An evaluation of paired motor unit estimates of persistent inward current in human motoneurons

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Vandenberk MS, Kalmar JM. An evaluation of paired motor unit estimates of persistent inward current in human motoneurons. J Neurophysiol 111: 1877–1884, 2014. First published February 12, 2014; doi:10.1152/jn.00469.2013.—Persistent inward current (PIC) plays an important role in setting the input-output gain of motoneurons. In humans, these currents are estimated by calculating the difference between synaptic input at motor unit recruitment and derecruitment (∆F) derived from paired motor unit recordings. The primary objective of this study was to use the relationship between reciprocal inhibition (RI) and PIC to estimate the contribution of PIC relative to other motoneuron properties that result in nonlinear motor unit firing behavior. This study also assessed the contribution of other intrinsic properties (spike threshold accommodation and spike frequency adaptation) to ∆F estimates of PIC in human motor units by using ramps with varying rates of rise and duration. It was hypothesized that slower rates of ramp rise and longer ramp durations would inflate ∆F estimates of PIC, and RI and PIC values would only be correlated during the ramp with the fastest rate of rise and shortest duration when spike threshold accommodation and spike frequency adaptation is minimized. Fourteen university-aged participants took part in this study. Paired motor unit recordings were made from the right soleus muscle during ramp contractions of plantar flexors with three different rates of rise and durations. ∆F estimates of PIC increased with decreased rates of ramp rise (P < 0.01) and increased ramp durations (P < 0.01), most likely due to spike frequency adaptation. A correlation (r = 0.41; P < 0.03) between ∆F and RI provides evidence that PIC is the primary contributor to ∆F in shorter ramps with faster rates of rise. 

persistent inward current; paired motor unit; spike frequency adaptation; spike threshold accommodation

PERSISTENT INWARD CURRENTS (PICs) are an intrinsic motoneuron property that increases the excitability of dendrites via voltage-sensitive L-type Ca"++" and persistent Na"+" channels (Lee and Heckman 1999; Li et al. 2004). Activated by brief excitatory input when near spike threshold, PICs amplify and prolong the effects of synaptic input (Lee and Heckman 1996), resulting in a 6- to 10-fold increase in motoneuron output (Lee and Heckman 2000). PIC is strongly dependent on the monoaminergic input (Hounsgaard et al. 1988; Hounsgaard and Kiehn 1985; Lee and Heckman 2000). Descending monoaminergic input is distributed diffusely across motor unit pools, biasing motor output toward agonist-antagonist coactivation (for review see Heckman et al. 2008). Because inhibitory input deactivates PIC (Hounsgaard et al. 1988; Kuo et al. 2003), local inhibitory circuits, such as reciprocal inhibition (RI), contribute to agonist-antagonist coordination (Heckman et al. 2004). Thus motoneuron excitability is increased across motor unit pools via neuromodulatory input and fine-tuned to set appropriate levels of gain in specific motor units through afferent inhibition (Heckman et al. 2008). The paired-motor unit technique is used to estimate PIC in human motoneurons (Gorassini et al. 1998; 2004). This technique uses the firing rate of a low threshold motor unit to estimate synaptic drive during a slowly increasing and decreasing “ramp” contraction. The difference between control unit firing rate at the recruitment and derecruitment of a higher threshold unit (∆F) provides an estimate of PIC. In humans, this technique has been used to assess PIC in spinal cord injury (Gorassini et al. 2004; Mottram et al. 2009), following amphetamine administration (Udina et al. 2010), and following stroke (Mottram et al. 2009). A recent simulation study suggests that two other motoneuron properties [spike threshold accommodation (STA) and spike frequency adaptation (SFA)] contribute to estimates of PIC made from paired motor unit recordings (Revill and Fuglevand 2011). Spike threshold accommodation is an increase in the current required to initiate a spike with decreasing rates of current rise (Bradley and Somjen 1961; Schlue et al. 1974; Wigton and Brink 1944). Spike frequency adaptation is a time-dependent decrease in motoneuron firing rate during a constant current input. Thus both properties could contribute to differences in firing rate at recruitment and derecruitment, thus inflating ∆F estimates of PIC in human studies.

