Evidence for Plateau Potentials in Tail Motoneurons of Awake Chronic Spinal Rats With Spasticity

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Bennett, David J., Yunru Li, Philip J. Harvey, and Monica Gorassini. Evidence for plateau potentials in tail motoneurons of awake chronic spinal rats with spasticity. J Neurophysiol 86: 1972–1982, 2001. Motor units of segmental tail muscles were recorded in awake rats following acute (1–2 days) and chronic (>30 days) sacral spinal cord transection to determine whether plateau potentials contributed to sustained motor-unit discharges after injury. This study was motivated by a companion in vitro study that indicated that after chronic spinal cord injury, the tail motoneurons of the sacrocaudal spinal cord exhibit persistent inward currents ($I_{\text{PIC}}$) that cause intrinsically sustained depolarizations (plateau potentials) and firing (self-sustained firing). Importantly, in this companion study, the plateaus were fully activated at recruitment and subsequently helped sustain the firing without causing abrupt nonlinearities in firing. That is, after recruitment and plateau activation, the firing rate was modulated relatively linearly with injected current and therefore provided a good approximation of the input to the motoneuron despite the plateau. Thus in the present study, pairs of motor units were recorded simultaneously from the same muscle, and the firing rate ($F$) of the lowest-threshold unit (control unit) was used as an estimate of the synaptic input to both units. We then examined whether firing of the higher-threshold unit (test unit) was intrinsically maintained by a plateau, by determining whether more synaptic input was required to recruit the test unit than to maintain its firing. The difference in the estimated synaptic input at recruitment and de-recruitment of the test unit (i.e., change in control unit rate, $\Delta F$) was taken as an estimate of the plateau current ($I_{\text{PIC}}$) that intrinsically sustained the firing. Slowly graded manual skin stimulation was used to recruit and then de-recruit the units. The test unit was recruited when the control unit rate was on average 17.8 and 18.9 Hz in acute and chronic spinal rats, respectively. In chronic spinal rats, the test unit was de-recruited when the control unit rate (re: estimated synaptic input) was significantly reduced, compared with at recruitment ($\Delta F = -5.5$ Hz), and thus a plateau participated in maintaining the firing. In the lowest-threshold motor units, even a brief stimulation triggered very long-lasting firing (seconds to hours; self-sustained firing). Higher-threshold units required continuous stimulation (or a spontaneous spasm) to cause firing, but again more synaptic input was needed to recruit the unit than to maintain its firing (i.e., plateau present). In contrast, in acute spinal rats, the stimulation did not usually trigger sustained motor-unit firing that could be attributed to plateaus because $\Delta F$ was not significantly different from zero. These results indicate that plateaus play an important role in sustaining motor-unit firing in awake chronic spinal rats and thus contribute to the hyperreflexia and hypertonus associated with chronic injury.

INTRODUCTION

Motoneurons in anesthetized animals respond relatively simply to injected current or synaptic excitation with proportional depolarizations and firing, somewhat like a passive summation device (Binder et al. 1996; Granit et al. 1966). In contrast, in unanesthetized deccerbrate cats, motoneuron behavior is more complex because the motor output can be substantially augmented and prolonged by voltage-dependent persistent inward currents ($I_{\text{PIC}}$) (Hounsgaard and Kiehn 1989; Hounsagard et al. 1988; Hultborn and Kiehn 1992; Lee and Heckman 1998b). That is, a depolarizing input that brings the potential above a critical threshold, even relatively briefly, can cause the $I_{\text{PIC}}$ to regeneratively activate and thus produce a sustained depolarization (plateau) and discharge (self-sustained firing) that outlasts the input (Bennett et al. 1998a,b; Hounsagard et al. 1988). Studies of motor units in awake animals (Eken et al. 1989; Gorassini et al. 1999a) and humans (Gorassini et al. 1997, 1998, 2001a; Kiehn and Eken 1997) have provided evidence for self-sustained firing in normal motor function. Furthermore, indirect calculations suggest that the $I_{\text{PIC}}$ (i.e., plateau) contributes a surprisingly large portion (50%) of the estimated depolarizing current to sustain moderate repetitive firing (Gorassini et al. 2001a).

Plateaus are facilitated by neuromodulatory inputs from the brain stem [e.g., serotonin (5-HT) and norepinephrine (NE)] (Hultborn and Kiehn 1992) and are thus eliminated by acute spinalization (acute spinal rat, Bennett et al. 2001; and cat, Conway et al. 1988; Hounsagard et al. 1988). However, over the months after injury, plateau return in motoneurons of chronic spinal rats (Bennett et al. 2001; see also Eken et al. 1989), indicating that plateaus can also occur in motoneurons by mechanisms intrinsic to the spinal cord. These results were obtained by intracellular recordings in an in vitro chronic spinal preparation (sacrocaudal spinal cord), where it was difficult to study the motoneurons’ natural firing behavior, cell type (flexor vs. extensor), or role in spasticity following injury (Bennett et al. 2001). The purpose of the present study was to record from the associated motor units in awake chronic spinal rats to verify the existence of plateaus and further quantify their function.

