REVIEW | *Spinal Control of Motor Outputs*

The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons

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Johnson MD, Thompson CK, Tysseling VM, Powers RK, Heckman CJ. The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons. *J Neurophysiol* 118: 520–531, 2017. First published March 29, 2017; doi:10.1152/jn.00018.2017.—Motoneurons are unique in being the only neurons in the CNS whose firing patterns can be easily recorded in human subjects. This is because of the one-to-one relationship between the motoneuron and muscle cell behavior. It has long been appreciated that the connection of motoneurons to their muscle fibers allows their action potentials to be amplified and recorded, but only recently has it become possible to simultaneously record the firing pattern of many motoneurons via array electrodes placed on the skin. These firing patterns contain detailed information about the synaptic organization of motor commands to the motoneurons. This review focuses on parameters in these firing patterns that are directly linked to specific features of this organization. It is now well established that motor commands consist of three components, excitation, inhibition, and neuromodulation; the importance of the third component has become increasingly evident. Firing parameters linked to each of the three components are discussed, along with consideration of potential limitations in their utility for understanding the underlying organization of motor commands. Future work based on realistic computer simulations of motoneurons may allow quantitative "reverse engineering" of human motoneuron firing patterns to provide good estimates of the relative amplitudes and temporal patterns of all three components of motor commands.

electrode array; motoneuron; motor unit

THE MOTOR UNIT provides the primary output for the CNS, converting all motor commands into the forces required for movement (Heckman and Enoka 2012). Each motor unit has two elements, the spinal motoneuron and the muscle fibers that it innervates. Normally there is a one-to-one match between the action potentials in each motoneuron and in its muscle fibers. Because there are many muscle fibers in each motor unit, every motoneuron action potential generates a greatly amplified signal that can be readily recorded in human muscles by appropriate electrodes. Thus motoneurons are the only cells in the CNS whose firing patterns can be routinely measured in humans, and, moreover, these firing patterns contain detailed information about the structure of motor commands. The potential of using motor unit firing patterns for understanding motor commands has long been appreciated, but for many years recording techniques were limited to identification of only one to three motor units at a time. To deal with this limitation, recently developed methods use array electrodes that, when placed on the skin surface above a muscle, allow simultaneous recording of the activity of many motor units (Farina et al. 2010; Holobar et al. 2014; Nawab et al. 2010). The availability of these highly detailed population data for motor units potentially provides a basis for a transformative advance in our understanding of the structure of motor commands in humans.

Essentially, the approaches reviewed here provide a form of "reverse engineering," using the rich set of details provided by the array data to identify the organization of motoneuron inputs. This reverse engineering approach would not be possible without a comprehensive understanding of the input-output functions of motoneurons. Systematic studies in many laboratories over the past 30 years (reviewed in Heckman and Enoka 2012; Hounsgaard 2002; Hultborn et al. 2004; Powers and Binder 2001) have provided a deep and quantitative understanding of this key issue, allowing the development of remarkably realistic computer simulations of motoneurons (e.g., Bui et al. 2008; Elbasiouny et al. 2006; Powers et al. 2012). Surprisingly, this body of work has clearly demonstrated that motoneuron input-output functions are highly malleable, with their characteristics varying depending on the composition of their motor commands. The high sensitivity of motoneuron behavior to different components of motor commands induces clear "signatures" in their firing patterns that allow estimation of the characteristics of these commands based on recordings of the discharge of multiple motor units.

In this review we discuss the key parameters of motor unit firing patterns that we have identified as being highly likely to represent important features of the synaptic organization of motor commands. The necessary foundation for understanding this approach requires a brief review of the methods for decomposing the discharge patterns of multiple motor units recorded with surface EMG arrays, as well as a summary of what is known about motoneuron intrinsic properties and the synaptic inputs to motoneurons based on recordings in reduced animal preparations. Perhaps the most important component of inputs to motoneurons is the neuromodulatory input, which has the capacity to alter motoneuron electrical properties that provide the unique features to unit firing patterns that population recordings from multielectrode arrays are particularly well suited to identify.

Array Recordings of Motor Unit Populations

The technical basis underlying the surface EMG array approach to recording multiple motor units in human subjects has been extensively reviewed (De Luca 1985; Farina et al. 2010; Holobar et al. 2014; Nawab et al. 2010; Zwarts and Stegeman 2003). Here we highlight its advantages and briefly consider its limitations. Several algorithms have been developed to decompose surface EMG signals into the discharge times of individual motor units (Holobar et al. 2010; Nawab et al. 2010; Negro et al. 2016). We focus on the approach developed by Holobar, Merletti, Farina, and colleagues (Farina et al. 2010), as this approach is the primary technique for ongoing studies in our laboratory. Figure 1 shows examples of these array electrodes and also illustrates a raster of 19 concurrently active motor unit spike trains in the tibialis anterior of one subject (unpublished data, Heckman laboratory), recorded with a 64-channel surface array (Fig. 1*Ai*) in combination with an automated decomposition approach (Holobar et al. 2010).

This array and automated decomposition approach has now been successfully used in a number of studies, and systematic assessments of its accuracy in comparison to the standard intramuscular fine wire methods typically show a correspondence close to 90% for a variety of muscles and contractions (e.g., Farina and Negro 2015; Holobar et al. 2014; Martinez-Valdes et al. 2016; Miller et al. 2014; Muceli et al. 2015). This degree of accuracy is more than sufficient for the analyses of motor unit firing patterns that we propose below. Limitations are not dissimilar to those of intramuscular fine wires. The array method is not as yet well suited to recording during movements, and recording at high force levels, above 50% of maximum, is difficult. However, these limitations are being addressed (Martinez-Valdes et al. 2016; Muceli et al. 2015), and for this review we focus on isometric conditions at relatively low input-output levels.

