculation of ΔF. A subject generates force by an isometric ramp contraction. The control unit spike times are converted to a firing rate in the upper panel. The recruitment rate is the firing rate of the control unit when the test unit starts spiking. The derecruitment rate is the firing rate of the control unit when the test unit stops spiking. Recruitment rate is subtracted from derecruitment rate to obtain an estimate of the change in synaptic drive required to start and stop spiking in the test unit.
unit firing rate, is compared when a higher threshold, “test” unit is recruited and subsequently derecruited. If only synaptic currents are maintaining the test unit, then the firing rate of the control unit should be similar at test-unit recruitment and derecruitment. Now, if a PIC is instigated in the test unit once it has been recruited, then there are two sources of current sustaining the test unit; that associated with synaptic drive, and that arising from the PIC. Consequently, a lower synaptic drive (reflected in a lower firing rate of the control unit) will be associated with derecruitment of the test unit because of the added contribution of the PIC. The difference in firing rates of the control unit at recruitment compared to derecruitment is thought to reflect the added contribution of the PIC and is called $\Delta F$. There are many observations of positive $\Delta F$ values (Gorassini et al. 2002a; Gorassini et al. 2004; Mottram et al. 2009; Oya et al. 2009; Powers et al. 2008; Stephenson et al. 2011; Stephenson and Maluf 2011; Udina et al. 2010). There is also evidence that changing the neuromodulatory state can change the magnitude of $\Delta F$ (Udina et al. 2010; however see Stephenson and Maluf 2010), which these authors conclude is support for the notion that $\Delta F$ is related to PIC activation. Therefore, $\Delta F$ is a consistent, robust phenomenon that may be explained by PIC activation. One of the goals of this dissertation, however, is to determine whether other non-linear intrinsic mechanisms could also account for $\Delta F$ (see Chapters III, IV, and V). Here, I refer to non-linear in terms of intrinsic properties that disrupt the otherwise relatively linear relationship between synaptic current input and firing rate output.
ii. Spike threshold accommodation

Another non-linear property of motor neurons is spike-threshold accommodation. Accommodation has been both defined as an increase in the current threshold (Vallbo 1964a) or in the voltage threshold (Schlue et al. 1974b) for motor neuron spiking as the rate of rise of depolarizing current decreases. Experimental evidence demonstrates a shift in the current threshold (Schlue et al. 1974a; b; c; Vallbo 1964a; Sasaki and Otani 1961) as well as a shift in the voltage threshold (Homma et al. 1970) (Fig. II.7). The magnitude of accommodation in motor neurons appears to be modest compared to some other neuron types, with current threshold increasing to a maximum of two-fold above rheobase current during low rates of current injection (Sasaki and Otani 1961; Burke and Nelson 1971; Schlue et al. 1974c).

Figure II.7. Accommodation in motor neurons. For each panel, the upper trace is an intracellular recording while the lower sloping line indicates muscle stretch (which provided Ia synaptic input to the recorded motor neuron). Dashed line indicates firing level for slower stretch illustrated in upper panel. Arrows indicate firing threshold. At faster muscle stretch rates in lower panel, voltage threshold decreased. From (Homma et al. 1970)
Accommodation has most typically been studied during relatively short ramp durations (generally less than 500 ms). Indeed, one report suggested that most accommodation of threshold had taken place in the first 40-80 ms of current injection (Bradley and Somjen 1961). However, during longer ramp current injections (2 s), threshold accommodation was not complete until nearly the end of the current ramp in some motor neurons (Ushiyama et al. 1966). It would be interesting to investigate motor neuron accommodative properties during longer ramps; perhaps those neurons with a relatively fixed threshold at rapid ramp rates might show accommodation during longer ramps. Sasaki & Otani demonstrated a tendency for threshold to increase in otherwise fixed threshold motor neurons at the longest intervals they could test (see their Fig 5, Sasaki and Otani 1961).

a. Mechanisms of accommodation

The mechanism underlying threshold accommodation seems to be related to sodium channel inactivation (Bradley and Somjen 1961; Schlue et al. 1974b; Vallbo 1964b). In order to elucidate this, properties of the action potential were investigated by Schlue (1974b). The action potential rising phase is predominantly mediated by activation of fast sodium channels. It turns out that the action potential rate of rise is related to the external concentration of sodium; a lower external sodium concentration led to a slower action potential rate of rise in the squid giant axon (Hodgkin and Katz 1949; Huxley and Stampfli 1951). Functionally, changing the driving force for sodium could
be achieved with fewer sodium channels available to be activated, i.e. by decreasing the sodium conductance (Hodgkin and Huxley 1952a) thereby decreasing the maximum possible sodium current (also reviewed in Schlue et al. 1974b). Thus, the observation of Schule (1974b) that action potential rate of rise was lower in the motor neurons exhibiting accommodation supports the idea that accommodation is related to sodium channel inactivation.

b. Prevalence of accommodation

The prevalence of threshold accommodation varies. In some reports, all motor neurons showed a shift in threshold (Homma et al. 1970; Schlue et al. 1974c), but in other experiments, only a subset of neurons demonstrated accommodation (Sasaki and Otani 1961; Burke and Nelson 1971). Low threshold motor neurons may show less accommodation than high threshold motor neurons (Burke and Nelson 1971; Sasaki and Otani 1961). Conversely, however, other reports do not show a relationship between motor neuron characteristics and accommodation magnitude (Schlue et al. 1974c). Therefore, the extent of accommodation in different motor neuron populations remains to be fully elucidated. Further to this point, to my knowledge, accommodation has not been studied in cranial motor neuron populations. Cranial motor neurons undergo sodium channel inactivation (Powers et al. 1999), and therefore they might also exhibit threshold accommodation. Finally, accommodation may not be limited to the very first spike of a
motor neuron. During sustained activity the threshold for spiking can depolarize and this also appears to be related to sodium channel inactivation (Schwindt and Crill 1982).

iii. Firing rate adaptation

Early observations of spike frequency adaptation were published by Granit and colleagues (1963). Spike frequency adaptation is defined as a decrease in firing rate while the motor neuron is receiving a suprathreshold constant level of current injection or stimulus intensity. Adaptation has been observed in both spinal motor neurons (Granit et al. 1963; Kernell and Monster 1982), cranial motor neurons (Powers et al. 1999) as well as in many other neuron types. It is now generally recognized that there are at least two phases of adaptation: early and late adaptation (Powers et al. 1999; Brownstone 2006). An additional initial linear adaptation phase has also been described for rat hypoglossal motor neurons that takes place over the first few interspike intervals (Sawczuk et al. 1995). Adaptation has been be quantified in terms of the drop in firing rate over a certain period of time (Kernell and Monster 1982; Viana et al. 1995; Button et al. 2006), or by the time constant(s) describing the exponential decrease in firing rate (Sawczuk et al. 1995; Gorman et al. 2005). Both approaches provide valuable information about how motor neuron firing rate profiles change with time.
a. Characteristics of adaptation

Early adaptation takes place during the first second of spiking (Granit et al. 1963; Kernell 1965b) and typically features a rapid decay in firing rate from a high rate (Sawczuk et al. 1995). Indeed, the time constant of firing rate decay for early adaptation is \( \sim 200 \text{ ms} \) (Sawczuk et al. 1995; Gorman et al. 2005) but possibly briefer for higher threshold motor neuron (Gorman et al. 2005). There is also a decrease in the current-frequency gain as early adaptation progresses (Granit et al. 1963; Kernell 1965b). Also, over 90% of the total adaptation in firing rate takes place in the first 2s of the train, which could represent a decrease in firing rate of 100 imp/s or more (Sawczuk et al. 1995).

Sawczuk and colleagues (Sawczuk et al. 1995) did not find any correlation between the extent of early adaptation and membrane properties (and therefore recruitment threshold or cell size) but the magnitude of early adaptation was positively linked to the initial firing rate.

Analysis of late adaptation starts after the first second of spiking and lasts for many tens of seconds (Kernell and Monster 1982; Sawczuk et al. 1995). Late adaptation has a longer time constant of \( \sim 22-23 \text{ s} \) (Sawczuk et al. 1995; Gorman et al. 2005) (Fig. II.8). The magnitude of firing rate decrease is smaller for late compared to early adaptation, but there is still approximately a 40% decrease in firing rate over \( \sim 30 \text{ s} \) of spiking (Button et al. 2006; Kernell and Monster 1982; Sawczuk et al. 1995). In fact, the absolute magnitude of firing rate decrease is closely linked to the initial firing rate (Kernell and Monster 1982; Sawczuk et al. 1995; Gorman et al. 2005) (Fig. II.8). In
some instances, a relationship between the recruitment threshold and the magnitude of late adaptation has been reported (Kernell and Monster 1982; Gorman et al. 2005), although this relationship was weaker than that associated with initial firing rate (Kernell and Monster 1982). In other experiments, contrary to the results discussed above, no correlations between membrane properties and late adaptation were found (Sawczuk et al. 1995).

As discussed above, adaptation has been characterized with constant current injection (Kernell and Monster 1982; Sawczuk et al. 1995; Powers et al. 1999; Button et al. 2007) but adaptation may also contribute to the firing rate pattern of a lower firing rate on the descending arm of a ramp current injection, i.e. “clockwise hysteresis” (Bennett et
al. 2001b; Button et al. 2006; Cotel et al. 2009; Hamm et al. 2010; Meehan et al.). Additionally, in some human motor unit studies, a lower firing rate at derecruitment compared to recruitment has been observed (Milner-Brown et al. 1973b; Clamann 1970; De Luca et al. 1982) and has been attributed to adaptation (De Luca et al. 1982; Barry et al. 2007; Moritz et al. 2005). Finally, the processes that underlie adaptation might be able to be modulated; the overt decay in firing rate consistent with adaptation appeared to disappear during fictive locomotion (Brownstone et al. 2010).

b. Mechanisms underlying adaptation

More challenging to assess, however, has been the actual mechanisms underlying adaptation. Since the decay in firing rate can usually be fit by two exponentials, there are likely at least two distinct mechanisms underlying the adaptive process. There is some evidence to suggest summation of the AHP may lead to early adaptation (Granit et al. 1963; Sawczuk et al. 1995). However, there are also changes in spike shape as firing rates slow (Kernell and Monster 1982; Miles et al. 2005; Powers et al. 1999; Viana et al. 1995), which has led authors to conclude that sodium channel inactivation may contribute to early (Miles et al. 2005; Powers et al. 1999), and possibly to late adaptation (Brownstone et al. 2010; Powers et al. 1999). However, late adaptation appears to be quite robust in the face of many perturbations (Powers et al. 1999; Zeng et al. 2005) so mechanisms other than sodium channel inactivation are probably involved. In particular, a recent study suggested that adaptation in rat subthamalic nucleus neurons could be due
to activation of a slow potassium current that is neither dependent on calcium nor sodium entry for activation (Barraza et al. 2009).

Finally, for as much as is known about adaptation, the functional role remains obscure. Initially high firing rates have been suggested to be useful for quickly attaining a high force output (Brownstone 2006) but firing rates typical during early adaptation (that is, in excess of 100 imp/s) are rarely seen during voluntary behavior. As such, early adaptation may not be present during voluntary behavior. Late adaptation might have a role in fatigue by matching motor neuron spiking behavior to the changing muscle fiber contraction properties (Nordstrom et al. 2007; however see Fuglevand et al. 1999).

4. Summary

Overall, there is the potential that these non-synaptic properties of motor neurons influence motor unit activity during natural behaviors. Should this be the case, this would change fundamentally how researchers consider the input-output functions of motor neurons. In the following chapters, I will discuss experiments that I have carried out to address the possible contribution of PICs, accommodation and adaptation to motor unit activity during voluntary activity. Since it is not possible to measure these properties directly during natural behaviors, I have used a variety of indirect methods to infer their presence. Chapter III presents simulations of a motor unit population model to identify the possible contributions of each of these non-linear properties to the ΔF measure. Chapter IV presents the results from in vivo experiments that test the simulation
predictions, which in turn provides some evidence for which non-linear property likely makes a predominant contribution to the ΔF measure. Because it is not clear whether hypoglossal motor neurons generate PICs, Chapter V investigates the ΔF measurement in genioglossus motor units (innervated by hypoglossal motor neurons) to assess whether the evidence might support PIC activation. Chapter VI uses a different approach (inhibition-mediated abeyance of PIC activity) to probe the possible contribution of PICs to firing rate profiles during voluntary contractions.
III. EFFECTS OF PERSISTENT INWARD CURRENTS, ACCOMMODATION AND ADAPTATION ON MOTOR UNIT BEHAVIOR: A SIMULATION STUDY

1. Introduction

Mammalian motor neurons have long been considered as prototypical neurons (e.g. Eccles 1950) that integrate synaptic inputs from many sources and are thought to generate action potentials at rates proportional to the overall excitatory input (Granit et al. 1963). There are, however, a number of properties of motor neurons that may disrupt a simple relationship between synaptic input and firing rate output. Here, we consider three such properties and their effects on motor neuron output, namely, persistent inward currents (PICs), spike-threshold accommodation, and spike-frequency adaptation.

First described in the 1970s (Schwindt and Crill 1977), PICs have now been established as a robust intrinsic property of motor neurons (Bennett et al. 1998; Bennett et al. 2001b; Conway et al. 1988; Hamm et al. 2010; Hounsgaard et al. 1984; 1988a; Hounsgaard and Kiehn 1989; Hultborn et al. 2003; Lee and Heckman 1996; 1998a; b; Schwindt and Crill 1980; 1982; Turkin et al. 2010). PICs are thought to be mediated by voltage-activated dendritic (Bennett et al. 1998; Booth et al. 1997; Carlin et al. 2000b; Hounsgaard and Kiehn 1993; Lee and Heckman 1996) and somatic (Ballou et al. 2006; Moritz et al. 2007) channels that require neuromodulators, such as serotonin, to be enabled (Hounsgaard et al. 1988a). PICs may last for many seconds (Lee and Heckman 1998a) and predominantly comprise an L-type calcium current (Carlin et al. 2000a; Hounsgaard and Kiehn 1989) but persistent sodium currents may play a role in specific
circumstances (Li et al. 2004a; Manuel et al. 2007). PICs have been implicated in synaptic input amplification (Hultborn et al. 2003; Lee et al. 2003; Prather et al. 2001), as well as self-sustained firing, whereby motor neuron activity continues even after the cessation of excitatory synaptic drive (Conway et al. 1988; Crone et al. 1988; Hounsgaard et al. 1984; 1988a; Lee and Heckman 1998b). As such, PICs have been proposed to facilitate prolonged activity in motor neurons needed for maintenance of posture (Heckman et al. 2003; Kiehn and Eken 1998), to boost excitability of motor neurons during locomotion (Heckman et al. 2003), and to provide the primary source of depolarizing current to motor neurons during normal motor behaviors (Heckman et al. 2005).

Spike-threshold accommodation refers to a progressive increase in the amount of depolarizing current required to bring a neuron to action potential threshold as the rate of rise of current decreases (Hill 1936; Wigton and Brink 1944). In motor neurons, the current required to initiate activity can increase by as much as 2-fold with slow rates of rise of injected (Araki and Otani 1959; Bradley and Somjen 1961; Burke and Nelson 1971; Sasaki and Otani 1961; 1962; Schlue et al. 1974a; b) or synaptic current (Eccles 1946; Homma et al. 1970). The mechanisms underlying accommodation have not been completely elucidated but appear to be primarily related to sodium channel inactivation (Bradley and Somjen 1961; Hodgkin and Huxley 1952b; Kernell 2006; Schlue et al. 1974c; Vallbo 1964b).

Finally, spike-frequency adaptation represents a time-dependent diminution in firing rate in response to a constant current input, and in motor neurons can be divided
into early and late phases (Gorman et al. 2005; Powers et al. 1999; Sawczuk et al. 1995; Spielmann et al. 1993). Early adaptation involves the first few spikes at the outset of activity in response to a step increase in depolarizing current and is responsible for a marked decay in firing rate from initial high values, often in excess of 100 impulses/s (e.g. Sawczuk et al. 1995). Late adaptation, on the other hand, is characterized by a slow exponential drop in firing rate proportional to the magnitude of the injected current leading to decreases in firing rate by as much as 40 - 60% over a period of 30 s of continuous activity (Button et al. 2007; Kernell and Monster 1982; Sawczuk et al. 1995). While a variety of mechanisms have been proposed to account for early (Granit et al. 1963; Miles et al. 2005; Powers et al. 1999; Sawczuk et al. 1995) and late adaptation (Barraza et al. 2009; Partridge and Stevens 1976; Sawczuk et al. 1997), definitive mechanisms underlying adaptation are yet to be fully identified.

Because intrinsic properties such as those described above can markedly affect the way neurons transform input current into spiking output (e.g. Hamm et al. 2010), it is essential to characterize these properties in order to fully understand how neurons process information. And while such intrinsic properties have been studied extensively in motor neurons of reduced preparations, little is known about how these properties actually interact with synaptic input to shape the output response of a motor unit pool during natural behaviors. This is because it is presently impossible to quantify the extent of synaptic input to motor neurons in awake behaving animals (Fuglevand et al. 2006; Heckman et al. 2009).
In an attempt to overcome this difficulty, Kiehn and Eken (1997) and Gorassini and colleagues (Gorassini et al. 1998; Gorassini et al. 2002b; a) developed a clever method that involved using changes in firing rate of a low threshold motor unit to represent changes in synaptic drive to a population of motor neurons. This method was then used to assess the presence and magnitude of PICs in motor neurons using the following approach. The activities of pairs of human motor units were recorded during voluntary ramp increases and decreases in muscle force. The firing rate of the first recruited, “control”, unit was used as an indicator of synaptic drive. Because PICs are thought to be activated rapidly and fully near the time a motor neuron reaches spiking threshold (Bennett et al. 1998) and to remain relatively stable for several seconds thereafter (Lee and Heckman 1998a), any subsequent changes in firing rate that occur in the control unit after recruitment were taken to reflect changes in the distributed synaptic input acting on the motor neuron pool.