Motor-unit recordings in the awake rat can be used to test for the presence of self-sustained firing and associated plateaus by examining whether a transient input (e.g., skin stimulation or...
muscle vibration) can trigger a sustained motor-unit discharge that continues when the estimated synaptic input to the motoneuron is held constant or increased (Gorassini et al. 1998, 2001a,b). That is, if a plateau helps maintain the firing of the motoneuron intrinsically, then more synaptic input should be required to initiate firing (and activate the plateau) than to maintain the firing. Further, the amount by which the synaptic input can be reduced before de-recruitment occurs corresponds to the contribution of the plateau to sustaining firing. The main difficulty with this is in estimating the synaptic input to the motoneuron. However, we have recently developed a paired motor-unit method in which the firing rate of a second lower-threshold motor unit (control unit) recorded simultaneously to the first (test unit; from same muscle) is used to estimate the common synaptic input to both units (Gorassini et al. 1998, 2001a). That is, the firing profile of the control unit is taken as an estimate of the synaptic input to the test unit, assuming that both units receive the same synaptic input or at least linearly related synaptic input (common drive) (DeLuca and Erim 1994) and that the control unit responds relatively linearly to the common synaptic input (Bennett et al. 1998a, 2001).

Conceptually, it might be difficult to understand how the second assumption could hold if the control unit has an active plateau. However, the term plateau does not indicate a fixed depolarization or firing rate of the motoneuron but simply that there is an added depolarization produced by a persistent inward current, \( I_{\text{PIC}} \) (perhaps the term plateau current might be less misleading than plateau potential) (see Bennett et al. 1998b). Synchronization or injected current can still produce changes in potential and firing rate modulation during a plateau by summating with the \( I_{\text{PIC}} \) (i.e., membrane potential changes ride on top of the plateau) (Bennett et al. 1998a,b; Hounsgaard et al. 1988). Another potential difficulty is that nonlinear behavior (e.g., bistable firing) may occur during a plateau activation in the control unit. For example, with intracellular current injection in cat motoneurons, the \( I_{\text{PIC}} \) (plateau) often has a high threshold and is abruptly activated during firing, causing a nonlinear jump in firing rate (bistable firing) (Bennett et al. 1998a; Hounsgaard et al. 1988). Thus the control unit firing might not be linearly related to input if a plateau is present. Fortunately, abrupt jumps in firing (bistable firing) are not common during more physiological activation by synaptic inputs in cat motoneurons (vs. intracellular) (see discussion in Gorassini et al. 1998) likely because the plateau threshold is much lower in this situation (re dendritic origin) (Bennett et al. 1998a). Instead the \( I_{\text{PIC}} \) is thought to be activated near recruitment (Bennett et al. 1998a), contributes to the initial recruitment step (or initial steep increase in rate), and is not further activated during increases in synaptic input; thus it does not interfere with linear rate modulation. There are conditions where bistable firing is seen with synaptic inputs in some motoneurons (Hounsgaard et al. 1988), and in these cases, the control unit might give an overestimation of the common synaptic input, and thus an underestimation of the plateaus. In chronic spinal rat motoneurons, the plateau is often activated before recruitment even with intracellular current injection (Bennett et al. 2001). Shortly after recruitment, during a slowly graded intracellular current injection, the frequency-current (\( F-I \)) relation is again relatively linear for most cells (Bennett et al. 2001). Thus the firing rate of a motor unit (re control unit) should accurately reflect the input to the motoneuron, as per the second assumption in the preceding text, even if this motoneuron has a plateau.

In the present study, we have thus applied the paired-motor-unit method to examine plateaus in awake chronic spinal rats. As anticipated, we found that a brief synaptic excitation, such as skin stimulation, caused sustained firing in a test unit that continued even when the estimated synaptic input (control unit rate) was decreased substantially compared with the input that was required to recruit the test unit (self-sustained firing and plateau). Remarkably, our estimate of the effect of the plateau on sustaining motor-unit firing (from change in control unit rate) was quantitatively very similar to that obtained with intracellular recording in vitro in the same population of motoneurons (Bennett et al. 2001). The consistency of the results from intracellular recording and motor-unit recordings provides a validation of paired-motor-unit method. Indeed, this validation was the second major purpose of the paper and is important because the paired-motor-unit method has begun to be used to study normal (Gorassini et al. 1998, 2001a,b) and spinal-cord-injured humans (Gorassini et al. 1999b).

