Contribution of intrinsic motoneuron properties to discharge hysteresis and its estimation based on paired motor unit recordings: a simulation study

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Powers RK, Heckman CJ. Contribution of intrinsic motoneuron properties to discharge hysteresis and its estimation based on paired motor unit recordings: a simulation study. J Neurophysiol 114: 184–198, 2015. First published April 22, 2015; doi:10.1152/jn.00019.2015.—Motoneuron activity is strongly influenced by the activation of persistent inward currents (PICs) mediated by voltage-gated sodium and calcium channels. However, the amount of PIC contribution to the activation of human motoneurons can only be estimated indirectly. Simultaneous recordings of pairs of motor units have been used to provide an estimate of the PIC contribution by using the firing rate of the lower threshold unit to provide an estimate of the common synaptic drive to both units, and the difference in firing rate (ΔF) of this lower threshold unit at recruitment and de-recruitment of the higher threshold unit to estimate the PIC contribution to activation of the higher threshold unit. It has recently been suggested that a number of factors other than PIC can contribute to ΔF values, including mechanisms underlying spike frequency adaptation and spike threshold accommodation. In the present study, we used a set of compartmental models representing a sample of 20 motoneurons with a range of ΔF values, including mechanisms underlying spike frequency adaptation and spike threshold accommodation. We drove the models with linearly increasing and decreasing noisy conductance commands of different rate of rise and duration and determined the influence of different intrinsic mechanisms on discharge hysteresis (the difference in excitatory drive at recruitment and de-recruitment) and ΔF. Our results indicate that, although other factors can contribute, variations in discharge hysteresis and ΔF values primarily reflect the contribution of dendritic PICs to motoneuron activation.

motor unit; discharge hysteresis; persistent inward current

The input-output properties of motoneurons are strongly influenced by the presence of persistent inward currents (PICs) carried by voltage-activated calcium and sodium channels (Heckman and Enoka 2012; Powers and Binder 2001). Although PIC characteristics and their neuromodulatory control have been well described in a variety of animal preparations (e.g., Hounsgaard et al. 1984, 1988; Hounsgaard and Kiehn 1985; Lee and Heckman 1998a, 1998b; Bennett et al. 2001a, 2001b), their exact contribution to the control of human motoneurons remains controversial. The primary evidence for the contribution of PICs to the discharge patterns of human motoneurons is the presence of certain discharge characteristics that have been associated with PICs in animal preparations (Heckman et al. 2008b). One such characteristic is hysteresis.

When motoneurons are driven with a triangular injected current command, firing rates tend to be higher on the descending limb of the command, and the motoneuron stops firing at a lower level of injected current than was required to recruit it (Hounsgaard et al. 1988; Lee and Heckman 1998b; Bennett et al. 2001a, 2001b). The difference in discharge on the ascending and descending phase of the command is thought to reflect the PIC contribution to the total current (intrinsic + injected) reaching the spike initiation zone, and that PIC contribution is thought to be mediated primarily by dendritic calcium channels. Gorassini and colleagues (2002) proposed that an indirect measure of hysteresis could be used to estimate the PIC contribution to human motoneuron discharge. Since the level of excitatory input to motoneurons cannot be measured directly in humans, they used the discharge rate of a lower threshold motor unit (reporter unit) to estimate the level of excitatory drive at the time of recruitment and de-recruitment of a higher threshold (test) unit. A higher firing rate value at recruitment is thought to reflect the fact that PIC is not yet fully active in the test unit; its subsequent activation allows the test unit to continue firing until synaptic drive (and the discharge rate of the lower threshold unit) has reached a lower value. They argued that the difference in firing rate (ΔF) of the reporter unit at recruitment and de-recruitment of the test unit reflects the PIC contribution to total current (synaptic + intrinsic) driving the test unit. Subsequent studies have provided evidence that factors other than PIC can contribute to variation in ΔF values, such as the amount of rate modulation in the reporter unit, and the difference in recruitment times between the reporter and test unit (Powers et al. 2008; Stephenson and Maluf 2011). More recently, Revill and Fuglevand (2011) have suggested that other intrinsic properties of motoneurons, such as those underlying spike threshold accommodation and spike frequency adaptation, can lead to positive ΔF values, even in the absence of a PIC contribution. They also proposed that, by varying contraction duration and speed, it might be possible to distinguish the contribution of PICs, accommodation and adaptation to ΔF. Their simulations suggested that low contraction speeds led to larger ΔF values if accommodation was the primary contributor, whereas larger ΔF values for longer duration contractions occurred when adaptation was the primary contributor.

In the present study, we use a set of compartmental models representing a sample of 20 motoneurons with a range of thresholds to investigate how several different intrinsic motoneuron properties can potentially contribute to variations in...
ΔF values. We found that, to obtain ΔF values in the range reported for human motor units (~4 imp/s), the models had to express relatively high PIC levels compared with those reported in the animal literature (Lee and Heckman 1998a, 1998b, 1999). Overall, our simulation results suggest that, although ΔF values are not linearly related to PIC levels and can be affected by other factors, ΔF values >1 imp/s require a significant level of PIC generated by dendritic channels, and increases in PIC above this minimum level should lead to higher ΔF values.

**METHODS**

**The Basic Model Population**

The basic model population is similar to the set of single cable compartmental models described in Powers et al. (2012). In that study, each model consisted of a multicompartment tapering dendritic cable, a soma compartment and a series of compartments representing the axon hillock and initial segment. The ion channels in the model are based on the available experimental data, and include spike generating conductances (Na and K) on the soma and axon, afterhyperpolarization (AHP)-generating conductances (high threshold Ca and Ca-activated K channels) on the soma and proximal dendrites and low-threshold calcium (Ca1.3) channels on the dendrite (Powers et al. 2012). The dendritic cable also has Ca-activated K channels (Li and Bennett 2007), Na channels (Jones and Lee 2006), and hyperpolarization-activated, cyclic-nucleotide-gated channels (Manuel et al. 2007; Zhao et al. 2010). To produce a range of recruitment thresholds and discharge properties in the population of models that mimics that found experimentally (Powers and Binder 2001), low-threshold models had a smaller surface area, a higher specific membrane resistance, longer AHPs and lower hyperpolarization-activated cyclic-nucleotide-gated channel densities than higher threshold models.

