Role of motoneurons in the generation of muscle spasms after spinal cord injury

Monica A. Gorassini, Michael E. Knash, Philip J. Harvey, Dave J. Bennett and Jaynie F. Yang

Summary

Motoneurons in the spinal cord have intrinsic voltage-dependent persistent inward currents (PICs; e.g., persistent calcium currents) that amplify synaptic inputs by three- to five-fold in addition to providing a sustained excitatory drive that allows motoneurons to fire repetitively following a brief synaptic excitation. In this study, we examined whether prolonged involuntary muscle spasms in subjects with long-term injury to the spinal cord are mediated by the activation of PICs in the motoneuron. To examine this in the human, we used a paired motor unit analysis technique where the firing frequency of one motor unit of the pair (control unit) was used to estimate the synaptic drive to the motoneuron pool, including the drive to a second higher-threshold motor unit of the pair (test unit). The degree to which a motoneuron PIC helped to sustain the discharge of a test motor unit (self-sustained firing) was determined from the reduction in control unit firing at de-recruitment (ΔF) compared with recruitment of the test unit. This ΔF value corresponds to the reduction in synaptic drive needed to counteract the intrinsic PIC and, thus, was used as an indirect measure of this current. In the nine motor unit pairs studied, the average estimated synaptic drive, or control unit firing rate, required to recruit a test motor unit at the onset of a muscle spasm was significantly higher (by 43%) than the estimated synaptic drive during de-recruitment at the end of a muscle spasm. This indicated that a motoneuron PIC, and associated self-sustained firing, facilitated the firing of the test units during the prolonged muscle spasms. In addition, in all subjects tested (seven out of seven), we observed that following a muscle spasm or voluntary contraction, spontaneous and self-sustained firing of motor units could continue for many seconds, even minutes, at very low discharge rates (average 5.2 ± 1.6 Hz) with extremely low spike-to spike variability (coefficient of variation = 5.4 ± 1.6%). Moreover, increases in synaptic drive (noise) to the spontaneously firing units with voluntary muscle contractions or muscle spasms increased both the mean firing rate of the motor units in addition to their firing variability. This suggests that the slow spontaneous firing commonly observed in chronic spinal injury likely occurs without appreciable synaptic noise and is likely driven to a substantial degree by PICs intrinsic to the motoneuron because it is self-sustained and very regular.

Keywords: muscle spasms; plateau potentials; motoneurons; spinal cord injury; human

Abbreviations: AHP = after hyperpolarization; CV = coefficient of variation; F = firing; FDI = first dorsal interosseous; HAM = hamstrings; I = current; PIC = persistent inward current; QUAD = quadriceps; SCI = spinal cord injury; SOL = soleus; TA = tibialis anterior.


Introduction

The purpose of this study was to examine the neuronal mechanisms contributing to the activation of involuntary muscle spasms in subjects with long-term injury to the spinal cord. Involuntary muscle spasms, which last for 8–10 s on average (Kawamura et al., 1989), can be evoked by brief noxious or innocuous stimuli and form part of a spastic syndrome that also includes exaggerated tendon reflexes and muscle tone (Young, 1994). Changes in the excitability of several neuronal pathways, including recurrent inhibitory circuits (Mazzocchio and Rossi, 1997), Ia transmission to α-motoneurons (Mailis and Ashby, 1990), reciprocal inhibition (Boorman et al., 1996), flexor reflex afferent pathways (Baldissera et al., 1981; Roby-Brami and Bussel, 1987; Remy-Neris et al., 1999) and reciprocal facilitatory pathways (Crone et al., 2003) have been demonstrated in subjects exhibiting a spastic syndrome. However, the degree of excitability...
changes in these neuronal pathways was not well correlated to the degree of spasticity assessed clinically (Faist et al., 1994; Hiersemenzel et al., 2000), although Crone et al. (2003) did find an impressive relationship between the emergence of reciprocal facilitatory pathways and hyperactive tendon tap reflexes. Moreover, the relationship between the enhanced excitability of these pathways and the generation of prolonged muscle spasms remains unknown, as these reflex pathways were not studied during spasm activity but only during resting conditions or voluntary contractions.

Because muscle spasms can continue for many seconds following a brief sensory stimulation and they are non-volitional, Hans Hultborn and colleagues (University of Copenhagen, Denmark) have proposed that muscle spasms are produced by voltage-dependent persistent inward currents (PICs) in motoneurons that generate sustained depolarizations or ‘plateau potentials’ (reviewed in Eken et al., 1989; Powers and Binder, 2001). Motoneuron PICs not only amplify synaptic inputs 3–5 fold (Lee and Heckman, 2000; Prather et al., 2001; Binder, 2002), but they also provide a sustained excitatory drive that allows motoneurons to fire repetitively following brief or reduced synaptic excitations (self-sustained firing; Conway et al., 1988; Hounsgaard et al., 1988). PICs and the self-sustained firing they produce are facilitated by the monoamines serotonin and noradrenaline (Hounsgaard et al., 1988; Lee and Heckman, 1999) that are released by descending fibres originating in the raphe and locus coeruleus nuclei of the brainstem. The activation of PICs and associated self-sustained firing are regulated by controlling the activity of these descending monoaminergic pathways in addition to controlling descending and segmental inhibitory inputs to the motoneuron (Heckman et al., 2003). In intact systems, therefore, PIC activation in the motoneuron can be controlled to prevent excessive and unwanted muscle contractions, i.e. muscle spasms.