METHODS

Single motor-unit action potentials were recorded in awake rats following an \( S_2 \) sacral spinal cord transection, either within 1–2 days (acute spinal; \( n = 38 \) units from 4 rats) or after \( >1 \) month (chronic spinal; \( n = 32 \) units from 5 rats). The details of the sacral spinal cord transection surgery are provided elsewhere (Bennett et al. 1999). The motor units were from the distal segmental tail muscles, which are controlled by motoneurons in the sacrocaudal spinal cord below the \( S_2 \) sacral transection (Bennett et al. 1999). The purpose was to examine plateau behavior from tail motor-unit firing, employing the paired-motor-unit method of Gorassini et al. (1998, 2001a). One month after sacral transection, rats develop a syndrome of spasticity in the tail muscles affected by the injury (hypertonus, coiling spasms, etc.) (Bennett et al. 1999), and all chronic spinal rats in the present study had such spasticity. All procedures were approved by a local animal-welfare committee.

Preparation for motor-unit recording

Just prior to motor-unit recording, acute and chronic spinal rats were briefly anesthetized with isoflurane (1.5–3% in \( O_2 \)), and the segmental tail muscles were exposed by making a 5-cm longitudinal incision in the skin at the midpoint of the tail, slightly ventral to the lateral dorsal vein in the left side. The segmental muscles are three small intrinsic tail muscles (1 cm each) that span between successive vertebra of the tail: the dorsal muscle is involved in dorsolateral extension of the tail (lifting the tail), while the ventral and ventrolateral muscles are involved in ventrolateral flexion of the tail (see Fig. 1) (see also Steg 1964; Thompson 1970). These muscles are covered by tendons projecting from larger muscles at the base of the tail (Thompson 1970), and some of these tendons were cut and removed for better access. Sterile gauze and oil were then placed over the exposed muscles to prevent drying.

The rat was transferred to a standard holding tube (Bennett et al. 1999) with the tail sticking out a hole in the end of the tube. The tail was stabilized horizontally to minimize movements during recordings. The anesthetic was then removed, and the rat remained in the tube for better access. Sterile gauze and oil were then placed over the exposed muscles to prevent drying.

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MOTONEURONS. As described in INTRODUCTION, we have used the VALIDATION USING INTRACELLULAR RECORDINGS FROM TWO motor units.

The stimulus to get a slow, graded recruitment and discharge of the single hair could often trigger a sudden sustained motor-unit discharge (Bennett et al. 2001), we attempted to produce a slowly increasing, polynomial (thick line on figures). For display purposes, the instantaneous firing rate of the motor unit was often plotted with the firing rate smoothed using a fifth-order text). For display purposes, the instantaneous firing rate of the motor unit was often plotted with the firing rate smoothed using a fifth-order polynomial (thick line on figures).

To follow the companion intracellular studies as closely as possible (Bennett et al. 2001), we attempted to produce a slowly increasing, then decreasing, synaptic input by applying a slowly graded manual stimulation to the skin, hair or muscles of the tail. The most common stimulation was to gently lift the tail hairs by entangling them in the fibers of a cotton swab. In chronic spinal rats, touching a single hair could often trigger a sudden sustained motor-unit discharge or even a full spasm, and it was thus necessary to very carefully apply the stimulus to get a slow, graded recruitment and discharge of the motor units.

**Motor-unit recording and activation**

Fine tungsten microelectrodes (usually 2; 12 MF; No. 5754, A-M Systems) were manually inserted into one of the segmental tail muscles and held in place by a coarse manipulator. Motor-unit activity was recorded with a custom-built preamplifier (Dr. K. Yoshida) and an Axoscope data-acquisition system (low-pass filter: 10,000 kHz; high-pass filter: 100 Hz; gain: 2000; sampling rate: 20 kHz; Axon Instruments). Activity was played through a speaker to aid in locating motor units during electrode insertion. At least two similar threshold motor units from the same muscle were recorded at a time (often from the same electrode). The high impedance of the electrodes assured pickup from only motor units in the same muscle, which was verified by comparing how distant two electrodes could be when recording from the same unit (0.2–0.5 mm).

The recorded data were analyzed off-line using custom software on a Linux system. Spikes were sorted manually by their shape, and the instantaneous frequency was computed. The frequency profile was at times averaged in 200-ms bins to facilitate analysis (see following text). For display purposes, the instantaneous firing rate of the motor unit was often plotted with the firing rate smoothed using a fifth-order polynomial (thick line on figures).