As a consequence, recruitment and derecruitment of the second, higher threshold, “test” unit could be registered in terms of changes in firing rate of the control unit. Once the test unit is recruited, and if a PIC is then instigated, there are two sources of depolarizing current sustaining activity in the test unit – that arising from synaptic input and that due to the intrinsic PIC. Subsequently, in order to terminate activity in the test unit, the total depolarizing current must drop below that associated with spiking threshold. Accordingly, the derecruitment threshold of the test unit should be associated with a lower overall synaptic drive (reflected in a lower firing rate of the control unit) compared to that at recruitment because of the added contribution of the PIC. As such, a
difference in firing rate of the control unit at the times of recruitment and derecruitment of the test unit, referred to as $\Delta F$, should be related to the magnitude of PIC activation.

An expanding number of reports consider positive $\Delta F$ measurements (i.e. higher firing rate of control unit at recruitment compared to at derecruitment of a test unit) as prima facie evidence for PIC activation in motor units (ElBasiouny et al. 2010; Gorassini et al. 2002b; a; Gorassini et al. 1998; Gorassini et al. 2004; Heckman et al. 2005; Heckman et al. 2008; Heckman et al. 2009; Kiehn and Eken 1997; Mottram et al. 2009; Oya et al. 2009; Powers et al. 2008; Stephenson and Maluf 2010; Udina et al. 2010). It seems feasible, however, that other nonlinear properties of motor neurons, such as accommodation or adaptation, might also lead to positive $\Delta F$ values. To evaluate this possibility, we used an updated version of a motor unit pool model (Fuglevand et al. 1993) in which we selectively included or omitted each of these nonlinear properties in simulations of muscle contractions. Because these individual properties depend differentially on rate of rise of excitation and on time, we also carried out simulations in which we varied contraction speed or duration while selectively enabling each nonlinear property. For each simulated condition, the magnitudes of $\Delta F$ were measured across populations of active motor units. Our results indicate that positive $\Delta F$ values can arise due to any one of these nonlinear properties, and therefore, a positive $\Delta F$ should not be considered exclusive evidence of PIC activation. However, we go on to show that changing the duration of ramp contractions may provide a means to distinguish among the probable causes of $\Delta F$. 
2. Methods

Motor unit spike times and isometric muscle force were simulated using a motor unit population model (for details, see Fuglevand 1993). To this model, we added representations of PICs, spike-threshold accommodation, and spike-frequency adaptation. Our overall approach involved simulating the phenomena, rather than the detailed biological mechanisms that shape the output behavior of a population of motor units. In this way, each motor neuron and motor unit could be represented individually with relatively few parameters. After a brief description of the original model, the physiological justification and procedures used to simulate the nonlinear features are described. The simulations presented here involved motor unit activities associated with isometric forces up to about 15% of the maximum force of the simulated muscle. This was done to simulate the conditions typically used in human motor unit experiments to assess ΔF. As such, the simulations primarily involved activities of low threshold motor units only. The model was implemented in the MATLAB (MathWorks Inc, Natick, MA) environment, and the software is available upon request.

i. Motor neuron pool

A population of 120 motor neurons was modeled to represent the pool innervating a typical human muscle. Each motor neuron received the same level of excitatory drive. Excitatory drive can be considered more or less equivalent to the net synaptic current
reaching the soma and spike-initiating zone. Motor neuron spike thresholds were determined by an exponential function where many motor neurons had low thresholds and few had high thresholds for recruitment. The range of recruitment thresholds \((RTE)\) across the pool was 50-fold. The minimum firing rate \((MFR)\) was set to 8 impulses/s \((\text{imp/s})\) for all motor neurons (Monster and Chan 1977; however see Moritz et al. 2005). If excitatory drive exceeded the assigned threshold for a particular motor neuron, \((i)\), then that motor neuron generated action potentials based on the following firing rate \((FR)\) equation:

\[
FR_i = g \times [E(t) - RTE_i] + MFR \quad (1),
\]

where \(g\) is a constant gain factor for all motor neurons and \(E(t)\) is a time-varying excitatory drive function. The units of excitatory drive are normalized such that 1.0 units of excitation represents the approximate level of excitation needed to recruit the lowest threshold motor neuron. For all simulations reported here, \(g\) was set to 1.0 imp/s/excitation unit. According to this equation, motor neuron firing rate increased linearly until a pre-determined maximum rate was reached: 35 imp/s for the first recruited motor neuron and down to 25 imp/s for the last recruited motor neuron. The normal variability in motor neuron discharge was emulated by adjusting the spike times to simulate a Gaussian random process with a 20% coefficient of variation (however, see Moritz et al. 2005).
ii. Force

Motor unit twitch force was modeled as an impulse response of a second order critically damped system. The lowest threshold motor unit innervated the smallest number of muscle fibers and generated the smallest twitch force, whereas the last recruited motor unit innervated the largest number of muscle fibers and produced the largest twitch force. Motor unit twitch forces varied exponentially as a function of recruitment order over a 100-fold range, such that most motor units were assigned relatively small twitch forces and relatively few, high-threshold units produced very large forces. Twitch contraction times were inversely related to twitch force and ranged from 30 to 90 ms such that the first recruited motor unit had the longest contraction time. A variable gain factor amplified motor unit twitch forces based on discharge rate in order to mimic the well-known sigmoidal relationship between discharge rate and isometric force. The total force produced by the muscle was a linear sum of the individual motor unit forces.

To this basic model, three modifications to the spiking behavior of the neurons were added: PICs, spike-threshold accommodation, and spike-frequency adaptation. These parameters could be selectively activated for each simulation.
iii. Persistent inward currents

A PIC provides an intrinsic source of excitation to motor neurons that is thought to boost the synaptic drive the motor neuron receives (Lee et al. 2003; Prather et al. 2001). Because calcium-mediated PICs appear to be the dominant form of PIC in motor neurons (Hounsgaard and Kiehn 1989), we focus our simulations primarily on this type, and hereafter refer to them simply as PICs. In the simulations, PICs were added as an excitatory ‘current’ that summed linearly with excitatory drive once a motor neuron was active. Because the activation threshold for PICs occurs near the time that motor neurons reach spiking threshold (Lee and Heckman 1998a; Li and Bennett 2003; Li et al. 2004b), particularly if motor neurons are activated synaptically (Bennett et al. 1998), PICs were therefore modeled to activate in each motor neuron at the moment the neuron was recruited (Fig. III.1).

Figure III.1. Schematic of PIC simulation. PICs (grey line) were simulated as a step increase in current added to the overall excitatory drive (“synaptic current”) (grey dashed line) at the time the motor neuron reached recruitment threshold. The “total current” (black) driving the motor neuron was the linear sum of the synaptic and PIC currents.
Intracellular recordings from motor neurons indicate that PICs may remain active for ten seconds or more (Carlin et al. 2000a; Lee and Heckman 1998a; Li and Bennett 2003), and a hyperpolarizing pulse may be needed to stop the self-sustained firing attributed to plateau potentials mediated by PICs (Hounsgaard et al. 1988a). While PICs recorded in vitro in high threshold motor neurons may decrement with time, PICs in low threshold motor neurons remain relatively stable over several seconds (Lee and Heckman 1998b). Therefore, for purposes of the present simulations involving mostly low threshold motor neurons, PICs were initially simulated as a steady level of excitatory current that remained active until the motor neuron was derecruited (see Fig. III.1).

The reported magnitudes of PICs (in nanoamperes) vary widely. Certainly, this is likely due to differences in experimental preparations used, particularly in terms of the neuromodulatory state of the preparation. However, this can also occur for motor neurons within a pool under similar experimental conditions. For example, Lee and Heckman (2000) showed only a weak tendency for higher threshold motor neurons supplying cat triceps surae to exhibit larger PICs. On the other hand, Hamm et al. (2010) found no systematic relationship between PIC magnitude and an indicator of recruitment threshold in rat hindlimb motor neurons. However, when PIC magnitude is expressed relative to recruitment threshold current (Hamm et al. 2010), low threshold motor neurons systematically exhibit larger normalized PICs than do high threshold motor neurons (Hamm et al. 2010; Lee and Heckman 2000). For example, depending on how PICs were measured, the lowest threshold motor neurons in the Hamm et al. study had PICs on the order of $1.5 - 2.5 \times$ threshold current whereas PICs for the highest threshold
motor neurons were $\sim 0.2 \times$ threshold current. Likewise, assuming rheobase currents of 3 nA and 30 nA for the lowest and highest threshold motor neurons, respectively (Gustafsson and Pinter 1984), and a roughly uniform PIC current of 7.8 nA across all motor neurons in the standard neuromodulatory state in the Lee and Heckman study (2000), the normalized PIC would be $\sim 2.6 \times$ threshold current for the lowest threshold neurons, and $0.26 \times$ threshold current for the high threshold neurons.

To partially mimic these values in our simulations, the absolute magnitude of the PIC was assigned the same value of 2.0 excitation units for all motor neurons. Therefore, for the lowest threshold motor neuron (with a recruitment threshold of 1.0 excitation units), this value represented a normalized PIC amplitude of $2.0 \times$ threshold excitation, whereas for a motor neuron with a 10-fold higher threshold, normalized PIC amplitude was $0.2 \times$ threshold excitation. These values, therefore, are similar to those associated with the experimental findings of Hamm et al. (2010) and Lee and Heckman (2000) (however, see Discussion).

It is not entirely clear as to how quickly a PIC activates. Some evidence suggests PICs activate in more or less an all-or-none manner (Bennett et al. 1998; Heckman et al. 2005; Hounsgaard and Kiehn 1989; Lee and Heckman 1999b) whereas other findings indicate that PICs are engaged more slowly, sometimes requiring a second or more to activate fully (Bennett et al. 2001a; Bennett et al. 2001b; Li and Bennett 2003; Li et al. 2004a). Furthermore, activation characteristics may depend on the magnitude of excitation (Hounsgaard and Kiehn 1989; Hultborn et al. 2003). Since there is no established consensus in the literature, our nominal simulations modeled PICs in the
simplest way, i.e. as being fully active at their recruitment (Fig. III.1). This assumption has also been adopted by Gorassini and colleagues associated with the $\Delta F$ measurement (Gorassini et al. 2004).

iv. Spike-threshold accommodation

The threshold for spiking may accommodate, that is, it may vary based on the rate of excitation delivered to the motor neuron (Araki and Otani 1959; Bradley and Somjen 1961; Burke and Nelson 1971; Homma et al. 1970; Sasaki and Oka 1963; Sasaki and Otani 1961; 1962; Schlue et al. 1974a; b; c). While some reports have shown higher threshold motor neurons to accommodate most (Burke and Nelson 1971; Sasaki and Otani 1961), others show low threshold motor neurons accommodate most (Bradley and Somjen 1961) or for accommodation to not vary systematically across motor neurons of differing thresholds (Schlue et al. 1974a). The magnitude of accommodation, namely, the current needed to bring a neuron to threshold during ramp current injection compared to that during a step current injection (i.e. the rheobase current, $I_{rh}$), ranges between $1.1$ and $3.3 \times I_{rh}$ with an average value of $\sim 1.8 \times I_{rh}$ during 1 s ramps (Schlue et al. 1974a). Note too that accommodation has also been reported based on an increase in the actual voltage threshold (Homma et al. 1970) at the onset of spiking. For our model, we defined accommodation based on the amount of additional current needed to bring a neuron to action potential threshold.
Figure III.2. Example accommodation-related changes in recruitment threshold associated with different rates of rise of excitation for two motor neurons. Solid lines indicate recruitment thresholds for motor neuron 5 (MN5) and 40 (MN40) that were derived from a simple inverse function of rate of rise of excitation. Dashed lines represent the nominally assigned values of recruitment threshold excitation for the two motor neurons, and can be considered equivalent to the rheobase current ($I_{rh}$) based on a step increase in current. Circles indicate the specific values of recruitment threshold excitation for three different rates of rise of excitation used in the simulations. For the slowest rate of rise, accommodation was modeled to increase threshold by ~63%.

Accordingly, we simulated accommodation in the following way. For every motor neuron, threshold increased above the nominally assigned recruitment threshold excitation (equivalent to rheobase current) as a simple inverse function of the rate of rise of excitatory drive (Fig. III.2). Because there is no clear consensus as to whether there are systematic differences in accommodation with threshold, we assigned the relative increase in threshold with increased rate of rise of excitation to be the same for all motor neurons. As described below, we tested three different rates of rise of excitation in these simulations: ~1.6, 3.2, and 6.4 excitation units/s. As shown in Figure 2 for two example
motor neurons (MN5 and MN40), the slowest rate of rise tested here would lead to about a 63% increase in threshold for both these neurons. This level of accommodation is in agreement with experimental findings, and may even partially underestimate the extent of accommodation because only high rates of rise of excitation have been tested experimentally. Last, it should be pointed out that accommodation as modeled here only affected the first spike in a train of spikes. Presumably, the rapid repolarization and afterhyperpolarization immediately following the first spike relieves most fast sodium channels from inactivation and thereby returns the spike-initiating zone to its near full complement of activatable sodium channels for the subsequent spike (however see Discussion).

v. Firing rate adaptation

Motor unit activity rarely exhibits the dramatic drop in firing rate at the outset of muscle contractions consistent with early adaptation. Because of this, and also because our simulations involved gradual rather than abrupt increases in excitatory drive, we did not consider early adaptation in the model. Instead, we focused on late adaptation, namely, a progressive, and relatively slow, reduction in firing rate in response to a steady level of depolarizing current. Late adaptation, which we refer to hereafter simply as adaptation, is a prominent feature of motor neuron activity (Button et al. 2007; Gorman et al. 2005; Hounsgaard et al. 1988b; Kernell and Monster 1982; Sawczuk et al. 1995; Spielmann et al. 1993; Turkin et al. 2010; Viana et al. 1995). The magnitude of
adaptation tends to be larger with greater levels of depolarizing current (Kernell and Monster 1982; Gorman et al. 2005; Sawczuk et al. 1995). Whereas some research suggests that the degree of adaptation is more pronounced in larger compared to smaller motor neurons (Kernell and Monster 1982), others suggest that recruitment order has no effect on adaptation magnitude (Gorman et al. 2005; Sawczuk et al. 1995). Therefore, adaptation magnitude was simulated here to be independent of excitation threshold.

While it is not clear what elicits late adaptation (Brownstone 2006), it may be due to a non-calcium dependent, slowly increasing potassium conductance (Barraza et al. 2009; Partridge and Stevens 1976; Sawczuk et al. 1997). Partridge and Stevens (1976) simulated this conductance as a simple time-dependent rising exponential with a relatively long time constant. Accordingly, we included an exponentially rising outward ‘current’ that was subtracted from the excitatory drive function to yield the net excitation acting on a modeled neuron (Fig. III.3A). The extent of this adaptation current, $A$, for any neuron $i$ was a function of both the time since motor neuron recruitment, $rt_i$, and the excitation level, $E(t)$, namely:

$$A(t, E) = q \times (1 - e^{-(t - rt_i)/\tau}) \quad (2),$$

where $e$ is the base of the natural logarithms and $\tau$ is the time constant. Based on the experimental observations of Sawczuk et al. (1995) and Gorman et al. (2005), we assigned $\tau$ to have a value of 22 s. The parameter $q$ in equation 2 designates the
maximum value of the adaptation current, and it depended on excitation level above threshold (i.e. $E(t) - RTE_i$) as:

$$q = \varphi \times [E(t) - RTE_i + d] \quad (3).$$

The parameter $\varphi$, was selected in order to match the magnitude of adaptation for different levels of excitation as reported by Kernell and Monster (1982) and was assigned a value of 0.67. Finally, the parameter $d$ was included to account for the observation that the minimum firing rate at recruitment appears not to be the absolute minimum firing rate that a motor unit can sustain (Barry et al. 2007). Since a lower firing rate at derecruitment relative to recruitment in human motor units may be due to adaptation (De Luca et al. 1982; Barry et al. 2007), the firing rate was allowed to decay with time below the initially specified minimum firing rate by a small amount determined by $d$. In the present simulations, $d$ was set to a value of 2 imp/s, similar to the values reported by Moritz et al. (2005) and Barry et al. (2007). Equation 3 was evaluated at each time step of the simulation (see below) to solve for $q$ based on the level of excitatory drive at each moment. The resulting instantaneous value of $q$ was then substituted into Equation 2 to estimate the adaptation current associated with that time step.

Figure III.3B shows examples of the simulated instantaneous firing rates of a motor neuron in response to three different levels of steady excitatory drive when adaptation, as represented by equations 2 and 3, was included in the model.
Figure III.3. Simulation of firing-rate adaptation. (A) Adaptation current, $A$ (top trace) was simulated as an exponentially rising outward current with a time constant of $\tau$. This current was subtracted from the excitatory drive, $E$, to yield the net excitation acting on a neuron. The adaptation current was constrained to a maximum level $q$ that depended on the magnitude of $E$. In the case of steady excitatory drive (middle trace), such an adaptation current would lead to a decrease in firing rate (FR) over time (bottom trace). (B) Example instantaneous (dots) and average firing rates (solid lines) for three different levels of steady excitatory drive (5, 10, and 20% of maximum excitation) in one motor neuron (motor neuron 5) when adaptation was enabled.
vi. Simulation protocol

A time resolution of 1 ms was used for all simulations. Isometric force and motor unit firing were initially simulated under four different conditions: control (no nonlinearities added to the model), inclusion of PICs, inclusion of accommodation, and inclusion of adaptation. In each case, the excitatory drive function linearly increased for 5 s and then decreased linearly back to baseline in 5 s giving rise to a triangularly-shaped force profile. The peak value of excitatory drive was selected such that the peak force under the control condition reached a value of about 15% of maximum force and was associated with recruitment of about 80 motor units. Maximum force (MF) was determined as the average force generated when all motor units in the pool were driven at supramaximal excitation levels.