Review of Input-Output Processing in Motoneurons

Role of intrinsic electrical properties of motoneurons. Motoneuron recruitment and firing rate modulation are heavily shaped by the intrinsic electrical properties of the cell. For recruitment, the dominant feature in motor unit firing patterns is characterized by Henneman's size principle: in response to a uniform distribution of excitatory synaptic input, recruitment proceeds from small, slow (S) motor units through progressively larger and faster (F) units (Henneman and Mendell 1981). Intracellular studies confirm that the intrinsic current threshold increases progressively with motor unit size (Binder et al. 1996). Rate modulation is also grounded in the intrinsic electrical behavior of motoneurons. Initially just above threshold, the motoneuron afterhyperpolarization (AHP) keeps firing rates low, but as input increases firing rate increases proportionally with the amount of injected or synaptic current (Powers and Binder 2001).

A Example multi channel EMG array electrodes

i. Small muscle, rigid (2.4 mm IED)

ii. Large muscle, flexible (8 mm IED)

iii. Medium muscle, flexible (4 mm IED)

B Dorsiflexion torque and 19 motor units
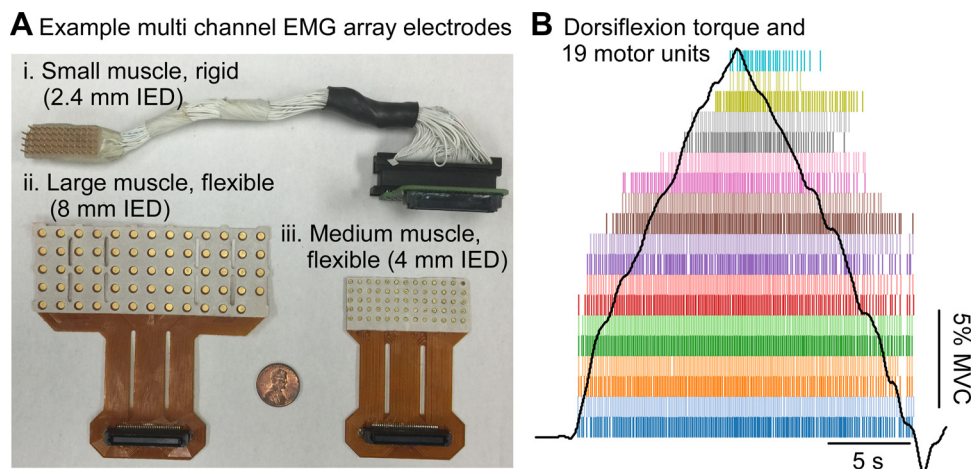
5% MVC

5 s

Fig. 1. Illustration of the 2 types of electrode arrays that are currently being used for high-density motor unit recordings of the muscle. *Ai*: rigid arrays are used for multiunit recordings directly on the muscle surface in vivo in animal experiments. *Aii* and *Aiii*: flexible arrays are used on the surface of the skin for multiunit recordings in humans. *B*: spike trains from a human subject experiment illustrating the spike trains from 19 motor units during an increasing/decreasing torque ramp. IED, interelectrode distance.

Role of synaptic input. Subsequent studies have shown that simple patterns of excitation and inhibition coupled with intrinsic electrical properties of motoneurons are far from sufficient to fully explain human motor unit firing patterns. Two major complications emerged. First, systematic studies of synaptic potentials (Burke 1981) and synaptic currents (Powers and Binder 2001) showed that excitatory synaptic inputs to motoneurons are not distributed uniformly to all motoneurons (see *Parameters to estimate the excitatory component of motor commands*). The second complication emerged as studies progressively revealed the existence of strong neuromodulatory inputs (Alaburda et al. 2002; Heckman et al. 2003). Unlike ionotropic inputs, which open ligand-gated ion channels to generate synaptic currents, neuromodulatory inputs act on G protein-coupled receptors to activate signal transduction pathways that alter the behavior of a variety of voltage- and calcium-activated ion channels. In motoneurons, neuromodulatory inputs modify the cells' intrinsic electrical properties and thus control how they process their excitatory and inhibitory inputs (Binder and Powers 2001; Heckman and Enoka 2012).

Neuromodulatory inputs are an essential component of normal motor commands. The two most powerful neuromodulatory inputs to motoneurons are mediated by axons descending from the brain stem and releasing either serotonin (5-HT) or norepinephrine (NE) (Heckman and Enoka 2012; Powers and Binder 2001). Their effects on motoneuron excitability are extraordinary. Recruitment thresholds are greatly reduced by depolarization of the resting potential and hyperpolarization of the spike voltage threshold as a result of neuromodulation (Krawitz et al. 2001; Power et al. 2010). Rate modulation is transformed because of facilitation of persistent inward currents (PICs) mediated by voltage-sensitive Na and Ca channels (Lee and Heckman 1998a; Svirskis and Hounsgaard 1998) and reduction of the spike AHP. It should be noted that the effects of 5-HT and NE on the AHP appear to be small in the adult state (Lee and Heckman 1999; Li et al. 2007); the primary control of the AHP in the adult is likely mediated by neuromodulatory actions of cholinergic input originating from spinal interneurons near the central canal (Miles et al. 2007; Zagoraiou et al. 2009). It is, however, the effect of the PIC on firing rates that provides the greatest potential for identifying the structure of motor commands from motor unit firing patterns, as emphasized in the next section. It is now generally accepted that motor behavior cannot be achieved without a strong neuromodulatory component (Heckman et al. 2003, 2008; Heckmann et al. 2005, Hultborn 2002; Hultborn et al. 2004). Moreover, there exists a steady neuromodulatory drive from the brain stem to the cord during the waking state, which is increased during sustained motor output (Aston-Jones et al. 2000; Jacobs et al. 2002).

Three Components to Motor Commands

Given the potent effect of neuromodulatory input on motoneurons, it is clear that there are three major components to all motor commands: excitation, inhibition, and neuromodulation. The temporal pattern of a movement is probably set by the pattern of ionotropic excitation, as is assumed by the many studies that rely on whole muscle EMG as an index of neural drive (but see below). We have hypothesized that the role of neuromodulatory input is instead to set the excitability of the

motor pool to a level appropriate for the motor behavior, being high for tasks requiring high forces or speeds and low for tasks requiring precision (Johnson and Heckman 2014). Essentially, this concept asserts that the brain stem serotonergic and noradrenergic inputs provide gain control for motor output; experimental support for this gain control hypothesis has recently been obtained (Wei et al. 2014).