One purpose of this study was to methodically examine the contaminating effects of STA and SFA on paired motor unit estimates of PIC in human motoneurons by using the ramp profiles modeled in the simulation study by Revill and Fuglevand (2011). This was accomplished by varying the rates of rise and durations of ramp contractions. The primary objective, and most novel aspect of this study, was to use the pronounced effect of RI on PIC (Hyngstrom et al. 2007) to estimate the contribution of PIC to the ∆F values derived from paired motor unit recordings. We hypothesized that slower ramp rates of rise and longer ramp durations would inflate ∆F estimates of PIC, and RI and PIC values would only be correlated during the ramps with the fastest rate of rise and shortest duration, when the effects of SFA and STA should be minimized.

METHODS

Participants

Fourteen healthy, university-aged individuals (9 men, 21.6 ± 2.2 yr) participated in this study. Exclusion criteria included a diagnosed neurological disorder, acute or chronic ankle injury, and medications that would alter central concentrations of monoamines such as serotonin reuptake inhibitors and methylphenidate. Participants were asked not to consume caffeine or perform rigorous exercise 12 h prior to the experi-
mental session and were told to have a full night of rest and to eat prior to experimentation. Written, informed consent was obtained from each participant. The study was approved by the institutional research ethics board and is in accordance with the Declaration of Helsinki.

Apparatus

Participants were seated upright in a modified automobile seat. The right leg was positioned in a custom-made leg dynamometer with a knee, ankle, and foot brace, and with the hip and knee joints each in 90° flexion. Plantar flexion and dorsiflexion range of motion were recorded for the purpose of calculating relative joint angles used during the experiment. An ankle and foot brace was used to maintain leg position in the dynamometer. Finally, a padded clamp over the distal femur was used to prevent the heel from rising during isometric plantar flexion contractions.

Protocol

Participants attended one 3-h experimental session to complete all parts of the experiment. All contractions were isometric and performed with the ankle in neutral, dorsiflexed (DF), and plantar flexed (PF) joint positions. For DF and PF conditions, the ankle was passively rotated and held at 10° less than maximal plantar flexion and 20° less than maximal dorsiflexion, respectively.

Part A: maximal voluntary contractions and surface EMG. Participants performed maximal voluntary plantar flexion contractions at the beginning of the experimental session. They were instructed to maximally plantar flex and to refrain from using hip flexors and trunk muscles to aid them during the contractions. A 1-min rest was provided in between each contraction to prevent the onset of fatigue. Maximum voluntary contraction (MVC) force was recorded when the peak torque achieved across several contractions was consistent (contractions were within 10% of each other). Participants performed approximately four MVC contractions before a consistent torque was achieved (Fig. 1A).

Surface electromyography (EMG) was used to record tibialis anterior and soleus muscle activation via two bipolar silver-silver chloride recordings electrodes, each with a 0.8-cm recording surface and an interelectrode distance of 2.0 cm (EQ, Chalfont, PA). Skin under each electrode was shaved and swabbed with 99% isopropyl alcohol. Signals were preamplified 40× (soleus) and 60× (tibialis anterior), and passed through a custom-built variable gain second-stage amplifier (20×).

Part B: estimating PIC and intramuscular EMG. Participants performed triangular-ramp contractions of the plantar flexors to a peak force of ~10% MVC to recruit at least three motor units. To estimate the effect of STA on ΔF values, participants performed ramp contractions with different rates of rise through manipulation of the time to peak (5 s, 7.5 s, and 10 s to peak). To estimate the contribution of SFA to ΔF, participants performed ramp contractions with different durations by inserting a plateau in the middle of the contraction (5-s and 10-s plateau), and with a constant rate of rise of 5 s to peak (note: a plateau of 0 s is the same as the 5 s to peak ramp) (Fig. 1B). To increase the accuracy and consistency of contractions, a template for each type of ramp contraction was drawn on transparencies and placed over the force feedback screen, which participants were asked to trace. Because PIC is known to “warm-up” during repetitive activation (Bennett et al. 1998), the order in which these six types of ramp contractions were performed was randomized, and were separated by 30 s of rest. Ramps were repeated if the participant was unable to accurately trace the ramp template.

Contraction profile measurements were made for each ramp contraction, averaged across participants and placed in Table 1. Values include the force of ramp contractions relative to individual MVCs (%), duration of rise and decline (s), rate of rise and decline (% target force per second (%TF/s)), the duration of plateaus (s), and the root mean square error (RMSE) of the force tracings (%TF).