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**Paired motor-unit method**

VALIDATION USING INTRACELLULAR RECORDINGS FROM TWO MOTONEURONS. As described in INTRODUCTION, we have used the firing rate of two simultaneously recorded motor units to study plateaus: one lower-threshold unit (control unit) to estimate the common synaptic input, and a second higher-threshold unit (test unit) to examine the involvement of the plateau in sustaining firing as the synaptic input was decreased. Specifically we measured the difference between the control unit’s firing rate (estimated synaptic input) at recruitment and de-recruitment of the test unit to determine the amount by which the plateau helped maintain the firing of the test unit.

To validate this method, we have applied it to the firing of two tail motoneurons recorded intracellularly, both of which we know have plateaus a priori (Fig. 2). The intracellular recordings were obtained from a chronic spinal rat in the companion study and the methods are detailed there (Bennett et al. 2001) (any of the 35 motoneurons from this study could have been used in Fig. 2, with similar conclusions). The two motoneurons were recorded at different times, but had an identical input (intracellular current injection; Fig. 2, A and B), as would presumably occur if they were synaptically activated and recorded simultaneously.

Both the test and control motoneurons in Fig. 2, A and B, had plateaus that maintained firing after recruitment as can be seen by the asymmetrical responses to the injected current (Bennett et al. 2001). That is, after recruitment (at a particular current; left dashed lines), firing was aided by the activation of I_{	ext{PIC}} because the injected current could be reduced substantially (by $\Delta I = -0.5 \text{nA}$) before the unit was de-recruited (right dashed lines). Accordingly, we have taken $\Delta I$ as an estimate of the persistent inward current that helped sustain the firing ($I_{\text{PIC}}$) (Bennett et al. 2001). Importantly, even though the control neuron (Fig. 2B) had a plateau, its firing profile closely reflected the input current (plotted in Fig. 2B above the potential), and indeed the F-I profile is remarkably linear (Fig. 2C). Thus we can treat the control motoneuron’s firing profile as the input (current) and again compare this input at recruitment and de-recruitment of the test unit (dashed lines in Fig. 2D), exactly as described in the preceding text for Fig. 2, A and B. That is, we can compute the amount by which the plateau contributed to sustaining the test neuron’s firing from the reduction in input possible after recruitment ($\Delta F = -2 \text{Hz}$; Fig. 2D).

Interestingly, because we know the slope of the frequency-current relation ($F-I$; slope $= 4 \text{Hz nA}$; Fig. 2C), we can also compute the current provided by the input from the control unit firing rate ($\Delta F = \Delta I / \text{slope} = -2/4 = -0.5$), which corresponds nicely to the current measured directly ($-0.5 \text{nA}$). Finally, note that the activation of the plateau in the control neuron occurred at its recruitment (see steep rise in rate at asterisk in Fig. 2C) (see Bennett et al. 2001) and thus did not affect the analysis because we only need to know the control neuron rate at a much later time (during the firing of the test neuron).

**SUMMARY AND REQUIRED CONDITIONS.** Returning to motor-unit recordings, if two motor units are recorded simultaneously and we assume that they have the same synaptic input (current or at least linearly related input), then the input to the higher-threshold unit can be estimated from the firing profile of the lower-threshold control unit. The degree of self-sustained firing and plateau current ($I_{\text{PIC}}$) can be estimated, as in Fig. 2D. For demonstration purposes, suppose that the data in Fig. 2D were from two such motor units. If the test unit was recruited at a particular estimated input level (8-Hz control unit rate in Fig. 2D; left dashed line), then the plateau and $I_{\text{PIC}}$ contribute to maintaining firing if the estimated input has to be reduced below this level before de-recruitment occurs (to 6-Hz control unit rate; right dashed line; $\Delta F = -2 \text{ Hz}$). This analysis is possible even if the
rate-dependent dynamics cause the firing rate to slow over time (see DISCUSSION). Thus spike frequency adaptation (Kernell and Monster 1982) and low-amplitude, slowly graded synaptic inputs must be noted. First, while the F-I relation is linear, we can only suppose that the current from synaptic input will produce a similar linear input/output relation (consistent with Granit et al. 1966; although see Lee and Heckman 2000). Second, a low-amplitude slow ramp was used (in Fig. 2C); with larger or faster current ramps, the motoneuron’s F-I relation becomes nonlinear (Bennett et al. 2001). That is, spike frequency adaptation (Kernell and Monster 1982) and rate-dependent dynamics cause the firing rate to slow over time (see DISCUSSION). Thus low-amplitude, slowly graded synaptic inputs must be used during the paired-motor-unit examination of plateaus. Third, fast increases or decreases in inputs were sometimes hard to avoid, causing the test unit to be recruited or de-recruited prematurely, and these data cannot be used. Finally, in some motoneurons, the plateau was only activated gradually over the first second or so of firing, slightly after recruitment (20%) (Bennett et al. 2001). Thus the control unit must fire for at least 1 s before it can be assumed to be on a plateau and in a state where it will be modulated linearly with input currents (as in Fig. 2C).

