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Intrinsic Activation of Human Motoneurons: Possible Contribution to Motor Unit Excitation

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Gorassini, Monica, Jaynie F. Yang, Merek Siu, and David J. Bennett. Intrinsic activation of human motoneurons: possible contribution to motor unit excitation. J Neurophysiol 87: 1850–1858, 2002; 10.1152/jn.00024.2001. The main purpose of this study was to estimate the contribution of intrinsic activation of human motoneurons (e.g., by plateau potentials) during voluntary and reflexive muscle contractions. Pairs of motor units were recorded from either the tibialis anterior or soleus muscle during three different conditions: 1) during a brief muscle vibration followed by a slow relaxation of a steady isometric contraction; 2) during a triangular isometric torque contraction; and 3) during passive sinusoidal muscle stretch superimposed on a steady isometric contraction. In each case, the firing rate of a tonically firing control motor unit was used as a measure of the effective synaptic excitation (i.e., synaptic drive) to a slightly higher-threshold test motor unit that was recruited and de-recruited during a contraction trial. The firing rate of the control unit was compared at recruitment and de-recruitment of the test unit. This was done to determine whether the estimated synaptic drive needed to recruit a motor unit was less than the amount needed to sustain firing as a result of an added depolarization produced from intrinsic sources. After test unit recruitment, the firing rate of the control unit could be decreased significantly (on average by 3.6 Hz from an initial recruitment rate of 9.8 Hz) before the test unit was de-recruited during a descending synaptic drive. Similar decreases in control unit rate occurred in all three experimental conditions. This represents a possible 40% reduction in the estimated synaptic drive needed to maintain firing of a motor unit compared with the estimated amount needed to recruit the unit initially. The firing rates of both the control and test units were modulated together in a highly parallel fashion, suggesting that the unit pairs were driven by common synaptic inputs. This tight correlation further validated the use of the control unit firing rate as a monitor of synaptic drive to the test motor unit. The estimates of intrinsically mediated depolarization of human motoneurons (~40% during moderate contractions) are consistent with values obtained for plateau potentials obtained from intracellular recordings of motoneurons in reduced animal preparations, although various alternative mechanisms are discussed. This suggests that similar intrinsic conductances provide a substantial activation of human motoneurons during moderate physiological activity.

INTRODUCTION

During voluntary contractions, the firing behavior of single motor units can vary in a nonlinear manner in relation to the exerted torque measured around the limb joint. For example, the torque at which a motor unit starts to fire during the ascending phase of an isometric contraction (recruitment threshold) is usually higher than the torque at which a motor unit stops firing during the descending phase of the contraction (de-recruitment threshold) (De Luca et al. 1982; Denier van der Gon et al. 1985; Milner-Brown et al. 1973; Person and Kudina 1972; Romaiguere et al. 1989, 1993). Likewise, firing rates at recruitment are, on average, higher than at de-recruitment. The lowered torque thresholds at de-recruitment have been postulated to involve fatigue, potentiation, and/or nonlinear summation of motor unit twitches (Binder-Macleod and Clamann 1989; Denier van der Gon et al. 1985), whereas the lowered firing rates at de-recruitment have been considered to be a consequence of rate adaptation during prolonged motor unit activity (De Luca et al. 1982).