Single motor unit recordings were obtained from the soleus muscle using 50.8-μm Formvar-insulated stainless steel wires (California Wire). Three wires were inserted into the soleus muscle near the musculotendinous junction using a sterilized 27 gauge (1¼ in.) hypodermic needle. Two of the three wires (selected based on the quality of intramuscular recording) were input to a preamplifier (40×; EQ, Chalfont, PA), which passed through a custom-built variable gain second-stage amplifier (20×), and further amplified (5×; Neurolog, Digitimer, Greenvale, NY).

To estimate PIC, one contraction was selected for each of the six types of ramps based on smoothness and adherence to the template. The following analysis was performed in accordance with the procedure outlined by Gorassini and others (2004). The instantaneous firing rate of the low-threshold control unit was plotted on a X–Y scatter plot, and fit to a fourth- or fifth-order polynomial to achieve the best fit. The times at test unit onset and offset were used in the polynomial equation of the line fit to the firing rate of the control unit. To allow for full PIC activation of the control unit, test units that were recruited <1 s after control unit onset were not used in this analysis (Bennett et al. 2001; Powers et al. 2008). The control unit firing frequency at test unit offset was then subtracted from the control unit firing frequency at test unit onset. The resultant value is an estimate of PIC amplitude and was denoted as ΔF (Fig. 2).
Values are means ± SD. The characteristics of all ramp contractions analyzed, averaged across joint angles and participants, showing that participants adhered to the contraction profiles outlined for them are shown. Values include the force of ramp contractions relative to individual maximum voluntary contractions (MVCs; %), duration of rise and decline (s), rate of rise and decline [%target force per second (%TF/s)], the duration of plateaus (s), and the root mean square (RMS) error of the force tracing (%TF). *5 s to peak ramp is the same as 0-s plateau.

In addition to ensuring the test unit was recruited at least 1 s after the control unit, supplementary criteria were analyzed for each motor unit pair to establish the assumptions of the paired motor unit technique were met, and a valid estimate of ΔF was obtained. First, rate-rate correlations were performed to provide an estimate of common synaptic drive. The smoothed firing frequencies (4th-order polynomial) of each motor unit pair were correlated, excluding the first three to four motor unit discharges to remove firing rate acceleration at the time of recruitment. Motor unit pairs with an r > 0.7 were accepted as receiving common synaptic drive (Gorassini et al. 2004; Stephenson and Maluf 2011; Udina et al. 2010). Second, ΔF estimates were subtracted from the range of the control unit firing frequency (ffmax – ffmin), and resultant values ≥0.5 confirmed estimates were not a result of control unit saturation (Stephenson and Maluf 2011). Motor unit pairs that met all of the aforementioned criteria were incorporated into final analyses (see Table 2).

Peak RI values and ΔF values obtained from DF and PF ankle joint positions were made relative to the values obtained during the NL joint angle via subtraction (DF – NL and PF – NL). As a result, positive values denote less inhibition and a greater ΔF compared with a NL joint angle, and negative values denote more inhibition and a smaller ΔF compared with a NL joint angle. Normalized values were then plotted on an X–Y scatter plot and fit to a linear trend line. Separate X–Y scatter plots correlating RI and ΔF were created for each type of ramp condition (see Fig. 5).

This study used the same ramp profiles as simulated by Revill and Fuglevand (2011). The changes in rate of ramp rise used to estimate the effects of STA on ΔF resulted in a change in total ramp duration. Increased ramp duration may result in greater SFA. Therefore, ΔF values obtained during the two ramp conditions with a total duration of 15 s (7.5 s to peak and 5-s plateau) were correlated with each other. A similar correlation was performed with the ramps that had a total duration of 20 s (10 s to peak and 10-s plateau). Each correlation was then compared with a line of identity (see Fig. 6).

Part C: RI. RI was studied using 80 stimulations, just below motor unit threshold, applied to the common peroneal nerve approximately every 3 s, while participants maintained a low-level contraction of the soleus (Fig. 1C). A bipolar electrode was used to stimulate the common peroneal nerve at the head of the fibula. The stimulating electrode was connected to a stimulator (Digitimer DS7A) with an adjustable current intensity and duration. Motor threshold of the tibialis anterior motoneurons was obtained using a 1-ms stimulating duration. The timing of the stimulations was dependent on the firing rate of the motor unit being recorded and was set to 60 ms prior to the next expected motor unit discharge. The resultant time intervals used for stimulations were used to create a control marker after a motor unit discharge to provide data when the pathway was not activated.