These four conditions were further tested at two other rates of rise and fall of excitation, such that peak force (i.e. 15% MF) was achieved in 2.5 s (fast condition) or in 10 s (slow condition). Furthermore, we also carried out simulations for which we 1) varied the parameters characterizing PICs, 2) varied contraction duration independently of rate of rise of force, and 3) concurrently included all three nonlinear features in the model.
vii. Data analysis

The dependent measure of primary focus here was $\Delta F$ (see Introduction), which is calculated as the difference in firing rates of a low threshold motor unit between the instances at which a higher threshold unit is recruited and then derecruited during a triangular-shaped contraction. For each simulation condition tested here, ten low threshold motor units were used as control units (the first ten recruited units) and ten higher threshold motor units were used as test motor units (the 31st through 40th units recruited). By comparing each lower threshold control unit with each higher threshold test unit, 100 motor unit pairs were created for each simulation condition. Firing rates of control units were calculated as five-point moving averages. Linear interpolation of the five-point moving average was then used to identify the firing rate associated with the specific times at which higher threshold units were recruited and derecruited. The firing rate value at derecruitment was subtracted from that at recruitment to determine $\Delta F$ for each low threshold – high threshold motor unit pair. In addition, we also determined the recruitment and derecruitment forces for every unit as the muscle force at the instant of the first or last spike, respectively, during each triangular contraction. And lastly, motor unit firing rates at recruitment and derecruitment were calculated as the average firing rate over the first, and last five interspike intervals, respectively, for each simulated contraction.
viii. Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine if $\Delta F$ differed across the four primary simulation conditions (control, PICs included, accommodation included, adaptation included). In addition, ANOVA was carried out to determine if $\Delta F$ varied with simulated rates of rise or duration of contraction. Paired t-tests were performed to determine if recruitment and derecruitment firing rates and forces were different within simulation conditions. Significance was set at $p < 0.05$.

3. Results

The goal of these simulations was to determine whether properties of motor neurons other than PICs could lead to a positive $\Delta F$ and, should this be the case, to ascertain a way to distinguish which of these properties might best account for $\Delta F$ observed experimentally. Figure III.4A shows results from the primary set of simulations for each of the four test conditions. For each condition, in addition to the predicted whole muscle force, the firing patterns associated with a representative motor unit pair (a low threshold “control” unit and a higher threshold “test” unit) are shown. These units indicate the firings of unit 5 and unit 40 in the recruitment order.

Even though the excitatory drive function was identical for each trial, the peak force varied somewhat across the four conditions (15.2, 17.8, 13.8, and 14.4% maximum force, for the control, PIC, accommodation, and adaptation conditions, respectively, Fig.
III.4A. As would be expected, the PIC condition produced the largest force because every motor neuron, once recruited, received an additional source of excitation in the form of the simulated PIC. On the other hand, the lowest peak force was produced under the accommodation condition. This occurred because for the intermediate rate of rise of excitation used in these simulations, the recruitment threshold of all motor neurons was slightly increased above $I_{rh}$ when accommodation was enabled (see Fig. 2). Therefore, for the accommodation condition, fewer total motor units were recruited (75) compared to the other three conditions (84).

For the example pair of units shown in Figure III.4A, positive ΔF values, although small, were evident for the PIC, accommodation, and adaptation conditions, but not for the control condition. Across all 100 pairs of motor units, ANOVA indicated a significant effect (p < 0.001) of simulation condition on ΔF (Fig. III.4B). Post hoc analyses (Holm-Sidak method) indicated that individual mean ΔF values for each condition were significantly different (p < 0.05) from that of all other conditions. Furthermore, a simple location t-test indicated that while ΔF for the control condition was not significantly different from zero (p > 0.05), ΔF was positive and significantly different (p < 0.001) from zero for the PIC, accommodation, and adaptation conditions.
Figure III.4. Simulation results across the four main test conditions. (A) Muscle force and example discharge patterns for two motor units (units 5 and 40) under the control condition (no added nonlinearities), and with persistent inward currents (PICs), spike-threshold accommodation, and spike-frequency adaptation enabled separately. For each condition, the excitatory drive increased linearly to the same peak level in 5 s and then decreased symmetrically. Tick marks on force trace indicate spike times of the 5\textsuperscript{th} (lower set) and 40\textsuperscript{th} (higher set) motor units. The associated instantaneous (dots) and mean discharge rates (solid traces) are shown in the upper two panels for the lower threshold ‘control’ unit 5, and the higher threshold ‘test’ unit 40. Dashed vertical lines extending from spike times indicate times of recruitment and derecruitment of the test unit. Horizontal lines extending from the mean rate trace of the control unit indicate the firing rate of the control unit at the times of recruitment and derecruitment of the test unit. For the control condition, these two lines overlap. The difference between the firing rate of the control unit at the times of recruitment and derecruitment of the test unit is indicated as ΔF. (B) Mean (SD) ΔF values across 100 pairs of motor units for each simulation condition. * Significantly different from zero (p < 0.001), one-way t-test.
We also examined firing rates and force levels at recruitment and derecruitment of the motor unit population for each simulation condition. In the control condition, firing rates at recruitment and derecruitment were not different from one another (p > 0.05, Fig. III.5A). By comparison, the firing rates of simulated motor units under PIC, accommodation, or adaptation conditions were all lower at derecruitment compared to recruitment (Fig III.5A, p < 0.001 for all comparisons). Experimentally, a lower firing rate at derecruitment compared to recruitment is often observed in human motor units (e.g. Clamann 1970; De Luca et al. 1982; Milner-Brown et al. 1973b; Pascoe et al. 2011) and has been explained as a result of adaptation (De Luca et al. 1982) or as a result of PIC activation (Gorassini et al. 2002a). As far as we are aware, the contribution that accommodation might play in different firing rates at recruitment and derecruitment has not been discussed in the literature. In terms of the present simulations, higher firing rates at recruitment occurred with accommodation because the onset of spiking was delayed, and as a consequence, was associated with a higher level of excitatory drive compared to the control condition. Because firing rate was directly related to excitatory drive (Equation 1), this led to higher firing rates at the outset of spiking. Accommodation had no effect, however, on motor unit firing during the descending phase of excitation.
Figure III.5. Recruitment and derecruitment firing rates and force thresholds. Mean (SD) recruitment and derecruitment firing rates (A) and forces (B) for all units activated for each simulation condition. % MF – percentage of maximum force. * p < 0.001, paired t-test.

Additionally, we compared recruitment to derecruitment force thresholds. Under control simulations, the average force at recruitment was lower than that at derecruitment (p < 0.001, Fig. III.5B). A similar result was also found for the simulation that included adaptation. On the other hand, recruitment and derecruitment forces were not different
from one another when PICs or accommodation were included in the simulations \((p > 0.05, \text{Fig. III.5B})\). These disparate results parallel to some degree findings in experimental work. For example, some investigators (De Luca et al. 1982; Fuglevand et al. 2006; Milner-Brown et al. 1973a; Patten and Kamen 2000) found smaller forces at recruitment of human motor units compared to derecruitment – similar to the results reported here for the control and adaptation conditions. This finding has been explained primarily on the basis of muscle mechanics (De Luca et al. 1982; Fuglevand et al. 2006; Milner-Brown et al. 1973a). Other studies, however, have shown recruitment force to be larger than derecruitment force (Gorassini et al. 2002a; Person and Kudina 1972; Romaiguere et al. 1989; 1993). This type of result has been provisionally explained as due to PIC activation (Gorassini et al. 2002a) or to enhanced activity of antagonist muscles during the descending phase of triangular contractions (Fuglevand et al. 2006; Patten and Kamen 2000).

It might be noted that when examining average recruitment force values across experimental conditions (black bars, Fig III.5B), there was no difference between the accommodation and control conditions. At first glance, this result might seem unexpected given that accommodation was associated with higher excitation thresholds needed to recruit motor units compared to control. This, however, did not translate into higher forces at recruitment for the following reasons. With accommodation enabled, and at a given level of excitation during the rising phase, fewer motor units were recruited compared to control conditions. With fewer units recruited, the overall muscle force was diminished. Indeed, for this reason, peak muscle force was lowest for the
accommodation condition (see Fig. III.4A) despite identical excitatory drive across conditions. Therefore, lower muscle force at a given level of excitation in the accommodation condition tended to offset the higher level of excitation needed to recruit a unit, such that the net effect was little average change in recruitment thresholds (in terms of force) across the population.

i. Effect of changing simulated contraction speed on $\Delta F$

While the PIC condition yielded the largest $\Delta F$ values (see Fig. III.4B), the physiological significance of differences in the absolute magnitudes of $\Delta F$ across simulation conditions should be considered with caution. This is because the specific parameter values selected to represent the different nonlinear properties, while influenced by experimental findings, were chosen somewhat arbitrarily. Perhaps the most salient finding thus far is that positive $\Delta F$ values were observed for all three conditions involving nonlinear properties. Therefore, we next sought to determine whether it might be possible to distinguish among these properties as contributors to $\Delta F$ by varying characteristics of the muscle contraction. Firing-rate adaptation, for example, is a time dependent property of motor neurons, so if the total duration of the contraction changes, adaptation-related $\Delta F$ should also vary as a function of total contraction duration. Spike-threshold accommodation, on the other hand, depends on the rate of rise of excitation. As such, if the excitation rate of rise changes, accommodation-related $\Delta F$ should also be affected. PICs, however, as they were simulated here, should be relatively insensitive to
changes in contraction speed or contraction duration. Therefore, to test these ideas, we re-ran the simulations using slower and faster rates of rise of excitation than used in the initial simulations. The ‘slow’ simulations involved symmetric triangular excitation profiles that peaked at 10 s (rate of force rise = 1.5 % MF/s), ‘moderate’ speed peaked at 5 s (rate of force rise = 3.0 % MF/s), whereas for the ‘fast’ speed peak excitation occurred in 2.5 s (rate of force rise = 6.0 % MF/s). In all cases, the value of the peak excitation was the same. While the moderate speed conditions were the same as that used for Figure III.4, we reran those simulations nevertheless. Because of the imposed randomness in the specific discharge times for individual motor units, the average values of ΔF varied to some degree for each simulation under identical conditions.

Figure III.6A illustrates simulated muscle force and activities of an example motor unit pair for the control condition at the three different contraction speeds. For the example motor unit pair in Figure III.6A, there was a small positive ΔF for the slow condition, and negligible ΔF for the moderate and fast conditions. For all 100 motor unit pairs, there was no significant effect of contraction speed on ΔF for the control condition (p > 0.05, Fig. III.6B). Likewise, when PICs were enabled in the model, ΔF was not significantly different across contraction speeds (p > 0.05). However, when
Figure III.6. $\Delta F$ across simulation speeds. (A) Example simulations for the control condition only in response to triangular excitatory drive function that increased to the same peak value in 10 s (slow), 5 s (moderate), and 2.5 s (fast) and then decreased symmetrically. Motor units 5 and 40 are illustrated, as in Fig. 4A. See Fig. 4A legend for details. (B) Mean (SD) $\Delta F$ values for 100 pairs of motor units for each simulation condition across three simulation speeds. For the PIC condition, PICs were simulated to have with instantaneous onset and constant amplitude. There was a significant effect of contraction speed on $\Delta F$ for the accommodation and adaptation conditions. * $p < 0.05$ Post-hoc analysis (Holm-Sidak method).
accommodation was included in the model, there was a significant effect of contraction speed on $\Delta F$ ($p < 0.001$). Post-hoc analysis (Holm-Sidak method) indicated that $\Delta F$ decreased significantly for each increase in contraction speed in the accommodation condition. Likewise, simulations with adaptation enabled also showed a significant effect of contraction speed on $\Delta F$ ($p < 0.001$) with a significant diminution with each increase in contraction speed (Fig. III.6B). Overall, these results suggest an experimental means to distinguish the possible contribution of PICs versus accommodation or adaptation to $\Delta F$. If $\Delta F$ remains stable across contraction speeds, then this would be consistent with a primary role of PICs. If on the other hand, $\Delta F$ diminishes with contraction speed, then this would be more likely due to the contributions of accommodation or adaptation. Such a reduction in $\Delta F$ with increased contraction speed has recently been demonstrated experimentally in human motor units (Stephenson and Maluf 2011).

ii. Effects of varying PIC parameters

While these results seemed promising to distinguish among possible contributors to $\Delta F$ during voluntary contractions, it is possible that PIC behavior is more complex than our initial assumptions. In particular, there is evidence to suggest that the magnitude of PICs diminish over time (Hounsgaard and Kiehn 1989; Lee and Heckman 1998a) or might require a few hundred milliseconds to be fully expressed (Bennett et al. 2001a; Bennett et al. 2001b; Li and Bennett 2003; Li et al. 2004a). Therefore, we varied PIC
activation and decay parameters to test their effect on $\Delta F$ with two further sets of simulations involving variations in contraction speed and duration.

Figure III.7. Effect of modulating PIC activation characteristics. A) Mean (SD) $\Delta F$ values for 100 pairs of units across three contraction speeds for simulations in which PICs activated instantaneously but decay at a fixed rate of 50% over 10 s. There was a significant effect of simulated contraction speed on $\Delta F$ ($p < 0.05$) yet post-hoc analyses were not significant. B) Mean (SD) $\Delta F$ values for simulations in which PICs activated in 500 ms and decay at a fixed rate of 50% over 10 s. * $p < 0.05$ Post-hoc analysis (Holm-Sidak method).

In the first, PICs were activated instantaneously but then decayed linearly such that there would be a 50% reduction in PIC amplitude over 10 s, similar to the maximum rate of decay shown by Lee and Heckman for partially bistable motor neurons (Lee and Heckman 1998a). In this case, ANOVA indicated a significant ($p < 0.05$) effect of contraction speed on $\Delta F$ (Fig. III.7A). While post-hoc comparisons were not significant,
overall there was a tendency for $\Delta F$ to increase with contraction speed, opposite to the effect when accommodation or adaptation were activated (Fig. III.6B).

For the second set of simulations, we addressed the possibility that PICs do not activate instantaneously. For these simulations, PICs increased linearly from zero to their assigned maximum value in 500 ms and then decayed at the same rate as above. Interestingly, this modification also resulted in a significant effect of speed on $\Delta F$ ($p < 0.05$), yet in this case $\Delta F$ decreased as simulation speed increased (Fig. III.7B).

The results of these simulations indicated that PICs could have a variable effect on $\Delta F$ depending on the specific form of PIC activation. In particular, the results of the latter simulation demonstrated that such PIC activation could lead to a similar $\Delta F$ profile as seen with accommodation and adaptation (Fig. III.6B). This result could be seen to complicate the interpretation of $\Delta F$ as obtained experimentally. Therefore, we next sought to determine whether there was an additional test we could use to better distinguish among the motoneuron properties most likely contributing to $\Delta F$ observed experimentally.

iii. Effect of varying contraction duration

Because accommodation is dependent on rate of rise of excitation whereas adaptation is a time dependent phenomenon, we carried out simulations for which the total duration of the simulations was varied without changing the rate of rise. It seemed reasonable to expect, therefore, that varying the duration of the contraction in this way
might lead to differential effects on $\Delta F$ when each of these nonlinear properties were activated.

Figure III.8. $\Delta F$ across simulation durations. (A) Example simulations for the control condition only for three simulation durations: short (no plateau, 10 s total simulation time), medium (5 second plateau, 15 s total simulation time) or long (10 s plateau, 20 s total simulation time). Motor units 5 and 40 are illustrated, as in Fig. 4A. See Fig. 4A legend for details. (B) Mean (SD) $\Delta F$ values across 100 pairs of motor units for each simulation condition and across the three durations. Simple PIC condition involved simulating PIC with instantaneous onset and constant amplitude. The complex PIC condition involved a 500 ms activation time and 50% decay rate in 10 s. $a$ - significant difference from the short simulation duration ($p < 0.05$). $b$ - significant difference from the medium simulation duration ($p < 0.05$).
To vary the total duration of the simulation without changing the rate of rise, we inserted a plateau of constant excitation between the rising and falling phases of excitation. We used the moderate rate of rise (i.e. 5 s to reach 15% maximum force) and tested three simulation durations (Fig. III.8A): short (no plateau, 10 s total simulation time), medium (5 second plateau, 15 s total simulation time) or long (10 s plateau, 20 s total simulation time). For PICs in the simulations shown in Figure III.8A, we used the same characteristics as used for the simulations associated with Figure III.7B, namely, a 500 ms activation time and 50% decay rate in 10 s. In addition, we also carried out a set of simulations using the simpler form of PIC with instantaneous onset and constant amplitude (example not shown in Fig. III.8A).

As shown in Figure III.8B, for the control condition, there was no significant effect (p > 0.05) of contraction duration and $\Delta F$ values were small. Likewise, when using the simple form of PIC, there was no effect of contraction duration on $\Delta F$ (p > 0.05, Fig. III.8B). However, when the more complex form of PICs was enabled, there was a significant effect of contraction duration ($P < 0.05$) and post-hoc analysis indicated a significant decline in $\Delta F$ between the brief and long contractions (Fig. III.8B). When accommodation was included in the simulations, $\Delta F$ was not different across simulation durations (p > 0.05, Fig. III.8B). Finally, when adaptation was activated, $\Delta F$ increased significantly (p < 0.001) as simulation duration increased (Fig. III.8B). Therefore, by using contraction patterns that vary in duration only, it might be possible to differentiate among the contributions of these properties to $\Delta F$ recorded in vivo.
iv. Effects of multiple nonlinearities on $\Delta F$

Ultimately, motor neurons may operate with all of these nonlinearities activated concurrently. Therefore, to gain insight into how these properties might interact, we ran simulations with all three properties (PICs, accommodation, and adaptation) simultaneously activated. This was done for simulations that varied in contraction speed (Fig. III.9A) and contraction duration (Fig. III.9B). The excitatory drive functions associated with slow, moderate, and fast speeds (Fig. III.9A) were the same as those used in the simulations shown in Figure III.6A. Likewise, the excitatory drive functions for brief, medium, and long contractions (Fig. III.9B) were the same as those associated with Figure III.8A. It should be pointed out that the moderate speed condition in Figure III.9A is the same as the brief duration condition in Figure III.9B. In all cases, we used the more complex form of PIC with a 500 ms activation time, and 50% decay in 10 s.

The overall magnitudes of $\Delta F$ were larger in these simulations compared to previous simulations, indicating a general additive effect of these properties on $\Delta F$. Furthermore, as shown in Figure III.9A, there was a significant effect of contraction speed on $\Delta F$ ($p < 0.001$) with a significant decrement (Holm-Sidak test, $p < 0.05$) in $\Delta F$ for each tested increment in speed. Similarly, there was a significant effect of contraction duration (Fig III.9B) on $\Delta F$ ($p < 0.001$) with a significant increase (Holm-Sidak test, $p < 0.05$) in $\Delta F$ for each increase in duration. In both cases, the pattern of change in $\Delta F$ with speed or duration were dissimilar to that occurring when PICs alone were enabled (cf. Fig. III.6B and Fig. III.8B) but were generally comparable to that arising from
simulations in which accommodation or adaptation operated (also, Fig. III.6B and Fig III.8B).