The differences in firing behavior of motor units during the ascending (contraction) and descending (relaxation) phases of an isometric contraction may also be mediated by differences in the amount and type of synaptic inputs during the two phases of the contraction. However, recordings from peripheral sensory afferents, for example, show that in the presence of electromyographic (EMG) activity, afferent firing profiles are very symmetrical during contraction and relaxation of an isometric contraction (Edin and Vallbo 1990; Ribot-Ciscar et al. 1991; Vallbo 1971; Wilson et al. 1995). Another intriguing possibility that could mediate the observed rate-torque nonlinearities is the activation of intrinsic conductances in the parent motoneuron. For example, following the administration of neuromodulators such as serotonin or norepinephrine, motoneurons in reduced turtle, cat, and rat motoneurons display hysteretic firing profiles, i.e., continued activation of a cell at levels of depolarizing synaptic or intracellular inputs that are lower than the levels needed to recruit the cell initially (Bennett et al. 1998a, 2001b; Hougaard and Kiehn 1989; Hougaard et al. 1988; Lee and Heckman 1996). Continued firing of motoneurons at these lower levels of depolarizing input have been shown to be mediated by an intrinsic, voltage-dependent persistent inward current (I_{hpc}) activated during the ascending portion of a synaptic or intracellular current ramp (Hultborn and Kiehn 1992; Lee and Heckman 1998, 1999; Schwindt and Crill 1984). Thus the I_{hpc} and the resulting self-sustained depolarization (i.e., plateau potential) help to maintain firing of
the cell at the lower levels of extrinsic excitation during the descending phase of the synaptic or intracellular current ramp. Although similar intrinsically facilitated (i.e., self-sustained) firing has been inferred from paired motor unit recordings in humans and conscious rats (human: Gorassini et al. 1997, 1998; Kiehn and Eken 1997; rat: Eken and Kiehn 1989; Gorassini et al. 1999, 2000), to date no one has estimated the actual contribution of intrinsic mechanisms (e.g., IPSC and plateau potentials) to motoneuron activation in the human. To do so, an estimate of the synaptic drive (i.e., level of effective synaptic activation) to the motoneuron is required so that any discharge of a motor unit that cannot be accounted for by synaptic inputs alone can be attributed to the activation of intrinsic conductances (e.g., IPSC). Since obtaining such a direct measure in the human is technically impossible, we have estimated the synaptic input to a “test” motor unit by using the firing rate of a slightly lower threshold “control” motor unit recorded simultaneously in the same muscle (see also Crane et al. 1988; Eken and Kiehn 1989; Gorassini et al. 1998, 1999; Kiehn and Eken 1997). Thus if a control unit is firing throughout a contraction, increases or decreases in synaptic drive from descending and/or peripheral inputs to the test unit should be reflected in the rate profile of the control unit (see following text and discussion).

We have compared the spike frequency of a control motor unit (i.e., estimate of synaptic drive) at the time of recruitment and de-recruitment of a test unit during triangular, isometric contractions. We used moderately slow contraction speeds (~10 s/contraction) to match the activity patterns obtained during triangular current injections in decerebrate cat and in vitro rat motoneurons (Bennett et al. 1998a, 2001b; Lee and Heckman 1998) and moderately low contraction amplitudes to avoid recruitment of very high-threshold motor units (~40% MVC) (Nardone et al. 1989). The difference between the control unit frequencies at recruitment and de-recruitment of the test unit provided an estimate of the strength of the presumed intrinsic activation in maintaining firing of the test unit after recruitment. By estimating the synaptic drive (i.e., input) to the test unit in terms of control unit firing rate, rather than in terms of joint torque (i.e., estimated muscle output), we were able to investigate the input-output properties of human motoneurons in a more direct manner without the added variables introduced by torque measurements (e.g., contributions from agonist and/or antagonist muscles, Romaiguere et al. 1989, 1993). We have also estimated the strength of the presumed intrinsic activation under two additional experimental paradigms where the test unit was activated by more peripherally mediated synaptic activation (e.g., by sinusoidal muscle stretch or by muscle vibration). Finally, we have compared the rate modulation patterns of both the control and test motor units to provide evidence that in each of the unit pairs examined, both units were activated in a similar manner and, therefore probably responded to similar common synaptic drives.

Although we are quantifying the potential contribution of intrinsic mechanisms (for simplicity, we will use the example of plateau potentials from here on) in the activation of the test motor unit, we are also assuming that a plateau potential could be activated in the control motor unit as well. However, this should have no bearing on the ability of the control unit to provide an indication of synaptic drive to the test unit. For example, during synaptic activation of decerebrate cat motoneurons (in contrast to activation by intracellular current injection), the threshold for the IPSC and associated plateau potential are usually at or near the threshold for action potential generation (Bennett et al. 1998a, 2001b; Lee and Heckman 1998). At this low threshold, the IPSC rapidly depolarizes the cell during recruitment to help boost the initial firing rate of the motoneuron. However, immediately thereafter, the motoneuron responds linearly to any increase or decrease in extrinsic activation, especially during moderate and physiological firing rates in low-threshold cells. For example, during symmetrical triangular current ramps when plotting current against spike frequency, the firing frequencies during the descending phase of the current ramp tend to overlay the firing frequencies on the ascending phase of the current ramp (e.g., Bennett et al. 2001b; Lee and Heckman 1998). Thus during activation of a second test motor unit, the control unit should be firing within its linear range (after the initial high firing rate) during moderate, voluntary (i.e., synaptic) contractions.

Parts of the present study have been presented in abstract form (Gorassini et al. 1997).

**Methods**

Single motor unit activity was recorded in the tibialis anterior (TA, n = 12 unit pairs) or soleus (n = 4 unit pairs) muscle in 11 subjects with no previous history of neuromuscular disease or injury (6 females, 5 males; mean age, 28 yr). Informed written consent was obtained from each subject with the study approved by the Faculty of Rehabilitation Medicine Ethics Committee at the University of Alberta.