To quantify RI, poststimulus time histograms (PSTHs) were derived from the motor unit discharges within the soleus after stimulation and control events and placed in 5-ms bins. PSTH values were normalized to the number of stimulations and control markers present after analysis. A rightward shift in the stimulation PSTH denotes inhibition; however, to quantitatively assess the degree of inhibition, difference PSTHs were made by subtracting the counts of the control

Table 2. Paired motor unit criteria values

<table>
<thead>
<tr>
<th>Ramp Condition</th>
<th>Time Between Control and Test Unit Onset, s</th>
<th>Test Unit ΔF-Control Unit Firing Rate, (Max-Min Firing Rate), impulses/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 s to peak*</td>
<td>2.1 (1.0–4.1)</td>
<td>3.7 (1.3–7.5)</td>
</tr>
<tr>
<td>7.5 s to peak</td>
<td>3.0 (1.1–5.0)</td>
<td>3.1 (0.5–8.4)</td>
</tr>
<tr>
<td>10 s to peak</td>
<td>3.6 (1.0–7.0)</td>
<td>2.9 (0.5–7.7)</td>
</tr>
<tr>
<td>5-s plateau</td>
<td>2.3 (1.0–3.9)</td>
<td>2.8 (0.5–6.1)</td>
</tr>
<tr>
<td>10-s plateau</td>
<td>2.2 (1.0–4.2)</td>
<td>2.5 (0.5–8.8)</td>
</tr>
</tbody>
</table>

The criteria required for control and test units to meet before being included in the final analysis are shown. Values [mean (minimum and maximum)] show time between control and test unit onset for each ramp condition (s), and the difference between the control unit range of firing rates (maximum − minimum) and test unit difference between control unit firing rate at the recruitment and derecruitment of a higher threshold unit (ΔF; impulses/s). *5 s to peak ramp is the same as 0-s plateau.
PSTH from the stimulation PSTH. The counts within the difference PSTH were then cumulatively added to form a cumulative sum, which permits an examination of the amount of inhibition, and the times at which it is most pronounced (Ellaway 1978). Post hoc analysis revealed a more pronounced effect of RI on the second interspike interval. As a result, peak values for RI were obtained from the cumulative sums of the second interspike interval (Fig. 3).

**Data Processing**

All signals were amplified, digitized (micro1401–3, Cambridge Electronics Design) and stored on a hard drive for offline filtering and analysis (Spike2, version 7, Cambridge Electronics Design). Force recordings were digitized at a sampling rate of 1000 Hz and low-pass filtered at 50 Hz. Values are expressed as a percentage of MVC (%MVC) and individual TF (%TF). The surface EMG was digitized at a sampling rate of 2,000 Hz (Micro3 1401, Cambridge Electronics Design), and high-pass filtered offline at 15 Hz. The root mean square of a selected duration was used to quantify the filtered EMG recordings. Intramuscular EMG was band-pass filtered between 300 Hz and 30 kHz (Neurolog, Digitimer, Greenvale, NY) amplified up to \times 3,200, and digitized at a sampling rate of 20 kHz (Micro3 1401, Cambridge Electronics Design). Motor unit action potentials (spikes) were extracted from intramuscular EMG recordings, and automatically and manually sorted, online and offline, based on shape and size using a template-matching feature in Spike2 software.

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**Fig. 3.** Quantifying RI. Control and stimulus poststimulus time histograms (PSTHs) were created using 5-ms bins, with a 4-s width to incorporate two spikes. A difference PSTH was made of the second interspike interval (ISI) by subtracting counts in the control PSTH from the stimulus PSTH. A cumulative sum (CUSUM) was made by cumulatively adding the values in the difference PSTH. The peak value in the CUSUM graph was used for analysis.
Statistics

Fifteen motor units were recorded at the NL and DF joint angles. Fourteen units were recorded at the PF joint angle because a shift in intramuscular electrode position during plantar flexion prevented spike discrimination in one experiment. Two-way (ankle joint angle × ramp type) repeated-measures ANOVA were performed to estimate the effects of STA (ramp rise times of 5 s, 7.5 s, and 10 s to peak), and SFA (ramps with 0-s, 5-s, and 10-s plateaus) on estimates of PIC during NL, DF, and PF joint angles. Post hoc analyses (Tukey honest significant difference) were performed on one-way ANOVA (ramp type with joint angle collapsed) when there was a main effect of ramp type, but no interaction or main effect of joint angle. Six Pearson correlations were performed to test the relationship between RI and ΔF during each ramp condition. Two additional Pearson correlations were performed to test the relationships between ΔF values obtained during ramp contractions with different rates of rise and plateau durations, but the same total ramp duration. Significance was set at an α-value of $P < 0.05$. All values are presented as means ± SD.