What then is the role of inhibition—what pattern or amplitude does it follow during a motor command? Motoneurons likely receive a significant amount of tonic inhibition during normal motor behaviors, based on the high levels of spontaneous activity in premotor spinal interneurons in awake, behaving primates (Prut and Perlmutter 2003a, 2003b), many of which are inhibitory. The decerebrate cat preparation also has substantial levels of tonic inhibition (Hyngstrom et al. 2007; Johnson et al. 2012). A steady background of inhibition potentially allows for several different temporal patterns for inhibition vs. excitation, as illustrated in Fig. 2 (green triangles indicate a linear increase and decrease in excitation; red shapes indicate the different patterns of inhibition). *1*) Inhibition could be held approximately constant while excitation varies; *2*) inhibition could decrease while excitation increases (a reciprocal pattern referred to as "push-pull") (Johnson et al. 2012), or *3*) excitation and inhibition could covary, with inhibition increasing in proportion to excitation ("balanced" inhibition) (Berg et al. 2007). Each pattern may be used across a range of neuromodulatory levels. All three patterns would produce a linear increase and then decrease in net depolarizing synaptic drive, thus linearly increasing and decreasing muscle forces and EMGs. As a result, these global output variables provide insufficient information to distinguish between these very distinct underlying command patterns. The potential for instead using the details of motor unit firing patterns for this purpose is considered below.

Distinctive Features of Motor Unit Firing Patterns That Identify the Structure of Motor Commands

Given the existence of three components of motor commands, the question then becomes, can their effects on motor
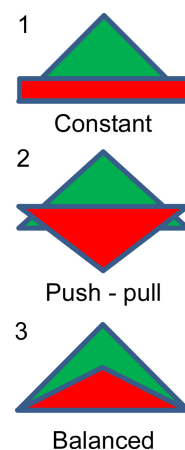

Fig. 2. Three basic interaction patterns for excitatory and inhibitory inputs to motoneurons: *1*) Excitation (green) can vary against a background of tonic (constant) inhibition (red); *2*) excitation and inhibition can vary out of phase of each other in a "push-pull" fashion, producing a larger net change in excitability than varying each input individually; and *3*) excitation and inhibition can vary in phase in a "balanced" fashion.

output be identified? The initial efforts to recreate the structure of motor commands from motor unit firing patterns relied only on the ionotropic components, i.e., excitation and inhibition, coupled to the intrinsic properties of motoneurons as revealed in anesthetized preparations that lack significant levels of neuromodulation (reviewed in Binder et al. 1996). These efforts failed (Binder et al. 1993; Heckman and Binder 1991b, 1993a; Heckman, unpublished results). In retrospect, it is clear that the lack of the neuromodulatory component was the primary problem, as detailed in the next section. Studies of the effects of 5-HT and NE on adult motoneuron properties were well underway at this point, led by the classic work of Hounsgaard, Hultborn, Kiehn, and colleagues (Hounsgaard et al. 1988a, 1988b). They showed that the PIC first described in motoneurons by Schwindt and Crill (Schwindt and Crill 1980a, 1980b) emerged as a physiological result of activation of the brain stem 5-HT and NE systems. Subsequent work in several laboratories over the next 20+ years (Heckman and Enoka 2012; Powers and Binder 2001), including the development of in vivo voltage-clamp methods to quantify the PIC and its interaction with ionotropic synaptic inputs (Hyngstrom et al. 2007, 2008a, 2008b; Kuo et al. 2003; Lee et al. 2003; Lee and Heckman 1996, 1998a, 2000), has provided a much fuller understanding of how motoneurons process synaptic inputs and generate their firing patterns.

Importance of PICs in Understanding Human Motor Unit Firing

Perhaps the most important result to emerge from studies of brain stem neuromodulation on spinal motoneurons is the critical impact PICs have on firing patterns (Heckman et al. 2008, 2009). Figure 3 illustrates the key effects of the PIC on the firing patterns of motoneurons in the cat. The input is a linearly rising and falling pattern of current injected by the microelectrode. This triangular input generates an approximately linear firing response in the motoneuron when PICs are not activated (red trace in Fig. 3). In contrast, a cell with a strong PIC responds very differently (Heckman and Enoka 2012; Powers and Binder 2001): *1*) There is an initial acceleration that imparts a very steep slope to the rate modulation. This acceleration has been shown to be due to the activation of the PIC. *2*) Next, a rapid transition occurs to a much lower slope for rate modulation. Now the PIC is fully active. *3*)
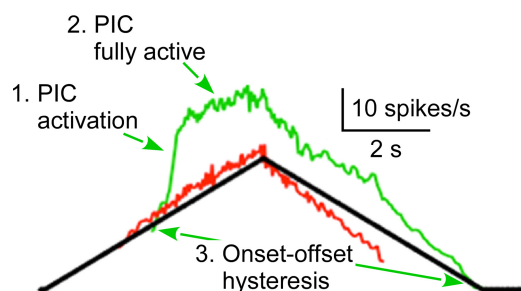

Fig. 3. Intracellular recording of a spinal motoneuron. A triangular current ramp is injected via the intracellular recording electrode. In the absence of persistent inward currents (PICs) (low neuromodulatory state) the change in firing frequency is linear and follows the pattern of current injection (red trace). When PICs are present (high neuromodulatory state) the firing behavior is nonlinear and shows strong initial acceleration and on-off hysteresis (green trace).

Finally, there is a more or less linear decline in firing rate as input levels decline, with derecruitment occurring at a much lower input level of current injection than recruitment. This onset-offset hysteresis is due to very slow inactivation of the PIC.