There are also several conditions on the firing of the test unit. First, because it sometimes takes 1 s to activate a plateau (as just mentioned), we only analyzed data where the test unit was activated for >1 s. Second, because the plateau is often activated just below the recruitment threshold (60% of cells) (Bennett et al. 2001), we must be sure that the plateau is off before testing for its activation. It does not suffice to assume the plateau is off if the test unit is not firing. We have instead only tested for plateau activations starting from rest, when all units were initially silent. Third, repeated activation of a plateau separated by <5 s can markedly lower the plateau threshold (due to warm-up and associated residual subthreshold plateau activation) (Bennett et al. 1998b). Thus we have only analyzed test motor-unit activations separated by at least 5 s. Fourth, sometimes de-recruitment was hard to define because irregular bursts in activity caused randomly isolated spikes. Those bursts were not considered to be repetitive firing, and de-recruitment of the test unit was assumed to occur only when the firing rate became irregular and dropped below 1 Hz (lowest repetitive firing rate in Bennett et al. 2001).

These conditions, particularly those on the control unit firing, were at times difficult to meet given that the synaptic input was controlled by manual stimulation (skin/hair stimulation) and spontaneous muscle spasms at times altered this input. However, by repeating the stimulation many times (sometimes over an hour), the appropriate control-unit firing conditions were usually obtained, and the mean value of ΔF was obtained from all acceptable trials. In some units, recruitment occurred with an abrupt spasm, and to avoid the rate dependent effects that would overestimate the plateaus (higher control rate), the control unit rate just prior to recruitment was in these cases used instead of the rate just at recruitment (as in Fig. 3 at asterisk, described in RESULTS).

In the text and figures, means ± SD are quoted. Statistical differences were computed with a Student’s t-test with a 95% confidence level.
RESULTS

Coordination of tail muscles in sacral spinal rats

The chronic spinal rats included in this study had spasticity in their tail muscles with associated hyperreflexia and hypertonus (Bennett et al. 1999). Single motor-unit recordings from the different muscle types revealed that all three segmental tail muscles (ventral, ventrolateral, and dorsal) had considerable spontaneous or reflex-evoked activity involved in this spastic behavior. This activity was often well coordinated, which was convenient for the purposes of experimentally controlling the motor units (see following text). For example, a light touch on the dorsal surface of the tail caused activation of the ventral muscles and deactivation of the dorsal muscles, which resulted in a contraction that produced a ventral flexion of the tail, withdrawing it from the skin contact point. Likewise, a light touch on the ventral surface caused reciprocally controlled dorsal muscle activation and a contraction that tended to extend the tail (dorsally) away from the contact point. The ventral muscles were usually most active, and this corresponds to the previous observation that the tail often flexed ventrally in these spastic rats, especially during spasms (which coils the tail under the body) (Bennett et al. 2001). We have thus mostly focused on the ventrolateral and ventral segmental muscles. Acute spinal rats had much weaker muscle contractions (no spasms) although similar patterns of muscle activity could be evoked with more forceful skin stimulation.

Plateaus and sustained firing of low-threshold motor units in chronic spinal rats

A striking feature of the chronic spinal rats was that low-threshold motor units often produced very long-lasting discharges in response to a relatively brief skin stimulation. In the ventral muscles, this discharge could go on for hours (up to 3 h tested); in the dorsal muscles, it lasted for many seconds. Further, the discharge could only be stopped by a substantial inhibitory skin stimulation to the opposite skin surface. Figure 3 demonstrates the firing of a pair of such low-threshold motor units recorded from the same muscle (ventral muscle). Although both units had a very similar threshold, making them difficult to recruit separately, we were able to recruit unit 1 without unit 2 by carefully touching the tail (not shown). Interestingly, although it was difficult to recruit unit 1 alone (because of their close threshold), once unit 1 was activated, it produced sustained firing that was easy to modulate by a further brief stimulation (Fig. 3A, asterisk), without recruiting the second unit. If the firing of unit 1 depended on a sustained increase in synaptic input to the motoneuron pool, then one would expect that unit 2 would instead be recruited by even the slightest stimulation and associated increase in the firing rate of unit 2, considering their closeness in threshold. The fact that this did not occur suggests that the firing of unit 1 was instead intrinsically maintained by the activation of a plateau at recruitment, as previously described for these motoneurons in Bennett et al. (2001).