RESULTS

Assumptions of the Paired Motor Unit Technique

Pearson correlations of each motor unit pair met the $r \geq 0.7$ requirement ($r = 0.88 \pm 0.38$) to fulfill the assumption that the control and test unit of each pair received common synaptic drive. To ensure complete PIC activation of the control unit, test unit recruitment occurred ≥1 s after control unit recruitment ($2.58 \pm 1.22$ s). Last, the difference between the range of control unit firing frequency ($f_{\text{max}} - f_{\text{min}}$) and the ΔF of each ramp was ≥0.5 (3.09 ± 1.69) to ensure each paired motor unit estimate was not saturated by the maximum and minimum firing frequencies of the control unit. Averages and ranges for each ramp condition can be found in Table 2.

Main Effect of Ramp Condition on ΔF Estimates of PIC

Participants were able to track all ramp profiles with minimal deviations from the TF, as indicated by the RMSE (Table 1). RMSE was slightly higher for the shortest ramps with the greatest rate of rise, but there was no correlation between RMSE and the ΔF values for these ramps ($r = 0.18, P = 0.25$). Rate of ramp rise [$F(2,22) = 6.76; P < 0.01$] and ramp duration [$F(2,20) = 7.74; P < 0.01$] had a significant effect on ΔF estimates of PIC. When ankle angle was collapsed, Tukey’s honest significant difference post hoc analyses of one-way ANOVA revealed an increase ($P < 0.01$) in ΔF from the fastest rate of ramp rise (5 s to peak) to the slowest rate of ramp rise (10 s to peak) (Fig. 4A), and an increase ($P < 0.01$) in ΔF from the shortest ramp duration (0-s plateau) to the longest ramp duration (10-s plateau) (Fig. 4B). There were significant correlations between ΔF estimates of PIC during the two ramps that lasted 15 s (7.5-s to peak and 5-s plateau ramp conditions, $r = 0.79; P < 0.001$), and between ΔF estimates of PIC during the two ramps that lasted 20 s (10 s to peak and 10-s plateau ramp conditions, $r = 0.76; P < 0.001$). In both cases, the line of best fit falls on the line of identity (see Fig. 6).

RI and ΔF Estimates of PIC

There is a large range in the absolute measures of RI between participants. Consequently, changes in joint angle did not result in significant differences in mean RI values when comparing the negative peaks in the CUMSUM for DF (~1.05 ± 0.58), NL (~0.68 ± 0.85), and PF (~0.54 ± 0.84). Because we sought to determine how changes in RI would alter estimates of PIC, we plotted relative data (change in estimated PIC vs. change in RI when ankle joint angle was moved from NL to DF and and NL to PF) and report a correlation between the change in RI and the change in estimated PIC. A decrease in RI was correlated ($r = 0.41; P = 0.03$) with an increase in ΔF estimates of PIC, but only in the shortest ramps with the fastest rate of rise (5 s to peak/0-s plateau). There were no significant correlations between RI and estimated PIC when ramp rate of rise was increased (7.5 s to peak, 10 s to peak, Fig. 5, B and C) or when ramp duration was increased (5-s plateau, or 10-s plateau, Fig. 5, D and E).

DISCUSSION

It is well established that PIC contributes significantly to motor output by increasing motoneuron excitability (Lee and Heckman 1996) to amplify and prolong the effects of synaptic input (Gorrasini et al. 1998; Hounsgaard and Kiehn 1985). PIC has been extensively studied in an animal model (Hounsgaard et al. 1988;
Hyngstrom et al. 2007; Li et al. 2003), but the study of PIC in humans is important to understand changes in intrinsic motoneuron excitability in healthy, diseased, and aging populations. Paired motor unit recordings are used to provide estimates of PIC in human motoneurons (Gorassini et al. 2004; Powers et al. 2008; Stephenson and Maluf 2011). This method utilizes a low-threshold control unit and a higher threshold test unit. Estimates of PIC amplitude are quantified by subtracting the control unit firing frequency at test unit offset from the control unit firing frequency at test unit onset (Gorassini et al. 2004; Udina et al. 2010) with the assumption that synaptic drive to the control and test unit are the same (Gorassini et al. 2004). This technique has provided important insights into the contribution of PIC to muscle activation in humans. For example, the paired motor unit technique has been used to estimate PIC in stroke and spinal cord injured patients as a potential mechanism underlying spasticity observed in these clinical populations (Gorassini et al. 2004; Harvey et al. 2006; Mottram et al. 2009).