Figure III.9. $\Delta F$ across simulation speeds and durations with multiple nonlinearities enabled. (A) Mean (SD) $\Delta F$ values for each simulation speed when PICs, accommodation, and adaptation were simulated simultaneously. PICs were simulated with a 500 ms activation time and a decay rate of 50% over 10 s. (B) Mean (SD) $\Delta F$ values for each simulation duration when PICs, accommodation, and adaptation were simulated simultaneously. PICs were simulated as in (A). * $p < 0.05$ Post-hoc analysis (Holm-Sidak method).
4. Discussion

Experiments involving reduced preparations in many species show motor neurons exhibit a variety of nonlinear spiking behaviors. Here, we have focused on three possible contributors to nonlinear spiking responses: PICs, which are thought to provide an intrinsic source of depolarizing current that boosts firing rates; spike-threshold accommodation, which alters the current needed to recruit a neuron depending on the rate of rise of current; and spike-frequency adaptation, which leads to a decrease in firing rate over time. Because it is experimentally difficult to characterize how these intrinsic properties influence motor neuron activity in awake behaving animals, we opted to address this issue using computer simulations. Furthermore, we restricted our focus here to the intriguing ΔF phenomenon, which has been reported experimentally (Gorassini et al. 2002b; a; Gorassini et al. 2004; Kiehn and Eken 1997; Mottram et al. 2009; Oya et al. 2009; Powers et al. 2008; Revill and Fuglevand 2009; Revill et al. 2010; Stephenson and Maluf 2010; Stephenson and Maluf; Udina et al. 2010) and is attributed to PICs (Gorassini et al. 2002a; Mottram 2009; Oya 2009). Our results indicate that a positive ΔF can arise only by the inclusion of nonlinear intrinsic properties; and as such, previous models of motor unit populations (Fuglevand et al. 1993; Heckman and Binder 1991b) cannot account for this physiological feature of motor unit behavior. The results of our simulations suggest that any one of the aforementioned nonlinear properties can lead to positive ΔF. Nevertheless, by examining the pattern of change in ΔF as a function of
contractions of varying duration, it may be possible to identify the predominant nonlinear property contributing to ΔF.

i. ΔF estimation of PICs – confounding effects of adaptation and accommodation

The original motivation for measuring ΔF is that it is considered an indicator of the presence and magnitude of PICs influencing firing of human motor units during natural behaviors (Gorassini et al. 1998; Gorassini et al. 2002b; a; Kiehn and Eken 1997). The ΔF calculation considers the firing rate of a lower threshold, “control” motor unit as an index of synaptic drive to the entire motor unit pool. If, however, the motor unit pool is subject to adaptation, then control-unit firing rate may not reflect changes in synaptic drive. Nevertheless, it has been argued that if adaptation magnitudes were similar across motor units, then adaptation would have little influence on ΔF (Gorassini et al. 2002a; Udina et al. 2010). In our simulations adaptation values were matched to experimentally observed values (Kernell and Monster 1982) and all motor units adapted in the same way, although the absolute magnitude of adaptation varied based on the extent of excitation above threshold for each motor unit. Despite being implemented in an identical way across the simulated motor unit population, adaptation had a marked effect on ΔF.

Therefore, in the absence of PICs, a test unit could be recruited and derecruited at the same level of synaptic drive, but will appear to be derecruited at a lower level of synaptic drive because the control-unit firing rate has diminished due to adaptation. Indeed, our
simulations suggest that this effect will be magnified in contractions of increasing duration.

An additional argument has been put forth that adaptation might not be present during voluntary contractions (Gorassini et al. 2002a; Udina et al. 2010) or during fictive locomotion (Brownstone et al 2010). Evaluating the presence of adaptation within the context of volitional movement is difficult since it is not possible to measure synaptic drive. Nonetheless, there is indirect evidence for firing-rate adaptation from studies in human subjects. For example, Moritz et al. (2005) and Barry et al. (2007) demonstrated that firing rates at recruitment are not the lowest level of steady firing motor units can achieve. Indeed, during threshold contractions, the minimal firing rate of human motor units decreased by 2 – 3 imp/s over a period of 5 – 20 s. These results were interpreted as being consistent with the effects of firing rate adaptation (Barry et al. 2007). Likewise, Johnson and colleagues (2004) showed a significant increase in surface EMG amplitude while subjects attempted to hold constant the firing rates of individual motor units. The increase in EMG was thought to reflect an overall increase in synaptic drive to the motor unit pool needed to offset the tendency for firing rate to decline caused by adaptation in the target motor unit. On this basis, it seems probable that firing rate adaptation may shape the activities of motor units during voluntary contractions.

As far as we are aware, accommodation has not been previously considered as a factor that influences ΔF. Bawa and colleagues, however, have suggested that spike-threshold accommodation might partially underlie rotation in the activities of low-threshold motor units during prolonged contractions (Bawa et al. 2006; Bawa and
Murnaghan 2009). Accommodation might also be responsible for the observed reduction in motor unit recruitment threshold at increased contraction speeds (Budingen and Freund 1976; Desmedt and Godaux 1978). As implemented in our simulations, accommodation shifted the first spike of the test unit toward a higher threshold, leading to a greater level of synaptic drive (as assessed by the firing rate of the control unit) at test unit recruitment compared to test unit derecruitment. Thus we found significant positive values of $\Delta F$ when accommodation was enabled. Therefore, this result also suggests that positive $\Delta F$ values can arise for reasons other than the presence of PICs.

ii. Limitations

While our model used here simulated each nonlinear property as distinct and non-interacting, this is unlikely to be the case in life (Hamm et al. 2010; Iglesias et al. 2011). For example, sodium-channel inactivation likely contributes both to accommodation (e.g. Schlue et al. 1974c) and adaptation (e.g. Miles et al. 2005). Thus the relative contribution of each may not be as easily distinguished.

Second, our simulations related to the effects of accommodation were limited to the first spike. It should be recognized, however, that with increasing stimulus strength, membrane potential between spikes can achieve relatively depolarized values (Schwindt and Crill 1982; Turkin et al. 2010), leading to sodium-channel inactivation. As such, accommodation might be expected to affect motor neuron discharge at time points after spiking onset. Whether this happens or not will depend critically on the magnitude and
perseverance of afterhyperpolarization (AHP) conductances needed to alleviate sodium-channel inactivation over the course of motor neuron discharge (Iglesias et al. 2011).

Another limitation with the present study has to do with the parameters of PICs used in the simulations. For example, the absolute amplitude of PICs was assigned the same value for all motor neurons in the pool. Whereas this formulation is consistent with experimental data obtained in cat motor neurons (Lee and Heckman 1998a) it is at variance with more recent findings in the rat (Hamm et al. 2010). Moreover, the magnitudes of PICs relative to rheobase for the simulated population were modest: approximately twice rheobase for the lowest threshold neurons and close to zero for the highest threshold neurons. Such low values of assigned PICs might partly account for the relatively small values of $\Delta F$ ($\sim 2$ imp/s) in simulations during which PICs were enabled. Experimentally, average values of $\Delta F$ in human subjects are $\sim 4$ imp/s (Gorassini et al. 2002a; Mottram et al. 2009; Stephenson et al. 2011; Stephenson and Maluf 2010). It should be noted, however, that when multiple nonlinearities were included in the model (Fig. III.9), simulated $\Delta F$ values did approach 4 imp/s.

Last, only relatively simple temporal patterns of PICs were simulated and the same patterns were imposed across the entire motor neuron population. The permutations tested ranged from the simplest case of instantaneous onset and fixed magnitude (Gorassini et al. 2004) to more complex forms of PIC behavior where the PIC needed time to activate fully and then decayed over time (Bennett et al. 2001a; Bennett et al. 2001b; Lee and Heckman 1998a; Li and Bennett 2003; Li et al. 2004a). PIC magnitudes, however, may vary with the magnitude of current injected (Hultborn et al.}
2003) and amount of inhibition present (Bui et al. 2006; Hultborn et al. 2003; Hyngstrom et al. 2008; Kuo et al. 2003). Furthermore, our model does not take into account the diverse nature of PIC activation observed across motor neurons within a pool. For example, PICs appear to decay more markedly in high-threshold compared to low-threshold motor neurons (Lee and Heckman 1998a). Despite these limitations, the model as implemented here provided a coarse overview of nonlinear properties and their effect on motor unit firing activity and the development of muscle force. To address each of the limitations of the model would require a more mechanistic and spatially complex neural models (e.g. Booth et al. 1997; Bui et al. 2006; Carlin et al. 2000b; Elbasiouny et al. 2006; Manuel et al. 2007; Miles et al. 2005; Powers 1993; Taylor and Enoka 2004).

iii. Other intrinsic properties

Our consideration of three intrinsic motor neuron properties (PICs, accommodation, and adaptation) should not be considered exhaustive. For example, Wienecke and colleagues (2009) have recently shown that the AHP in motor neurons elongates over the course of a ramp current injection. The mechanisms underlying AHP elongation were not elucidated, but a functional outcome of this elongation was that firing rate at derecruitment was lower than at recruitment. Similarly, Li and Bennett (2007) and Hamm and colleagues (2010) reported a voltage-activated, sustained outward current during triangular voltage-clamp experiments in rat motor neurons. Furthermore, Iglesias et al. (2011) recently demonstrated that slow inactivation of sodium channels can
lead to hysteresis in the current-frequency relationship. Each of these phenomena likely contribute to ΔF, and thus future investigations of ΔF should incorporate these findings.

iv. Predictions

The results presented here ultimately lead to testable predictions for human subject experiments. If motor unit pairs are compared across different contraction speeds and ΔF remains relatively constant, then PICs are likely a primary contributor to ΔF. If, however, ΔF is inversely modulated with contraction speed, then ΔF could be a result of accommodation, adaptation, or complex PIC behavior. To better distinguish among these three properties, varying contraction duration should be useful. If ΔF decreases as contraction duration increases, then PICs that activate slowly and show inactivation are likely dominate. If ΔF remains stable across contraction durations, PICs of fixed magnitude or accommodation are the most likely explanations. Finally, if ΔF increases as contraction duration increases, then adaptation is probably the dominant property contributing to ΔF. Indeed, recent experimental work by Stephenson and Maluf (2011) showed a progressive increase in the magnitude of ΔF as contraction duration increased up through 20 s (the longest duration tested here). This finding, therefore, is consistent with a primary role of adaptation rather than PICs in mediating ΔF.

In summary, we modified an existing motor unit pool model to test the effect of PICs, accommodation, and adaptation on the output of a motor pool. The results of our
simulations studies suggest that any of these properties lead to positive ΔF values. On this basis, it seems caution must be exercised when interpreting positive ΔF as it does not appear to be an exclusive indicator for the presence of PICs. Varying contraction duration and speed could provide experimental means to distinguish between these mechanisms as contributors to ΔF.
IV. EVALUATION OF AN IN VIVO METHOD TO DETECT PERSISTENT INWARD CURRENTS DURING VOLUNTAR BEHAVIOR

1. Introduction

Motor neurons were long thought to integrate synaptic inputs passively and to generate spikes at rates proportional to this input (e.g. Granit et al. 1963). It is now recognized, however, that motor neurons have active processes, such as persistent inward currents (PICs), that can modulate the relationship between synaptic input and firing rate output. PICs are thought to be predominantly mediated through L-type calcium channels (Carlin et al. 2000b; Hounsgaard and Kiehn 1989), which require depolarization in the presence of neuromodulators such as serotonin (Hounsgaard et al. 1988a; Hounsgaard and Kiehn 1989 or noradrenaline (Conway, 1988 #721; Lee and Heckman 1999a) to be activated.

While there is ample evidence in reduced preparations that motor neurons can generate large calcium mediated PICs (e.g. Bennett et al. 1998; Conway et al. 1988; Hamm et al. 2010; Hounsgaard et al. 1988a; Hultborn et al. 2003; Lee and Heckman 1998a), it is not clear in what context these currents might become active in life (Fuglevand et al. 2006; Hornby et al. 2002b; Nordstrom et al. 2007; Powers 2009; Powers et al. 2008). Although it is not possible to measure PIC activation in awake behaving animals, an innovative approach, developed by Kiehn and Eken (1997) and Gorassini and colleagues (Gorassini et al. 2002b; a; Gorassini et al. 1998), has been used to estimate their activation during voluntary muscle contractions. Since PICs are thought
to activate near recruitment threshold (Bennett et al. 1998) and to be relatively stable for many seconds thereafter (Lee and Heckman 1998a), changes in firing rate of a low threshold motor unit, once the initial effects of PICs have stabilized, may reflect changes in the overall synaptic drive acting on a pool of motor neurons (see Bennett et al. 2001a; Gorassini et al. 2004; Revill and Fuglevand 2011; Wienecke et al. 2009 for assumptions). As such, the firing rate of a lower threshold ‘control’ unit associated with recruitment, and then derecruitment of a higher threshold ‘test’ unit can be compared; any differences in firing rate at these two instances, called $\Delta F$, may be related to PIC activation in the test unit (Gorassini et al. 2002a).

To date, most experiments focused on the $\Delta F$ measure have been conducted in human subjects, but at least two reports examined the validity of $\Delta F$ in animal experiments. In each case, $\Delta F$ was readily observed in motor unit pairs from motor neuron populations previously identified as having large PICs (sacral motor neurons, Bennett et al. 2001a; and lumbar motor neurons in the decerebrate cat, Powers et al. 2008). However, it was also noted that $\Delta F$ can be quite variable and the $\Delta F$ value may not necessarily correspond closely to the activation of PICs (Powers et al. 2008). Additionally, Wienecke and colleagues (2009) suggested that elongation of the afterhyperpolarization, leading to a lower firing rate at derecruitment compared to recruitment, also may contribute to $\Delta F$.

Based on computer simulations, we have recently shown that intrinsic properties of motor neurons other than PICs should lead to significant $\Delta F$ values (Revill and Fuglevand 2011). One such property is spike threshold accommodation, whereby the
amount of depolarizing current needed to bring a neuron to spike threshold increases with slower rates of rise of current (Bradley and Somjen 1961; Burke and Nelson 1971; Homma et al. 1970; Sasaki and Otani 1961; 1962; Schlue et al. 1974a; b; c). Another property is spike-frequency adaptation that contributes to a time-dependent diminution in firing rate in response to a constant current input (Button et al. 2007; Gorman et al. 2005; Hounsgaard et al. 1988b; Kernell and Monster 1982; Powers et al. 1999; Spielmann et al. 1993; Viana et al. 1995). While PICs, accommodation, and adaptation led to positive $\Delta F$ values (firing rate of control unit was greater when the test unit was recruited compared to when it was derecruited), our simulations indicated that the effect of each on $\Delta F$ might vary as a function of contraction speed and duration.

In one set of simulations from that study (Revill and Fuglevand 2011), the excitatory drive to the modeled population of motor units increased and then decreased symmetrically giving rise to a triangularly-shaped muscle force profile. The time to peak excitation, however, was varied across conditions. Therefore, both the rate of rise of force, and the total contraction duration varied for this set of simulations. When PICs alone were enabled for these simulations, $\Delta F$ did not vary with contraction speed. However, when either accommodation or adaptation was included in the simulations, $\Delta F$ decreased significantly as contraction speed increased. Because accommodation is dependent on rate of rise of excitation whereas adaptation is a time dependent phenomenon, we also carried out another set of simulations for which the total duration of the simulations was varied without changing the rate of rise. This was accomplished by inserting a plateau of constant excitation but of varying durations between rising and
falling phases of excitation. In this case, when PICS were engaged, ΔF either did not change or decreased slightly with increased contraction duration, depending on the specific form of PIC simulated. When accommodation was simulated, ΔF did not change with variation in contraction duration. However, when adaptation was enabled in the simulations, ΔF increased with increased contraction duration.

Therefore, on this basis, we designed a series of experiments to evaluate changes in ΔF as a function of contraction speed and duration. We performed these experiments in two muscles: extensor digitorum, a hand muscle used predominantly in fine motor control, and tibialis anterior, an ankle dorsiflexor used for posture and locomotion. We selected these muscles because PICs are thought to be useful for posture (Heckman et al. 2009), whereas no role for PICs in fine motor control has yet been proposed. For both muscles, ΔF decreased with increasing contraction speed. Furthermore, ΔF increased with increasing duration of contraction, consistent with adaptation being the predominant mechanism underlying ΔF.

2. Methods

A total of 14 healthy human subjects (age range: 21-51 years) volunteered to participate in these experiments. Some subjects were tested on more than one occasion. Eight subjects participated in experiments involving motor unit recording from the extensor digitorum (ED) while 9 subjects participated in experiments involving the tibialis anterior (TA), three of whom participated in both sets of experiments. The
University of Arizona Human Subjects Committee approved all experimental procedures and every subject gave informed consent.

i. Experimental set-up

For all experiments, subjects were seated comfortably in a dental chair. The experimental set-up details for ED recordings have been published previously (Keen and Fuglevand 2003) with some minor changes and are described briefly here. All subjects were right handed. Each subject’s right forearm and hand rested on a metal platform where padded posts attached to magnetic stands secured the hand in a mid-supinated orientation with the wrist in a neutral position (~180 degrees) while the fingers were flexed approximately 90 degrees at the metacarpophalangeal (MCP) joint. This position was chosen to minimize the potential contribution of antagonist muscle activity to the measured force during the ramp contractions (Fuglevand et al. 2006). The subject’s third digit was attached to a force transducer (FT-10, Grass Instruments, Warwick, Rhode Island, USA) and a leather finger cuff placed around the proximal interphalangeal (PIP) joint. The other fingers were placed in similar finger cuffs so that all fingers were maintained at the same position.