Recent studies in neurologically intact subjects have suggested that a significant amount of the excitatory drive required for cell firing comes from intrinsic (e.g. PIC) sources in the motoneuron (Kiehn and Eken, 1997; Gorassini et al., 1998, 2002a). In these studies, a paired motor unit analysis technique was used where the firing rate profile of a relatively lower threshold control motor unit was used to estimate the synaptic drive to a slightly higher threshold test motor unit under conditions of common synaptic drive (see Methods for rationale). From this technique, we have estimated that during moderate volitional or reflex muscle activity, 40–50% of a motoneuron’s depolarizing current comes from PICs (Gorassini et al., 2002a). This is because the amount of estimated synaptic drive (i.e. control unit firing rate) required to recruit a test motor unit was 40–50% higher than the amount required to sustain test unit firing at its minimum rate. We propose that a motor unit can continue to discharge at levels of synaptic drive that are much lower than the levels needed to recruit the unit because of the added depolarization from an intrinsic PIC activated in the motoneuron.

In animal models, after an acute and complete spinal cord injury (SCI), motoneuron PICs and self-sustained firing are largely eliminated due to the removal of descending monoaminergic inputs (Conway et al., 1988; Bennett et al., 2001b). However, after chronic SCI, the development of involuntary muscle spasms, hyperreflexia to non-noxious stimuli and clonus are paralleled by the re-emergence of calcium and sodium-mediated-PICs that drive self-sustained firing in motoneurons, even without exogenous application of serotonin or noradrenaline (Bennett et al., 2001b; Li and Bennett, 2003). Importantly, brief dorsal root stimulation can trigger PICs and self-sustained firing in motoneurons of chronically injured spastic animals to generate long-lasting, spastic-like reflexes in affected musculature. Elimination of the voltage-dependent PIC by hyperpolarizing the motoneuron eliminates the long-lasting portion of the reflex response and demonstrates that intrinsic PICs are the main source of excitatory drive that generates long-lasting muscle spasms in chronically injured animals (Bennett et al., 2001b; Li et al., 2004).

Considering that PICs contribute significantly to normal motoneuron activation in human subjects and that they re-appear in animal models of chronic SCI, we examined whether the activation of motoneurons during non-volitional muscle spasms in chronically injured subjects was also driven by PICs intrinsic to the motoneuron, as opposed to being maintained solely by elevated synaptic inputs. We did this by looking for evidence of self-sustained firing during involuntary muscle spasms using the paired motor unit analysis technique described above (Gorassini et al., 2002a). In addition, during the course of the experiments, we noticed that following a muscle spasm or voluntary contraction when there was little surface EMG activity, self-sustained firing of some motor units often continued for long periods at very low (≤5 Hz) and steady rates (see also Davey et al., 1990; Zijdewind and Thomas, 2001). Similar slow and regular discharge is also observed in motoneurons below a chronic spinal transection in rats and this self-sustained discharge has been shown to be driven by the repetitive activation of a sub-threshold, sodium-mediated PIC (Li and Bennett, 2003; Li et al., 2004). By increasing the level of synaptic noise to these spontaneously active units and measuring changes in discharge variability, we examined whether this involuntary activity was occurring in the absence of appreciable synaptic drive and hence was mediated by the activation of PICs intrinsic to the motoneuron (Jones, 1995; Matthews, 1996; Powers and Binder, 2000).

Methods

Patients

Fifteen subjects with damage to the spinal cord due to trauma (n = 13), vascular infarct (n = 1) or viral encephalitis (n = 1) with an average post-injury time of 7.1 ± 7.3 years were examined (see Table 1). Three subjects were classified as motor complete, i.e. not able to voluntarily activate muscles innervated by the spinal cord below the lesion. The remaining 12 subjects were motor incomplete, i.e. able to voluntarily activate muscles below the lesion as
motoneuron Plateaus in muscle spasms

Summary of injury and medication history for each subject tested and description of muscles, number of motor units in each muscle studied and stimulus used to evoke muscle spasms or spontaneous unit activity. *Motor unit pairs recorded during involuntary muscle spasms.