Recent work (Revill and Fuglevand 2011) suggests that motoneuron properties, such as SFA and STA, may also contribute to differences between recruitment and derecruitment firing rates, and contribute to paired motor unit estimates of PIC. Spike frequency adaptation is a time-dependent decrease in motor unit firing frequency due to slow inactivation of Na$^{+}$ channels and increased Ca$^{2+}$-dependent potassium conductance (Fleidervish et al. 1996; Miles et al. 2005; Powers et al. 1999; Sawczuk et al. 1997). Spike threshold accommodation is an increase in motoneuron firing threshold the slower the rate of current application and is most likely due to sodium channel inactivation. In the present study, we assessed the effects of SFA and STA on ΔF estimates of PIC indirectly by incorporating ramps of increasing durations (to increase SFA), and ramps with decreasing rates of rise (to increase STA), into the standard paired motor unit technique (Gorassini et al. 2004; Powers et al. 2008; Udina et al. 2010). It was expected that SFA, which decreases motoneuron firing rate (Sawczuk et al. 1997), and STA, which increases motoneuron threshold (Bradley and Somjen 1961), would inflate estimates of PIC in humans. The results of this study show that longer ramp durations increase estimates of PIC amplitude in human motoneurons.

**Fig. 5.** Relationship between RI and ePIC estimated with ramps of varying rates of rise and durations. Scatter plots displaying the relationships between peak inhibition (lowest CUSUM value) and ePIC (ΔF values) during ramps contractions with 5 s to peak (A), 7.5 s to peak (B), 10 s to peak (C), 5-s plateau (D), and 10-s plateau (E). Plantar flexion and dorsiflexion data are plotted relative to neutral, such that a positive value on the X-axis indicates less RI compared with the neutral joint angle and a positive value on the Y-axis indicates a larger ΔF value compared with neutral, as denoted by the compass inset. Significance was set at an α of 0.05.
The increase in ΔF estimates of PIC appears to be largely driven by SFA. Applying a hyperpolarizing stimulus would increase the effect of SFA (Partridge and Stevens 1976) and result in a further decrease in motor unit firing rate the longer it is active. As a result, when using the paired motor unit analysis, an increase in the amount of inhibition would produce a larger ΔF value due to a steeper decline in firing rate of the control unit. In summary, SFA would produce larger ΔF values with increased inhibition, and smaller ΔF values with less inhibition during ramp contractions longer than 10 s (i.e., 5 s to peak). These data points would fall in the top left and bottom right quadrants, respectively, of the graphs in Fig. 5. Results show that correlated values obtained during ramps longer and slower than 5 s to peak are found in both of the aforementioned quadrants, which supports the notion that SFA is most likely the primary motoneuron property contaminating estimates of PIC in this study. Accordingly, the correlations between ΔF values from ramps with an overall duration of 15 s (7.5 s to peak and 5-s plateau), and 20 s (10 s to peak and 10-s plateau) demonstrate lines of best fit that fall on the line of identity. If STA contributed significantly to ΔF, a hyperpolarizing stimulus would decrease the rates of accommodation and allow for earlier motor unit onset. As a result, an increased amount of inhibition would produce smaller ΔF values, which would cause the relationship seen during a 5-s to peak ramp contraction (Fig. 5A) to persist during ramps with slower rates of rise (Fig. 5, B and C). Additionally, if STA produced effects independent of SFA, the correlations seen in Fig. 6 would deviate from the line of identity. Therefore, because ΔF values are very similar for ramps with the same duration but different rates of rise, it would appear that SFA is the primary motoneuron property contaminating estimates of PIC in the present study. It should be noted, however, that the rhythmic firing properties examined in this study may share underlying mechanisms and, therefore, be somewhat interdependent. Sodium channel inactivation, for example, contributes to both SFA (Miles et al. 2005) as well as STA (Bradley and Somjen 1961), and it has been suggested that slow inactivation of the sodium PIC could contribute to SFA (Powers and Binder 2001), although this has not yet been clearly demonstrated.