**Intramuscular electrodes**

The intramuscular electrodes were fabricated similar to that described by Eken and Kiehn (1989) and Dr. A. Prochazka (personal communication). For each electrode, three 50-μm stainless steel wires (California Fine Wire, 304, H-ML), ~12 cm long, were twisted tightly together. At 1 cm from the end of the wire bundle, a 90° bend was made, and a small bead of oven-cure epoxy was applied at the bend. The electrodes were then baked overnight and after hardening, the epoxy bead was cut flush, ~0.5 mm lateral from the bend so that the three exposed recording surfaces lay perpendicular to the long axis of the electrode. A small burr made from the cut epoxy acted as an anchor to help stabilize the recording end of the electrode once inserted into the muscle. For muscle insertion, the distal end of the electrode was housed in a 1½-in., 24-gauge needle that was removed after insertion. All electrodes were gas-sterilized before use and discarded after the experiment.

**Experimental protocol**

Subjects were seated comfortably in a chair with their left foot strapped securely onto a metal rest plate that was fixed to a rotary shaft. Knee and ankle angles were fixed to ~120 and 90°, respectively. The foot plate was coupled to a force transducer to monitor both dorsiflexion and plantarflexion torque about the ankle joint, which were scaled to the maximum voluntary contraction (%MVC) torque. Subjects had a visual display of their exerted torque on the computer screen using AxoScope 1.1 acquisition software and were asked to track a triangular line drawn on a transparency overlay on the screen. The horizontal scale on the computer display was adjusted to modify the speed of the contraction, which ranged from 2 to 5% MVC/s. Faster contractions were not studied given the variability in recruitment and de-recruitment thresholds of motor units at faster speeds (Desmedt and Godaux 1977; Freund 1983). The initial level and the
size of the contraction were controlled by changing the horizontal offset and vertical scale, respectively, of the torque display. The strength of the contraction was adjusted so that at least two units were identified in the intramuscular EMG signal. All trials were separated by ≥1 min to avoid frequency-dependent facilitation of the motor units (see companion paper, Gorassini et al. 2002).

In other trials, recruitment and de-recruitment of motor units in response to vibration or sinusoidal muscle stretch were examined to determine if similar reductions in de-recruitment threshold occurred during more peripherally mediated synaptic excitation. In these experiments, subjects were asked to maintain a constant level of dorsiflexion or plantarflexion torque. A brief muscle vibration or sinusoidal muscle stretch was then applied to either the TA or soleus tendon to reflexively recruit new (test) motor units, similar to that described in Gorassini et al. 1998, 1999. Following muscle vibration, subjects were instructed to slowly decrease their contraction effort to zero. The sinusoidal muscle stretches that were used to phasically activate the test motor unit were applied with a rotary motor that was coupled to the foot rest plate in a cam-type arrangement.

Data recording and analysis

The compound single motor unit action potentials (MUAPs) were recorded differentially, using the pair of wires that gave the best waveform discrimination. Surface EMG was recorded with electrodes placed over the TA and soleus muscles. The surface EMG was used to ensure that subjects did not co-contract the antagonist muscle, since this markedly affected the torque about the ankle joint at the contrac-
tion levels employed. All EMG signals were fed to custom-built preamplifiers that were electrically isolated from ground. The intramuscular EMG was typically amplified by 5,000–10,000 and band-pass filtered between 300 and 10 kHz. Surface EMG was amplified by 10,000–20,000 and band-pass filtered between 30 and 3 kHz. All signals were digitized at a sampling rate of 20 kHz using AxoScope 1.1 hardware and software.

Data were analyzed off-line using Linux-based software (Spinal Cord Research Center Analysis Software written by G. R. Detillieux, University of Manitoba). Single MUAPs were selected off-line by setting a horizontal trigger. Each selected MUAP was then inspected by eye, verifying that the discriminated potentials were of similar shape and belonging to the same motor unit. Once all units were selected for a single trial, each successive waveform was superimposed to compare the shape of each MUAP and to examine how it changed throughout a recording trial.

A pair of clearly distinguishable MUAPs was then selected from a given contraction. The relatively higher threshold unit of this pair (i.e., ~2–5% MVC higher than the control unit) was considered the test unit, and its recruitment and de-recruitment were observed. The lower threshold (control) unit fired during recruitment and de-recruitment of the test unit. The firing rate of the control unit was used as a monitor of the effective synaptic input to the motoneuron pool, and specifically to the test motor unit, as outlined in the INTRODUCTION (see also Gorassini et al. 1998). To detect firing rate changes reliably, a fifth-order polynomial was used to smooth the spike-frequency profiles of the control units. We found that a fifth-order polynomial was high enough to reflect the slow changes in the mean rate of the units during the 10-s contractions used in this study. Smoothing the spike-frequency profiles decreased the subjectivity in selecting the recruitment and de-recruitment values, especially when there were occasional extraneous frequencies or when the control unit rate occasionally varied more than 3 Hz about its mean.