Table 1
Clinical, motor unit recording and muscle spasm summary

<table>
<thead>
<tr>
<th>Code/sex</th>
<th>Level: type</th>
<th>Cause of injury</th>
<th>Years post injury</th>
<th>Anti-spastic medicines</th>
<th>Muscle (no. of units recorded)</th>
<th>Control of muscle</th>
<th>Spontaneous unit activity</th>
<th>Stimulus used to evoke spasm/spontaneous unit activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M</td>
<td>T8: C</td>
<td>T</td>
<td>3.5</td>
<td>None</td>
<td>TA (2)</td>
<td>No</td>
<td>Yes</td>
<td>Manual rubbing of thigh</td>
</tr>
<tr>
<td>2M</td>
<td>C6: C</td>
<td>T</td>
<td>2.5</td>
<td>Baclofen, Valium</td>
<td>SOL (3)*</td>
<td>No</td>
<td>Yes</td>
<td>Movement of torso</td>
</tr>
<tr>
<td>3M</td>
<td>C5/6 IC</td>
<td>T</td>
<td>18</td>
<td>None</td>
<td>SOL (1)</td>
<td>Yes</td>
<td>Yes</td>
<td>Rip tape off leg</td>
</tr>
<tr>
<td>4M</td>
<td>C6/7: IC</td>
<td>T</td>
<td>1.5</td>
<td>Baclofen</td>
<td>FDI (2)*</td>
<td>Yes</td>
<td>Yes</td>
<td>Vibrate medial arch of foot</td>
</tr>
<tr>
<td>5M</td>
<td>C6/7: IC</td>
<td>T</td>
<td>2.5</td>
<td>Baclofen</td>
<td>TA (2)</td>
<td>Yes</td>
<td>Yes</td>
<td>Ice applied to forearm, Vibrate medial arch of foot</td>
</tr>
<tr>
<td>6M</td>
<td>T4/5: C</td>
<td>T</td>
<td>1.0</td>
<td>None</td>
<td>SOL (2)*</td>
<td>No</td>
<td>No</td>
<td>Vibrate medial arch of foot</td>
</tr>
<tr>
<td>7M</td>
<td>C5/6: IC</td>
<td>T</td>
<td>3.5</td>
<td>Baclofen</td>
<td>HAM (2)*</td>
<td>No</td>
<td>Yes</td>
<td>Movement of torso, Ice applied to inner thigh</td>
</tr>
<tr>
<td>8M</td>
<td>T5/10: IC</td>
<td>VE</td>
<td>0.8</td>
<td>None</td>
<td>TA (1)</td>
<td>Yes</td>
<td>Yes</td>
<td>Attempt to move legs</td>
</tr>
<tr>
<td>9M</td>
<td>T3/5: IC</td>
<td>VI</td>
<td>4</td>
<td>None</td>
<td>HAM (1)</td>
<td>Yes</td>
<td>Yes</td>
<td>Voluntary contraction</td>
</tr>
<tr>
<td>10F</td>
<td>C6/7: IC</td>
<td>T</td>
<td>6.5</td>
<td>Baclofen</td>
<td>None</td>
<td>No</td>
<td>NT</td>
<td>Movement of torso</td>
</tr>
<tr>
<td>11M</td>
<td>T6/7: IC</td>
<td>T</td>
<td>1</td>
<td>Baclofen</td>
<td>None</td>
<td>No</td>
<td>NT</td>
<td>Extend legs while lying prone</td>
</tr>
<tr>
<td>12M</td>
<td>T12-L1: C</td>
<td>T</td>
<td>5</td>
<td>Emipra-mine</td>
<td>None</td>
<td>No</td>
<td>NT</td>
<td>Movement of torso</td>
</tr>
<tr>
<td>13M</td>
<td>C5/6: C</td>
<td>T</td>
<td>17</td>
<td>Baclofen, Dantrim</td>
<td>None</td>
<td>No</td>
<td>NT</td>
<td>Push legs together</td>
</tr>
<tr>
<td>14M</td>
<td>C4-7: IC</td>
<td>T</td>
<td>20</td>
<td>None</td>
<td>SOL (1)</td>
<td>Yes</td>
<td>Yes</td>
<td>Voluntary contraction</td>
</tr>
<tr>
<td>15M</td>
<td>C5-6: IC</td>
<td>T</td>
<td>19</td>
<td>None</td>
<td>HAM (2)</td>
<td>TA (2)*</td>
<td>Yes</td>
<td>Voluntary contraction</td>
</tr>
</tbody>
</table>

Involuntary muscle spasms

Subjects were seated in their wheelchairs with their limbs free to move since limb immobilization interfered with generating involuntary muscle spasms. We asked subjects to describe the type of stimuli that best evoked muscle spasms in their upper or lower limbs. Based on this information, we then evoked spasms by applying ice to the skin, vibrating the medial arch of the foot or having subjects extend their torso (see Table 1). Two disposable surface EMG electrodes (3.3 × 2.2 cm, Kendall Soft-E, Kendall-LTP, Chicago, IL, USA), separated by at least 1 cm, were placed over muscles that appeared to be most active during the muscle spasms. Intra-muscular fine-wire EMG electrodes (described by Gorassini et al. 2002a) were then inserted into one or two of these muscles to record single motor unit action potentials.

If able, subjects were instructed to maintain a constant but weak contraction, either against a mechanical stop or gravity, to recruit a single ‘control’ motor unit. Auditory feedback of the intramuscular EMG was used to help subjects maintain a constant contraction. Once steady firing of the control unit was established, the experimenter applied the appropriate stimulus to evoke a muscle spasm. The strength of the applied stimulus was graded in order to produce a mild spasm so that one or two additional motor unit action potentials could still be discriminated in the intra-muscular EMG. A hard, over-cured epoxy burr at the recording end of the intra-muscular electrode stabilized the position of the electrode during the muscle spasms. Motor units that were recruited at the onset of a muscle spasm were considered as ‘test’ motor units. In cases where subjects were not able to contract voluntarily, the first motor unit that was recruited during the muscle spasm was used as the control unit and later recruited units (by at least 3 s) were used as test motor units. Control unit profiles with slow symmetrical increases and decreases in firing rate were selected as rapid changes in synaptic drive can affect both recruitment and de-recruitment thresholds (Freund, 1983).

Spontaneous unit activity

Motor units in the ‘relaxed’ muscle would continue to discharge in seven subjects tested (1, 3, 7, 8, 9, 14 and 15M; Table 1) following a
muscle spasm or voluntary contraction. During this spontaneous activity, subjects were instructed to remain relaxed and not to voluntarily intervene if they had control over that muscle. In six of the seven subjects, single motor unit action potentials were distinguished in the surface EMG with the spontaneously active unit appearing to be the only one active in that muscle, although there may have been activity in other units that were not detected by the surface EMG electrodes. These same units were also recorded during involuntary muscle spasm activity or during mild voluntary contractions to examine how superimposed increases in synaptic drive (noise) changed firing variability.

Data recording and analysis

Intra-muscular EMG signals were fed to a custom-built pre-amplifier and the surface EMG was fed to an eight-channel Octopus pre-amplifier (Bortec Inc., Calgary, Alberta, Canada), which was electrically isolated from the ground. EMG signals were amplified by 5000 and band-pass filtered between 100 and 10 kHz (intra-muscular EMG) or 10 and 1 kHz (surface EMG). All signals were digitized at a sampling rate of 20 kHz using AxoScope hardware and software (Axon instruments, Union City, CA, USA). Data were analysed off-line using Linux-based analysis software developed by the Spinal Cord Research Center, University of Manitoba, Canada. Single motor unit action potentials were selected off-line by setting an amplitude threshold. Each selected potential 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 potential and to examine how it changed throughout a recording trial.

Motor unit pair analysis

Calculation of ΔF

The contribution of PICs to the activation of human motoneurons during involuntary muscle contractions was obtained from paired motor unit analysis techniques (Gorassini et al., 2002a). The rationale for the use of this technique is based on intracellular recordings from rat motoneurons and is summarized as follows (Bennett et al., 2001a).