When we first uncovered firing patterns of this form in cat motoneurons in our studies of the effects of 5-HT and NE in the decerebrate cat preparation (Lee and Heckman 1998b), we were struck by their similarity to firing patterns exhibited by human motor units. At this time several studies (De Luca et al. 1982a; Monster and Chan 1977; Person and Kudina 1972) had demonstrated a sharp transition from steep to reduced slope during linearly increasing force, but this rate saturation (or rate limiting) proved very difficult to simulate based on the intrinsic properties of motoneurons in the absence of neuromodulation (Heckman and Binder 1993a). In contrast, this transition follows naturally from PIC activation (*steps 1* and *2* in Fig. 3). The marked hysteresis in recruitment and derecruitment (*step 3* in Fig. 3) had been clearly demonstrated in deltoid motor units in human subjects by De Luca and colleagues (De Luca et al. 1982a), but hysteresis proved impossible to simulate based on motoneurons without PICs (Heckman and Binder, unpublished data). Subsequent studies in humans clearly demonstrated that acceleration, saturation, and hysteresis are standard features of human motor unit firing patterns during linearly rising and falling isometric contractions (e.g., De Luca et al. 1982b; De Luca and Contessa 2012; Fuglevand et al. 2015; Mottram et al. 2009, 2014; Revill and Fuglevand 2017).

An important related result in studies of cat motoneurons was that PICs are much more effectively activated by excitatory synaptic input than by injected current (Bennett et al. 1998; Lee and Heckman 1996, 2000). This reduction of PIC threshold occurs because most of the channels that generate PICs are located in the dendrites, just as are synaptic contacts. As a result, PICs tend to activate right at recruitment threshold of motoneurons, even when their amplitudes are modest because of modest levels of neuromodulatory input. Moreover, these studies showed that the PIC saturation was even stronger for excitatory synaptic inputs than for injected currents. Unlike current injected from microelectrodes, current flowing into the cell from synaptic boutons is subject to the effects of driving force. The dendritic location of many PIC channels means that PIC activation induces large depolarizations in dendritic regions that greatly reduce this excitatory driving force (Elbasiouny et al. 2005, 2006; Powers et al. 2012; Powers and Heckman 2015). Thus once the PIC becomes fully activated, the efficacy of excitatory synaptic input is considerably reduced (Hyngstrom et al. 2008b; Lee and Heckman 2000; Powers et al. 2012).

The final necessary step in understanding how PICs and neuromodulation influence motoneuron firing patterns came from the development of highly realistic computer simulations of motoneurons (e.g., Bui et al. 2008; Elbasiouny et al. 2006; Powers et al. 2012). Figure 4 shows computer simulations of motoneuron firing patterns (Powers et al. 2012) and compares the result to those in human subjects. In both cases, the input to the motoneurons was triangular, with a similarly slow time course. In the simulations, input was generated by synaptic
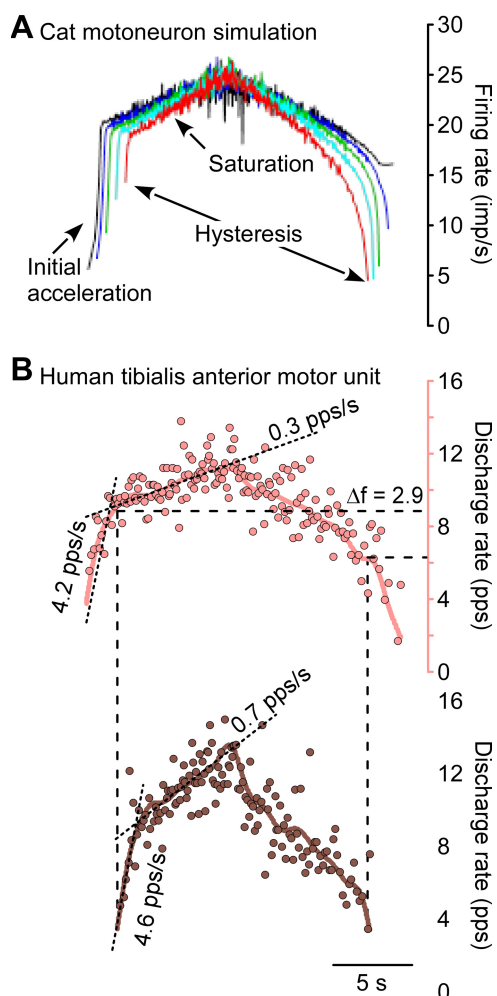
Fig. 4. *A*: computer simulations of motoneuron firing patterns in response to slowly increasing and decreasing synaptic conductances. The acceleration, saturation, and hysteresis typically seen in motoneurons with prominent persistent inward currents in vivo are evident. Firing rate is presented in impulses per second (imp/s). *B*: motor unit discharge patterns from 2 motor units from the tibialis anterior muscle from a human subject in response to slow isometric contractions. Discharge rate is presented in pulses per second (pps). The firing behavior is strikingly similar to the simulations in *A*, showing the key characteristics imparted by persistent inward currents. The "ΔF" technique compares the firing rates between a higher (test)- and a lower (control)-threshold motor unit. The control unit's firing rate is used to estimate the synaptic drive to the motor pool. During slowly increasing and decreasing contractions the control unit firing rate at recruitment and derecruitment is compared to the corresponding firing rates of the test unit. This technique measures changes in the recruitment/derecruitment thresholds of the test unit to detect changes in intrinsic excitability (presence of persistent inward currents).

conductances instead of current injection. The simulations (Fig. 4*A*) clearly show the acceleration, saturation, and hysteresis. For comparison, Fig. 4*B* shows two motor units recorded by array electrodes in a human tibialis anterior muscle (unpublished data, Thompson and Heckman). The similarity between simulated and real data is striking and strongly supports the fundamental role of PIC activation and deactivation in shaping human motor unit firing patterns in slow isometric contractions. This figure shows only two examples from a human subject; however, the occurrence of acceleration, saturation, and hysteresis in human motor unit firing patterns is a widespread phenomenon.