The pattern of firing rate modulation during each control and test unit pair was compared to determine whether both units were responding to common synaptic drives during the triangular isometric contractions (see De Luca and Erwin 1994 for different technique). The mean firing rate (calculated every ~500 ms) of the control unit was plotted against the mean firing rate of the test unit. In some cases, the first two or three mean frequency values were omitted due to low start-up firing rates at the beginning of the contraction (Kiehn and Eken 1997), especially for slow contractions. Other data manipulations such as fitting linear regression lines to data and calculating paired t-tests (at 95% confidence level) were also performed. Means ± SD are presented throughout the article.

RESULTS

Motor unit firing at estimated subthreshold levels of synaptic drive

When recording the firing behavior of single motor units during tonic contractions, a striking observation was that, after a unit was recruited by muscle vibration, subjects could substantially reduce their contraction effort (and resulting torque) and yet the unit would continue to discharge. This relationship is illustrated in Fig. 1, where a subject was initially instructed to maintain a constant dorsiflexion effort, as reflected by the spike-frequency profile of a tonically activated control unit (bottom panel). The smoothed frequency profile of this control unit was used as an estimate of the synaptic drive to a second “test” motor unit (see following text). The TA muscle was briefly vibrated (hatched rectangle, top trace) to transiently increase the synaptic drive to the TA motoneuron pool, most likely by vibration-sensitive spindle afferents. A second, slightly higher-threshold unit (test unit, middle panel) was recruited toward the end of the muscle vibration at the dashed,
vertical line. The test unit continued to fire even after the vibration was removed (see also Gorassini et al. 1998; Kiehn and Eken 1997). Moreover, sustained discharge of the test unit occurred even though the firing rate of the control unit decreased from \( \approx 10 \) Hz before the test unit was recruited (as shown by the solid horizontal line, bottom panel) to \( \approx 5 \) Hz. In six motor unit pairs recorded from five different subjects, the average decrease in the firing rate of the control unit at de-recruitment versus recruitment of the test unit was \( 5.0 \pm 1.80 \) Hz (mean \( \pm \) SD, \( n = 16 \) vibration trials).

The response of the test unit to the brief muscle vibration demonstrated the following: 1) the test unit sustained its discharge even though the peripheral synaptic input that recruited the unit was removed (self-sustained firing), and 2) the test unit continued to discharge tonically at estimated levels of synaptic drive that were too low to recruit the unit initially. Both these phenomena may be explained by an intrinsic activation (e.g., plateau potential) in the parent motoneuron (see INTRODUCTION).

The estimated level of synaptic drive required to voluntarily recruit a motor unit (as opposed to a reflexive recruitment with muscle vibration) was also higher than the levels needed to sustain unit firing. This is shown in Fig. 2, where the test unit (middle panel) was recruited when the smoothed firing rate of the control unit was 8.8 Hz (horizontal line in bottom panel). As shown for the previous figure, the test unit continued to fire even when the subject markedly decreased the contraction effort, as related by a decrease in the firing rate of the control unit below 8.8 Hz (below horizontal line). Thus the test unit continued to fire at levels of estimated synaptic drive that were too low to recruit the unit initially: i.e., at subthreshold levels of synaptic drive.

**Difference in estimated synaptic drive at recruitment and de-recruitment**

We are assuming that the motor unit discharge at the lower levels of estimated synaptic drive was maintained mainly by an added depolarization produced from the sustained activation of intrinsic currents (e.g., \( I_{\text{PIC}} \)). If so, then the difference between the synaptic drive needed to recruit a unit, and the level required to sustain its firing at its minimal discharge rate (i.e., just before de-recruitment), can be considered as an estimate of the strength or amplitude of the intrinsic activation of the motoneuron. Hence, we instructed subjects to perform symmetrical, isometric contractions with triangular torque profiles. This approach allowed us to compare the spike-frequency value of a control unit when a test unit was initially recruited during the ascending phase of the torque ramp to the spike-frequency value of a control unit when the test unit was de-recruited during the descending phase of the torque ramp. The firing profile of a typical unit pair is shown in Fig. 3A, where a test unit (middle panel) was recruited at a control unit frequency of 7.8 Hz (1st dashed vertical line) and de-recruited at a lower control unit frequency of 3.3 Hz (2nd dashed vertical line). Thus compared with recruitment, there was a 58% drop in the firing rate of the control unit just before the test unit stopped firing. In total, 16 unit pairs were tested in 11 subjects and all unit pairs showed similar firing behavior during the triangular isometric torque contractions performed in this study.