When a slowly increasing current is injected through an electrode into a motoneuron (lower trace in Fig. 1A; ramp), the cell depolarizes proportionally until a PIC is activated, as reflected by an abrupt depolarization of the membrane potential (at left arrow in Fig. 1A, middle trace). PIC activation typically occurs at recruitment, especially during synaptic excitation, and helps to depolarize the cell and initiate firing (see below and Bennett et al., 1998; Li et al., 2004). After recruitment, the PIC provides a steady excitatory drive to help maintain firing and remains activated until just after de-recruitment (as reflected in the after-potential at right arrow in Fig. 1A, middle trace). Thus, cell firing can only be stopped when the injected current is decreased sufficiently to counteract the intrinsic PIC. The PIC that helps to sustain firing can therefore be estimated indirectly from the reduction in injected current required to stop firing after the PIC and cell firing are initiated (ΔI = 1.3 nA in Fig. 1A). Surprisingly, even in cells with large amplitude PICs (Fig. 1A and B), once a PIC is activated, subsequent firing is smoothly graded in precise proportion to the slowly graded current (Fig. 1A and B, top trace), as shown for the linear firing (F)–current (I) relationship in Fig. 1C from the cell in Fig. 1B. Thus, the modulation in the firing rate of a cell (ΔF) can be used to estimate the amount of current injected into the motoneuron given the linear F–I relation of the cell (Powers and Binder, 1995).

Fig. 1 Demonstration of paired motor unit analysis technique from intracellular recordings in adult rat sacral motoneurons (see Bennett et al., 2001b for details of experimental set-up). Changes in membrane potential (middle traces) and firing rate (upper traces) in response to a somatic triangular current injection (lower traces) in a higher threshold test (A) and lower threshold control (B) motoneuron. In both motoneurons, activation and de-activation of a PIC (at left and right arrows, respectively) occurs just sub-threshold to cell firing. During PIC activation, the firing rate of both units linearly reflects the profile of the underlying depolarizing input as shown for the IF current relation of the control motoneuron in (C). Estimation of the PIC amplitude in the test motoneuron in (A) is calculated by measuring the reduction in injected current that is required to counteract the added depolarization from the PIC and stop cell firing (lower solid horizontal line) after the PIC and cell firing are initiated (upper solid horizontal line) to give a ΔI value of 1.3 nA. Because the firing rate is proportional to injected current as shown in (C), the firing rate of a lower threshold control motoneuron can also be used as a measure of input to the test motoneuron when both cells receive common inputs, as occurs in (A) and (B). The PIC amplitude can then be estimated by the reduction in firing rate of the control motor unit that is required to stop firing after the PIC and cell firing are initiated to give a ΔF value of 5.7 Hz, as in (D). The ΔF measurement can then be converted to current by the known F-I slope, 4.2 Hz/nA from (C), to verify that the ΔF estimate is accurate; i.e. ΔI (estimate) = 5.7/4.2 = 1.4 nA, which is remarkably close to the measured ΔI of 1.3 nA in (A).

This linearity in firing occurs because the PIC is activated and de-activated sub-threshold to firing (at arrows) and thus does not, by itself, cause abrupt changes in firing rate (bistable firing).

Given this linearity, if a pair of motoneurons are recorded simultaneously and if each cell receives the same input as occurs in Fig. 1, then the firing rate of the lower threshold motoneuron (control cell in
Fig. 1B) can be used to estimate the injected current received by the higher threshold motoneuron (test cell in Fig. 1A). The size of the PIC can then be estimated as described above, but in this case from the firing rate information alone. If we only know the cell’s firing rate (Fig. 1D), as is the case with human motor unit recordings, then the firing rate profile of the control unit can be viewed as the injected current or input to the cell. Then, the size of the PIC in the test unit of Fig. 1A can be determined from the reduction in control unit firing measured at the time of de-recruitment of the test unit, compared with at recruitment (ΔF = 5.7 Hz in Fig. 1D). This ΔF estimate of the PIC is not in units of current (nA), but nevertheless reflects the amplitude of the PIC. In the case of Fig. 1, the ΔF measurement can be converted to current by the known F–I slope (4.2 Hz/nA from Fig. 1C), to verify that the ΔF estimate is accurate; i.e. ΔI (estimate) = 5.7/4.2 = 1.4 nA, which is remarkably close to the actual measured ΔI of 1.3 nA (in Fig. 1A).

In humans, we are limited to measuring the firing rate profiles of motoneurons (motor units) that are activated by synaptic inputs rather than with intracellular current injection. Thus, in calculating PIC amplitude by using the firing rate of a control motor unit to estimate inputs to a test motor unit, we need to make four key assumptions which are supported by previous animal and human work.

Assumption 1: During a gradually increasing synaptic input, a PIC in a motoneuron is activated just before or at recruitment in an all-or-nothing manner (Bennett et al., 1998, 2001b; Li et al., 2004) due to its dendritic origin (Hounsgaard and Kiehn, 1993; Lee and Heckman, 1996). Following this, the PIC provides a discrete depolarizing bias current that helps sustain cell firing and does not produce abrupt accelerations in firing rate (i.e. no bistable firing). Consistent with this, secure bistable firing is not seen in human motor units during normal motor behaviour (Kiehn and Eken, 1997; Gorassini et al., 1998, 2002a). In contrast, during intracellular current injection, graded PIC activation can occur supra-threshold to produce firing rate accelerations not related to the input profile (Bennett et al., 1998; Hultborn et al., 2003).

Assumption 2: After recruitment and PIC activation from synaptic inputs, the firing rate of a motor unit varies smoothly in proportion to the current reaching the cell soma (i.e. effective synaptic current) and thus can be used to estimate the input to the cell (Lee et al., 2003). During muscle spasms, motor units fire between 5 to 15 Hz, well within rates where the F/I relation is linear, both with injected current and synaptic activation of the cell (Bennett et al., 1998, 2001b; Lee et al., 2003; Li et al., 2004).