If the recruitment and derecruitment ΔF derived from paired motor unit recordings is due, at least in part, to PIC, then factors that modulate PIC should alter ΔF. PIC is activated via excitatory input, and the extent of their activation is increased in the presence of monoamines within the spinal cord, such as serotonin and norepinephrine (Hounsgaard et al. 1988; Lee and Heckman 2000). Increased concentration of these central monoamines via administration of amphetamine is associated with increased paired motor unit estimates of PIC amplitude (Udina et al. 2010). An inhibitory stimulus, such as RI, is required to deactivate PIC (Hounsgaard et al. 1988). Thus, in the present study, we used the relationship between RI and PIC to assess the relative contribution of PIC to ΔF during ramps of different rates of rise and duration. We expected that RI would inactivate PIC (Hounsgaard et al. 1988; Kuo et al. 2003) and result in a decrease in ΔF. Because STA and SFA contaminate estimates of PIC, the strongest relationship between ΔF estimates of PIC and RI should be seen during the shortest ramps with the fastest rate of rise (5 s to peak force with no plateau), when other motoneuron properties contribute the least to ΔF values. As predicted, higher levels of RI were associated with smaller ΔF estimates of PIC during the ramps with the fastest rate of rise and shortest overall duration (5 s to peak, no plateau ramps). In contrast, there was no relationship between ΔF estimates of PIC and RI when rate of ramp rise was slower (7.5 s and 10 s to peak) or when ramp duration was increased (5-s and 10-s plateau). In other words, the effect of inhibitory input on PIC is most evident with the least contaminated estimate of PIC. Additionally, because a relationship exists between ΔF and RI during the shorter ramp contractions, we can conclude that, during this torque profile, ΔF is, at least in part, due to PIC.

Results of the present study suggest that other motoneuron properties are likely to contribute to paired-motor unit estimates of PIC amplitude. It should be noted that the present study relies on some assumptions, which may be overly simplistic. First, our interpretation of data from the present study rests on the assumption that the measure of inhibition made at the end of each ramp profile reflects baseline inhibition throughout the ramp protocol at each joint angle. A shortcoming of the PSTH approach to assessing inhibition at a single motor unit level is that it takes ~3 min and, therefore, cannot be used to assess changes in inhibition over the course of each ramp. It is certainly possible that inhibition changes during contractions as it does in other experimental protocols, such as Johnson et al. (2012). However, in contrast to Johnson et al. (2012) which utilized cyclical passive changes in ankle position, the ramp contractions made in the present study were...
intermittent and isometric. Thus it is not possible to predict how inhibition changes during the different isometric ramps in the present study. Interpretation of the data also relies on the assumption that motor units receive similar inputs during ramps with different rates of rise and durations. Although our experiment was not designed to test this possibility, the small differences in RMSE across ramp types and the lack of correlation between RMSE error and PIC estimates in the fastest and shortest ramps suggest that the strategies were similar across ramps. This is, nonetheless, an assumption that was not rigorously tested.

The strong relationship between inhibitory input and PIC amplitude when STA and SFA is minimized (fastest rate of rise and shortest overall ramp duration) provides evidence that these paired-motor unit estimates are due at least, in part, to PIC. While self-sustained firing of human motor units has been used to demonstrate the presence of PIC (Gorassini et al., 1998, 2004; Walton et al. 2002), this approach does not provide an estimate of PIC amplitude. Thus the paired-motor unit technique is currently the best procedure for estimating PIC amplitude in human motoneurons; however, we would suggest that ΔF values obtained from this technique might better be considered an estimate of overall intrinsic motoneuron excitability. In future paired-motor unit studies, assessing effects of inhibition on ΔF and varying ramp rate of rise and duration may yield more information when estimating PIC in human motor units.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.S.V. and J.M.K. conception and design of research; M.S.V. performed experiments; M.S.V. analyzed data; M.S.V. and J.M.K. interpreted results of experiments; M.S.V. prepared figures; M.S.V. and J.M.K. revised manuscript; M.S.V. and J.M.K. approved final version of manuscript.

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