The firing rates of the control and test units in Fig. 3A were plotted against one another to examine whether they were modulated in a similar manner (Fig. 3B). To do this, the firing rate profile for each unit pair was averaged every \( \approx 500 \) ms so that corresponding mean frequencies of the control and test unit could be plotted against one another. The coefficient of determination for the linear regression line fit through the
averaged rate-rate plot was very high ($R^2 = 0.92$), indicating that both units were modulated in a highly parallel manner. Averaging the firing frequencies every 100 ms produced a similar, but slightly lower, $R^2$ value (i.e., 0.83). The slope of the linear regression line was 1.2, indicating that the control and test units were firing at very similar rates.

**Analysis of reduction in synaptic drive to maintain firing after recruitment**

Only those trials where the rate of rise and fall of the triangular isometric contraction were smoothly controlled were used for analysis. Specifically, trials were not used if the rate of change of torque, at either recruitment or de-recruitment, was >16% MVC/s (see METHODS for rationale). Further, only trials in which the rate of relaxation was equal to or faster than the rate of contraction were included. This last criterion assured that we erred on the side of underestimating the reduction in rate of contraction were included. This last criterion assured in which the rate of relaxation was equal to or faster than the averaged rate-rate plot was very high ($R^2 = 0.92$), indicating that both units were modulated in a highly parallel manner. Averaging the firing frequencies every 100 ms produced a similar, but slightly lower, $R^2$ value (i.e., 0.83). The slope of the linear regression line was 1.2, indicating that the control and test units were firing at very similar rates.

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This value reflected an average reduction of ≈40% in the estimated synaptic drive required to initiate versus maintain a test unit’s discharge [i.e., (9.8 – 6.2)/9.8 × 100]. Figure 4B shows that the corresponding firing rate differences (i.e., for recruitment – de-recruitment) for our full sample of TA versus soleus units were 3.9 ± 2.7 versus 3.1 ± 1.5 Hz, $P = 0.1$, an insignificant difference.

When comparing torque values during recruitment and de-recruitment of the test unit (rather than control unit rate), the torque at de-recruitment of the test unit was also lower than at recruitment (by 25.3 ± 23.2%, see also Romaiguere et al. 1993 for similar results). However, the torque values were more variable compared with values measured by the control unit firing rate (i.e., the torque mean and SD were almost equal) and further suggests that ankle torque may not be the best indicator of input to the test motor unit (see DISCUSSION).

The mean firing rate of each of the nine control and test units in Fig. 4 were plotted against one another as for the example unit in Fig. 3. The average $R^2$ values for the linear regression lines fit through the rate-rate plots was $0.83 ± 0.07$ (range $0.71–0.96$), indicating that the firing rates of the control and test units in Fig. 4 were modulated in a very similar manner. In other motor behaviors (e.g., during tonic contractions and sinusoidal muscle stretch, see following text), these same unit pairs were also modulated in a parallel fashion. The mean slope of the linear regression line through the rate-rate plots was $0.95 ± 0.26$ (range $0.58–1.3$), indicating that the control and test units fired at very similar rates during the moderate, isometric contractions in this study. This is in contrast to the onion effect observed by De Luca and Erim (1994), where, during stronger isometric contractions, higher-threshold units actually fired at much lower rates than lower-threshold units. The lack of onion effect in this study suggests that the control and test units recruited during the moderate isometric contractions were similar with respect to their firing properties and recruitment thresholds.

Higher firing rates at recruitment for individual units

When considering individual motor units, as opposed to control and test unit pairs, a rather consistent finding during
these moderately slow (i.e., 2–5% MVC/s) triangular torque contractions was that the spike-frequency at recruitment was significantly higher than that at de-recruitment (De Luca et al. 1982). This is shown for 159 contraction trials in 16 randomly selected units (control or test) from 11 subjects (Fig. 5), where the firing rate of a unit at recruitment is plotted against the firing rate of a unit at de-recruitment. The majority of points fall below the 45° line (149 of 159 trials), and the mean de-recruitment rate is significantly lower (by 3.0 Hz) than the recruitment rate (6.3 ± 3.8 Hz vs. 3.3 ± 1.9 Hz, \( P < 0.0001 \)).

Again, only trials without abrupt torque changes and with rates of relaxation equal to, or slightly faster than, the rate of rise of a contraction were used.