Assumption 3: The processing of synaptic inputs is similar between the control and test motor units. This would be expected for similar threshold units having similar firing frequency profiles under conditions of moderate synaptic drive (Booth et al., 1997). Although there may be non-linearities in the transform from synaptic inputs to currents reaching the cell soma, especially for large synaptic inputs, this process appears to be linear for moderate inputs in mammalian motoneurons (Prather et al., 2001).

Assumption 4: The synaptic drive to both units of a pair is similar (common drive; De Luca and Erim, 1994) and, thus, the firing rate of the lower threshold unit (control unit) provides an estimate of the synaptic drive seen by the higher threshold unit (test unit) before and after its recruitment. For each motor unit pair, this assumption can be verified by computing the correlation between the instantaneous firing rate profiles of the two units (see Measure of common drive below).

To calculate the firing rate of the control unit when the test unit was recruited/de-recruited in a reliable way, a 10th order polynomial was used to smooth the spike-frequency profiles. We found that a 10th order polynomial was high enough to reflect the slow changes in the mean rate of the units during the ~10 s spasms. 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 >3 Hz about its mean. In some cases when the firing rate profile of the control motor unit changed too rapidly (e.g. Fig. 2B), we simply used the raw frequency values to calculate ΔF.

Measure of common drive

The firing rate profiles of the control and test motor unit pairs were compared to determine whether both units were responding to common synaptic drives during involuntary muscle spasms or isometric voluntary contractions. To do this, the mean firing rates of the control and test units were calculated by binning the data every 500 ms (so there would be at least 3–5 frequency points in each bin) and averaging the frequency values in each bin. The mean firing rate of the control unit was then plotted against the mean firing rate of the test unit at the same time points (rate–rate plot) and a linear regression was fitted to the data. In some cases, the first mean rate was excluded due to unstable start-up frequencies at the onset of a contraction (Kiehn and Eken, 1997). For each rate–rate plot, the correlation coefficient (r), slope and P-value of the linear regression was calculated with significance set at P < 0.05.

Spike train analysis

Inter-spike interval values for spike trains of stationary unit discharge lasting >10 s were calculated during spontaneous and superimposed voluntary unit activity. Inter-spike interval histograms were then constructed by counting the number of intervals that fell into time bins that were separated by 5 ms and expressing these values as a percentage of the total number of intervals (Person and Kudina, 1972; Matthews, 1996). The mean, standard deviation (SD) and coefficient of variation (CV = SD/mean × 100%) for both firing rate and inter-spike interval values were calculated for each spike train using Sigma Plot 8.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance of all measures was compared using Student’s t-test (at the 95% confidence level).

Results

Paired motor unit activity during involuntary muscle spasms

Following the application of a spasm-triggering stimulus, the firing rate profiles of the control and test motor units during the involuntary muscle spasm were modulated in a graded manner. In Fig. 2A, for example, a small amount of ice applied to the subject’s forearm triggered an involuntary muscle spasm that lasted for ~20 s, as reflected by the increased firing rate of the control motor unit from its baseline of 7 Hz. As the estimated synaptic drive, or control unit firing...
rate (see Methods for rationale) increased during the spasm, a second higher-threshold motor unit was recruited. This test motor unit was recruited when the firing rate of the control motor unit reached 11.0 Hz and, as the muscle spasm subsided, the test unit was de-recruited when the firing rate of the control motor unit decreased to 7.0 Hz to produce a $\Delta F = 4.0$ Hz. Thus, after recruitment, less synaptic drive was required to sustain firing of the test unit, indicating that a PIC intrinsic to the motoneuron was activated to help sustain firing. A similar response was recorded in the TA muscle during a spasm that was triggered by a brief vibration applied to the medial arch of the foot (Fig. 2B). In this case, the firing of the test motor unit actually continued well beyond the activation of the control motor unit (de-recruitment reversal; see also Gorassini et al., 2002b), even though the firing rate of the control motor unit decreased to frequencies well below the frequency at test motor unit recruitment ($\Delta F = 6.5$ Hz).

Muscle spasms and self-sustained motor unit activity indicative of motoneuron PIC activation were easier to evoke if diffuse cutaneous, rather than localized muscle, afferent stimulation was used (see Table 1). This is demonstrated in Fig. 3 where vibrations were applied to the TA tendon at the base of the lower leg. The tendon vibration caused a transient increase in the firing rate of the control motor unit (Fig. 3, bottom trace) and only a few action potentials in the higher-threshold test motor unit that was recruited by the tendon vibration (Fig. 3, middle trace). In contrast, a similar duration of vibration applied to the medial arch of the foot (skin stimulation, see below) produced an appreciable involuntary muscle spasm in the TA muscle which lasted for >10 s. Vibration to the foot produced a larger increase in firing rate of the control unit and, in addition, the same test motor unit which was only briefly activated in response to the TA tendon vibration now continued to discharge for many seconds after the medial arch stimulation was removed. Moreover, this test unit still fired even though the firing rate of the control unit was much lower than the rate when the test unit was recruited initially ($\Delta F = 6.2$ Hz), indicating that a PIC was activated as described above. Additional motor units also continued to fire at this lower rate of estimated synaptic drive as seen in the surface EMG recording (Fig. 3, top trace). Similar spasm-inducing activity could be evoked by lightly touching the inside of the foot, suggesting that excitation of low-threshold cutaneous afferents mediated this response.