When these single-cable models are driven by a triangular dendritic excitatory conductance command, they can reproduce a number of features of human motor unit discharge during linearly increasing and decreasing voluntary isometric contractions (Powers et al. 2012). However, on the descending limb of the command, they exhibit a sharp drop in discharge rate just prior to de-recruitment, reflecting the collapse of a plateau of dendritic depolarization (see Fig. 3 in Powers et al. 2012), whereas human motor units typically show a smoother decline in firing rate prior to de-recruitment (e.g., Fig. 3 in Gorassini et al. 2002). Motoneurones have a number of extensively branching dendritic trees, allowing the possibility of multiple plateau-generating regions that can potentially have different thresholds for activation and de-activation (Carlin et al. 2009), and this variation may act to smooth out the decline in motoneuron firing rate as the synaptic command decreases. However, simulations and sensitivity analyses using a population of motoneuron models with completely represented dendritic trees would be quite computationally expensive. In the present study, we compromised by replacing our single cable models with three dendritic cables attached to the soma compartment. The three cables had the same length and diameter profile (representing one-third of the surface area of the original cable), but different densities of Ca1.3 channels, leading to different depolarization profiles when activated by the same synaptic input.

Figure 1 shows the response of a medium threshold three-cable motoneuron model to a noisy, triangular conductance command (Fig. 1D) applied to the dendritic cables. Figure 1B shows the somatic voltage (black) and the voltage in the middle of the dendritic cables with high (red), medium (green) and low (blue) Ca1.3 channel densities. The firing rate (Fig. 1A) shows an initial steep rise, reflecting the onset of a plateau depolarization in the cable with the highest density of calcium channels. The subsequent, shallower increase in firing rate reflects the almost complete saturation of depolarization in the high-density cable and partial saturation in the cable with a medium Ca1.3 channel density. On the descending limb of the conductance command, the graded decline in depolarization in the medium- and low-density cables leads to a relatively smooth decline in firing rate. Figure 1C shows the proportion of Ca1.3 channels activated in each of the dendrites. Only the dendrite with the highest density of Ca1.3 channels shows nearly complete channel activation. The red trace in Fig. 1D shows the mean level of excitatory conductance, and the arrows show the mean level at recruitment and de-recruitment. The lower level at de-recruitment reflects the PIC contribution to the hysteresis in the onset and offset of discharge.

This discharge hysteresis is shown more clearly in Fig. 1E, which plots firing rate as a function of excitatory conductance (expressed as a percentage of the maximum level). The arrows indicate the direction of change of conductance. The motoneuron is recruited when the excitatory conductance reaches 26.5% of its maximum value, but is only de-recruited when the conductance falls to 9.4% of its maximum, giving a difference (ΔG) of ~17%. Other than this difference, the firing rate responses are similar over most of the ascending and descending limb of the conductance command, indicating that, for the standard model, the firing rate provides a similar estimate of the relative excitatory drive, regardless of whether conductance is increasing or decreasing. Somewhat different behavior is observed if the model is driven with a triangular injected current command applied at the soma (data not shown). In this case, there is a counterclockwise hysteresis in the firing rate, in addition to the difference in recruitment and de-recruitment thresholds, i.e., firing rates are higher on the descending limb of the command for the same level of injected current, as seen in experimental recordings (see Introduction). This difference presumably reflects the earlier activation of PIC by synaptic input on the dendrites, as opposed to current injected into the soma. In the remainder of the paper, we refer to a ΔG at discharge onset and offset as discharge hysteresis to distinguish this from firing rate hysteresis.

Figure 1F compares firing rate vs. conductance relations when the peak amplitude of the excitatory conductance command is reduced to 50% of the standard value (blue) or increased to 200% (red). For all three commands, the drive is sufficient to almost fully activate Ca1.3 channels in the most excitable dendrite (data not shown), whereas Ca1.3 channels in the other two dendrites reach nearly full activation only for the largest command. Nonetheless, ΔG is about the same in all three cases (see inset in Fig. 1F), showing that it is largely dependent upon current flowing from the most excitable dendrite.

The standard model also contains some other minor modifications from the Powers et al. (2012) model, including higher sodium and calcium half-activation voltages to more closely approximate the experimental data in decerebrate cats (Lee and Heckman 1998a, 1998b, 1999). As before, simulations were run in NEURON 7.3 (Hines 1989), and the NEURON files for running most of the simulations will be made available at http://senselab.med.yale.edu/modeldb.

**Additional Model Mechanisms**

**Spike frequency adaptation.** Spike frequency adaptation in motoneurones may depend upon a number of mechanisms (Powers et al. 1999). We incorporated three different additional mechanism that have been suggested to contribute to spike frequency adaptation: 1) slow sodium inactivation; 2) activity-dependent lengthening of the AHP; and 3) a slow, low-threshold voltage-dependent potassium current. The basic model has sodium channels mediating both transient and persistent sodium currents, and the transient currents exhibit only fast inactivation (time constant of a few milliseconds). We introduced an additional slow inactivation of both transient and persistent sodium currents as described in Fleidervish et al. (1996), so that classic Hodgkin-Huxley (Hodgkin and Huxley 1952) formulation for sodium channel activation and inactivation was supplemented with
an additional slow inactivation state (s), i.e., the proportion of open channels was given to be \( m_{3hs} \). The forward and backward rate constants (\( k_{f1} \) and \( k_{b2} \), in units of ms\(^{-1}\)) for slow inactivation are given as follows:

\[
\begin{align*}
k_{f1} &= 0.001 \exp\left(\frac{-85 - V_m}{30}\right) \\
k_{b2} &= 0.0034/\{\exp\left[\frac{-17 - V_m}{10}\right] + 1\}
\end{align*}
\]

where \( V_m \) is membrane potential. The slow inactivation state variable had a nonzero minimum value (Fleidervish et al. 1996). For the models with slow inactivation, the minimum value was 0.2 (regardless of model threshold), whereas in the standard models the minimum value was 1 (i.e., no slow inactivation). For models with inactivation, the formula for steady-state inactivation (s) is as follows: \( s = 0.2 + 0.8 \left[ \alpha/(\alpha + \beta) \right] \). With the parameter values given above, the half-maximal inactivation voltage \( (s = 0.5) \) was \(-38.5 \) mV, and the voltage-dependent time constant of inactivation had a peak value of about 2.3 s. Revill and Fuglevand (2011) modeled a much slower inactivation process, so we also looked at the effects of slow inactivation with a 10-fold reduction in the rate constants (i.e., with a maximal time constant of 23 s).

Recent evidence suggests that an increase in AHP duration contributes to decreased firing rates during prolonged motoneuron activity (Wienecke et al. 2009). Since the AHP in motoneurons is mediated by a Ca-activated K conductance (e.g., Viana et al. 1993), an increased AHP duration may reflect some saturation of calcium buffering and/or extrusion. Our model represents effective calcium concentration as a simple first-order process reflecting influx through calcium channels and decay with a single time constant, so we represented the net effect of activity on AHP duration by increasing the time constant of calcium decay by up to 50% of its base value based cumulative channel activity with a forward rate constant of \( n \times 0.002 \) ms\(^{-1}\) and a backward rate constant of 0.0005 ms\(^{-1}\), where \( n \) is the state variable reflecting the proportion of open AHP channels.