**Reflexive recruitment and de-recruitment of motor units by muscle stretch**

Motor units that were reflexively recruited by sinusoidal muscle stretch were also de-recruited at estimated levels of synaptic input that were lower than the levels at recruitment. To demonstrate this, subjects were instructed to maintain a weak, tonic contraction while their ankle joint was passively rotated. Again, the firing rate of a tonically active control unit was used to monitor the synaptic drive to a higher-threshold test motor unit. The frequency profile of the control unit (e.g., TA control unit, middle panel in Fig. 6) was smoothly modulated during the applied muscle stretches, and this modulation likely reflected the underlying synaptic profile of the stretch-activated afferent inputs. For example, the peak firing rate of the control unit was slightly phase advanced to the peak of ankle dorsiflexion (ankle angle, top panel) as would be expected from primary spindle afferent activation (Hulliger et al. 1977).

A second TA test unit (bottom panel) was recruited during muscle stretch (plantarflexion) and de-recruited during muscle shortening. There was an average difference of 3.4 Hz (14.9 ± 1.0 – 11.5 ± 1.2 Hz, statistically significant \( P < 0.001, n = 10 \) stretches) between the control unit frequency at test unit recruitment (large, rightward arrows) and de-recruitment (small, leftward arrows). The average difference in control unit rate for four unit pairs tested during sinusoidal stretch was 3.9 ± 1.4 Hz (\( n = 4 \) subjects). This difference was very similar to that seen for motor units activated during the slower, volitional contractions (i.e., 3.6 Hz). Note also the very high firing rates (doublets) of the test unit reached at recruitment, compared with at de-recruitment, during the smooth muscle stretches.

**Discussion**

The results of this study show that in the human, the estimated minimum synaptic drive needed to sustain firing of a test was much lower (by \( \approx 40\% \)) than the amount required to recruit the unit initially. This finding consistently occurred under three different experimental conditions: 1) during recruitment of units by brief muscle vibration followed by de-recruitment during relaxation of a steady isometric contraction (Fig. 1); 2) during recruitment/de-recruitment of units by triangular isometric torque contractions (Figs. 3 and 4); and 3) during recruitment/de-recruitment of units by passive sinusoidal muscle stretch superimposed on a tonic, isometric contraction (Fig. 6). We have used the firing rate of the control unit to estimate synaptic drive to the test unit under study since we believe this is a better indicator of input to the motoneuron than contraction torque (see following section). The important functional implication is that maintained discharge of human motor units can occur at significantly lower levels (\( \approx 40\% \)) of estimated synaptic drive than the levels needed to recruit the motor unit initially.

**Firing rate of control unit as an accurate measure of synaptic drive to the test motor unit**

There are several important assumptions that we have made concerning the profile of the synaptic drive during the isometric contractions performed in this study and the ensuing response of both the control and test units to this input. First, we are assuming that both the control and test motor units responded in a similar manner to increases and decreases in net excitatory synaptic drive. This is supported by the finding that the mean firing rate profiles of the simultaneously recorded unit pairs increased and decreased together in a highly linear manner, especially during the triangular isometric contractions that were mainly used to calculate the contribution of the intrinsic activation of the parent motoneurons. The average coefficient of determination for all units when plotting the mean firing rate of the control and test units against one another was quite high \( (R^2 = 0.83) \), suggesting that the unit pairs were modulated by common synaptic inputs. However, we cannot rule out the possibility that synaptic inputs (descending or peripheral) were distributed differently to the control and slightly higher-threshold test motor units so that the firing rate of the control unit did not exactly reflect the synaptic drive to the test unit. It is unlikely, however, that the large difference in the estimated level of synaptic inputs at recruitment and de-recruitment of the test unit (\( \approx 40\% \)) can solely be explained by this mecha-
nism given the tight correlation between the firing rates of the control and test unit pairs. In addition, the TA motor units, which potentially come from more synaptically compartmentalized motoneuron pools (Hensbergen and Kernell 1997), had similar differences in control unit firing rates at test unit recruitment and de-recruitment as the more homogeneous soleus units (Fig. 4B). This suggests that the TA units were activated as a homogeneous population during the moderate contractions used in this study.