If we assume that the firing rate of unit 1 (control unit) provides an estimate of the synaptic input and this input was similar to the synaptic input received by unit 2 (test unit), we can compare the control rate (estimated synaptic input) before and after recruitment of the test unit and thus determine the effect of the plateau on sustaining the firing of this test unit (if present; see METHODS and INTRODUCTION). That is, before recruitment of the test unit (unit 2), the control unit reached 7–10 Hz (Fig. 3A, asterisk), following a brief dorsal stimulation (left arrow). A second dorsal stimulation caused a spasm that recruited the test unit. After recruitment of the test unit, the control unit rate could be reduced to as low as 3 Hz while the test unit continued to fire (Fig. 3A, double asterisk), suggesting that a plateau activated at recruitment provided a depolarizing current that allowed the input to be reduced without de-recruiting the test unit. In this case, the control unit firing rate was slowed by inhibitory stimuli to the ventral tail skin (Fig. 3A, at right-most arrows).

Even though the test unit (unit 2) required a moderately high estimated synaptic input to be recruited, as judged by the control unit firing rate (unit 1), it continued to fire when the estimated synaptic input was reduced so much that the control unit reached near its minimum rate (Fig. 3A, right; see summary in the following text). Occasionally, the control unit (unit 1) even stopped firing while the test unit (unit 2) continued (3/16 units; Fig. 3B, left) (de-recruitment reversal as in Gorassini et al. 2001b). If both units stopped firing and were then recruited again, the original recruitment order was restored (not shown; see DISCUSSION).

Plateaus in higher-threshold motor units in chronic spinal rats

Other motor units in chronic spinal rats required a continuous tail skin/hair stimulation to cause them to fire (except during muscle spasms). However, these higher-threshold motor units also exhibited pronounced plateau behavior. For example, during a slowly graded skin stimulus, the test unit (Fig. 4A) was recruited when the estimated synaptic input was moderately high (i.e., control unit rate of $F = 18$ Hz; left dashed line), whereas it was de-recruited when the stimulation was reduced and the estimated synaptic input was much lower (control unit rate $F = 4$ Hz; right dashed line). The drop in control unit rate, and thus estimated synaptic input, at test de-recruitment was $-14$ Hz (=$\Delta F$), which suggests that the plateau in the test unit contributed a substantial depolarization that sustained its firing even when the synaptic input was reduced. Given that the average frequency-current ($F-I$) slope for chronic spinal motoneurons was $\sim 5$ Hz/nA (Bennett et al. 2001), this 14-Hz drop corresponds approximately to a 2.8-nA persistent inward current ($I_{\text{PIC}} = -\Delta I = -\Delta F$/slope) that helped sustain the test unit’s firing (see Fig. 2 and METHODS for further explanation).

As already discussed, the paired-motor-unit method that we have employed assumes that the test and control unit received the same input. This assumption can be verified by examining the correlation between the firing rates of both units, as shown in Fig. 4B. Shortly after the onset of firing of the test unit, the firing rates of the test and control units were well correlated ($\rho = 0.77$), both tracking the spontaneous fluctuations in synaptic input. Interestingly, for the first couple seconds after recruitment, the test unit firing rate (Fig. 4A) lagged behind the control unit rate (Fig. 4B, ○). This could be explained by a slow activation of the plateau in the test unit, such that the plateau activation continued for a few seconds after recruitment (see Bennett et al. 2001). The steeper slope of this portion
of the plot in Fig. 4B is consistent with the activation of a plateau at this time. Similar delayed activation of plateaus has been seen during intracellular recordings from chronic spinal rat motoneurons, and indeed the plot in Fig. 4B looks remarkably similar to counterclockwise hysteresis of the F-I plot of Fig. 2D of Bennett et al. (2001). Finally, this delayed plateau activation was at times also manifested in low-threshold units that had self-sustained firing triggered by a brief stimulation. That is, it was at times necessary for these units to fire for a second before self-sustained firing was obtained. With shorter activations, the unit stopped firing immediately after stimulation (not shown).