Finally, we looked at the effects of adding slow, low-threshold potassium channels to the initial segment, axon hillock and soma compartments. The voltage-dependence of activation was taken from a recent model of the M-current in hippocampal cells (Hu et al. 2009),

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**Fig. 1.** Response of a medium-threshold three-cable model to a noisy excitatory conductance command. **A:** firing rate (FR). **B:** somatic voltage and middendritic voltage in each of the three cables. **C:** proportion of low-threshold calcium channel activation \( m_{Ca_{1.3}} \) in each of the three dendrites. **D:** excitatory conductance (G) command applied to the dendrites (black). The mean level of excitatory G is shown in red. Red arrows indicate the mean level of excitatory G at recruitment and de-recruitment. **E:** FR vs. the mean level of excitatory G, expressed as a percentage of the maximum level. **F:** comparison of FR vs. G for the standard case (green) and when the maximum G is one-half (blue) or twice (red) the standard level. The inset shows an expanded version of the rate-G relations near recruitment and de-recruitment.
but the kinetics of activation were made two orders of magnitude slower to mimic the outward current used by Revill and Fuglevand (2011) to produce spike frequency adaptation, which had a time constant of 22 s. Although an M-current has been reported in turtle motoneurons (Alaburda et al. 2002) and KCNQ channels are present on the initial segments of mouse motoneurons (Duftocq et al. 2011), there has been no report of an M-current with a time constant on the order of seconds: we have simply chosen this as a mechanism to produce a slow, outward current.

**Spike threshold accommodation.** This process has been extensively studied in motoneurons in anesthetized cats, primarily based on changes in the minimal current required to evoke a spike during ramp current injections with different rates of rise (Schlue et al. 1974a, 1974b, 1974c). The minimal current required to elicit a spike during 1-s ramps was found to be up to 3.3 times the threshold to a step of current, with an average value of ~1.8 (Schlue et al. 1974c). The increase in current threshold is associated with a decrease in the rate-of-rise of the evoked spike (Schlue et al. 1974a, 1974b), suggesting subthreshold sodium inactivation as the likely mechanism. More recent experiments suggest that a strong persistent sodium current can greatly reduce the amount of accommodation and that, in the absence of a persistent sodium current, motoneurons will not fire repetitively in response to a slow current ramp (Kuo et al. 2006). In neocortical pyramidal neurons, the persistent sodium current exhibits two time constants of inactivation (Aracri et al. 2006), and the faster time constants (hundreds of milliseconds) are consistent with the effects of current ramp rate on threshold reported by Schlue et al. (1974c). We used the same formalism as that used for slow inactivation to describe the voltage dependence and time constants of this intermediate inactivation, but set the parameters to produce considerable inactivation in the subthreshold range:

\[
\alpha = 0.0075 \exp\left(-90 - V_m\right)/10
\]

\[
\beta = 0.015 / \exp\left(-26 - V_m\right)/10 + 1\]

The minimum value of this intermediate inactivation variable was set to 0.15.

A subthreshold potassium current could also give to spike threshold accommodation (Higgs and Spain 2011; Platkiewicz and Brette 2011; Sciamanna and Wilson 2011; Wester and Contreras 2013). Computer simulations (e.g., Sciamanna and Wilson 2011) show that inserting voltage-gated potassium (Kv1) channels on the axon near the site of spike initiation increases the voltage attenuation from this site to the soma and thus increases the somatic threshold for spike initiation. However, the Kv1 kinetics are too fast to reproduce the characteristics of accommodation in spin motoneurons. We inserted KCNQ channels on the initial segment instead, since these mediate the slower M-current. We again used the model of Hu et al. (2009), with activation kinetics that were four times slower than in the original model.

**Slow decreases in net PIC.** Two mechanisms were used to produce a slow decrease in the net PIC: 1) increased levels of the calcium-activated potassium (KCa) conductance in the dendrite; and 2) voltage-dependent inactivation of the dendritic Ca1.3 channels. Since PICs show less persistence in higher threshold cells (Lee and Heckman 1999ab), the influence of these mechanisms was increased as a function of threshold. For the first mechanism, we increased dendritic KCa as a linear function of recruitment order so that the density in the highest threshold cell was 3.2 times that in the lowest threshold cell. For the second mechanism, the half-inactivation voltage (\(V_{1/2}\)) was decreased in a linear fashion from the lowest to highest threshold cell (from 15 to ~25 mV). The steady-state inactivation was described as 1/[1 + \(\exp(V - V_{1/2})/10\)], where \(V\) is voltage, with a voltage-independent time constant of 2 s, chosen to approximate the time course of PIC decay described by Lee and Heckman (1998a). The dendritic KCa current acted more quickly in keeping with the findings of Li and Bennett (2007).

**Change in spatial distribution of Ca1.3.** The hysteresis in dendritic current delivered to the soma is thought to arise in part from an appreciable electrotonic distance between the dendritic channels mediating PIC and the soma (e.g., Booth et al. 1997; Elbasiony et al. 2005), although other factors may contribute (Morizot et al. 2007). To reduce hysteresis, we moved the Ca1.3 channels to the proximal portion of the dendrite and increased their density by 10% to get a similar initial peak of inward current (see Fig. 2D) in response to a voltage-clamp command.

**Simulation Protocols.** The models were driven with noisy, excitatory conductance commands that increased and decreased linearly at different rates, reaching the same peak conductance in 10, 5, or 2.5 s, as in the simulations of Revill and Fuglevand (2011). The excitatory conductance is generated as described in Destexhe et al. (2001) and is meant to mimic the random arrival of multiple unitary synaptic events; for an increasing conductance command, the mean and variance increase in parallel (see Fig. 1D). The same mean time course of excitatory command (red trace in Fig. 1D) is applied to each of the 20 models, and their discharge rates are compared to calculate \(\Delta F\) as described below. The same protocol is then run for different rates of excitation and different dendritic Ca1.3 densities. To relate discharge behavior to PIC characteristics, we also measure the response of the models to a slow triangular voltage clamp, as described in Lee and Heckman (1999; see their Fig. 1).

**Data Analysis.** We calculated \(\Delta F\) values for all possible pairwise comparisons for our sample of 20 models, yielding a maximum of 190 \(\Delta F\) values for each set of simulations. (The total number was generally less than this, since the calculation of \(\Delta F\) requires that the higher threshold unit is both recruited later and de-recruited earlier than the lower threshold unit; due to the noisy input a pair of units with similar threshold could sometimes show a different recruitment vs. de-recruitment order.) \(\Delta F\) was calculated as the difference in the value of the smoothed (5-point binomial) firing rate value of the lower threshold unit when the higher unit was recruited and de-recruited. Figure 2A illustrates the calculation of \(\Delta F\) for one pair of units. The black dots show the instantaneous raw firing rate of the earlier recruited unit, and the solid trace is the smoothed firing rate. The lower black dots show the firing rate of the later recruited unit. The left upward arrow indicates the smoothed firing rate at the time of the higher threshold unit’s recruitment, and the right arrow the smoothed firing rate at the time of its de-recruitment; the difference in rate is 5.9 imp/s. For a given higher threshold unit, the exact value of \(\Delta F\) will depend upon which lower threshold unit is used for the pairwise comparison; thus the ability to make multiple pairwise comparisons can potentially provide a more robust estimate of the PIC contribution to the motoneuron discharge behavior. Figure 2B shows mean and standard deviation for \(\Delta F\) estimates for the entire set of pairwise comparisons as a function of the difference in recruitment time between the pair (in bins of 0.5 s).