Second, we are assuming that the firing rate of the control motor unit was a sensitive and linear indicator of net excitatory synaptic drive at the time of test unit recruitment and de-recruitment. In decerebrate cat and in vitro rat motoneurons, spike-frequency profiles of low-threshold motoneurons have been shown to vary linearly with moderate, symmetrically modulated synaptic inputs or triangular current injections (note, this is in contrast to high-threshold cells where firing rates can actually decline or stay flat following the activation of a plateau) (Bennett et al. 1998a, 2001b; Lee and Heckman 1998; Prather et al. 2001). This occurred either when no I_{PIC} was activated, or when the I_{PIC} was activated coincident with cell recruitment, as usually happens with synaptic activation of the motoneuron. However, in the human, firing rates of motor units have been shown to saturate at high levels of torque (De Luca and Erim 1994), and thus we may have underestimated the synaptic drive, particularly during recruitment of the test unit. The firing rate of the control units was, on average, 10 and 6 Hz during test unit recruitment and de-recruitment, respectively (Fig. 4A). At these rates, the control units were firing within the sensitive range of the torque-frequency relation: i.e., between 5 and 15 Hz (De Luca and Erim 1994). Toward the peak of the triangular torque contractions, firing rates could exceed 20 Hz; however, we did not take measurements of control unit firing rates during this period.

Lowered firing rates unrelated to low synaptic inputs may also have been produced by rate adaptation toward the end of the 10-s contractions. For example, slow inactivation of the I_{PIC} in decerebrate cat motoneurons has been postulated to mediate rate adaptation during somatic current injection (Lee and Heckman 1998). However, during synaptic activation of motoneurons (especially low-threshold motoneurons), the amount of I_{PIC} inactivation (and correspondingly rate adaptation) was minimal for activation periods of this duration (Bennett et al. 1998a, 2001b; Lee and Heckman 1999). However, if there was any appreciable amount of rate adaptation, it was probably similar in the control and test units considering both these units were relatively low-threshold (i.e., recruited at <30% MVC). As such, this would not have affected the calculation of the estimated intrinsic activation of the motor units in terms of control unit firing rate since the effect of rate adaptation in both units would have been the same and thus canceled out. If the amount of rate adaptation was greater in the higher-threshold test motor units (Lee and Heckman 1998), the size of the intrinsic contribution to motor unit firing would have been underestimated. Finally, the low firing rates reached during de-recruitment (∼5 Hz or less) may have been generated by a sustained subthreshold depolarization with fluctuations in background noise occasionally driving the cell to fire (Kudina 1999; Matthews 1996), rather than being determined by the afterhyperpolarization of the motoneuron during a suprathreshold synaptic drive. Thus there is a possibility that the decrease in synaptic drive, in terms of control unit firing rate, was slightly overestimated at these lower frequencies. However, similar decreases in control unit rate at recruitment versus de-recruitment of the test unit (4–5 Hz) were found for units whose de-recruitment occurred at much higher control unit firing rates (>10 Hz).

Interpretation of results in terms of I_{PIC} and associated plateau potentials

ESTIMATED PLATEAU STRENGTH. During intracellular recordings, the input to the soma of a motoneuron can be directly controlled by current injection, and the effects of the I_{PIC} and associated plateaus on motoneuron firing can be quantified in terms of their amplitude and duration (Bennett et al. 1998a; Lee and Heckman 1999). For example, in Bennett et al. (1998a), the I_{PIC} in cat motoneurons contributed to an average increase in firing rate of 20 Hz, or about 40–50% of the frequency that these cells fire during moderate physiological conditions (∼40–50 Hz during posture and walking) (Hoffer et al. 1987). Obviously, we cannot control inputs to human motoneurons in a similar manner. However, it is reasonable to assume that the firing rate of a control motor unit during moderate activity is proportional to the synaptic drive to the test motoneuron (see above and INTRODUCTION). If we further assume that the I_{PIC} was activated and de-activated when the test unit started and stopped firing, then the effective current provided by the I_{PIC} can be estimated from the difference in the control unit firing rate at test unit recruitment and de-recruitment if it indeed was the main source of this difference (see above). This is ∼4 Hz or about 40% of the frequency that motor units fire during moderate isometric contractions (∼10 Hz). In motoneurons with higher initial discharge rates (>10 Hz) the % contribution to motoneuron activation by intrinsic sources would be smaller given that the I_{PIC} is probably of fixed amplitude in cells with different thresholds (Lee and Heckman 1998). In summary, it is possible that during moderate, physiological activity intrinsic conductances can contribute significantly (about 40%) to the activation of human motoneurons in comparable amounts to that seen for I_{PICs} and plateau potentials in decerebrate cat motoneurons.