It was possible to clearly dissociate the activity of nine motor unit pairs during involuntary muscle spasms in five subjects (subject 4M was tested twice on separate days and in separate muscles). When grading the amount of sensory stimuli given by varying the amount and duration of ice or vibration application, we were able to evoke relatively mild spasms to produce slowly graded increases and then decreases in motor unit firing rates. For all subjects, the rate of the control unit when the test unit was recruited was plotted against the rate of the control unit when the test unit was de-recruited (Fig. 4). All points fell below the line of unity slope, denoting negative $\Delta F$ values that are indicative of PIC activation in the motoneuron. On average, a test unit was de-recruited at a control unit firing rate which was
4.1 ± 1.6 Hz lower than at recruitment (ΔF = 6.2 ± 2.0–10.3 ± 2.3 Hz; significantly different). The firing rate profiles of the motor units and the ΔF values obtained during the muscle spasms were similar to that produced during graded muscle stretches or mild voluntary contractions suggesting that similar graded and moderate synaptic inputs were present during the muscle spasms. As in volitional contractions, ΔF values were larger for stronger contractions (Gorassini et al., 2002a). When the control unit frequency was >8 Hz at the time of test unit recruitment, ΔF was 4.5 ± 1.2 Hz compared with 1.8 ± 1.0 Hz when the control unit frequency at test unit recruitment was <8 Hz (statistically different).

To obtain an indication of how similar the firing rate profile of a control motor unit followed the firing rate profile of a test motor unit (i.e. common drive), the mean firing rate of each unit (calculated every 500 ms) was plotted against one another at corresponding time points for 12 of the spasm trials (rate–rate plots; see Methods). In 75% of the spasm trials, the correlation coefficient (r) of the linear regression fit through the rate–rate plots was >0.75 and the P-values were <0.05 (the remaining three trials had P-values of 0.1). Therefore, on average, the correlation between the firing rate of a control and test unit during a muscle spasm was significant at the 95% confidence level. During slowly graded isometric contractions (subjects 4, 5 and 15M; n = 10 contractions), the r values for the linear regression of the rate–rate plots were higher with 80% of all trials having r values >0.75.

For muscle spasm trials, the average slope of the regression line fit through the rate–rate plots was 1.4 ± 0.60 Hz/Hz indicating that, on average, a test motor unit had larger changes in firing rate during a muscle spasm than the control motor unit. In a few rare cases, small changes in control unit firing rate during a muscle spasm (see asterisks in Fig. 2A) were accompanied by larger changes in test unit firing rates. However, for the most part, the firing rate profiles of the control and test motor units were modulated in a similar manner (e.g. Figs. 2B and 3). In nearly all spasm trials (18 out of 19), the peak firing rate of a control motor unit occurred within 500 ms of the peak rate of a test motor unit.

Slow and regular spontaneous motor unit activity

The firing rates of the test motor units towards the end of a muscle spasm during self-sustained firing were very low (average 3.0 ± 1.3 Hz; n = 19 spasms; e.g. Figs 2 and 3). This involuntary low frequency discharge could continue for many seconds or even minutes after being triggered by a muscle spasm or following a brief voluntary contraction. Figure 5B shows the firing rate profile of a spontaneously active HAM motor unit (filled symbols) recorded from an
even when spontaneous unit activity occurred at similar mean rates (e.g. 7.6 Hz histogram in Fig. 5A).

Spontaneous motor unit activity (n = 18 units) was recorded in seven subjects from the TA (n = 6 units), HAM (n = 5 units), SOL (n = 5 units) and quadriceps (QUAD) (n = 2 units) muscles. The average mean rate during spontaneous activity in all units was 5.2 ± 1.6 Hz and there were no statistical differences between the different muscles tested (QUAD units were not compared due to the low number of units recorded). Likewise, the CV did not differ between the muscle groups with a low average value of 5.4 ± 1.6%.

It has been proposed that, in non-SCI control subjects, slow and irregular motor unit firing (similar to that shown in Fig. 5B) is obtained when the membrane potential of the motoneuron between spikes rests below firing threshold and random synaptic noise triggers repetitive firing with intervals that are much longer (>200ms) than the duration of the motoneuron’s after-hyperpolarization (AHP) (Jones, 1995; Matthews, 1996; Powers and Binder, 2000). As synaptic drive to a motoneuron increases, to increase the mean depolarization level and firing rate, repetitive discharge is then more regulated by the duration of AHP. This results in more secure spiking and, thus, motor unit discharge paradoxically becomes less variable even though there is more synaptic noise. The slow spontaneous firing in injured subjects is not likely to be mediated by a similar synaptic noise driven mechanism because of its extremely low variability (see above). Instead, it is likely mediated by repetitive activation of PICs intrinsic to the motoneuron (see Discussion for mechanism). Thus increases in noisy synaptic drive should serve to increase, rather than decrease, firing variability (Jones, 1995; Matthews, 1996; Li et al., 2004). To examine this, the variability of the discharge rate during spontaneous unit activity was compared with the variability during superimposed voluntary contractions or during muscle spasms, i.e. during instances of increased synaptic drive (noise). Variability of discharge was measured as the SD of the firing rate calculated during periods (≥10 s) of stationary unit discharge. As shown in Fig. 6A for a SOL and HAM motor unit from subjects 1M and 7M respectively, when the mean firing rate was increased from 5 Hz (spontaneous) to 8 Hz (synaptic drive), the spike to spike variability (SD) also increased. This is demonstrated in Fig. 6B, where the mean rate of a unit is plotted against its corresponding SD during low (spontaneous) and high (voluntary contraction/muscle spasm) frequency discharge for seven units from the seven subjects studied (filled symbols). The variability of discharge (SD) increased as a function of mean rate with an average positive slope of 0.17 ± 0.11 SD/Hz, consistent with the idea that the slow firing seen with chronic injury is mediated by PICs intrinsic to the motoneuron rather than by synaptic noise. This relationship is in marked contrast to that observed for motor units in non-SCI control subjects where increases in mean rate from 5 Hz to 10 Hz during contractions of increasing strength were associated with decreases in SD from 1.8 to 1.3 Hz (Fig. 6B, open

**Fig. 5** (B) Instantaneous firing rate of spontaneously active HAM motor unit from subject 7M (filled symbols) compared with minimum volitional firing rate profile of HAM motor unit from non-injured control subject (open symbols). Although the mean discharge rate of the spontaneously active unit was lower than the volitionally activated unit (5.0 Hz versus 7.1 Hz), the CV was also lower (4.3% versus 27.8%). The number of inter-spike intervals as a percentage of the total number of intervals (inter-spike interval histogram) are plotted in (A), using 5 ms bin widths with spontaneous unit activity from SCI subjects in thick lines and volitional activity from a non-injured control subject in thin lines (n = 746 spikes for SCI; n = 524 spikes for non-SCI histogram). A third interval histogram is also plotted from a spontaneously active HAM motor unit firing at a similar mean discharge rate (7.6 Hz, n = 121 spikes, from SCI subject 9M) as the volitionally activated unit.