Figure 2C shows the voltage-clamp current (thick line) of a model of a medium threshold motoneuron to a simulated somatic voltage clamp (thin line). Figure 2D plots the voltage-clamp current as a function of voltage. The thick line shows the current recorded on the ascending phase of the voltage clamp, and the thin line that on the descending phase. The slope of the initial portion of the current-voltage (I-V) curve is used to calculate the input (leak) conductance, and the amplitude of the peak inward current on the ascending (initial) and descending phase (sustained) is calculated as the difference between the projected value of leak current and the actual peak inward current. The onset of the inward current is taken as the first zero-slope point on the I-V relation, and the offset as the final zero-slope point. The bistable current range is the difference between the current value at the onset point and the peak sustained inward current.
Comparisons between different sets of values were either based on t-tests, with the Bonferroni correction for multiple tests, or one-way ANOVA with a Tukey honestly significant difference test.

RESULTS

Influence of Spike Frequency Adaptation Mechanisms on ΔF

As described in Methods, we looked at the effects of including three different mechanisms of adaptation on ΔF values: 1) slow Na inactivation; 2) activity-dependent lengthening of the AHP; and 3) a slow, low-threshold outward current. Figure 3A shows the effects of these mechanisms on the response of a medium-threshold motoneuron to a 40-nA, 20-s injected current step. Since activation of dendritic PICs can distort the smooth, exponential decline in firing rate produced by these mechanisms, this panel shows the responses of models without dendritic PIC. The thin black trace shows the response of the standard model (no adaptation). After an initial, rapid drop in firing rate (the firing rate for the first two interspike intervals is off scale), the firing rate is constant. (This initial rapid drop in rate is seen in all models and probably reflects AHP summation; cf., Powers et al. 1999.) Slow Na inactivation (dashed trace) and AHP lengthening (gray trace) both produce a slower decline in firing rate of about 6–7 imp/s. It proved difficult to achieve greater amounts of adaptation using these mechanisms. Increases in sodium inactivation led to spike failure at higher firing rates. The AHP duration increased by about 17% over the course of the response, which is slightly larger than the average AHP prolongation reported by Wienecke et al. (2009) in motoneurons studied in unanaesthetized decerebrate cats. When the maximum amount of AHP lengthening was doubled, the drop in firing rate was 10 imp/s (not shown), but the percentage increase in AHP duration over 20 s was 34%, significantly larger than that generally found by Wienecke et al. (2009). In contrast, activation of a slow, outward current (thick black trace) produced a large drop in firing rate (~13 imp/s).

Figure 3B shows the response of the medium-threshold models (now with the full complement of Ca PIC) to a
triangular excitatory conductance command with a 10-s rise time. The recruitment times and initial firing rates are indicated by the upper oval in Fig. 3B and are similar for all of the models. As conductance increases, all three adaptation mechanisms lead to lower firing rates compared with the standard model (thin black), and, as expected from the responses to injected current, firing rates are lowest for the model with the slow outward current (thick black). Unlike the standard model, firing rates are lower on the descending limb of the conductance command for the models with adaptation, particularly for the models with the lengthening AHP (gray) and slow outward current, i.e., there is a clockwise firing rate hysteresis. Also note that the models with slow Na inactivation or AHP lengthening are de-recruited at about the same time as the standard model, whereas the model with the slow outward current is de-recruited significantly earlier. As a result, the discharge (onset-offset) hysteresis is significantly less in the model with the slow outward current than in the other models. Figure 3C quantifies the difference in the discharge hysteresis (expressed as a percentage of the maximum level) between the models with adaptation mechanisms and the standard models. Each point represents the percentage ΔG at recruitment and de-recruitment vs. a standard (control) model with the same recruitment rank. The points for the models with AHP lengthening (open circles) and slow Na inactivation (open squares) are close to the line of identity, and the mean values are not significantly different than those for the standard model (paired t-test = 0.35, nonsignificant for slow AHP; 1.93, nonsignificant for slow Na inactivation). In contrast, the ΔG values for the models with slow outward current (solid circles) mostly lie below the line of identity, and the mean value is significantly lower than that of the standard model (paired t-test = 3.57, P < 0.01). Although these results show how adaptation mechanisms can affect the difference in the level of conductance input at recruitment and de-recruitment, their effects on initial and final firing rates also will determine their influence on ΔF.

Figure 3D compares the firing rates at de-recruitment of the three models with adaptation mechanisms to those of standard models with the same recruitment rank. Most of the points for the three adaptation models lie below the line of identity,
indicating lower firing rates at de-recruitment than in the standard model. However, these differences only reached statistical significance for the model with the slow outward current (paired \( t = 4.69, P < 0.005 \)). Nonetheless, these differences can potentially affect estimates of \( \Delta F \), as pointed out by Wienecke et al. (2009). Depending upon the time of recruitment and de-recruitment of a higher threshold unit, lower firing rates near de-recruitment of the lower threshold (reporter) units could lead to higher \( \Delta F \) values. In contrast, less discharge hysteresis in the higher threshold unit should lead to lower \( \Delta F \) values. For the models with Na inactivation as well as those with the slow outward current, the effects of less hysteresis and lower de-recruitment firing rates appeared to cancel one another, as \( \Delta F \) values were not significantly different from those of the standard model (standard: 4.78 ± 2.12 imp/s, \( n = 168 \); Na inactivation: 4.62 ± 1.89 imp/s, \( n = 173 \); outward current: 4.44 ± 1.71 imp/s, \( n = 186 \)). In contrast, lower de-recruitment firing rates and similar amounts of discharge hysteresis led to \( \Delta F \) values for the AHP lengthening models that were significantly higher than those of the standard model (6.01 ± 2.23 imp/s, \( n = 167 \); unpaired \( t = 5.18, P < 0.001 \)). Nonetheless, AHP lengthening alone could not produce \( \Delta F \) values comparable to those seen in the presence of PIC. For the slow conductance command, the mean \( \Delta F \) value for the AHP lengthening model with no PIC was 0.72 ± 0.39 imp/s, and the \( \Delta F \) values for the other adaptation models without PIC were even smaller. Overall, these results indicate that adaptation mechanisms alone are not likely to be responsible for the \( \Delta F \) values measured in human subjects.