LOWER DE-RECRUITMENT VERSUS RECRUITMENT RATES. The de-recruitment rate of both the control and test motor units during the 8- to 10-s triangular contractions was, on average, 48% lower than the initial recruitment rate (Fig. 5) (see also Christova and Kossev 1998; De Luca et al. 1982; Romaiguere et al. 1993). This is very similar to the results obtained for motoneurons in the decerebrate cat activated by muscle stretch or intracellular current injection of similar duration (∼5–8 s) (Bennett et al. 1998a). In the decerebrate cat studies, it was inferred that this effect resulted from the rapid activation of a I_{PIC} coincident to recruitment, which boosted the initial firing rate obtained by the cell. Further, the I_{PIC} was often not de-activated until after the cell was de-recruited (particularly with synaptic activation), so there was no corresponding rapid drop in firing rate as the excitation to the motoneuron was decreased, allowing lower rates of firing at de-recruitment. Alternatively, the failure to initiate firing at the minimum rate obtained at de-recruitment may also be explained in terms of the spike initiation mechanisms and associated fast-Na+ channels themselves, rather than the I_{PIC}. However, in low-
threshold cat motoneurons when the $I_{\text{PIC}}$ was not active at recruitment, cell firing was very symmetrical, with firing rates and input currents (or muscle stretch) very similar at recruitment and de-recruitment (e.g., Fig. 2A in Bennett et al. 1999b; see also Hounsgaard et al. 1988). The preceding statement also argues against the possibility that the lower firing rates at de-recruitment were produced by time-dependent changes in the spike-generating mechanism of the motoneuron (i.e., late adaptation) (Granit et al. 1963; Kernell and Monster 1982; Sawczuk et al. 1995). Rather, the persistent activation of the $I_{\text{PIC}}$ and associated plateau potential most likely allows the motoneuron to continue to discharge at lower levels of synaptic input than at recruitment and, therefore, at lower firing rates.

**Force generation**

In contractions where subjects were told to smoothly increase and decrease their effort (torque not controlled as in Fig. 2), motor units were de-recruited at a much lower torque than at recruitment. This often resulted in a marked asymmetry in the firing rate profile in relation to the peak torque: i.e., the unit fired much longer after the peak than before (asymmetrical to the right) (De Luca and Erim 1994; Gorassini et al. 1999). The asymmetrical torque profile may be explained by the presence of $I_{\text{PIC}}$, as follows. With the synaptic drive ramped up and down symmetrically at equal rates (see control unit in Fig. 2), the motor unit firing and associated force from each unit should be asymmetrical. This is due to the fact that once a unit is recruited, it should be more difficult to de-recruit because of the sustained depolarization produced by the $I_{\text{PIC}}$ at recruitment. Thus once all units are recruited (near peak torque) with a ramp increase in synaptic drive, motor unit firing and force should persist longer during a symmetrical decrease in synaptic drive.

With the motor unit force generated in this way, where the $I_{\text{PIC}}$ adds a sustained excitation as each unit is recruited, the torque not only reflects the synaptic input to the motoneuron pool, but also the asymmetrical effects of the $I_{\text{PIC}}$. For this reason, we have not used torque as the primary indicator of synaptic drive to the test motor unit. Furthermore, if antagonist muscles are activated, the torque produced about the ankle joint can vary substantially at these low contraction levels (e.g., Fig. 2, although co-contractions were monitored and minimized; see methods). Thus joint torque may not truly represent the muscle force of the motor unit–bearing muscle and can make interpretation of recruitment and de-recruitment torque thresholds problematic (Romaiguere et al. 1993).

**Functional implications**

Regardless of whether the rather indirect interpretation in terms of $I_{\text{PIC}}$ and plateaus is correct, the results demonstrate that the extrinsic excitation (reflexive or voluntary) required to recruit a motor unit is greater than the extrinsic excitation needed to maintain tonic firing of the unit. Thus less synaptic inputs (e.g., descending drive) are needed after voluntary recruitment of a motor unit to sustain its firing, and this must be due to some type of intrinsic depolarization of the motoneuron (see Hounsgaard et al. 1988). This may explain the common observation made by many subjects that it takes more effort to recruit a motor unit than it does to keep a unit firing tonically.

This amplification of synaptic inputs seems to be associated with recruitment of the motoneuron since after recruitment, the firing rate increases and decreases smoothly with effort to amplify synaptic inputs without disrupting motor unit recruitment and force generation mechanisms.

If the nonlinearities in motor unit firing observed are indeed mediated by similar mechanisms to that in cat and rat motoneurons ($I_{\text{PIC}}$ and plateau potentials), then the regulation of associated brain stem–derived neuromodulators, such as serotonin and norepinephrine, provides a powerful gain control mechanism over force generation (Hounsgaard et al. 1988; Hultborn and Kiehn 1992; Jacobs and Fornal 1993; Lee and Heckman 1999, 2000). It would be interesting to know how this proposed mechanism changes with motor state, injury, or disease that influence descending neuromodulatory systems.