For TA experiments, the right foot was secured to a custom-built footplate that rotated freely about an axis aligned collinear with the talocrural joint of the ankle. An isometric force transducer (FT-10, Grass Instruments, with custom built stiff springs) was attached to the distal end of the footplate at a fixed distance from the axis of rotation to
measure dorsiflexion force. A counterweight system was used to counterbalance the weight torque produced by the foot and footplate. The subject was positioned so that the knee was slightly flexed and the foot was plantarflexed approximately 70 degrees. Plantarflexing the ankle shortens the antagonist muscles and thereby minimizing their potential contribution to measured force (Fuglevand et al. 2006).

ii. Electromyography recordings

Motor unit action potentials were recorded using tungsten microelectrodes (Frederick Haer and Co. Bowdoinham, Maine, USA; 1–5 µm tip diameter, 5–10 µm uninsulated length, 250 µm shaft diameter, ∼200 kΩ impedance at 1000 Hz after insertion). Surface electrodes placed over bony prominences served as reference electrodes. We verified electrode location in the digit 3 compartment of ED by using low threshold electrical stimulation through the tungsten electrode. In some cases for ED, intramuscular fine wire electrodes (double stranded 50 µm) wires) were used in an attempt to increase recording stability. Intramuscular EMG signals were differentially amplified (x 1000), band pass filtered (0.3 – 3 kHz, Grass Instruments), displayed on a computer monitor as well as routed to an audio amplifier. EMG signals were digitized at approximately 25 kHz and force signals at 1 kHz (Cambridge Electronic Design Ltd., Cambridge, UK).

To locate one, or a few, motor units on an electrode, subjects were instructed to hold a low force level while the electrodes were gently manipulated until at least one
motor unit was clearly distinguishable on each electrode. Peak force for the contraction task was then estimated based on the recruitment thresholds of the motor units as well as the clarity of the recording at higher force levels.

iii. Experimental protocol

Initially, subjects were asked to generate three isometric maximum voluntary contractions (MVC) of the target muscle, and maximal force was defined as the largest force of the three trials. Subjects then performed a series of isometric triangular contractions with symmetric ramp increases and decreases in force. Two types of ramp contractions were performed: those that varied in contraction rate and those that varied in duration. Subjects were provided with an online template to guide their activity and they had the opportunity to practice the tasks prior to collecting data.

For the first type, we tested three different contraction rates, referred to as: slow (1.5% MVC/s), moderate (3% MVC/s) and fast (6% MVC/s). The contraction rates used in the present study generally span the range of values reported by others (Gorassini et al. 2002a; Mottram et al. 2009; Stephenson et al. 2011; Stephenson and Maluf 2011; Udina et al. 2010). In some ED experiments and all TA experiments for this type of task, a brief plateau (2 s) was inserted at the peak of the contraction. This served three purposes: it seemed easier for subjects to follow the shape of the contraction when a plateau was added, it ensured the test unit was active for a period thought to be sufficient for full PIC
activation (Mottram et al. 2009; Udina et al. 2010), and it tended to minimize the relative differences in contraction duration across the three contraction rates.

In experiments where contraction duration varied, the moderate contraction rate was used with brief (2 s), medium (5 s) and long (10 s) duration plateaus inserted between the rising and falling phases. The peak force of the contractions could vary to accommodate the recruitment characteristics of the motor units recorded.

For each type of contraction, the order of contraction speeds or durations was chosen randomly. Subjects performed five contractions at each speed or duration, with at least 10 seconds rest between each contraction. After all tasks were completed for a motor unit pair, subjects were given a few minutes to rest and then a new motor unit pair was sought. This involved moving the electrode within the current electrode tract or inserting the electrode at a new location. Experiments lasted up to two hours.

iv. Data analysis

Data were analyzed offline using Spike2 software and custom-designed scripts (Cambridge Electronic Design Ltd, UK). Recruitment threshold was measured as the force at the time of the first spike of the train for which the interspike interval was less than 500 ms (Fuglevand et al. 2006). Derecruitment threshold was similarly measured as the force at the time of the last spike where the preceding interspike interval was less than 500 ms. Recruitment and derecruitment firing rates were calculated based on the first (or last in the case of derecruitment) three spikes of the unit (Mottram et al. 2009).
For accurate $\Delta F$ measurements, the control unit firing rate should reflect changes in synaptic drive at the moment of test unit recruitment and derecruitment (e.g. Gorassini et al. 2002a). In particular the control unit firing rate could become saturated, such that its firing rate is no longer modulated with force, thus preventing it from indicating changes in synaptic drive (Fuglevand et al. 2006). To address this concern, Stephenson and Maluf (2011) proposed a calculation that would determine whether the control unit firing rate was likely saturated when the test unit was recruited and derecruited. We have adopted their method for our analysis, rather than the more commonly used rate-rate plot that compares the firing rate of two concurrently active motor units (Gorassini et al. 2002a; Mottram et al. 2009; Powers et al. 2008).

$\Delta F$ was calculated by using linear interpolation of a five-point moving average to determine control unit firing rate at the time of test unit recruitment and then derecruitment. The control unit firing rate at test unit recruitment was subtracted from that at test unit derecruitment to obtain $\Delta F$. Occasionally, the control unit did not generate sufficient spiking activity around recruitment or derecruitment of the test unit to calculate a five-point average; in these cases, the instantaneous firing rate was used instead. Furthermore, in rare events, a recruitment or derecruitment reversal occurred (i.e. the higher threshold unit started spiking prior to the lower threshold unit, or the lower threshold unit stopped spiking before the higher threshold unit) (e.g. De Luca et al. 1982; Henneman et al. 1974; Tanji and Kato 1973). When this occurred, the averaged control unit firing rate was linearly extrapolated to the time of test unit recruitment, or derecruitment, by using the first, or last, two averaged firing rate values for extrapolation.
However, if the extrapolated firing rate was less than zero, the firing rate defaulted to zero since the lowest firing rate a motor neuron can exhibit is 0 imp/s.

$\Delta F$ was calculated for each motor unit pair for each contraction speed and then averaged so that every motor unit pair had one averaged $\Delta F$ value for every contraction speed. After excluding motor unit pair activity from some trials because the control unit likely showed firing rate saturation when the test unit was active, between one and six contractions were used for each average $\Delta F$ value.

Contraction rates of rise and fall were compared with a paired t-test. A one way repeated measures analysis of variance (RM ANOVA) was used to determine whether $\Delta F$ varied across contraction speeds or contraction durations. Additionally, one way RM ANOVA was used to determine whether firing rates or forces measured at recruitment and derecruitment varied as a function of recruitment and derecruitment. Data is presented as mean (standard deviation). Significance was set at 0.05.

3. Results

We report here on firing rate behavior of 82 ED motor units and 72 TA motor units during isometric triangular ramp contractions at varying contraction speeds and durations. The recorded motor unit population was likely comprised of predominantly lower threshold motor units because the peak force for contractions was relatively low. For ED, the mean (SD) peak force attained was 12.3 (9.1) % MVC (range 5 – 50% MVC)
and for TA, the mean (SD) peak force attained was 8.0 (2.7) % MVC (range 5-15% MVC).

An example contraction is illustrated for each of three speeds for an ED experiment (Fig. IV.1A) and for a TA experiment (Fig. IV.1B). In Fig. IV.1A, each electrode recorded one ED motor unit: a lower threshold control unit (bottom trace) and a higher threshold test unit (upper trace). In this example, the firing rate difference of the control unit at test unit recruitment and subsequent derecruitment yielded a difference in firing rate ($\Delta F$) of 8.2 imp/s at the slow speed, 4.7 imp/s at the moderate speed and 4.6 imp/s at the fastest speed. In Fig. IV.1B, it was possible to discriminate 2-3 TA motor units on each electrode, but we have illustrated firing rate values and associated $\Delta F$ values from one motor unit pair – the smaller unit on the lower recording served as the control unit and the larger unit on the upper recording was the test unit. $\Delta F$ values from this motor unit pair were 1.8 imp/s for the slow contraction, 1.5 imp/s for the moderate speed, and 0.3 imp/s for the fast speed. Finally, for the ED motor unit pair illustrated in Fig. IV.1A, the peak goal force was 12% of the subject’s MVC, therefore the total contraction duration is longer than that observed for the TA motor unit pair illustrated in Fig. IV.1B, where the peak goal force was only 6% MVC.
Figure IV.1. Example recording of motor units from the third compartment of extensor digitorum (A) and from tibialis anterior (B) during isometric triangular ramp contractions at three different speeds. Traces from bottom to top (A and B): raw intramuscular EMG signal for control unit, instantaneous firing rate of control unit, raw intramuscular EMG signal for test unit, instantaneous firing rate of test unit, exerted isometric force (thick black line) and goal force (thin grey line). Insets are an overlay of discriminated motor units. $\Delta F$ calculation is illustrated by dashed lines.
Overall, subjects generally produced rates of contraction close to the goal force traces, i.e. 1.5% MVC/s (slow), 3.0% MVC/s (moderate) and 6 %MVC/s (fast). For ED, on average, the slow contraction force rate of rise (1.55 (0.29) %MVC/s) was similar to the force rate of decay (1.63(0.28) %MVC/s) (p > 0.05, paired t-test). At the moderate speed, the average force rate of rise (2.80 (0.30) % MVC/s) was significantly slower than the average rate of decay (3.06 (0.39) %MVC/s) (p < 0.01, paired t-test). At the fast speed, the average force rate of rise (5.10 (0.94) %MVC/s) was not significantly different from the average force rate of decay (5.34 (1.21) %MVC/s) (p > 0.05, paired t-test). For the slow contraction in TA, the average force rate of rise (1.43 (0.13) % MVC/s) was significantly slower than the average force rate of decay (1.83 (0.56)% MVC/s) (p < 0.001, paired t-test). For the moderate speed, the average force rate of rise (2.74 (0.33)% MVC/s) was also significantly slower than the average force rate of decay (3.07 (0.42)% MVC/s) (p < 0.01, paired t-test). Finally, for the fast speed, the average force rate of rise (5.25 (0.64)% MVC/s) was not different from the average force rate of decay (5.26 (0.90)% MVC/s) (p > 0.05, paired t-test).
Figure IV.2. Threshold behavior for ED and TA motor units. Recruitment (black bars) and derecruitment (grey bars) force (A, B) and firing rate (C,D) for 82 ED motor units (A,C) and 72 TA motor units (B,D). Data is presented as mean (SD). * p < 0.05, Holms-Sidak method.

As well as ΔF, we examined threshold and firing rate at recruitment and derecruitment to consider population motor unit firing behavior. For ED motor units, recruitment force was significantly smaller than derecruitment force at the moderate and fast contraction speeds (p < 0.05, Fig IV.2A). A similar result was found for TA motor units (p < 0.05, Fig IV.2B). A lower recruitment compared to derecruitment threshold has been previously reported in the literature (De Luca et al. 1982; Fuglevand et al. 2006; Milner-Brown et al. 1973b; Patten and Kamen 2000; however see Gorassini et al. 2002a; Person and Kudina 1972; Romaiguere et al. 1989; 1993) and may be explained by muscle
mechanics (De Luca et al. 1982; Fuglevand et al. 2006; Milner-Brown et al. 1973b).

Additionally, motor unit firing rate was lower at derecruitment compared to recruitment across all contraction speeds in both ED and TA motor units (p < 0.05, Fig. IV.2C & D). A lower firing rate at derecruitment compared to recruitment has been previously reported and has either been attributed to spike frequency adaptation (Barry et al. 2007; De Luca et al. 1982; Milner-Brown et al. 1973b) or to activation of PICs (Gorassini et al. 2002a).

Of the recorded motor units, 76 motor unit pairs were compared from ED and 64 pairs from TA. If more than two motor units in a trial were clearly discernable, motor units were combined to make multiple pairs. In ED, approximately 11% of motor unit pair activity was excluded from further $\Delta F$ analysis at each contraction speed because the control unit firing rate may have saturated (Stephenson and Maluf 2011). On average, positive $\Delta F$ was observed at every contraction speed (Fig IV.3A). Furthermore, average $\Delta F$ decreased significantly from the slow to fast contraction speed (p < 0.05, Fig. 3A).

From the TA recordings, data from approximately 24% of motor unit pair activities were excluded from further $\Delta F$ analysis because the control unit firing rate may have saturated (Stephenson and Maluf 2011). $\Delta F$ values from TA motor unit pairs also decreased as contraction speed increased (p < 0.05, Fig. 3B). In addition to demonstrating a similar pattern across muscle types, the average $\Delta F$ values from TA motor units were consistently smaller than the average $\Delta F$ values observed in ED motor units.
Figure IV.3. Mean (SD) $\Delta F$ for different contraction rates. A) $\Delta F$ measured from ED motor unit (MU) pairs (slow: 75 MU pairs, moderate: 76 MU pairs, fast: 71 MU pairs). B) $\Delta F$ measured from TA MU pairs (slow: 63 MU pairs, moderate and fast: 64 MU pairs), * $p < 0.05$, Holms-Sidak method. Overall, $\Delta F$ decreased as contraction speed increased.

For the second type of ramp task, different duration hold-phases were maintained between the ramp increases and decreases in force (at 3% MVC/s). This task type was only carried out in TA. Examples of each contraction duration are illustrated in Fig. 4. In this case, two motor units were clearly distinguishable on one electrode – a smaller amplitude, low threshold control unit and a larger amplitude, higher threshold test unit.
In this example, $\Delta F$ was 1.5 imp/s for the brief contraction, increased to 3.6 imp/s for the medium contraction and increased further to 6.4 imp/s during the long contraction.

Overall, based on 40 - 64 TA motor unit pairs, there was a significant increase in $\Delta F$ as contraction duration lengthened ($p < 0.05$, Fig. IV.5).

Figure IV.4. Example recording of TA motor units during isometric triangular ramp contractions at three different durations. Traces from bottom to top: intramuscular EMG signal, instantaneous firing rate of control unit, discriminated motor unit action potentials of test unit, instantaneous firing rate of test unit, exerted force (thick black line) and goal force (thin grey line). Insets indicate overlay of discriminated motor unit shapes. $\Delta F$ calculation is illustrated by dashed lines.
4. Discussion

We have evaluated a technique that was originally developed to estimate the activation of PICs during natural behaviors. Like many other researchers (Gorassini et al. 2002b; a; Mottram et al. 2009; Oya et al. 2009; Powers et al. 2008; Stephenson et al. 2011; Stephenson and Maluf 2010; 2011; Udina et al. 2010), we observed positive ΔF values, i.e. the control unit firing rate was lower when a higher threshold test unit was derecruited compared to when it was recruited (see Fig. 1). ΔF was robustly observed in two motor unit pools: extensor digitorum (used predominantly in fine motor control) and tibialis anterior (used for postural control and during locomotion) across different contraction speeds and durations. Based on predictions from computer simulations
(Revill and Fuglevand 2011), the pattern of ΔF we observed (i.e. ΔF increased as contraction duration increased) is most consistent with spike frequency adaptation dominating the ΔF measurement.

i. Comparison to other experimental results

We report on ΔF values for the first time in a hand muscle, extensor digitorum. The ED ΔF values obtained in this study are similar to ΔF values presented for other muscles, i.e. ~4 imp/s (e.g. tibialis anterior and soleus, Gorassini et al. 2002a; biceps brachii, Mottram et al. 2009); trapezius, Stephenson, 2010 #860; tibialis anterior, Udina, 2010 #689). By contrast, our average TA ΔF values are lower by ~2 imp/s than those values reported previously (Gorassini et al. 2002a; Udina et al. 2010), although our average values fall within the range of ΔF values reported by Stephenson and Maluf (2011).

It is not immediately clear why this discrepancy in TA ΔF values across studies might exist. ΔF appears to depend on a variety of contraction parameters beyond contraction speed and duration. In particular, there is a relationship between ΔF and the duration of control unit spiking prior to test unit activation (Stephenson and Maluf 2011; Udina et al. 2010), as well as extent of rate modulation of the control unit, duration of test motor unit activity, rate of discharge increase in the control unit, and also recruitment threshold of the test unit (Stephenson and Maluf 2011). Therefore, a variety of contraction parameters may influence ΔF. Previous studies achieved a higher peak force
than we were able to obtain (i.e. 12-18% MVC compared to ~8% MVC in the present study) (Stephenson and Maluf 2011; Udina et al. 2010). As such, other studies might have used longer duration contractions, or recorded higher threshold test units, or also achieved a greater separation between control and test unit recruitment time. Any combination of these factors could have resulted in larger ΔF values in these studies compared to our present results.

ii. ΔF across muscles

In the current results, ΔF was greater for ED compared to TA motor units. One interpretation could be that ED motor units generate larger PICs than TA motor units (Gorassini et al. 2002a). ED is an extensor muscle whereas TA is a flexor muscle. Results from two experiments suggest that PICs may be more prominent in extensor compared to flexor motor neuron populations (Cotel et al. 2009; Hounsgaard et al. 1988a). This would be consistent with the present ΔF results, if indeed PICs are the main contributors to ΔF.

iii. Possible contributors to ΔF

Previous modeling results (Revill and Fuglevand 2011) demonstrated that non-linear mechanisms were required for motor unit pairs to demonstrate positive ΔF. PICs are thought to lead to ΔF because they contribute an ongoing source of excitation that
activates near recruitment threshold (Bennett et al. 1998) which allows the motor unit to continue spiking below the level of synaptic excitation usually associated with derecruitment (Gorassini et al. 2002a; Gorassini et al. 2004). Thus, test unit recruitment is only determined by synaptic drive, but test unit derecruitment occurs when the combined excitation from PICs and synaptic drive drops below that unit’s derecruitment threshold. Therefore, the control unit firing rate, the proxy for synaptic drive, would be lower at test unit derecruitment compared to recruitment, leading to positive $\Delta F$.

We also showed in simulations that accommodation could contribute to positive $\Delta F$ (Revill and Fuglevand 2011). As we simulated it, spike threshold accommodation leads to a larger synaptic drive needed for recruitment (Burke and Nelson 1971; Homma et al. 1970; Sasaki and Otani 1961; 1962) but has no influence on derecruitment. Thus, the control unit firing rate, as the proxy for synaptic drive, would reflect a higher firing rate (i.e. synaptic drive) at test unit recruitment compared to derecruitment, therefore leading to positive $\Delta F$. Changing the contraction rate should influence $\Delta F$ magnitude, if accommodation contributes to $\Delta F$.