Incomplete C5/6 subject (7M; Table 1), who could not contract this muscle voluntarily. This unit fired with a mean rate of 5.0 ± 0.21 Hz (SD) and CV of 4.3%. The inter-spike interval histogram constructed from this data had a Gaussian distribution with a very narrow width of 50 ms (Fig. 5A, thin lines). This is in contrast to the interval histogram that was also produced from a HAM motor unit, but recorded in a non-injured control subject (non-SCI) instructed to maintain as low a stationary discharge as possible. The firing profile of the unit from the non-injured subject (Fig. 5B, open symbols) was seven times more variable than the spontaneously firing unit from the injured subject (CV = 27.8%), even though the mean firing rate was almost 2 Hz higher (7.1 ± 1.6 Hz). This is reflected in the inter-spike interval histogram having a wider distribution (200 ms) and marked skew to the right (Fig. 5A, thick lines). Interval histograms of spontaneously active units from SCI subjects were still more narrow than histograms from volitionally activated units in non-injured controls

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Fig. 6. (A) Comparison of firing rate profile during spontaneous unit discharge (5 Hz discharge) versus superimposed synaptic activation (8 Hz discharge) for a SOL and HAM motor unit from subjects 1M and 7M, respectively. (B) Plot of mean rate versus standard deviation (SD) of mean rate during periods of stationary spontaneous and superimposed volitional/muscle spasm activity for subjects 1, 3, 4, 7, 8, 9 and 14M (closed symbols) from HAM (circle), SOL (square) and TA (triangle) muscles. As mean firing rate increased, so too did SD with an average slope for each line of 0.17 ± 0.11 SD/Hz. In contrast, similar increases in mean firing rate during increases in voluntary synaptic drive in non-injured controls (open symbols) were associated with reduced SD and an average negative slope relation of −0.13 ± 0.01 SD/Hz.

Discussion

Results from this study suggest that motoneuron activity during involuntary muscle spasms is facilitated by the activation of motoneuron PICs. We estimate that, on average, ~40% of the excitation to motoneurons during spasm activity comes from PICs given that the extrinsic synaptic drive required to keep a motor unit firing during a muscle spasm is significantly lower (by 40%) than the levels required to recruit the motor units initially. PIC-facilitated activation of the motoneuron and the self-sustained firing it produces can continue unchecked for several seconds most likely because of the reduced descending and segmental inhibition that develops after SCI (Tanaka, 1983; Boorman et al., 1996; Crone et al., 2003).

The pronounced degree of self-sustained firing during a muscle spasm is likely to be due to the activation of a large calcium-mediated PIC in the motoneuron, which has been shown in chronically spinalized adult rat motoneurons to be the primary cause of prolonged self-sustained firing (Li et al., 2004). The results also suggest that the spontaneous unit activity commonly observed after a muscle spasm or voluntary contraction was also a result of intrinsic activation of the motoneuron given that such triggered firing was self-sustained, had extremely low rates and firing variability, and became more variable with increases in synaptic drive. Similarly slow and regular firing in chronically injured rats is mediated by a sodium PIC as discussed below (Li et al., 2004). Thus, self-sustained motor unit activity in human subjects
with chronic SCI displays evidence of both calcium and sodium PIC activation—the two main intrinsic currents in animal models of chronic SCI that increase the excitability of motoneurons to facilitate long-lasting and spastic-like reflexes (Li and Bennett, 2003).

**Estimation of PIC amplitude (DF) from paired motor unit recordings**

Our estimation of the PIC contribution to self-sustained motoneuron firing, or $\Delta F$, relies on the assumption that the firing rate of the control motor unit closely reflects the depolarizing input to the parent motoneuron. As shown in Fig. 1, the firing rate profile of adult rat motoneurons closely parallels the profile of the depolarizing input they receive when a PIC is activated sub-threshold to firing (see Fig. 1 and Bennett et al., 1998, 2001a; Li et al., 2004). Sub-threshold activation of the PIC consistently occurs with synaptic excitation of the motoneuron (as opposed to intracellular current injection) due to the predominantly dendritic location of the PIC channels (reviewed by Heckman et al., 2003). Therefore, it is likely that motoneurons activated by synaptic inputs during muscle spasms had PICs that were activated sub-threshold to firing. This was evidenced by the fact that abrupt jumps in firing rate (i.e. bistable firing) in one unit but not the other, which is indicative of supra-threshold PIC activation (Eken and Kiehn, 1989), did not occur as firing rates gradually increased and decreased during the graded muscle spasms. Moreover, because motor unit firing demonstrated marked hysteresis with respect to its inputs (i.e. $\Delta F$), it is most likely that the PIC was fully activated in an all-or-nothing manner at recruitment and did not produce appreciable increases in firing rate after the cell started to fire. Substantial hysteresis in cell firing is produced by a strong PIC activation that, in turn, is associated with a steep negative slope region in the current voltage ($I-V$) relation of a motoneuron during triangular voltage commands (Heckman et al., 2003). This negative slope produces an unstable region where the membrane voltage jumps to a more stable region (bistability), with the PIC being activated in an all-or-nothing manner to produce a discrete and rapid jump in membrane potential. Thus, because PIC activation is sub-threshold (see above), rapid PIC-induced changes in firing rate are not likely to occur during synaptic activation; or if they do occur, only just after recruitment in the first few action potentials as the PIC and cell firing are being activated. Following this, firing rate changes in a motoneuron, such as the control motor unit only reflect changes in extrinsic synaptic activation (Li et al., 2004).