**FIG. 3.** Sustained firing and plateau in low-threshold motor units of a chronic spinal rat. A pair of low-threshold motor units from the ventral segmental tail muscles with a similar recruitment threshold. A: the lowest-threshold unit (unit 1, control unit) was recruited by a brief light stimulation to hair on the dorsal surface of the tail (not shown) and then fired for many seconds without stimulation. A further stimulation (left arrow and asterisk in A) increased the firing rate of unit 1 but did not recruit the 2nd unit. A stronger stimulation (2nd arrow) caused a muscle spasm and recruited the second unit (smaller-amplitude unit). This unit 2 (test unit) continued to fire even when the firing rate of unit 1 (control unit; see estimated synaptic input) was decreased to its minimal rate (at double asterisk; 3 Hz), well below the rate before the test unit recruitment (at asterisk; 7–10 Hz). This suggests that the firing of the test unit was intrinsically maintained (plateau). B: in some cases, unit 2 continued to fire even when the control unit stopped briefly (de-recruitment reversal). A brief stimulation to the hair of the ventral surface of the tail was used to inhibit or slow the firing, as indicated by the arrows in A and B.
The second assumption of the paired-unit analysis method was that the firing rate of the control unit was linearly related to its input. Although the linear correlation of the test and control units in Fig. 4B is consistent with a linear input-output relation, this assumption cannot be directly tested. It should, however, be noted that it is unlikely that nonlinearities associated with a plateau activation occurred in the control unit during the period of analysis (i.e., during the firing of the test unit). That is, the control unit fired for many seconds before the test unit was recruited, at which point the control unit’s plateau would be fully activated (if present) (Bennett et al. 2001).

Furthermore, there were no jumps in control unit firing rate during this period (i.e., no delayed plateau activation).

**Lack of plateaus in acute spinal rats**

Motoneurons of acute spinal rats usually required continuous stimulation to maintain their firing and had no clear indications of a plateau activation. For example, when the test motor unit (Fig. 5) was recruited during a slowly graded skin stimulation, the estimated synaptic input corresponded to a control unit firing rate of ~28 Hz (left dashed line). At de-recruitment (at right dashed line), the estimated synaptic input was similar, or even slightly higher ($\Delta F = +3$ Hz in this case; i.e., no plateau, and firing stopped early). We found that the acute spinal rat motor units were harder to study because the control unit rate was more variable and difficult to control, perhaps because recruitment of motor units in acute spinal rats often required more vigorous skin stimulation than in chronic spinal rats. Accordingly, we found considerable variability in the estimated change in synaptic input at de-recruitment compared with recruitment ($\Delta F$). For a given motor unit, in some trials, $\Delta F$ was positive and in others, negative (no plateau). On average, however, it was positive, suggesting that plateaus were not present (as in Fig. 5; see summary described in the following text).

**Summary of firing properties and estimation of plateau current**

On average, the control unit firing rate ($F$, estimated synaptic input) at test unit recruitment was $17.8 \pm 5.3$ Hz in acute spinal rats and $18.9 \pm 5.2$ Hz in chronic spinal rats. For acute spinal rats, the control units rates at de-recruitment simply covaried with the rates at recruitment ($E$; i.e., fell on $45^\circ$ line in Fig. 6A), suggesting that there was a common synaptic input to these 2 units. The control and test unit firing rates were averaged in 200-ms bins and plotted against one another. After a short period during the onset of the test unit firing ($\bullet$), the control and test unit firing rates were well correlated ($r^2 = 0.77$; ●), suggesting that there was a common synaptic input to these 2 units. * indicates the 1st and last points.

**FIG. 4.** Plateau in higher-threshold motor units in chronic spinal rats. A: firing rate of 2 ventrolateral segmental motor units during a continuously graded dorsal skin stimulation. Note that the firing rate of the low-threshold unit (control) was graded in a ramp-like profile (as in Fig. 2D), and this rate was assumed to provide an estimate of the common synaptic input to both units. The test unit was recruited when this estimated synaptic input was moderately high (16 Hz; left dashed line), whereas it was de-recruited when the estimated synaptic input was very low (2 Hz; right dashed line; drop in rate $\Delta F$), likely due to a plateau that aided in sustaining the test unit firing. B: the test and control unit firing rates were averaged in 200-ms bins and plotted against one another. After a short period during the onset of the test unit firing ($\bigcirc$), the control and test unit firing rates were well correlated ($r^2 = 0.77$; ●), suggesting that there was a common synaptic input to these 2 units. * indicates the 1st and last points.

**FIG. 5.** Lack of plateaus in acute spinal rats. Two ventral segmental motor units recorded in an acute spinal rat. Same format as in Fig. 4A. Note the symmetrical firing of the test unit with respect to the graded firing rate of the control unit (estimated synaptic input), suggesting that a plateau was not present.
with no significant drop in rate at de-recruitment ($\Delta F = +0.25 \pm 1.9 \text{ Hz}; n = 19$ motor-unit pairs; Fig. 6, B and C). In contrast, in chronic spinal rats, the control unit rate at de-recruitment nearly always fell below the recruitment rate (below $45^\circ$ line, Fig. 6A), with a significant drop at de-recruitment ($\Delta F = -5.5 \pm 4.0 \text{ Hz}; n = 16$ motor-units pair; Fig. 6, B and C). This drop in estimated synaptic input ($\Delta F$) suggests that, after recruitment, a persistent inward current ($I_{\text{PIC}}$; plateau) was present that aided in maintaining firing. De-recruitment only occurred when the current provided by the plateau was cancelled by the reduction in the effective current provided by the synaptic input ($\Delta I = -I_{\text{PIC}}$). Because we know the average slope of the $F-I$ relation in chronic spinal rats (from Bennett et al. 2001), we can convert $\Delta F$ into an equivalent change in current at de-recruitment ($\Delta I = \Delta F$/slope; where slope = $5 \text{ Hz}/nA$; see METHODS and Fig. 2). Thus the persistent inward current that aided in sustaining the motoneuron firing can be directly computed ($I_{\text{PIC}} = -\Delta I = -\Delta F$/slope). The average computed $I_{\text{PIC}}$ is $\sim 1.1 \text{ nA}$ (Fig. 6C) (compared with 0.8 nA in Bennett et al. 2001).