Human Motor Unit Firing Pattern Parameters That Can Be Used to Estimate the Neuromodulatory, Excitatory, and Inhibitory Components of Motor Commands

In this section, we consider motor unit firing rate parameters that can be used to identify the relative contributions of neuromodulatory, excitatory, and inhibitory components of motor commands. This focus is distinct from the well-developed statistical methods that use the discharge times of multiple motor units to estimate cross-correlations and coherence (e.g., De Luca et al. 1982b; Farina et al. 2013; Keen and Fuglevand 2004; Moritz et al. 2005; Negro and Farina 2012). These methods are fundamentally important for understanding functional connectivity but are not designed to distinguish the relative contributions of the three main components of motor commands. More recently, statistical analyses of the interspike interval distributions of single motor units have been used to estimate the duration of the motoneuron's AHP (Gossen et al. 2003; Macdonell et al. 2008; Matthews 1996) and have shown how the AHP is altered in disease states (Piotrkiewicz and Hausmanowa-Petrusewicz 2011; Suresh et al. 2014). Neuromodulatory modification of human motoneuron AHPs is an area that needs further research, but a potential problem is that this input may be primarily involved in the genesis of locomotion via cholinergic inputs arising within the spinal cord (Miles et al. 2007). The presently available AHP estimation techniques require long-duration, stationary firing patterns, conditions very unlike the phasic activity of locomotion. It is likely that the central pattern generator involves both local and descending neuromodulatory inputs (Power et al. 2010). Additionally, there are DC shifts within the spinal cord during fictive scratch that may be related to neuromodulatory drive (Cuellar et al. 2009; Tapia et al. 2013). The parameters discussed below for estimating the components of motor commands are also subject to a limitation: they are designed only to apply to slowly rising and falling isometric efforts. Nonetheless, as we argue in the final section, systematic comparison of this simple task across many muscles has the potential to identify how the basic organization of motor commands varies with the structure and function of the human musculoskeletal system.

Parameters to estimate neuromodulatory component of motor commands. The essential role of PICs in shaping firing patterns during slowly varying isometric contractions provides a quantitative means of estimating PIC properties in humans. Because PIC amplitude is directly proportional to the level of 5-HT and NE input from the brain stem, estimation of PIC properties provides an estimation of a major portion of the neuromodulatory component of motor commands in humans. Potentially all three effects of PICs on firing could be used for this purpose: acceleration, saturation, and hysteresis. In practice, the acceleration component is so rapid that it often generates only a few spikes, resulting in high variability from trial to trial (Bennett et al. 1998; Hultborn et al. 2003; Lee and Heckman 1998b). Thus there are two parameters of human motor unit firing that potentially provide effective estimates of the PIC: hysteresis and saturation.

Gorassini and colleagues developed a method that has now become standard to quantify the hysteresis generated by PIC activation (Gorassini et al. 2002). This method estimates hysteresis in one motor unit by comparison with the change in

firing rate of a lower-threshold motor unit (as illustrated in Fig. 4*B*) and is thus referred to as the delta frequency, or ΔF, technique. The ΔF technique has been used extensively (Herda et al. 2016; Mottram et al. 2009, 2014; Stephenson and Maluf 2010, 2011; Udina et al. 2010; Wilson et al. 2015) and has been thoroughly validated by experiments in animal preparations (Gorassini et al. 2002; Powers et al. 2008). Multielectrode array recordings are ideally suited to the ΔF analysis technique, as they provide many potential unit comparisons. Systematic assessments of ΔF using realistic computer simulations (Powers and Heckman 2015) have alleviated recent concerns about potential effects of firing rate adaptation (Revill and Fuglevand 2017; Vandenberk and Kalmar 2014).

Although saturation has not yet been used for estimates of PICs and neuromodulatory input, both experimental results (Hyngstrom et al. 2008b; Lee and Heckman 1999, 2000) and recent computer simulations (Powers and Heckman, unpublished) show that firing rate saturation increases with PIC amplitude. Saturation is easy to measure, as it is just a slope assessment for firing rate once acceleration is complete, as illustrated in Fig. 4*B*. It is important to note that this slope is typically expressed as a function of whole muscle or joint variables and muscle EMG is inherently noisy and potentially subject to cross talk, while joint torque is usually generated by multiple muscles. The ΔF method avoids these difficulties by comparing the relative firing patterns of two units (Gorassini et al. 2002). It is thus best to use saturation in conjunction with ΔF: all other things being equal, the animal experimental data and the computer simulation results clearly predict that larger ΔF values correspond to greater saturation (i.e., lower firing rate slopes vs. EMG or force).

Parameters to estimate excitatory component of motor commands. The first question to consider is whether the temporal trajectory of the excitation component of the motor command can be estimated. The usual assumption is that the overall envelope of EMG or torque is a good estimate of this trajectory. EMG has long been used as an approximate estimate of motor commands. How accurate is this assumption? The initial simulations of the input-output function of a motor pool (defined as the set of motoneurons innervating a single muscle), which were based on the properties of motoneurons in deeply anesthetized preparations without significant levels of neuromodulation, revealed an approximately sigmoidal form (Fuglevand et al. 1993; Heckman and Binder 1991b). Yet this function is unlikely to apply to normal motor behavior where significant levels of neuromodulation are present. Recently we have reinvestigated this issue, using a pool of simulated motoneurons that realistically recreates the effects of PICs on the input-output functions of individual motoneurons (Powers and Heckman 2017). For each individual motoneuron, this resulting input-output function is manifestly nonlinear, because of the acceleration and saturation effects of the PIC illustrated in Fig. 3. Our simulations suggest that this nonlinearity is attenuated, but still present, when the effects of recruitment and rate modulation of the whole pool are taken into account. Thus a linear output in terms of EMG and torque may, in these conditions of slowly rising and falling isometric tasks, require a nonlinear input. Much further work is required, but for the present it is possible that EMG and torque are somewhat distorted indicators of the temporal pattern of excitatory motor commands.