Finally, spike frequency adaptation could also lead to positive $\Delta F$ (Revill and Fuglevand 2011). As a function of time, adaptation influences the relationship between the level of synaptic drive and the rate of spiking (e.g. Kernell and Monster 1982; Sawczuk et al. 1995). Thus, at test unit recruitment the control unit firing rate would be higher than at test unit derecruitment due to the time-dependent effects of adaptation. This would also result in positive $\Delta F$. Changing contraction duration should influence $\Delta F$ if adaptation is responsible for $\Delta F$. 
The present results demonstrated that $\Delta F$ decreased as contraction speed increased and contraction duration decreased (c.f. Fig. IV.3). This result would be most consistent with accommodation or adaptation contributing to $\Delta F$ (Revill and Fuglevand 2011). Furthermore, the present results also demonstrated that $\Delta F$ increased as contraction duration increased. Therefore, the most parsimonious explanation is that adaptation is the predominant contributor to $\Delta F$ (Revill and Fuglevand 2011).

iv. Evidence for spike frequency adaptation

While it is presently not possible to measure directly spike frequency adaptation during voluntary behavior, certain motor unit behaviors have been attributed to this process. For example, a lower firing rate at derecruitment compared to recruitment has been suggested to be due to adaptation (Barry et al. 2007; De Luca et al. 1982; Milner-Brown et al. 1973b; however see Gorassini et al. 2002a). Also, when subjects are instructed to maintain the firing rate of a motor unit constant, there is a gradual increase in whole muscle surface EMG (Johnson et al. 2004). Such an increase in surface EMG was interpreted as an overall increased synaptic drive needed to counteract adaptation in the target motor unit (Johnson et al. 2004). Furthermore, there is some evidence that adaptation and PICs may even be concurrently active in motor neurons. Hamm and colleagues (Hamm et al. 2010) measured PIC magnitude in voltage clamp and frequency-current relationships in current clamp in the same motor neurons. Their results suggest that rat hindlimb motor neurons may have activated PICs even though the firing rate
profile during triangular current injections was stereotypical for adaptation (i.e. firing rates are lower on the descending compared to ascending limb, or clockwise hysteresis) (Hamm et al. 2010). Therefore, adaptation is likely an important feature of motor unit behavior and may coexist with expression of PICs.

Furthermore, an alternate interpretation of the higher $\Delta F$ values in ED compared to TA could be related to firing rate adaptation. The magnitude of adaptation is proportional to the initial firing rate of the motor neuron (Gorman et al. 2005; Kernell and Monster 1982; Sawczuk et al. 1995). ED motor units tended to fire at a higher rate than TA motor units during the ramp contraction tasks, for example, average ED motor unit recruitment firing rates were greater than TA motor unit recruitment firing rates (Fig. IV.2). Therefore the observed larger $\Delta F$ values in ED motor unit pairs could reflect a larger extent of spike frequency adaptation compared to TA motor units.

v. Limitations of $\Delta F$

Our results are also not the first to suggest that $\Delta F$ may have some limitations as a metric to estimate PIC activation during natural behaviors. Two recent publications have highlighted variability in $\Delta F$ that may be independent of PIC activation (Powers et al. 2008; Stephenson and Maluf 2011). For example, the magnitude of rate modulation in the control unit is positively correlated with $\Delta F$ magnitude, suggesting that rate modulation of the control unit sets an upper limit for $\Delta F$ values (Powers et al. 2008; Stephenson and Maluf 2011). Powers and colleagues (2008) further demonstrated
conceptually how the activation characteristics of a PIC in the control unit could affect dramatically the obtained $\Delta F$ value, even though PIC magnitude in the test unit was constant. As a consequence, each of these studies has created a rather stringent list of criteria to fulfill in order for $\Delta F$ to be attributed to PIC activation. However, our present results, coupled with our previous modeling work (Revill and Fuglevand 2011), suggest that even if these criteria are fulfilled, $\Delta F$ values may still not accurately reflect PIC activation.

In conclusion, $\Delta F$ is a robust phenomenon of motor unit behavior that is consistently observed among motor units in postural and hand muscles. While we cannot exclude the possibility that PICs are contributing to $\Delta F$, we suggest that $\Delta F$ may not be the best method to detect PIC activation during natural behaviors. Based on the increase in $\Delta F$ with contraction duration seen in the present experiments, and the results of our previous simulations (Revill and Fuglevand 2011), we conclude that spike frequency adaptation is likely the predominant mechanism underlying $\Delta F$. Therefore, further work will be needed to develop a metric to assess possible PIC activation during natural behaviors.
V. EVALUATING GENIOGLOSSUS MOTOR UNIT ACTIVITY FOR THE PRESENCE OF PERSISTENT INWARD CURRENTS

1. Introduction

Persistent inward currents (PICs) are a source of long lasting excitatory current (Conway et al. 1988; Hounsgaard et al. 1988a; Lee and Heckman 1998a) that can add to synaptic currents to influence motor neuron activity (Hultborn et al. 2003; Prather et al. 2001). The primary current underlying PICs in spinal motor neuron pools is mediated by a long-lasting L-type calcium current (Carlin et al. 2000b; Hounsgaard and Kiehn 1989), which requires serotonin (Hounsgaard et al. 1988a) or noradrenaline (Conway et al. 1988) as well as depolarization (Bennett et al. 1998) for activation. Recent evidence suggests that hypoglossal motor neurons which reside in the brain stem and innervate tongue muscles, may also generate PICs (Lamanauskas and Nistri 2008; Moritz et al. 2007; Nani et al. 2010; Powers and Binder 2003). However, the prolonged depolarizations ('plateau potentials') associated with activation of PICs in spinal motor neurons are absent in hypoglossal motor neurons (Greer and Funk 2005; Powers and Binder 2003). This may be due to the relative paucity in hypoglossal motor neurons of the L-type calcium channel thought to mediate PICs (Haddad et al. 1990; Viana et al. 1993b; a). As such, PIC activation in hypoglossal motor neurons should be modest in comparison to spinal motor neuron pools. Therefore, we decided to examine activity from genioglossus muscle units innervated by hypoglossal motor neurons for evidence of PIC activation.
To pursue this question, we used the paired motor unit technique developed by Kiehn and Eken (1997) as well as by Gorassini and others (2002a). For this method, the firing rate of a lower threshold, “control”, unit is used to estimate changes in synaptic drive to the entire motor unit pool. The relative level of synaptic drive, as assessed by control unit firing rate is compared at the times when a higher threshold, “test”, unit is recruited and subsequently derecruited (see Gorassini et al. 2002a; Revill and Fuglevand 2011 for more information). If a PIC (i.e. a non-synaptic source of depolarizing current) is instigated in the test unit once recruited, then two sources of current (i.e. synaptic and intrinsic) support the firing of the test unit. As a consequence, in order to terminate firing in the test unit, the synaptic drive needs to be less than that required to initiate firing because of the added contribution of the PIC. Accordingly, the firing rate of the control unit (as a proxy for synaptic current) should be higher when the test unit is recruited compared to when derecruited. Such a difference in firing rate, referred to as $\Delta F$, is considered as evidence for PIC activation (Gorassini et al. 2002a). Many studies have demonstrated positive $\Delta F$ values in a variety of limb muscles (Gorassini et al. 2002a; Gorassini et al. 2004; Mottram et al. 2009; Oya et al. 2009; Powers et al. 2008; Stephenson et al. 2011; Stephenson and Maluf 2010; 2011; Udina et al. 2010).

Therefore, we sought to evaluate $\Delta F$ in genioglossus (GG) motor units and compared these values to those recorded previously in limb muscles. To modulate motor unit activity in GG, human subjects protruded and retracted their tongues in a symmetrical pattern while activity from a few motor units was tracked. Unexpectedly,
our results demonstrated that either large PICs are expressed in human GG motor units or that ΔF is influenced predominantly by other intrinsic properties.

2. Methods

4 subjects (3 males and 1 female, age range 23-51 years) participated in 6 experiments. The University of Arizona Human Subjects Committee approved all experimental procedures and all subjects gave informed consent.

i. Electromyography and displacement recordings

Tungsten microelectrodes (Frederick Haer and Co. Bowdoinham, Maine, USA; 1–5 µm tip diameter, 5–10 µm uninsulated length, 250 µm shaft diameter, ∼10 MΩ impedance at 1000 Hz after insertion) were used for intramuscular electromyography (EMG) recordings. Surface electrodes (4 mm diameter, Ag-AgCl) were placed over the left and right mastoid processes to serve as reference electrodes. Intramuscular EMG signals were differentially amplified (x 1000), band pass filtered (0.3 – 3 kHz, Grass Instruments), displayed on a computer monitor as well as routed to an audio amplifier. Intramuscular EMG signals were digitized at approximately 20 kHz (Cambridge Electronic Design Ltd.).

The subject’s tongue was coupled to an isotonic displacement transducer (Harvard Apparatus, Kent, UK) by a lever arm that was fixed to the tongue by a custom-made thermoplastic housing (see Bailey et al. 2007 for further details). This coupling still
allowed the subject to swallow or talk as necessary. Zero displacement was defined as the neutral tongue position just behind the front teeth with the jaw relaxed. Positive displacement indicated tongue protrusion from neutral and negative displacement indicated tongue retrusion from neutral. The displacement transducer output was routed to Spike2 (Cambridge Electronic Design Ltd., Cambridge, UK) and displayed on a computer monitor.

ii. Experimental protocol

Subjects were comfortably seated in a dental chair with their head supported. Once the displacement transducer was properly coupled to the tongue, subjects performed three maximum protrusions. The largest recorded protrusion was defined as the subject’s maximum.

Two tungsten microelectrodes were inserted into GG from the skin behind the chin. Electrodes were inserted ~1.5 cm either side of the midline and ~2-4 cm posterior in a rostral direction to a depth of at least 15 mm, which has previously been shown to be the approximate location of GG (Bailey et al. 2007). With electrodes in place, the subject slightly protruded the tongue while the experimenter gently manipulated each electrode in turn. Once a suitable pair of motor units was found, the subject performed 3-4 slow protrusion-retrusion tasks. Subjects were coached verbally to produce a relatively symmetrical displacement trajectory and their actual displacement was provided for visual feedback. The subject was instructed to maintain similar displacement rates for each trial. Additionally, because GG motor units are difficult to maintain on an electrode for more than a few minutes, only a brief rest (~2-3 s) was provided between
displacements. Once the subject completed 3-4 displacement contractions, they were
given a few minutes to rest before another motor unit pair was sought. Experiments
lasted approximately an hour.

iii. Data analysis

Data were analyzed offline using Spike2 software and custom-designed scripts
(Cambridge Electronic Design Ltd.). Motor unit recruitment was defined as the time of
the first spike when the subsequent interspike interval was 500 ms or less (Fuglevand et
al. 2006). Derecruitment was defined by the time of the last spike where the preceding
interspike interval was 500 ms or less (Fuglevand et al. 2006). Recruitment and
derecruitment firing rates were calculated based on the first, or last, three spikes
(Mottram et al. 2009). Peak displacement was the maximum displacement measured
during each contraction. Protrusion or retrusion rates were calculated as the time to go
from neutral position to peak displacement, or from peak displacement back to neutral
position.

For ΔF calculations, the control unit should be a sensitive indicator of changes in
synaptic drive (e.g. Gorassini et al. 2002a; Gorassini et al. 2004; Mottram et al. 2009;
Stephenson and Maluf 2011). To ensure that control unit firing rate was modulated with
tongue protrusion, we used a calculation proposed by Stephenson and Maluf (2011) that
tested whether the control unit firing rate was likely saturated during test unit activity.
Data were excluded if the control motor unit activity appeared to saturate during specific
protrusion-retrusion tasks.
During a given trial, often two, or more, GG motor units could be discriminated on a single microelectrode. In these trials we were able to combine motor units to obtain multiple motor unit pairs. To calculate $\Delta F$, a 5-point moving average firing rate was calculated from control unit spiking. Linear interpolation of this averaged firing rate was used to determine the control unit firing rate at test unit recruitment and derecruitment. The calculated control unit firing rate at test unit recruitment was subtracted from that at test unit derecruitment to obtain $\Delta F$. In some instances, the control unit did not spike sufficiently around test unit recruitment or derecruitment, so the instantaneous control unit firing rate was used instead. $\Delta F$ values for a particular motor unit pair were averaged across 2 to 7 displacement tasks. Therefore each motor unit pair had one averaged $\Delta F$ value that was used for further analysis.

To evaluate the effects of control unit firing rate on $\Delta F$, normalized $\Delta F$ values were also calculated as follows (Oya et al. 2009):

$$n\Delta F = (CFR - CFD) / CFR, \ (1)$$

where $n\Delta F$ is the normalized $\Delta F$, CFR is the control unit firing rate at test unit recruitment and CFD is the control unit firing rate at test unit derecruitment.

Due to the small sample size, recruitment and derecruitment firing rates were compared with a Wilcoxon signed rank test. Mean GG $\Delta F$ values were compared to the null hypothesis that $\Delta F$ would be zero by a one-way t-test. Due to the small sample size of GG motor units, $\Delta F$ across GG, ED, and TA motor unit pools were compared with an
analysis of variance on ranks. Data is presented as mean (SD), and significance was set at 0.05.

3. Results

A total of 40 GG motor units were recorded during triangular tongue protrusion-retrusion tasks. While subjects were instructed to perform the task symmetrically, mean (SD) protrusion rate (3.1 (1.9) mm/s) was slower than the mean retrusion rate (3.7 (1.9) mm/s) (p < 0.001, paired t-test). Peak displacement varied from trial to trial based on the motor unit characteristics as well as the clarity of the motor unit recordings. Overall, mean peak displacement was 19.4 (11.6) mm, which represented ~50% maximum displacement. One example protrusion-retrusion task, along with associated single motor unit activity, is illustrated in Figure V.1. In this example, the difference in control unit firing rate at test unit recruitment and derecruitment yielded a relatively large ΔF value of 9 imp/s.

In the recorded motor unit population, firing rate at recruitment was significantly greater than at derecruitment (Fig. V.2, p < 0.001). This result is similar to that observed in other motor unit pools and has been previously attributed to firing rate adaptation (Barry et al. 2007; De Luca et al. 1982; Milner-Brown et al. 1973a) or to PIC activation (Gorassini et al. 2002a).

For ΔF calculations, a total of 31 motor unit pairs were compared. Due to potential control unit firing rate saturation (see Methods), approximately 18% of
individual trials were excluded from further analysis, although no motor unit pairs were completely excluded from further analysis. ΔF from 30/31 GG pairs was greater than zero and mean ΔF was significantly greater than zero (p < 0.001, one-way t-test).

ΔF values obtained from GG were then compared to ΔF values from extensor digitorum (ED) and tibialis anterior (TA) motor units (data for ED and TA from Chapter IV). We used ED and TA data from the slowest rate of rise (~1.5 %MVC/s) because that data most closely matched GG contraction durations. Across these three motor unit populations, ΔF was significantly larger in GG than in ED and TA (Fig. V.3A, p < 0.05).
GG firing rates, however, were often higher than either ED or TA firing rates. For example, GG recruitment firing rate was ~15 imp/s whereas mean ED recruitment firing rate was ~9 imp/s and mean TA recruitment firing rate was only ~7 imp/s. As such, firing rate could be a confounding factor in \( \Delta F \), so we normalized \( \Delta F \) values by the control unit firing rate (see equation (1)). \( n\Delta F \) values were not different across motor unit populations (Fig V.3B, \( p > 0.05 \)).
4. Discussion

Here, we have presented results from activity of pairs of motor units from the tongue muscle genioglossus (GG) during voluntary activity in human subjects. In particular, we demonstrated that GG motor unit pairs generated large, positive $\Delta F$ values.
To our knowledge, this is the first time that $\Delta F$ has been evaluated in tongue motor units. Furthermore, $\Delta F$ values in GG were greater than that in either ED or TA.

$\Delta F$ was originally developed as a method to detect PIC activation during voluntary motor unit activity in human subjects (Gorassini et al. 2002a). While PICs are clearly present in spinal motor neurons (Bennett et al. 1998; Bennett et al. 2001b; Conway et al. 1988; Hamm et al. 1984; 1988a; Hounsgaard and Kiehn 1989; Hultborn et al. 2003; Lee and Heckman 1996; 1998a; b; Schwindt and Crill 1980; 1982; Turkin et al. 2010), their prevalence in hypoglossal motor neurons remains unclear (Greer and Funk 2005; Powers and Binder 2003; however see Lamanauuskas and Nistri 2008). Therefore, we would have expected to observe small $\Delta F$ values in GG motor units. Our results, however, were contrary to these expectations.

Through computer simulations, we have shown that positive $\Delta F$ values can arise due to the activation of PICs, but we also demonstrated that positive $\Delta F$ values could be elicited when spike threshold accommodation or spike frequency adaptation were included in the simulations (Revill and Fuglevand 2011). As such, the present results of positive $\Delta F$ values in GG motor units have two possible interpretations: 1) GG motor units generated large PICs, which implies that human motor units differ from rodent motor units in this aspect, or 2) other non-linear properties such as adaptation were activated in GG motor units. These two interpretations are not mutually exclusive, however, since PIC activation and spike frequency adaptation could be concurrently active (e.g. Hamm et al. 2010).
In support of the possibility of adaptation contributing to $\Delta F$, hypoglossal motor neurons are known to exhibit late adaptation (Powers et al. 1999; Sawczuk et al. 1995; 1997). Furthermore, the $\Delta F$ results across motor unit pools would also fit with this hypothesis. That is, the absolute magnitude of late adaptation depends on the initial motor neuron firing rate (Kernell and Monster 1982; Sawczuk et al. 1995). As such, motor units with the highest firing rate, namely GG motor units, should undergo the greatest adaptation and therefore generate the largest $\Delta F$ values, whereas motor units with the lowest firing rates, namely TA motor units, should have the smallest $\Delta F$ values because they underwent the least adaptation.