Interaction of Contraction Speed and Duration with the Effects of Adaptation Mechanisms on \( \Delta F \)

The simulation results of Revill and Fuglevand (2011) suggested that the drop in firing rate produced by adaptation mechanisms should increase with contraction duration, and that the resulting increase in firing rate modulation in the lower threshold reporter unit between recruitment and de-recruitment of the higher threshold test unit would lead to higher \( \Delta F \) values. Figure 4 compares \( \Delta F \) values for triangular contractions with three different rise times (short: 2.5 s, standard: 5 s, long: 10 s) as well as ramp and hold contractions with ramp rise times of 5 s, but different duration hold phases (5 and 10 s). In agreement with Revill and Fuglevand (2011), contraction duration had no significant effect on \( \Delta F \) values in the absence of adaptation mechanisms. However, the effects of contraction speed and duration on \( \Delta F \) values in the standard model (A), and models with different mechanisms of spike frequency adaptation (B–D) are shown in Figure 4. Box plots indicate median value (middle horizontal line), 75th and 25th percentiles (top and bottom of the box), and 90th and 10th percentiles (top and bottom vertical lines). Each of the five boxes in each panel represents the distribution of values for different contraction speeds [short: 2.5-s rise time; std (standard): 5-s rise time; long: 10-s rise time], and hold times for ramp and hold contractions (5 and 10 s). Horizontal lines and asterisks above the boxes indicate instances where the \( \Delta F \) values at different contraction speeds or durations were significantly different from one another based on a Tukey honestly significant difference test (*\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \)).
of any spike frequency adaptation (Fig. 4A, one-way ANOVA, \( F = 1.068 \), nonsignificant). In contrast, there were significant effects of contraction duration on \( \Delta F \) values when adaptation mechanisms were present (Fig. 4, B–D; one-way ANOVA, \( F = 3.860, 7.941, \) and \( 4.266; P < 0.05 \) in all cases). However, the effects of contraction duration were relatively small, and, depending upon the adaptation mechanism, \( \Delta F \) could either decrease or increase significantly with contraction duration. For models with either slow Na inactivation (Fig. 4B) or a slow, outward current (Fig. 4D), \( \Delta F \) values decreased significantly with contraction duration in some cases (Tukey honestly significant difference test), reflecting the fact that these mechanisms tended to lead to de-recruitment at a higher level of excitatory drive as duration increased (leading to less discharge hysteresis) without much change in the firing rate at de-recruitment. For models with slow increases in AHP duration (Fig. 4C), contraction duration had little effect on the excitatory conductance at de-recruitment, but did lead to lower firing rates at de-recruitment, leading in some cases to significant increases in \( \Delta F \) with increasing contraction duration.

**Influence of Spike Threshold Accommodation Mechanisms on \( \Delta F \)**

Revill and Fuglevand (2011) modeled accommodation as an increase in recruitment threshold that varied inversely with the rate of rise of excitation. The increase in threshold led to a higher level of excitatory drive at recruitment, leading to a higher initial firing rate without affecting the subsequent relation between excitatory drive and firing rate. As a consequence, the difference between the initial and final firing rates increased for all units, leading to positive \( \Delta F \) values that were greatest for the slowest contractions. In contrast, for the mechanisms of accommodation that we simulated, the firing rate for a given level of excitatory drive was reduced throughout the response (for reasons that are addressed in the DISCUSSION), leading to lower \( \Delta F \) values.

The effects of spike accommodation mechanisms depend upon the level of dendritic PIC. Figure 5, A–D, shows the relation between the spike threshold and the rate of rise of a current ramp injected into the soma of medium threshold

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**Fig. 5. Spike threshold accommodation. A:** effects of ramp speed on the voltage threshold of the first spike evoked by a ramp of injected current in the standard model, a model with intermediate inactivation of the persistent sodium current (NaP), and a model with axon KCNQ channels. B: effects of ramp speed on current threshold. C and D: same as A and B, except that the models have no Cav1.3 channels. The solid lines show best linear fits to the data, whereas the curved lines show the best power function fits. E: FR response of same models (with Cav1.3) to a slow, triangular excitatory G command. FR is plotted as a function of the relative level of excitatory G. The inset shows an expanded version of a portion of these rate-G plots to illustrate differences in recruitment (rec) and de-recruitment (de-rec). F: time course of variations in spike voltage threshold during the G-evoked discharge.
models with (A and B) and without (C and D) dendritic PIC. For the standard model with no accommodation, the spike voltage threshold (calculated as the voltage at which dV/dt first exceeded 10 mV/ms) in the presence of dendritic PIC (Fig. 5A, open circles) showed no dependence on ramp speed (r = 0.03, nonsignificant). Adding an intermediate time course of inactivation of the persistent sodium current (Fig. 5A, solid circles) produced a slight increase in spike voltage threshold that was not statistically significant (paired \( t = 2.40, P = 0.06 \)). The model with axonal KCNQ channels (Fig. 5A, open squares) had significantly higher spike thresholds (paired \( t = 6.73, P < 0.01 \)) that showed an inverse linear dependence on ramp speed (\( r = -0.93, P < 0.5 \)). Figure 5B shows that, when dendritic PIC was present, the current threshold showed a significant positive linear relation to ramp speed for all three models, instead of the expected inverse relation. The reason is that dendritic PIC shows some activation prior to spike threshold (particularly when spike threshold is elevated), and, since the apparent kinetics of PIC activation are slow (\( \tau = 60 \text{ ms} \) in all models), more PIC is activated at slower ramp speeds, leading to lower current thresholds.

The original experiments on accommodation in spinal motoneurons were performed under conditions in which descending neuromodulatory drive from the brain stem was minimal or absent (Bradley and Somjen 1961; Burke and Nelson 1971; Schlue et al. 1974a, 1974b, 1974c), so simulations in the models without dendritic PIC should more closely approximate these conditions. Figure 5, C and D, shows spike and current thresholds in the three different models without dendritic PIC. In the standard model, spike voltage threshold is independent of ramp speed, and current threshold shows a slight but significant positive linear relation to ramp speed. Both of the models with accommodation mechanisms now show a strong inverse dependence of both spike and current thresholds on ramp speed.

Figure 5E shows the firing rate responses of the three medium-threshold models (with dendritic PIC) to an excitatory conductance command with a 10-s rise time as a function of the relative level of excitatory conductance. Figure 5F shows the time course of spike voltage threshold. (Only one medium threshold model is shown for each model type, but similar results were obtained for all 20 models of each type.) Although both of the accommodation mechanisms produced an increase in spike threshold, recruitment thresholds were only slightly increased (upper oval in Fig. 5E), again reflecting the influence of dendritic PIC on threshold. The firing rates at recruitment were not significantly different between the three sets of models. The increase in spike threshold persisted throughout the response, resulting in lower firing rates compared with the model without accommodation, as well as earlier de-recruitment (lower oval in Fig. 5E).