There is, however, another important aspect to excitatory motor commands, which is that they are unlikely to be generated by a single source. Descending motor command systems include not only the corticospinal system but also the rubrospinal system as well as the vestibulospinal and reticulospinal systems (Kuypers 1981). There are of course many sensory inputs as well, with the monosynaptic Ia afferents being especially important (Matthews 1972). In fact, there are striking differences: Ia input favors activation of S units (Heckman and Binder 1988); all other excitatory inputs studied thus far favor activation of F units, either strongly [corticospinal, rubrospinal (Binder et al. 1998; Powers et al. 1993)] or modestly [vestibulospinal (Westcott et al. 1995)]. Inhibition from both descending and sensory inputs appears to be a bit simpler, being uniformly distributed (Heckman and Binder 1991a; Lindsay and Binder 1991). As yet several key sensory systems have not been measured in terms of synaptic currents, including Golgi tendon organ Ib afferents, muscle spindle group II afferents, cutaneous afferents, and the inputs from the flexion reflex/crossed extension system. However, it has been shown that the excitatory postsynaptic potentials (EPSPs) generated by Ib and cutaneous afferents are not correlated to the input resistance of motoneurons (Powers and Binder 1985). S motoneurons have much larger resistances than F motoneurons (Powers and Binder 2001), and thus these inputs are unlikely to generate larger currents in S motoneurons like Ia afferents do. Also, electrical stimulation of skin afferents has been shown to evoke EPSPs in F motoneurons (Burke et al. 1970). The corticospinal input also requires further study, as synaptic currents from this system have only been measured in the cat (Binder et al. 1998), which lacks the monosynaptic component present in primates. EPSPs generated by this system have been assessed in forearm motor pools in the primate, revealing weak or no correlations between their amplitudes and axonal conduction velocity (Clough et al. 1968). Conduction velocity correlates with input resistance (faster in lower-resistance type F motoneurons) (Powers and Binder 2001), and thus this input is also unlikely to be larger in S motoneurons. Overall, it is important to keep in mind that the studies of Powers, Binder, and colleagues were restricted to ankle extensor motor pools in the cat. Further work may reveal important differences in distributions of inputs to S and F motoneurons innervating different muscles (e.g., arm vs. leg vs. trunk).

Given the potential presence of nonuniform distributions of synaptic input across S and F motoneurons in motor commands, an important first step is to determine whether these nonuniformities can be detected in human subjects. This detection is in fact achievable, via two approaches, one based on rate modulation and one on recruitment. The rate modulation technique is the rate-rate plot, in which the firing rates of two motor units are plotted vs. each other, with the higher threshold unit on the *y*-axis. This method was first employed by Monster and Chan (1977) and is also a fundamental step in the ΔF technique, where a requirement is that the rate-rate plot of the two units have a high correlation and thus likely receive a similar pattern of input (Gorassini et al. 2002). The parameter that indicates input distribution is not the correlation coefficient of rate-rate plots but instead the slope. This rate-rate slope will be equal to 1.0 unless the two motoneurons generating the firing patterns have different slopes for their conversion of synaptic current to firing frequency or if they receive different

shares of the synaptic input. Data from intracellular recordings generally show similar current-frequency slopes in S vs. F motoneurons (Powers et al. 2012; Powers and Binder 2001), so it is likely that rate-rate slope is primarily an assessment of the distribution of synaptic input.
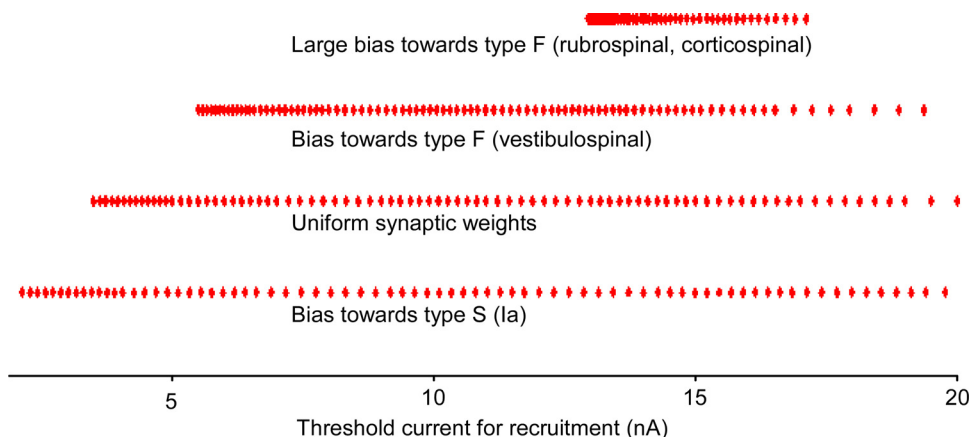
Recruitment thresholds of motoneurons are also highly sensitive to the distribution of synaptic input across S vs. F units, as illustrated in Fig. 5. Inputs that generate relatively greater synaptic current in high-threshold F motoneurons generate relatively stronger depolarizations in these cells, reducing the amount of additional current needed to depolarize them to threshold. The result is a compression of the range of recruitment thresholds. If the differential in input strength is large enough, F units can potentially be recruited before some S units, effectively reversing recruitment order. This information is highly quantitative: the polysynaptic components of corticospinal and rubrospinal inputs have the greatest tendency to generate larger currents in F motoneurons, vestibulospinal somewhat less so, and Ia input the opposite tendency (large in S motoneurons). These differences are readily evident in Fig. 5. The relations between input distribution, recruitment threshold, and variance in recruitment order were quantified in simulations by Heckman and Binder (1993b). Here, compression of the range of thresholds was shown to increase the percentage of recruitment reversals on a trial-to-trial basis, but overall recruitment remained orderly for all of the inputs tested. The advantage of assessment of recruitment range is that it is not complicated by any differences in slopes of the current-frequency transforms of motoneurons. Use of measurements of recruitment range in conjunction with rate-rate slopes potentially provides a very useful tool for identifying the composition of excitatory synaptic input, although this has yet to be applied to any data set.