In conclusion, we have demonstrated that pairs of GG motor units consistently generated large positive $\Delta F$ values. This implies that either potent PICs are activated in hypoglossal motor neurons or that $\Delta F$ is not a reliable indicator of PICs and that other intrinsic properties, such as spike frequency adaptation, are primarily responsible for $\Delta F$. 
VI. EVALUATING A POSSIBLE ROLE FOR PERSISTENT INWARD CURRENTS IN FIRING RATE SATURATION

1. Introduction

   In reduced preparations, the relationship between firing rate and excitatory drive is commonly observed to be linear, when measured during current injection (Granit et al. 1963; Kornell 1965b; Schwindt and Crill 1982) or synaptic excitation (Granit et al. 1963; Binder et al. 1999; Powers and Binder 2000b). Analogous recordings can be made during natural behaviors in human subjects, because total muscle force tends to be linearly related to synaptic drive (Fuglevand et al. 1993). As such, a reasonable prediction would be that single motor unit firing rates be linearly related to whole muscle force. Instead, however, when motor unit activity is recorded while human subjects progressively increase the strength of muscular contraction, firing rate initially increases steeply with modest increases in isometric force (Bailey et al. 2007; De Luca et al. 1982; Monster and Chan 1977; Moritz et al. 2005). However, after this initial phase, firing rate tends to level out even though force (and presumably synaptic drive) continues to increase. Such non-linear firing rate responses are a common feature of motor units and have been observed in a wide variety of muscles (Bailey et al. 2007; De Luca et al. 1982; Monster and Chan 1977; Moritz et al. 2005).

   One possible feature that might contribute to the initial steep rise in motor unit firing rate followed by saturation is activation of persistent inward currents (PICs). While the role for PICs in natural behaviors is still uncertain (Fuglevand et al. 2006;
Nordstrom et al. 2007; Powers 2009), they have been repeatedly characterized in reduced preparations. PICs are predominantly mediated by a voltage-gated L-type calcium channel (Carlin et al. 2000b; Hounsgaard and Kiehn 1989) that require neuromodulators such as serotonin or noradrenaline for full expression (Hounsgaard and Kiehn 1989; Conway et al. 1988). PICs tend to be activated at the same time as motor unit recruitment, especially when the motor neuron is activated synaptically (Bennett et al. 1998). Furthermore, PICs may need a few hundred milliseconds to activate fully (Bennett et al. 2001a; Bennett et al. 2001b; Li and Bennett 2003; Li et al. 2004a) and in some instances may be maintained at a relatively fixed amplitude for many seconds (Lee and Heckman 1998a). Also, PICs are highly susceptible to inactivation by inhibitory inputs (Bui et al. 2006; Hultborn et al. 2003; Hyngstrom et al. 2008; Kuo et al. 2003).

Some experimental evidence supports the idea that PIC activation could lead to firing rate saturation (Hornby et al. 2002b; a; see also Heckman et al. 2008; Lee et al. 2003). Hornby and colleagues (Hornby et al. 2002b; a) compared the firing rate-current (f-I) relationship of turtle motor neurons in the presence and absence of neuromodulators such as serotonin or muscarine. Under control conditions, the f-I relationship was linear, but when neuromodulators were added, firing rate initially increased rapidly and then saturated (Hornby et al. 2002a).

As such, the goal of the present experiment was to test whether the initial steep rise in firing rate observed during natural behaviors in human subjects might be explained by PIC activation. Figure VI.1 illustrates our hypothesis for how PICs might influence the relationship between single motor unit firing rate and whole muscle force. During an
increasing force contraction, synaptic drive ($I_{syn}$) presumably increases proportionately (Fig. VI.1A). At motor neuron recruitment threshold ($I_{th}$), a PIC is activated ($I_{PIC}$) that reaches its peak amplitude rapidly (Fig. VI.1A). Total somatic current ($I_{total}$), which determines spiking output, is a linear sum of synaptic currents and PICs (Fig. VI.1A). However, as muscle force and motor unit activity increases, there is also increased inhibition from peripheral sources (such as autogenic inhibition mediated through activation of Golgi tendon organs, and recurrent inhibition mediated through Renshaw cells) and from descending sources (Fig. VI.1B). As the contraction gets stronger, increased inhibition might progressively inactivate PICs, thus leading to a plateau in $I_{total}$ (Fig. VI.1B). Motor unit firing rate mimics the shape of $I_{total}$, that is, firing rates would increase rapidly and then reach a plateau. If PICs contribute to the initial steep rise and subsequent plateau in firing rate, we reasoned that increased inhibition to a motor unit pool would limit PIC activation (Fig. VI.1C), and thus would lead to total somatic current being linearly related to synaptic drive and whole muscle force (Fig. VI.1C).

To test the hypothesis that PICs contribute to the initial rapid increase in firing rate, we compared the relationship between motor unit firing rate and whole muscle force during isometric ramp contractions in the absence (control condition) and presence of increased inhibition (cutaneous stimulation condition). To add inhibitory input experimentally, the sural nerve was stimulated, which activated the peripheral pathway responsible for the cutaneous silent period (reviewed by Floeter 2003). Our results demonstrated that the relationship between firing rate and force became more linear in
the presence of additional inhibitory input. Therefore, we conclude that PICs may contribute to firing rate saturation.
Figure VI.1. Schematic to illustrate how PIC activation could lead to firing rate saturation. A) During a ramp contraction, synaptic drive ($I_{\text{syn}}$, dashed line) increases. At recruitment for a motor unit ($I_{\text{th}}$), a PIC is activated ($I_{\text{pic}}$, grey line) and the total current ($I_{\text{total}}$, black line) is a linear sum of $I_{\text{pic}}$ and $I_{\text{syn}}$. B) Increased synaptic inhibition (from reflexes and descending pathways) causes the net magnitude of $I_{\text{pic}}$ to decay. Overall, despite an increase in $I_{\text{syn}}$, the decay in $I_{\text{pic}}$ leads to a plateau in $I_{\text{total}}$. Thus firing rate, due to net somatic current, would increase rapidly and then plateau. C) In the presence of constant synaptic inhibition, $I_{\text{syn}}$ sums with synaptic inhibition and $I_{\text{total}}$ is thus directly proportional to $I_{\text{syn}}$. Therefore firing rate would now be linearly related to synaptic input.

2. Methods

In total, sixteen subjects volunteered to participate in 27 experiments. However, we were only able to obtain data from four subjects in six experiments (3 males and 1 female, age range: 24-52 years old) (see Results for more information). All subjects reported no neuromuscular deficits. The University of Arizona Human Subjects Committee approved all experimental procedures and every subject gave informed consent.

i. Experimental set-up

During all experiments, subjects were seated comfortably in a dental chair. The subject’s right foot was secured to a custom-built footplate that rotated freely about an axis aligned collinear with the talocrural joint of the ankle. The platform location was adjustable so that subjects could sit with their knee bent slightly and the foot plantarflexed approximately 70 degrees. The foot was attached to the platform by Velcro straps and a counterweight system was used to minimize inertia of the platform and foot.
ii. Force and electromyography recordings

A stiff force transducer (sensitivity of 43 N/mV, Grass Instruments, Warwick, Rhode Island, USA) was used to measure subjects’ maximum voluntary force output. A more sensitive force transducer (sensitivity of 15 N/mV) was used during motor unit recordings. Force signals were amplified (x1000), sampled and digitized at ~1000 Hz and displayed on a computer monitor.

Tungsten microelectrodes (Frederick Haer and Co. Bowdoinham, Maine, USA; 1–5 µm tip diameter, 5–10 µm uninsulated length, 250 µm shaft diameter, ∼200 kΩ impedance at 1000 Hz after insertion) were used to record motor unit action potentials. Manual palpation of tibialis anterior (TA) was used to ensure the electrode was inserted in the correct muscle. A surface electrode (4 mm diameter, Ag-AgCl) placed over the tibia served as the reference electrode. Two surface electrodes were placed over TA, and two surface electrodes were placed on the triceps surae muscle group to monitor global muscle activity of agonists and antagonists, respectively, during contractions. Surface electromyography (EMG) signals were also differentially amplified (x1000), band pass filtered (0.3 - 1 kHz), displayed on an oscilloscope, and recorded by Spike2 (Cambridge Electronic Design Ltd., Cambridge, UK) for further analysis. Intramuscular EMG signals were differentially amplified (x 1000), band pass filtered (0.3 – 3 kHz, Grass Instruments), displayed on a computer monitor as well as routed to an audio amplifier. Intramuscular EMG signals were digitized at approximately 25 kHz (Cambridge Electronic Design Ltd.).
iii. Cutaneous stimulation and the cutaneous silent period reflex

The sural nerve, which provides cutaneous innervation to the dorsum of the foot, was stimulated at a location just posterior and distal to the lateral malleolus by a surface electrode that supplied constant current from a stimulator (Multichannel Systems, Germany). To minimize skin impedance (and therefore maximize the range of currents that could be delivered through the electrode), the skin was gently abraded. The surface electrode placement was optimized for each subject by finding a location where the subject reported a particularly strong radiating sensation on the lateral aspect of the foot when a low frequency, low level of current was applied (3 mA at 1 Hz). The electrode was subsequently taped in place on the surface of the skin. Next, we measured sensation threshold by applying 500 ms pulses of current increasing by 0.1 mA increments. Threshold was defined as the moment that the subject reported any sensation. We aimed to find a location where threshold was ~1 mA, since that tended to predict whether or not we could elicit a cutaneous silent period (CSP).

Next, the threshold for the CSP was determined. Subjects maintained a weak contraction of 5% maximum voluntary contraction (MVC) while stimuli were delivered at a rate of 1 Hz at slowly increasing intensities. Activation of the reflex was clearly observed on an oscilloscope as almost complete silencing of the EMG signal at a latency of 80-100 ms, which lasted for ~40 ms (see Floeter 2003 and Fig. VI.2). If we could not observe a CSP, we attempted to find another stimulus location that would activate the reflex. If we were unsuccessful in eliciting the reflex, we did not continue with the
experiment due to uncertainty surrounding the amount of inhibition being supplied to the TA motor unit pool.

Figure VI.2. Example cutaneous silent period. The subject maintained a low level of force (5% MVC) while twenty 1 Hz stimuli (11 mA, 0.5 s duration) were delivered. Surface EMG signal was rectified and subsequently used for a stimulus-triggered average. 100 ms prior to stimulus shows baseline EMG levels, and the marked depression in surface EMG ~100 ms post-stimulus is the cutaneous silent period.

For stimulation during ramp contractions (detailed below) a 25 Hz input was used. This frequency delivered a pulse every 40 ms, which is a similar interval to the CSP duration associated with a single pulse (Floeter 2003), and thus should provide a fairly consistent level of inhibition to the motor unit pool. The magnitude of the 25 Hz input was determined by the following factors: the tolerance level of the subject (the level of stimulation needed to generate a CSP is painful), the effects the stimulation had on motor units (stronger stimuli sometimes caused dramatic shifts in recruitment threshold), and whether the subject could produce a contraction (in some cases, the subject tolerated a strong stimulus but was then unable to overcome the inhibition to generate the targeted force).
iv. Experimental Protocol

The experiment began with subjects performing three maximum voluntary contractions (MVCs). The peak force achieved by the subject during any contraction was defined as their MVC.

Once an appropriate stimulation site was located near the lateral malleolus (as detailed above), we inserted the microelectrode into the belly of TA and then attempted to locate single motor units for recording. While searching for a motor unit, subjects were instructed to hold a weak contraction while the electrode was gently manipulated until one, or a few, motor units were visually distinguishable. Peak force for the ensuing experimental trials was determined based on the motor unit recruitment threshold as well as the clarity of the motor unit recording.

Subjects performed triangular isometric ramp contractions at a rate of rise and fall of 0.75% MVC/s. Subjects were provided with an online template to guide their force production and were instructed to follow this template as closely as possible. This usually provided motor unit activity of sufficient duration for the curve fitting analysis (see below). Subjects performed at least three practice contractions before data collection began.

During data collection, subjects performed approximately four control contractions interleaved with four stimulation contractions. At least ten seconds elapsed between contractions. In rare instances, fewer contractions were obtained because the electrode moved and the unit was no longer clearly discernable, and in some cases, more
contractions were performed because the intramuscular recording was particularly clean. After each set of trials, subjects were given a few minutes to rest before a new motor unit was sought. Successful experiments lasted approximately two hours.

v. Data analysis

Data were initially analyzed offline using Spike2 software (Cambridge Electric Design Ltd.) and custom-designed scripts. Recruitment and derecruitment thresholds were defined as the force at the first and last, respectively, motor unit spike of continuous activity. The period of continuous activity was considered to have started, or ended, once interspike intervals were 500 ms or less (Fuglevand et al. 2006). Recruitment and derecruitment firing rates were calculated based on the first and last, respectively, three spikes (Mottram et al. 2009). Peak force was the largest force value around the midpoint of the contraction. Force rate of rise and fall was calculated as the slope from the contraction onset to peak force, and from peak force to the end of the contraction, respectively.

Further analysis was carried out using custom-written MATLAB scripts (The Mathworks Inc, Natick, MA). Instantaneous spike times were smoothed by calculating a five-point moving average. The force trace was similarly smoothed by calculating a five-point average force, based on the force at each spike time. The spike train and force data were then divided into ascending (all firing rate and force values that occurred prior to
peak force) and descending (all firing rate and force values that occurred subsequent to the peak force) portions.

Each phase of firing rate-force data was then fit in two ways. A firing rate trace that initially increases steeply with force and then saturates has a form similar to a rising exponential, and therefore the data were fit to:

\[ R(F) = R_m \times (1-e^{-\frac{(F-F_{th})}{\Phi}}) + R_{th}, \]  

where \( R(F) \) is the firing rate at a given force \( F \) level, \( R_m \) is the maximum firing rate, \( F_{th} \) is the force at recruitment threshold, \( R_{th} \) is the minimum firing rate, and \( \Phi \) represents the amount of force increase associated with a 63% \( (1-e^{-1}) \) increase in firing rate. We termed \( \Phi \) a force constant, similar to a time- or length-constant. The same data were also fit by a linear equation:

\[ R(F) = m \times F + R_{th}, \]  

The parameters are the same as equation (1), except that \( m \) represents the slope of the relationship between force and firing rate. For a spike train to be included in subsequent analysis, the best fit (i.e. exponential or linear) function had to have an \( R^2 \) value of at least 0.3. Low \( R^2 \) values were commonly associated with doublets or highly irregular spiking. These cases were more commonly observed during stimulation trials.
For each phase, we determined whether a rising exponential (equation 1) provided a significantly better fit than the linear function (equation 2) using the F-test. Then, we tallied the total number of spike trains best fit by each function for each condition (ascending control trial, descending control trial, ascending stimulation trial, descending stimulation trial). For motor unit activity best fit by the exponential function, we calculated the average force constant ($\Phi$) across trials. Similarly, for motor unit activity best fit linearly, we also calculated the average slope ($m$) across trials.

Finally, the symmetry of the ascending and descending phases of firing rate-force data were evaluated. To quantify symmetry, we calculated the area, or hysteresis, enclosed within the rising phase-falling phase firing rate-force loops. A smaller area would indicate that the ascending and descending spike trains were more symmetrical. We limited the analysis to ascending and descending spike trains that had a linear or exponential fit with $R^2 \geq 0.3$ and at least 15 overlapping data points between the ascending and descending trains. Finally, since the absolute area between curves could vary due to force range or firing rate characteristics, we normalized the hysteresis as a percentage of the maximum possible hysteresis.

Due to the small sample size, nonparametric statistics were predominantly used. A signed rank test was used to assess force rates of rise and fall. A two-way analysis of variance (ANOVA) was carried out to compare forces and firing rates at recruitment and derecruitment during control and cutaneous stimulation conditions. The counts of exponential and linear fits were compared using a Chi-square test. Force and slope constants as well as hysteresis values were compared across the control and cutaneous
stimulation conditions using a Mann-Whitney rank sum test. Data are presented as mean (SD). Significance was set at 0.05.

3. Results

The results we present are from 27 motor units that were active during both the control and cutaneous stimulation conditions. The reasons for a small data set were primarily due to difficulty in eliciting the cutaneous silent period (CSP) reflex in some subjects. We suspect that subjects’ individual anatomy prevented sufficient stimulation of the sural nerve, or that subjects’ CSP reflex threshold was higher than the maximum output of the stimulator (15 mA). Additionally, in some instances, motor unit activity was too irregular to perform curve fitting due to doublet firing, and in some cases motor units were not recruited during both conditions.

While the cutaneous inhibitory input affected subjects’ perception, subjects still performed the ramp contraction task well during control and cutaneous stimulation conditions. The force rate of rise was not different between the two conditions (control vs. stimulation: 0.70 (0.14) vs. 0.71 (0.14) % MVC/s, mean (SD), p > 0.05, paired t-test) but the force rate of fall was significantly greater during the stimulation trials (control vs. stimulation, 0.72 (0.19) vs. 0.86 (0.27), p < 0.05, Wilcoxon signed rank test). Additionally, the peak force that subjects attained was not different between the two conditions (control vs. stimulation, 5.7 (1.1) vs. 6.4 (2.4) % MVC, p > 0.05, Wilcoxon signed rank test).
One example of control and stimulation trials is illustrated in Figure VI.3. The left panel depicts motor unit activity during a control triangular contraction. On the ascending force phase, note the rapid increase in motor unit firing rate followed by firing rate saturation despite continued increases in force (Fig. VI.3A). This relationship between firing rate and force during the ascending phase is quantified in the left hand panel of Figure VI.3B. The right panels of Figure VI.3A illustrate an example of force and motor unit activity during cutaneous stimulation, which provided additional inhibitory input to motor units. In this one example, the firing rate-force relationship is best fit linearly, as illustrated by the linear fit in the right hand panel of Figure VI.3B. Overall, the firing rate-force relationship during the ascending phase was consistently best fit exponentially during control conditions (Fig. VI.4A, p < 0.0001) but was fit equally well linearly or exponentially during cutaneous stimulation (Fig. VI.4A, p > 0.05). However, in all cases, the firing rate-force relationship was best fit linearly during the descending phase of the contraction (Fig. VI.4B, p < 0.0001).
Figure VI.3. Example motor unit traces. A) Sample motor unit behavior during a control and a cutaneous stimulation trial. Traces from bottom to top: stimulus magnitude, force trace (thick black line) and goal force trajectory (light grey line), raw intramuscular EMG signal, instantaneous firing rate of discriminated motor unit. B) Illustration of the curve fitting process for the ascending phase of a control (left) and a stimulation (right) trial. The open circles are the 5-point moving average firing rate values as a function of average force values. The ascending phase was best fit exponentially under control conditions (p < 0.05, F-test), but best fit linearly under stimulation conditions (p > 0.05, F-test).
The force constant, $\Phi$, from equation (1), provides a measure of curvature of the rising exponential. A small force constant means that the curve rises steeply, thus motor unit firing rates tend to saturate quickly. A larger force constant occurs when the curve is shallow, meaning that motor unit firing rates do not saturate quickly (if at all). Therefore, considering only motor units that were best fit with a rising exponential, the ascending phase force constants were significantly longer during stimulation compared to control trials (Fig VI.5A, $p < 0.0001$). Cutaneous stimulation had no effect on the slope of the firing rate-force relationship during the descending phase (Fig VI.5B, $p > 0.05$). Therefore, our results are consistent with inhibitory input decreasing the tendency for motor units to demonstrate firing rate saturation.
Figure VI.4. Percentage of ascending (A) or descending (B) spike trains that were significantly better fit by a rising exponential or a linear function. A) 119 ascending spike trains were analyzed in the control condition and 72 ascending spike trains were analyzed in the stimulation condition. B) 122 descending spike trains were analyzed in the control condition and 83 descending spike trains were analyzed in the stimulation condition. *, p < 0.0001, chi-square test.
Figure VI.5. The effect of cutaneous stimulation on fit parameters. A) Mean (SD) force constant of rising exponential for motor units ascending trains that were, on average, best fit by rising exponential. 28 control and 13 stimulation motor unit traces were analyzed. B) Mean (SD) slope of the linear fit for motor unit descending trains that were, on average, best fit linearly. 29 control and 27 stimulation motor unit traces were analyzed. * p < 0.05, Mann-Whitney rank sum test

Additionally, cutaneous stimulation had effects on motor unit recruitment and derecruitment behavior. Motor unit recruitment and derecruitment force increased
significantly with cutaneous stimulation (Fig. VI.6A, p < 0.05). However, within each condition, recruitment and derecruitment forces were similar (Fig. VI.6A, p > 0.05). Average firing rates were higher at recruitment compared to derecruitment in both conditions (Fig. VI.6B, p < 0.05), but cutaneous stimulation had no further effects on recruitment, or derecruitment, firing rates (Fig. VI.6B, p > 0.05).