Given the above arguments, it is reasonable to assume that the firing rate of the control motor unit should provide a good measure of the synaptic input it receives (see also Lee et al., 2003). Thus, if a control and test motor unit are processing synaptic inputs in a similar manner and if they are receiving common inputs, then the firing rate of the control motor unit should reflect the input to the test motor unit. Similar synaptic processing may only occur during moderate to small synaptic inputs where the relationship between synaptic inputs and current reaching the soma is linear (Prather et al., 2001). The fact that the firing rate profiles of the control and test motor units were significantly correlated with one another strongly suggests that the two units were receiving and processing qualitatively similar inputs during the muscle spasms. Admittedly, the average rate–rate correlation during muscle spasm activity was not as strong as seen during volitional contractions. This was most likely because the firing rates of the newly recruited test units were more erratic at the onset of a muscle spasm and the range of firing frequencies reached during a spasm (7–12 Hz) was smaller than the range reached during volitional contractions (9–15 Hz). In addition, the slope of the rate–rate plot during spasm activity was 1.4 Hz/Hz and implies that, for a given synaptic input, the firing rate of the test unit changed more than the firing rate of the control motor unit. Because of this, we may have underestimated the magnitude of the PIC (or $\Delta F$) contributing to test unit firing during muscle spasms. Thus, imperfect rate–rate correlations coupled with potentially different input–output gains of the control and test units may lead to some inaccuracies in the quantification of PIC amplitude from paired motor unit recordings. Nevertheless, $\Delta F$ should provide a reasonable estimate of the contribution of the PIC to motoneuron excitation, especially when control unit firing rates traverse a relatively large range of firing frequencies.

Based on the supportive evidence from intracellular animal studies and corresponding behaviour of human motor units, it appears that prolonged motoneuron activation during involuntary muscle spasms is not maintained solely by elevated synaptic drive, but rather it is in large part facilitated by the activation of PICs in the motoneuron. A possible source of elevated synaptic drive may have come from contraction-induced increases in muscle spindle afferent inputs. However, these increases were not appreciable as reflected in the reduced firing rate of the control motor unit towards the end of a spasm.

We observed that the generation of muscle spasms and self-sustained motoneuron activity were easier to evoke when using more diffuse sensory inputs such as vibrating the foot or having subjects extent their torso compared with vibration of the homonymous muscle (e.g. Fig. 3). Further studies are required to understand how changes in different sensory inputs after SCI affect the activation and facilitation of involuntary muscle spasms and motoneuron PICs (Schmit et al., 2002).

**Mechanism of slow and regular discharge of motor units in chronic SCI**

As mentioned in Results, the very regular and low frequency discharge of the spontaneously active units from chronically injured subjects was most likely mainly driven by PIC activation of the parent motoneuron. Following chronic SCI in adult rats, motoneurons recover their ability to exhibit PICs that lead to prolonged self-sustained firing which can be triggered by
brief depolarizing inputs (Bennett et al., 2001b). Interestingly, this sustained firing is unusually slow and regular, very much like the spontaneous firing seen in injured humans with inter-spike intervals that appear to be much longer than the motoneuron’s AHP. Recent studies in rats have shown that this slow firing is caused by a repetitive activation of a sub-threshold sodium component of the PIC, which can activate and deactivate rapidly (Li and Bennett, 2003; Li et al., 2004). That is a sodium PIC is activated by a brief depolarization as usual, but then each AHP deactivates the sodium PIC. Following this deactivation, the sodium PIC is reactivated after the AHP to trigger a new spike and this repeated sodium PIC activation causes slow firing. In contrast, following acute injury when PICs are greatly reduced, motoneurons will only repetitively discharge during a depolarizing current pulse with inter-spike intervals limited to less than the AHP duration; this results in higher minimum discharge rates compared with chronic animals (Li et al., 2004). Thus, following chronic SCI, motoneurons below the lesion can repetitively fire at very low and stable frequencies without extrinsic excitation due to the repetitive and sub-threshold activation of the sodium-PIC.

It is possible that the spontaneous motor unit discharge recorded in chronically injured subjects is also mediated by a repetitive activation of the motoneuron’s sodium PIC(s). This repetitive unit activity is not due to muscle fibrillations or fasciculations commonly seen in amyotrophic lateral sclerosis patients because spontaneous unit activity can be recorded by surface EMG (unlike fibrillations) and they occur at higher rates than muscle fasciculations, which typically occur at frequencies that are <1 Hz (Sivak et al., 1993). An intrinsic motoneuron mechanism is likely to be responsible for slow firing given that:

(i) spontaneous unit activity can occur at levels of synaptic input that are lower than the levels required to recruit the unit initially (self-sustained firing);
(ii) secure low frequency firing occurs with extremely low discharge variability; and
(iii) increases in synaptic drive (noise) are associated with increases in discharge variability, unlike that in uninjured controls (see Results).

Increases in the activation of sub-threshold sodium PICs after long-term SCI may increase the ability of motoneurons to repetitively fire in the face of reduced descending synaptic drive.

**Conclusions**

Following a brief sensory stimulus, motor unit activity generated during involuntary muscle spasms can continue for several seconds at lowered levels of synaptic drive due to the activation of PICs intrinsic to the parent motoneuron. This self-sustained firing can continue unchecked given that descending and segmental inhibition are greatly impaired after SCI (Tanaka, 1983; Crone et al., 2003). Although the activation of PICs contributes to the generation of unwanted muscle spasm activity, PICs may also facilitate repetitive firing of motoneurons during reduced levels of synaptic drive.

**References**