Binder et al. (1998) noted in their studies of synaptic currents that corticospinal and rubrospinal inputs are nearly nine times larger in the highest-threshold F motoneurons compared with the lowest-threshold S motoneurons, while the vestibulospinal input is approximately two times larger in F motoneurons. The Ia pattern is approximately the inverse of the vestibulospinal pattern, being about twice as large in the smallest type S motoneurons vs. the largest type F motoneurons. These values are reflected in the recruitment ranges shown in Fig. 5 (note that there is not a simple relation between width of the recruitment ranges and range of the input because motoneuron thresholds are highly skewed toward low values).

Since Ia input is the only one that is relatively larger in S motoneurons, rate-rate slopes should generally be >1.0. Monster and Chan (1977), in fact, found an average value of 1.7 for their sample of motor units in the extensor digitorum communis muscle, suggesting a preponderance of inputs organized like the corticospinal and rubrospinal inputs. If a muscle has an average rate-rate slope much closer to 1.0, then it is likely such a muscle is more strongly controlled by vestibulospinal and Ia inputs. Moreover, this low average value for rate-rate slope should be accompanied by a relatively wide range of recruitment thresholds. In this context, it is interesting to note that distal arm muscles tend to have more compressed ranges of recruitment than proximal ones (De Luca et al. 1982a; Heckman and Enoka 2012; Klass et al. 2008; Kukulka and Clamann 1981), suggesting a relatively larger role for Ia and vestibular inputs in proximal muscles. The relation above between recruitment range and rate-rate slope predicts that proximal muscles should also therefore have values for rate-rate slopes that are closer to 1.0 than distal muscles. Strong Ia and vestibulospinal input in proximal muscles makes sense in terms of their stabilizer functions.

Parameters to estimate inhibitory component of motor commands. The different organizations of inhibition as compared with excitation illustrated in Fig. 2 are likely to have a strong impact on the firing patterns of motor units. The reason for this impact is that PICs are highly sensitive to synaptic inhibition (Kuo et al. 2003). Because of the PIC's largely dendritic origin, inhibitory synapses located either proximally or distally directly alter its activation (Bui et al. 2008; Powers et al. 2012). Our results (Powers et al. 2012) of computer simulations of these interactions are illustrated in Fig. 6. In brief, a push-pull organization produces a relatively gradual activation of the PIC that largely eliminates acceleration/saturation and thus imparts a relatively steep slope to rate modulation (red trace in Fig. 6). In contrast, a balanced organization tends to limit PIC activation and very much reduce rate modulation (green trace in Fig. 6). This severe form of rate saturation has a mechanism different from that induced by the PIC; essentially, the increase in inhibition in parallel with excitation reduces the net synaptic drive. In contrast to these large effects, a modest background of constant inhibition has a relatively small effect on rate modulation (blue trace in Fig. 6). If this background becomes too large, however, it will prevent PIC activation. A recent study by Revill and Fuglevand (2017) is strongly supportive of these simulation results: steady inhi-

Fig. 5. Schematic of the distribution of synaptic inputs across motoneuron types shows that synaptic sources are not uniformly distributed. Additionally, synaptic sources do not have a uniform effect on each type of motoneuron (S vs. F). Cortical and rubrospinal inputs produce much greater (up to 9 times) effects in F motoneurons than they do in S motoneurons. Vestibular inputs are about twice as strong in F motoneurons than in S. Ia inputs have the strongest effect in S motoneurons, being about twice that in F motoneurons. This nonuniform distribution and effect of inputs on motoneurons greatly affects recruitment by compressing threshold ranges across motoneuron type.
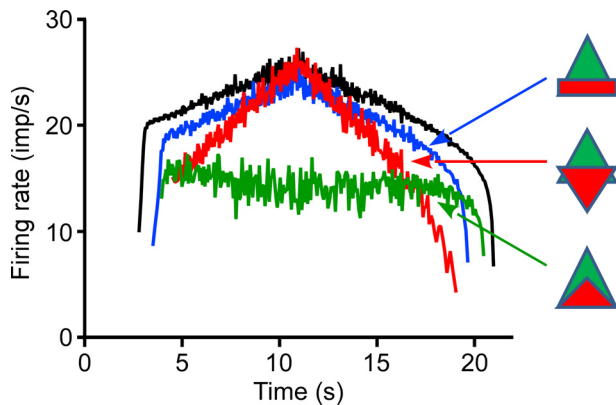

Large bias towards type F (rubrospinal, corticospinal)

Bias towards type F (vestibulospinal)

Uniform synaptic weights

Bias towards type S (Ia)

Threshold current for recruitment (nA)

Fig. 6. Computer simulation of 3 possible combinations of inhibition and excitation and the resulting firing patterns each produces: *1*) a background of steady inhibition (red) superimposed on increasing then decreasing excitation (green); *2*) inhibition changing out of phase with excitation, the "push-pull' arrangement; *3*) inhibition and excitation covarying in phase, the "balanced" arrangement. *Arrangements 1* and *2* closely resemble firing patterns seen in animal and human experiments.

bition derived from electrical stimulation of a cutaneous nerve eliminated most of the acceleration/saturation effects of the PIC. Note that this effect of the temporal pattern of inhibition (e.g., push-pull vs. balanced) should have roughly equal effects on all motoneurons, as the distribution of inhibitory inputs is approximately equal on S vs. F motoneurons. Thus push-pull organization will increase the slope of rate modulation of all units and should leave intact systematic differences between low- and high-threshold units (S vs. F) that result from the unequal distributions of excitatory synaptic current discussed in the previous subsection.

There is one further way of using PIC effects to assess inhibition. Because of its high sensitivity to inhibition, the PIC varies strongly with static changes in joint angle because of the degree of modulation induced by reciprocal inhibition from changes in muscle length (Hyngstrom et al. 2007). These results of Hyngstrom and colleagues clearly predict that ΔF will covary with variation in joint angle, but only if a steady background of Ia reciprocal inhibition is present. Moreover, the larger this background, the stronger the variation. This result has so far only been demonstrated in an animal preparation, but an inverse covariation in the strength of transient Ia reciprocal inhibition induced by electrical stimulation and the value of ΔF has been demonstrated by Vandenberk and Kalmar (2014).