The net effect of these changes led to \( \Delta F \) values that were significantly lower, both for the model with persistent sodium inactivation (3.78 ± 1.79 imp/s) and the model with axonal KCNQ channels (3.77 ± 1.50 imp/s) compared with the standard model (4.78 ± 1.77 imp/s). A similar pattern of effects (slightly higher recruitment threshold, lower firing rates, earlier de-recruitment) was observed, regardless of contraction speed or duration. As a result, as was the case for the standard model, there was no significant effect of the type of contraction on \( \Delta F \) values in the models with accommodation (data not shown).

Influence of Characteristics and Distribution of Dendritic Channels on \( \Delta F \)

The current delivered by dendritic channels to the spike generating conductances near the soma depends upon the characteristics and spatial distribution of dendritic channels. Since discharge hysteresis is thought to reflect the hysteresis of the current delivered by the dendrites (Lee and Heckman 1998a, 1998b), we examined the effects of mechanisms that would be expected to affect this hysteresis. Two broad types of mechanisms could reduce PIC-induced hysteresis, a progressive decline in PIC following its activation or a proximal shift in the channels mediating PIC. We studied the effects of two different mechanisms that produce a decline in the net inward current generated by dendritic channels: 1) an increase in outward current produced by dendritic KCa channels (Li and Bennett 2007); or 2) slow, voltage-dependent inactivation of Cav1.3 channels (see Methods).

Figure 6A shows the responses of a medium threshold model of the standard type (thin black trace), along with those of three different model variations to an excitatory conductance input with a 10-s rise time (gray: Cav1.3 inactivation, dashed: increased dendritic KCa, thick black: proximal Cav1.3 distribution). Changes in the properties or distribution of dendritic channels had relatively little effect on recruitment threshold, but in some cases did affect the firing rate at recruitment (upper oval in Fig. 6A). Figure 6B shows the firing rates at recruitment for each of the 20 models of each type vs. those of standard models with the corresponding recruitment rank. Cav1.3 inactivation has little effect on the initial firing rate (open circles), since inactivation only began to develop when the dendrites reached high levels of depolarization, which occurred after recruitment. Calcium entry through dendritic KCa channels began as soon as Cav1.3 channels started to open, and, in the case of high threshold motoneurons with higher levels of KCa, this could lead to lower firing rates at recruitment (open squares). For models with a proximal distribution of Cav1.3 channels (solid circles), their close proximity to the soma prevented an abrupt dendritic depolarization near the recruitment threshold, and firing rates at recruitment were lower than those of the standard models, regardless of their recruitment rank.

All three model variations exhibited earlier de-recruitment (i.e., they were de-recruited at high levels of excitatory conductance) compared with the corresponding standard models, and this led to lower levels of discharge hysteresis, as shown in Figure 6C. Both Cav1.3 inactivation and increasing KCa led to a decrease in the net PIC over time, resulting in early de-recruitment compared with the standard model that had a higher level of net PIC at de-recruitment. In the model with the proximal distribution of Cav1.3 channels, the response to the excitatory conductance input was symmetric, so that recruitment and de-recruitment occurred at approximately the same level of excitatory conductance, unlike the standard model where the electrotonic separation between the dendritic Cav1.3 channels and the soma resulted in a more prolonged dendritic depolarization that sustained discharge until the excitatory conductance had reached a lower level on the descending limb of the command.

Both lower firing rates at recruitment and earlier de-recruitment levels led to significantly lower values of \( \Delta F \) (\( t \)-test, \( P < 0.001 \)) for all of the model variants discussed above. For the conductance command with a 10-s rise time, \( \Delta F \) values were lowest for models with the proximal distribution of Cav1.3.
DISCHARGE HYSTERESIS IN MOTONEURONS

We explored the effects of changing the density of dendritic Ca$_{1.3}$ channels on the behavior of the four models considered above (the standard model and the three models with different dendritic channel properties or channel distribution). As would be expected, changing the density of Ca$_{1.3}$ channels changed the amount of inward current delivered to the soma. This can be seen most directly by comparing different characteristics of $I-V$ relations (see Fig. 2D) as Ca$_{1.3}$ channel density is varied. Figure 7 shows how two of these characteristics, the amplitude of the initial PIC peak amplitude and the bistable current range, vary as Ca$_{1.3}$ channel density is varied (density is expressed as a proportion of the value used in the previously described simulations). For all of the models, both the initial PIC peak amplitude and the bistable current range show a nonlinear relation to Ca$_{1.3}$ channel density, initially rising relatively slowly with increasing Ca$_{1.3}$ density and then more rapidly. (The nonzero initial PIC amplitude when Ca$_{1.3}$ density is zero reflects the contribution of the persistent sodium current.) The relation between Ca$_{1.3}$ density and initial PIC amplitude is similar for all of the models, whereas the bistable current range reaches higher values in the standard model than in the other models.

Figure 8 shows two measures of discharge behavior (average $\Delta F$ and average firing rate modulation) evoked by an excitatory conductance commands with a 5-s rise time as a function of $I-V$ characteristics. The relation of $\Delta F$ to the underlying PIC characteristics is very different for the different types of models. For matched levels of initial PIC peak amplitude, $\Delta F$ values are highest in the standard model, lower in the models with inactivating Ca$_{1.3}$ and increased KCa, and lowest in the model set with a proximal distribution of Ca$_{1.3}$ channels (Fig. 8A). The same trends are seen when $\Delta F$ is plotted against the average bistable current range (Fig. 8B), although the differences between the model sets are somewhat smaller. In contrast, the average peak firing rate shows very similar relations to PIC characteristics for the different model sets (Fig. 8, C and D). As discussed below, this indicates that $\Delta F$ values reflect the effects of PIC on discharge hysteresis rather than the total PIC contribution to firing rate modulation.

**DISCUSSION**

In the simulation results presented above, we examined the effects of a number of different mechanisms on the amount of discharge hysteresis, as well as estimates of discharge hysteresis based on paired motoneuron recordings ($\Delta F$). $\Delta F$ values based on paired recordings of motoneurons should accurately reflect the amount of discharge hysteresis in the higher threshold (test) unit, provided that the firing rate of the lower threshold (reporter) unit bears a consistent relation to the level of excitatory drive over the time period in which the higher threshold unit is active. This condition should generally hold for our standard model (no accommodation or adaptation), since firing rate vs. excitatory conductance relations for ascending and descending drive superimpose over much of their extent (Fig. 1E). However, mechanisms that change the slope of the relation between firing rate and excitatory drive should change the relation between $\Delta F$ and discharge hysteresis, and mechanisms that lead to different rate vs. conductance relations, depending on whether conductance is increasing or decreasing, can potentially lead to positive $\Delta F$ values in the absence of discharge hysteresis. Since there is no way to directly measure the level of excitatory drive in human motoneurons, we have simulated the effects of spike frequency adaptation, spike threshold accommodation and different characteristics of dendritic channels on the response of motoneu-
rons to excitatory conductance inputs. Our simulation results suggest that variations in dendritic PIC make the primary contribution to variations in discharge hysteresis, even though other factors may contribute.