Cutaneous stimulation had additional effects on spiking behavior and force generation. Subjects uniformly reported an increased effort for contractions when cutaneous stimulation was added. Furthermore, the force output was usually more variable during stimulation trials (e.g. Fig. VI.3). Stimulation also occasionally had dramatic effects on recruitment threshold. For example, motor units would not be recruited during the simulation trials, despite being routinely recruited during control trials (Fig. VI.7). Occasionally a new unit that had not been recruited during control trials was recruited during stimulation trials (Fig. VI.7). Sural nerve stimulation has non-uniform distribution to motor unit pools (Kanda et al. 1977), and this may be the reason for the varied effects on recruitment threshold.
Figure VI.6. Recruitment and derecruitment force (A) and firing rate (B) data for 27 motor units. A) Recruitment and derecruitment force presented as mean (SD) % MVC. Recruitment and derecruitment force both increased with cutaneous stimulation but were not different within a condition. B) Firing rates were not different between control and stimulation, but derecruitment firing rate was significantly lower than recruitment firing rate in both conditions, mean (SD). *, p < 0.05, Holm-Sidak method.
Figure VI.7. Example trace illustrating the variation in motor unit behavior during control and stimulation trials. Traces are the same as Fig. VI.2. Note that the middle motor unit is reliably recruited during the control trials but is not recruited during the cutaneous stimulation trial. Also note that the upper motor unit is only recruited during the stimulation trial.

Finally, we also examined the symmetry of spiking behavior between the ascending and descending firing rate-force curves by examining the hysteresis, or area between these two curves. As discussed in Methods, we calculated the area, or hysteresis, between the two curves and expressed it as a percentage of the maximum possible area between those curves. A positive area would indicate that the ascending firing rates were, on average, greater than the descending firing rates at comparable force levels (see Fig. VI.8A). For every control trial, hysteresis was positive, indicating that
Figure VI.8. Hysteresis calculations. A) Example hysteresis calculation. Open symbols indicate the original spike train, where squares are the ascending train and circles are the descending train. Solid circles represent the spike train used in the hysteresis calculation, black circles are the ascending spike train and grey circles are the descending spike train. Vertical grey dotted lines are the boundaries for the area calculation in the force dimension. The horizontal black dashed lines are the peak and minimum firing rates for this trace. The area of the rectangle bounded by the dotted and dashed lines represents the theoretical maximum possible hysteresis. In this example, hysteresis is 56% of maximum. B) Mean (SD) percentage hysteresis for 27 control and 18 stimulation trials. *, p < 0.05, Mann-Whitney rank sum test.
ascending firing rates were greater than descending firing rates at comparable force levels. With the addition of cutaneous inhibition, there was still generally positive hysteresis between ascending and descending firing rates, although the average magnitude decreased compared to control trials (Fig. VI.8B, p < 0.05). Therefore, cutaneous inhibition increased symmetry between ascending and descending firing rate-force loops during isometric contractions.

4. Discussion

During voluntary contractions of increasing force, motor unit firing rates often initially tend to increase rapidly and then saturate despite continued force increases (Bailey et al. 2007; De Luca et al. 1982; Monster and Chan 1977; Moritz et al. 2005, as well as present results). Here, we have presented data to demonstrate that the relationship between firing rate and force can be manipulated. In particular, the addition of inhibitory input to the TA motor unit pool caused firing rates to become more linearly related to force. Moreover, these results are consistent with the hypothesis that activation of PICs might contribute to the initial steep rise in firing rate as force increases (see Fig. VI.1).

i. Effects of inhibition

To add inhibition to the TA motor unit pool, we stimulated the sural nerve, which inhibits lower threshold motor neurons (Kanda and Hashizume 1992), and also provokes
a cutaneous silent period (CSP) if the stimulation is sufficiently strong (Floeter 2003). The pathway that mediates the CSP has not been fully elucidated (reviewed in Floeter 2003), but probably involves A-δ fibers (Floeter 2003; Uncini et al. 1991). Furthermore, it is not entirely clear if the CSP is purely an inhibitory reflex, or if it also has an excitatory component. Certainly, there is often increased surface EMG after the CSP consistent with increased excitation (e.g. Fig. VI.2). This increased EMG may be due to an artifact of motor unit synchronization after inhibition (Kahya et al. 2010; Kranz et al. 1973), but may also involve a longer-latency excitation related to the startle reflex (Kumru et al. 2009).

Additionally, the CSP does not appear to habituate (Uncini et al. 1991; Yoon et al. 2011). While there has been no quantification of the CSP at the interpulse intervals used in the present study (i.e. 40 ms), recent evidence demonstrated that paired stimuli delivered at 60 ms intervals continued to elicit CSPs (Yoon et al. 2011). In some subjects, these short interpulse intervals even led to a single longer, fused CSP (Yoon et al. 2011). However, these interstimulus intervals also led to shorter average second CSP durations, with increased latency (Yoon et al. 2011). Overall, based on the qualitative observations from subjects that contractions required more effort when the cutaneous stimulation was applied, as well as the available experimental evidence, we feel that the cutaneous stimulation was effective at providing a relatively constant level of inhibition to the motor unit pool.

To our knowledge, the effect of sural nerve stimulation on PIC activation has not been previously investigated in reduced preparations. Therefore, we cannot be certain
that sural nerve stimulation did indeed limit PIC activation. However, other sources of inhibition, such as reciprocal inhibition (Hyngstrom et al. 2007) or recurrent inhibition through Renshaw cells (Bui et al. 2008a; Crone et al. 1988), successfully inhibited PICs. Furthermore, while the effects of these peripheral pathways on PICs are known, it was not possible to use either of these pathways in the present experiment. We could have activated reciprocal inhibition pathways to TA by vibrating the Achilles tendon. However, this would have complicated the contraction task, since tendon vibration also excites homonymous motor units (in this case, triceps surae) through monosynaptic excitation (Mendell and Henneman 1971). Renshaw cells are excited through recurrent inhibition, that is, motor neuron axon collaterals synapse onto Renshaw cells that in turn have inhibitory synapses to motor neurons (Eccles et al. 1954). Therefore, Renshaw cells are probably normally activated during a contraction. It might be possible to increase Renshaw cell activity by increasing force (and presumably synaptic drive) to increase motor unit firing rates, although this would also make the interpretation of the results more complicated because the force tasks would not be identical under the two conditions.

Finally, sural nerve stimulation did not affect every motor unit studied equally. For example, the ascending firing rate profiles of some motor units during cutaneous stimulation were best fit linearly, while others were still best fit exponentially, albeit with a larger force constant. There are two possible interpretations of this result. Perhaps the inhibition delivered to the motor unit pool was insufficient to completely prevent PIC
activation in all cases. Alternately, there could be multiple mechanisms that underlie firing rate saturation. Each of these interpretations seems plausible.

ii. Firing rate-force hysteresis

We also examined the hysteresis, or area, between ascending and descending firing rates as a function of force. Consistently, but peculiarly, there was positive hysteresis between firing rate and force on the ascending and descending limb of the contractions. Since force is an outcome of motor unit spiking, one would probably predict that the ascending and descending limbs of a force-firing rate plot for a triangular, symmetric contraction would overlap. Hysteresis between ascending and descending firing rate-force loops has been investigated in only a few other instances, and in all cases there was positive hysteresis (Clamann 1970; Milner-Brown et al. 1973a; Vander Linden et al. 1991). However, there has been little speculation as to the mechanism underlying this hysteresis.

We propose that this hysteresis may be due to a combination of muscle mechanics and the activation of non-linear motor neuron properties such as PICs or spike-frequency adaptation. The relationship between stimulation rate and individual motor unit force is not symmetric, as has been demonstrated in reduced preparations (e.g. Binder-Macleod and Clamann 1989) as well as in humans during intraneural microstimulation (e.g. Macefield et al. 1996). In either case, the force produced during increasing stimulus ramps was less than the force produced at the same stimulus rate on decreasing stimulus
ramps (see Fig. 8 in Macefield et al. 1996). Stated another way, a lower stimulus rate would be necessary to attain the same force output on the descending, compared to ascending, portion of a triangular contraction. Furthermore, in each of these examples, the hysteresis can only be due to muscle mechanics, since motor units were electrically stimulated, thus preventing activation of any non-synaptic mechanisms such as PICs or spike frequency adaptation.

Additionally, when motor neurons in reduced preparations are subjected to a triangular current injection protocol, lower firing rates on the descending current injection ramp are often observed and have been attributed to spike frequency adaptation (Hamm et al. 2010; Meehan et al. 2010; Turkin et al. 2010). Thus, it is possible that firing rate adaptation may contribute to the lower firing rates on the descending portion of the contraction. Finally, based on the present results where hysteresis decreased when cutaneous stimulation was added, hysteresis may also be related to PIC activation. Overall, the hysteresis between motor unit firing rates and whole muscle force may be due to a combination of complex muscle mechanics as well as ongoing non-synaptic mechanisms such as spike frequency adaptation and PICs.

In conclusion, we have examined the tendency for motor unit firing rates to saturate during voluntary isometric ramp contractions. We manipulated motor unit spiking behavior by applying cutaneous stimulation, which added inhibition to the motor unit pool. With the addition of inhibition, motor unit spiking behavior became more linearly related to force. Thus, our results are consistent with the hypothesis that PICs could underlie the initial steep rise in firing rate during isometric contractions.
VII. CONCLUSIONS

As the final common pathway, motor neurons integrate synaptic inputs from many sources to generate appropriate firing rate responses. It was originally thought that most of this integration occurred passively, however, active intrinsic properties may make the process of synaptic integration more complex. Thus, the primary goal of this dissertation was to determine whether evidence for activation of intrinsic non-linearities in motor neurons could be observed during voluntary contractions in human subjects. The experimental work throughout this dissertation has sought to apply what is known about non-synaptic motor neuron properties from experimental work in reduced preparations to motor unit activity during natural behaviors in human subjects.

To understand the role that PICs, accommodation, and adaptation may play during voluntary activity, I used a combination of computer simulations as well as human experiments. Computer simulations were employed initially to understand how each of these non-linear properties could influence firing rate output separately. The main behavior considered was the difference in firing rate ($\Delta F$) of a lower threshold motor unit when a higher threshold motor unit was recruited and then derecruited (Gorassini et al. 2002a). $\Delta F$ was originally developed to estimate PIC activation during voluntary behaviors. However, the results from the computer simulations presented here suggested that accommodation or adaptation, as well as PICs, could lead to positive $\Delta F$ values. Importantly, $\Delta F$ was not observed in the control condition, implying that if $\Delta F$ is observed in vivo, there must be non-linear properties activated. Finally, by selectively
activating motor neuron properties and by also varying contraction duration and speed, I developed a set of testable hypotheses that I subsequently evaluated in human subjects.

I carried out experiments to test these hypotheses in two muscles serving different functions: extensor digitorum, which controls fine movements of the hand, and tibialis anterior, which is mostly used during posture and locomotion. \( \Delta F \) was observed in both motor unit populations. However, the results from these experiments together with the simulation results were most consistent with spike-frequency adaptation primarily contributing to \( \Delta F \). Therefore, \( \Delta F \) may not be the best metric to assess PIC activation during natural behaviors. With that said, these results do not preclude activation of PICs during natural behaviors.

Furthermore, the relative contribution of these intrinsic non-linear properties may vary across different motor unit pools. For example, a role for PICs has been proposed during posture and locomotion (e.g. Heckman et al. 2008), but no role has been suggested during other normal motor tasks. Therefore, I compared \( \Delta F \) from motor units pools with different functional roles: extensor digitorum used in fine motor control, tibialis anterior used during posture and locomotion, and also genioglossus used for volitional tasks like speech and non-volitional tasks such as breathing. \( \Delta F \) varied across different motor unit pools, but the absolute magnitude appeared to be related to the firing rate ranges in the different pools rather than the functional role of motor unit pools. Indeed, \( \Delta F \) was largest in the genioglossus motor unit pool, which also happened to have motor units that fired at the highest rate.
While $\Delta F$ may not be the best method to detect PICs, it remains an important question whether PICs contribute to motor unit activity during natural behaviors. Thus, for the final portion of my dissertation I considered whether other motor unit properties might be explained by PIC activation. In particular, I focused on the observation that motor unit firing rates tend to increase steeply and then saturate, despite continued increases in whole muscle force. Since PICs are particularly sensitive to inhibition, I examined motor unit activity in the absence and presence of additional inhibition, supplied by stimulation of the sural nerve. The results from this experiment demonstrated that with inhibition, the relationship between motor unit firing rate and force became more linear. Thus, this result is consistent with PICs contributing to firing rate saturation.

The interpretation of the results from the above experiments, however, relies strongly on what is known about motor neuron properties in reduced preparations, since it is not presently possible to measure these properties directly during voluntary behavior. It became apparent that some aspects of these intrinsic motor neuron properties remain to be fully elucidated. Thus, in order to further inform the interpretation of the data from the experiments comprising this dissertation, I propose that the following experiments should be conducted.

The first experiment that should be conducted would be to examine $\Delta F$ in a reduced preparation, perhaps using the preparation of Powers and Binder (2003), where PIC magnitude could be manipulated. That is, if PICs were blocked by a drug like nifedipine (Hounsgaard and Kiehn 1989), would $\Delta F$ still be observed? This type of
The experiment may also allow insight into the relative contribution of non-synaptic mechanisms, namely PICs, accommodation or adaptation, to $\Delta F$.

Furthermore, some molecular properties of PICs remain to be completely elucidated. For example, the process by which PICs inactivate is unclear. The persistence of PICs may depend on the recruitment threshold of the motor neuron (Lee and Heckman 1998a; however see Hamm et al. 2010), or also on the level of depolarization (Hounsgaard and Kiehn 1989), but to my knowledge it is not clear exactly what determines when PICs turn off once activated. Additionally, inhibition inactivates PICs (Bui et al. 2006; Hultborn et al. 2003; Hyngstrom et al. 2008; Kuo et al. 2003), but the mechanism behind this is not entirely clear. Inhibitory inputs appear to synapse in the same location as L-type calcium channels (e.g. Bui et al. 2008b), which may cause local hyperpolarization that then inactivates the channels. Computer modeling might be one way to gain insight into this question. Finally, to my knowledge, there has been little systematic study of the differences in PIC magnitude across a particular motor unit pool (Hamm et al. 2010; Lee and Heckman 1998a), or across different motor unit pools. For example, there is little evidence for PICs in flexor motor neurons (Cotel et al. 2009; Hounsgaard et al. 1988a), or in hypoglossal motor neurons (Greer and Funk 2005). Considering the variation in PIC magnitude in different types of motor neurons may provide further insight into the contribution of PICs during behavior.

There are also properties of accommodation that still need to be investigated. For example, most experiments investigating accommodation were completed over relatively brief ramps of current injection (usually no more than a few hundred milliseconds).
More recent experiments, particularly those concerning PICs, often use slow ramps of current injection over many seconds. Thus, understanding the extent to which motor neuron spiking threshold may be affected over longer duration current ramps would be important for interpreting the results. Furthermore, much like PICs, the extent of accommodation across motor unit in a pool or across different motor unit pools remains unclear (e.g. Burke and Nelson 1971; Homma et al. 1970; Schlue et al. 1974c). This too would provide information about the relative importance of accommodation in motor unit pools. Finally, there is some evidence that the behavioral state of the animal may influence the accommodative properties of motor neurons (e.g. Sasaki and Otani 1962). Comparing the extent of accommodation in a reduced preparation in the presence and absence of neuromodulators would provide information as to the likely extent of accommodation during natural behaviors.

Additionally, the mechanism underlying late adaptation has not been fully resolved in motor neurons. Recently, exciting work in the subthalamic nucleus demonstrated a novel potassium current that appears to underlie adaptation in this neuronal population (Barraza et al. 2009). Replicating this protocol in motor neurons might be a very good place to start to determine the mechanism underlying late adaptation in motor neurons. Additionally, some evidence suggests that adaptation may become less prevalent during fictive locomotion (Brownstone et al. 2010), thus it would be useful to determine how the neuromodulatory state of a preparation affects the magnitude of adaptation.
Overall, this dissertation highlights the role that some non-linear motor neuron properties, in particular PICs, accommodation, and adaptation, can play during natural behaviors. I have provided evidence that both PICs and adaptation may be activated during natural behaviors. Thus, the process by which motor neurons integrate synaptic inputs may be more complicated than originally thought.
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