Firing Patterns of Motor Units in Awake, Behaving Animals

The focus of this review is on understanding the genesis of human motor unit firing patterns, but these patterns have also been recorded in several species of animals (Eken and Kiehn 1989; Gorassini et al. 1999; Hoffer et al. 1987b; Palmer and Fetz 1985; Ritter et al. 2014). The patterns in the primate and the cat are similar to those in humans, though with typically higher firing rates. Studies recording units in mice during quiet standing revealed that they exhibit an extremely broad range, from 5 to 50 Hz (Ritter et al. 2014). This is in contrast to human unit rates, which are consistently low (10 Hz or less) during low-torque tasks (Heckman and Enoka 2012). The mechanism of this broad range is not clear. As yet, array methods have not been systematically deployed in awake

animal subjects, but the development of intramuscular arrays (Muceli et al. 2015) is likely to make this possible.

The Advantage of Population Data

One major advantage of having data from 10–30 motor units at once is that it provides a much more quantitative view of the variance in any measured motor unit parameter. Consider, for example, measurements of ΔF. In a sample of 20 simultaneously recorded motor units, each motor unit can be compared to all lower-threshold motor units, resulting in a large number of ΔF values. Even increasing the number of units to five or six greatly increases the set of ΔF measurements (Wilson et al. 2015). Measurements of rate-rate slopes or the range of recruitment thresholds require many data points to be meaningful; the array approach naturally fulfills this requirement. This in-depth information will allow both within- and between-subject comparisons for multiple muscles. Eventually, as arrays are increasingly used to study disease states, this population database per muscle and per subject may greatly aid subject-specific diagnosis and therapy. Recent studies are already producing encouraging results evaluating motoneuron intrinsic properties in stroke patients (Murphy et al. 2015).

The extensive data provided by arrays also has the potential to provide truly quantitative estimates of the amplitudes and patterns of the three components of motor commands. The existence of biologically realistic computer simulations provides a basis for "reverse engineering" motor unit firing patterns. Essentially, this approach would work as follows. A good-quality data set from array electrodes for a single human muscle is chosen as the target for reverse engineering. A pool of simulated motoneurons with properties that match those of human motoneurons is created. Then, simulations are run iteratively with different combinations of excitation, inhibition, and neuromodulation to discover the combinations that most accurately recreate the firing patterns of the target human data. These combinations will represent a solution space that will model the range of possible motor command structures for a particular set of firing patterns and motor tasks.

One major issue is, of course, whether there will be many redundant motor command structures that produce similar outputs or the solution space will be relatively restricted. Fortunately, there are many constraints on motor output that will tend to limit the solution space. Perhaps the most important constraints are *1*) that recruitment must be orderly and *2*) that all synaptic inputs must affect many motoneurons simultaneously instead of independently controlling individual cells. These constraints tend to ensure that the structure of motor commands, i.e., the pattern of excitation, inhibition, and neuromodulation, is approximately the same for all motoneurons in a pool. However, we expect that there will be exceptions, particularly when considering multijoint muscles (Hoffer et al. 1987a). Another important concern in addition to the size of the solution space is that a given level of depolarization can potentially be generated by many different combinations of excitation and inhibition. Fortunately, as shown in Fig. 6, the PIC is affected very asymmetrically by excitation, which simply activates it in a relatively uncontrolled manner (thus the sudden acceleration), and inhibition, which can control its activation (note the smooth modulation for push-pull organization). These considerations engender optimism that the re-

verse engineering approach will be feasible; however, considerably more study is required. We are presently examining the validity of reverse engineering in experiments in the decerebrate cat preparation (Silverman et al. 2005), where array recordings of motor units are easily obtained and where reverse engineering estimates of synaptic organization can be directly compared to intracellular data.

A further consideration is that there are several reasons to think that the three components of motor commands vary across different muscles. Different descending systems have anatomical projections that subserve different functional roles. The corticospinal system projects strongly to distal muscles and is important for precise control of discrete wrist, hand, and finger movements (reviewed in Alstermark and Isa 2012). Vestibular and reticulospinal systems project strongly to proximal muscles and have an important role in stabilization of the body and limbs (Matsuyama et al. 2004; Prentice and Drew 2001). Sensory input also varies across motor pools; reflexes mediated by muscle spindle Ia afferents appear to be strongest in stabilizer muscles (Pierrot-Deseilligny and Burke 2012). The overall pattern of neuromodulatory inputs has yet to be studied, but the brain stem neuromodulatory input is part of the reticulospinal system and thus may be strongest in proximal stabilizing muscles, providing an elegant functional role for the PIC's tendency to prolong motoneuron firing (as suggested in Lee and Heckman 1998a, 1998b). These considerations suggest that motor unit firing patterns may show significant differences in muscles across the body. Previous investigations have focused on a relatively small number of muscles, and so it is unclear whether there is an overall pattern. Array data have the potential to evaluate the overall concept that motoneuronal firing patterns vary systematically across the muscles of the human body and that this variation reflects fundamental connections between the synaptic organization of motor commands, the structure of the musculoskeletal system, and the diversity of motor tasks.

Summary

The chief points that this review has emphasized is that there are three main components to any motor command and that there exist parameters in the firing patterns of populations of motor units that provide important insights into the relative amplitudes and temporal patterns of these components. With the exception of the ΔF technique to estimate PIC amplitudes, these parameters have as yet been little used to further our understanding of the genesis of motor unit firing patterns. These parameters have limitations and may well interact—for example, the steepness of rate modulation will be affected by both PIC saturation and the organization of synaptic input (especially the balanced mode). It is thus likely that realistic computer simulations will continue to be fundamental for interpreting motor unit firing patterns.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

M.D.J., R.K.P., and C.J.H. conceived and designed research; M.D.J. interpreted results of experiments; M.D.J., V.M.T., R.K.P., and C.J.H. edited and revised manuscript; M.D.J., C.K.T., and C.J.H. approved final version of manuscript; C.K.T. and V.M.T. performed experiments; C.K.T. and R.K.P. analyzed data; C.K.T. prepared figures; R.K.P. and C.J.H. drafted manuscript.

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