**Model Limitations**

Although our models incorporate a number of features of real motoneurons, there is not sufficient experimental data to constrain the parameters describing channel kinetics and distribution, so our representations of the mechanisms underlying motoneuron discharge are obviously approximations. We have also simplified the geometry of our models, and a recent study suggests that this can lead to less hysteresis in the frequency-current and I-V relations for a given PIC amplitude (ElBaisouny 2014). Thus it is possible that our models overestimate the amount of PIC needed to produce a given amount of discharge hysteresis. However, the PIC amplitudes in our cable models are comparable to those simulated in model with a full representation of dendritic geometry (Bui et al. 2008; ElBaisouny et al. 2005), and the discharge hysteresis produced in a

![Fig. 7. Changes in average initial PIC peak amplitude (A) and average bistable current range (B) for four different model sets as Cav1.3 density (expressed as a proportion of the standard value) is varied.](image)

![Fig. 8. Relations between discharge behavior and PIC characteristics. Average ΔF as a function of initial PIC peak amplitude (A) and bistable current range (B) is shown. Peak FR as a function of initial PIC peak amplitude (C) and bistable current range (D) is shown.](image)
single cable model by synaptic input appears to be similar to that obtained in the full geometry model (see Fig. 3C in Elbasiouny 2014).

**Contribution of Different Spike Frequency Adaptation Mechanisms to Discharge Hysteresis and \( \Delta F \) Estimates of Hysteresis**

Spike frequency adaptation is reflected as a progressive decline in firing rate for a given level of excitation (Fig. 3A) that can lead to a clockwise hysteresis in the relation between excitatory drive and firing rate in response to a ramp increase followed by a ramp decrease in drive (Fig. 3B; and Bennett et al. 2001a, 2001b; Button et al. 2006; Turkin et al. 2010). In contrast to the counterclockwise firing rate hysteresis resulting from dendritic PIC activation, clockwise hysteresis leads to lowering firing rates on the descending limb of the conductance command. As pointed out by both Revill and Fuglevand (2011) and Wienecke et al. (2009), this alone will lead to positive \( \Delta F \) values, even if the higher threshold unit of a pair is recruited and de-recruited at the same level of excitatory conductance (\( \Delta G = 0 \)). For example, in the model with a slow outward current (thick black trace in Fig. 3B), firing rates are up to \( \sim 2.5 \) imp/s lower on the descending limb of the conductance command than they are at matched conductance levels on the ascending limb. Somewhat smaller differences are seen for the model with the lengthening AHP (gray trace, up to \( \sim 2 \) imp/s) and for the model with slow Na inactivation (dashed trace, up to \( \sim 1 \) imp/s).

In the absence of any effects on discharge hysteresis (\( \Delta G \)), these effects should lead to higher \( \Delta F \) values in the models with adaptation mechanisms. However, spike frequency adaptation could also lead to earlier de-recruitment and a decrease in \( \Delta G \). For the models with slow Na inactivation as well as those with a slow outward current, this latter effect was sufficient to counteract the effect of the progressive decline in firing rate, so that there was no net effect on \( \Delta F \) values. For the models with lengthening AHPs, the progressive decrease in firing rate was not coupled with a decrease in excitability, so that the models were de-recruited at similar levels of conductance as the standard models. This led to \( \Delta F \) values that were significantly higher than those of the standard models. This mechanism might explain the small (<1 imp/s) but significant increase in \( \Delta F \) values with decreasing ramp speed and increasing ramp duration that was recently reported in human soleus motor units (Vandenberk and Kalmer 2014). Nonetheless, our simulations indicate that spike adaptation alone is not likely to produce \( \Delta F \) values similar to those recorded experimentally; in the absence of dendritic PIC, models with lengthening AHPs yielded mean \( \Delta F \) values <1 imp/s, much less than those reported in the human literature.

**Contribution of Different Mechanisms of Spike Accommodation to Discharge Hysteresis and \( \Delta F \) Estimates of Hysteresis**

Spike accommodation could potentially lead to positive \( \Delta F \) values by increasing the recruitment threshold of the higher threshold (test) unit of the pair without changing its de-recruitment threshold, resulting in a higher firing rate of the lower threshold (reporter) unit at test recruitment than at de-recruitment (Revill and Fuglevand 2011). This scenario assumes that the activation or inactivation of conductances responsible for accommodation largely takes place prior to recruitment. For example, an increase in fast Na inactivation as the motoneuron is depolarized would lead to delayed recruitment, but, once repetitive discharge begins, the AHPs between spikes would relieve this inactivation, so that it would not affect subsequent discharge or de-recruitment threshold.

Although the amount of fast Na inactivation will certainly increase as the motoneuron is slowly depolarized toward threshold, Na channels show many time constants of inactivation, and the time constant of fast inactivation can be as short as \( \sim 1 \) ms (Barrett and Crill 1980). In contrast, the time constant of accommodation in cat motoneurons appears to be on the order of 100 ms (e.g., Fig. 2 of Schluhe et al. 1974b). This slower time constant of accommodation, together with the fact that much of the voltage range traversed during repetitive discharge is similar to that covered from resting potential to spike threshold, means that mechanisms of spike accommodation will continue to operate during repetitive discharge. We modeled spike threshold accommodation by including either a slowly inactivating persistent sodium current or a slowly activating axonal potassium current and found that both mechanisms produced increases in spike threshold that remained elevated throughout the period of repetitive discharge (Fig. 5F). Moreover, because the subthreshold activation of dendritic PIC acted to depolarize the membrane toward threshold, spike threshold accommodation had little effect on recruitment threshold, but did cause earlier de-recruitment (Fig. 5E). As a result, spike threshold accommodation led to significantly lower values of \( \Delta F \).

**Contribution of Different Types and Distributions of Dendritic Channels to Firing Rate Hysteresis and \( \Delta F \) Estimates of Hysteresis**

The presence of channels carrying voltage-activated PICs can lead to an N-shaped steady state \( I-V \) relation that has two stable equilibrium points (i.e., points where the net current is zero): one at the resting potential that is below the PIC activation threshold, and one at a depolarized potential that is above the PIC threshold (Gutman 1991). Activation of dendritic PIC channels can lead to a stable plateau of dendritic depolarization that provides an intrinsic inward current that adds to the depolarizing drive produced by excitatory synaptic input and can lead to discharge hysteresis (Gutman 1991; Heckman et al. 2008b; Houngaard et al. 1988; Powers and Binder 2001). Any mechanism that can lead to earlier deactivation of dendritic PIC channels should reduce the amount of discharge hysteresis. Our simulations suggest that either slow inactivation of dendritic calcium channels or slow activation of dendritic channels carrying outward current acts to decrease the net inward current delivered to the spike-generating conductances on and around the soma, reducing discharge hysteresis (\( \Delta G \)) and \( \Delta F \) values.

The duration of the dendritic plateau produced by PIC activation will also be influenced by the activation of conductances on or near the soma, and this influence is inversely proportional to the distance between the soma and the dendritic PIC channels (Manuel et al. 2014). During repetitive discharge, activation of perisomatic spike and AHP conductances acts as an imperfect voltage-clamp that limits the depolarization of the
somatic (and nearby) membrane as excitatory drive is increased (Schwindt and Crill 1982). Our simulations showed that, although the activation of PIC channels on the proximal dendrites acted to amplify the effects of excitatory synaptic drive, the partial voltage-clamp of these dendritic channels by the somatic conductances prevented a dendritic plateau potential and eliminated discharge hysteresis. Thus perisomatic channels alone do not produce hysteresis, which requires relatively distal dendritic channels.

**Using ΔF to Estimate Changes in Neuromodulation**

Increases in ΔF have been taken to indicate enhanced neuromodulatory effects on PICs following chronic spinal cord injury (Bennett et al. 2001a, 2001b) and following the administration of amphetamine (Udina et al. 2010). Conversely, the absence of significant differences in ΔF values between stroke patients and control subjects (Mottram et al. 2009) have been taken to indicate that factors other than increased neuromodulatory drive contributed to increased motoneuron excitability following stroke. There is a variety of evidence that PIC channel activation is enhanced through pathways mediated by the activation of receptors to neuromodulators, particularly the monoamines serotonin and norepinephrine (Heckman et al. 2008a, 2008b; Hounsgaard et al. 1988; Lee and Heckman 1999; Li et al. 2007; Powers and Binder 2001). Enhanced neuromodulatory effects can be mediated by increases in the activity of descending monoaminergic fibers (Hounsgaard et al. 1988), the administration of exogenous monoaminergic agonists (Lee and Heckman 1999; Li et al. 2007), or by injury-induced constituent activity in monoaminergic receptors (Murray et al. 2011).

Direct comparisons of the effects of spinal cord injury, and serotonin agonists or antagonists on PIC amplitudes and ΔF in the adult rat sacraudinal spinal cord preparation, support the association between PIC amplitude and ΔF values (Bennett et al. 2001a, 2001b; Li et al. 2007; Murray et al. 2011). In addition to their effects on calcium-mediated PICs, neuromodulators also enhance motoneuron excitability through their effects on a number of other channel types (reviewed in Heckman and Enoka 2012; Powers and Binder 2001). However, our simulations indicate these other effects would not counteract the effects of changes in PIC amplitude on discharge hysteresis and ΔF values. For example, neuromodulators have been shown to lower spike threshold (e.g., Fedirchuk and Dai 2004; Krawitz et al. 2001). Our simulations indicate that raising spike threshold through increases in accommodation reduces ΔF values; thus lowering spike threshold should enhance the effects of neuromodulatory drive on ΔF. Similarly, reductions in late spike frequency adaptation during fictive locomotion that are thought to be mediated by neuromodulatory pathways (Brownstone et al. 2011) would not be expected to affect ΔF values if this late adaptation is mediated by either slow sodium channel inactivation or a slow outward current. Our simulations indicate that lower firing rates at de-recruitment mediated by AHP prolongation could enhance ΔF values, but the differences in recruitment vs. de-recruitment firing rates appear to be similar in preparations with low and high levels of neuromodulatory drive (Wienecke et al. 2009). Thus both the experimental evidence and our simulation results support a correlation between ΔF values and the level of neuromodulatory drive.

**Variability of ΔF Values and Advantages of Multiunit Array Recordings**

Values of ΔF can exhibit considerable variability even for the same subject under similar conditions (e.g., Gorassini et al. 2002; Powers et al. 2008; Stephenson and Maluf 2011; Udina et al. 2010). Some of this variation may reflect a dependence of ΔF on the amount of rate modulation in the lower threshold reporter unit (Powers et al. 2008; Stephenson and Maluf 2011; see, however, Udina et al. 2010) or on the difference in the recruitment times of the reporter and test unit (Powers et al. 2008; Stephenson and Maluf 2011; Udina et al. 2010). Regardless of its source, variation in ΔF values can potentially limit the utility of the paired unit technique. Stephenson and Maluf (2011) found that the between-trial variation in ΔF values for the same pair of motor units under the same experimental condition implied that a difference of ΔF of at least 4.8 imp/s would be required to detect a change between experimental conditions. The reliability of the paired unit technique can be improved by averaging across several contractions (cf., Udina et al. 2010). In addition, averaging across several motor unit pairs in each condition will improve reliability. It is now possible to obtain simultaneous recordings of up to 20 motor units using arrays of surface electrodes (e.g., De Luca et al. 2006; Farina et al. 2010), which yields up to 190 pairwise comparisons. Our simulations show that this allows the detection of significant differences in ΔF across conditions of <1 imp/s (see Fig. 4).

**Summary**

Taken together, the results presented here and the considerations discussed above strongly support use of the ΔF technique of Gorassini and colleagues (2002) for estimation of PIC amplitudes in human motoneurons, but an important point is that absence of a positive ΔF value does not mean that PIC is absent. The ΔF value technique only detects PIC, if PIC amplitude is sufficient to produce some discharge hysteresis. If PIC amplitude is below a certain value, or if PIC inactivation or a counteracting outward current is prominent, PIC may contribute to synaptic amplification without producing discharge hysteresis. Since PIC inactivation has been reported to be prevalent in high-threshold motoneurons (Lee and Heckman 1998b), this may lead to lower ΔF values for high-threshold test units (Stephenson and Maluf 2011). Nonetheless, the frequent reports of positive ΔF values in human motoneurons (see above) suggest that PIC amplitudes are commonly fairly high. As PIC amplitude varies in proportion to neuromodulatory drive from the brain stem to motoneurons, ΔF is also a reasonable technique for estimation of their neuromodulatory state.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS
Author contributions: R.K.P. and C.J.H. conception and design of research; R.K.P. performed experiments; R.K.P. analyzed data; R.K.P. and C.J.H. interpreted results of experiments; R.K.P. prepared figures; R.K.P. drafted manuscript; R.K.P. and C.J.H. edited and revised manuscript; R.K.P. and C.J.H. approved final version of manuscript.

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