The firing rate of the control unit at the recruitment of the test unit approximately reflected the test unit’s recruitment threshold and its firing rate at de-recruitment (Fig. 6B). This suggests that the control unit de-recruitment rate was not significantly different (9.1 ± 5.1 and 9.1 ± 3.7 Hz, respectively; $n = 19$).

Anomalies in repetitive firing after chronic spinal cord injury

In our companion in vitro studies (Bennett et al. 2001), we noticed that the control of repetitive firing is significantly altered in motoneurons of chronic spinal rats with plateaus, in at least two respects: the firing often starts at a higher rate than the minimum repetitive firing rate is usually low (1 Hz). Motor units in awake chronic spinal rats also exhibited both of these characteristics, as shown in Fig. 7. That is, the test unit in Fig. 7 started firing at $\sim 15 \text{ Hz}$ and stopped at $<2 \text{ Hz}$ (the control unit de-recruitment is not shown). This test unit had a plateau, with a drop in estimated synaptic input at de-recruitment of $\Delta F = -5.0 \text{ Hz}$ (control unit rate), as described in the preceding text for Fig. 4. Possibly the high initial rate was related to an abrupt plateau activation that occurred at recruitment, as in Bennett et al. (2001). For all units tested, on average the firing rate at recruitment ($12.7 \pm 5.7 \text{ Hz}$) was significantly higher than at de-recruitment ($8.0 \pm 3.6 \text{ Hz}; n = 16$; Fig. 8). In contrast, in acute spinal rats, the firing rates at recruitment and de-recruitment were not significantly different (9.1 ± 5.1 and 9.1 ± 3.7 Hz, respectively; $n = 19$).
The present results contribute new concepts related to the functional role of plateaus after chronic injury: plateaus can be triggered by a brief skin stimulation and was often only stopped by applying an inhibitory stimulation to the opposite skin surface. Higher-threshold units required more stimulation to activate, but again once firing had begun, it was maintained even when the stimulation was reduced substantially. This sustained motor-unit firing could not be attributed to sustained increases in estimated synaptic input to the motoneuron pool (control unit rate; see following text); instead, we suggest that it resulted from the activation of $I_{\text{PIC}}$ and associated plateaus intrinsic to the motoneurons. This conclusion is consistent with the plateaus found in motoneurons of the sacrocaudal spinal cord in chronic spinal rats in our companion in vitro study (Bennett et al. 2001), other muscles innervated by the sacrocaudal cord likely also have plateaus in chronic spinal rats. However, we did not study these muscles, because they are situated in the base of the tail (with long tendons running down the length of the tail) and are thus less accessible for study in the awake animal.

In using motor-unit firing to study plateaus, one approach is to look for known anomalies in motoneuron firing that sometimes occur with plateaus, including a high initial firing rate due to plateau activation at recruitment (as in Fig. 7) (also see Bennett et al. 1998a; Gorassini et al. 1998, 1999, 2001a); a lowering of the plateau threshold with repeated activation, and associated lowering of the recruitment threshold (warm-up) (Bennett et al. 1998b; Gorassini et al. 1998, 2001b); bistable firing caused by plateau activation slightly after recruitment (Eken et al. 1989); and a very low minimum firing rate, possibly related to repeated activation and de-activation of the plateau currents ($I_{\text{PIC}}$) just subthreshold to the firing level (Bennett et al. 2001; Kernell 1999; see also similar low rate after injury in Carp et al. 1991; Powers and Rymer 1988; Thomas and Ross 1997). However, each of these anomalies in firing could also be explained by variations in synaptic input rather than plateaus, and thus while useful, they do not by themselves demonstrate the presence of plateaus intrinsic to the motoneurons (see discussions in Gorassini et al. 2001a,b).

It is critical that we have a reliable estimate of the synaptic input to the motoneurons so as to be able to clearly distinguish pre- and postsynaptic events and ultimately demonstrate the presence of plateaus intrinsic to the motoneurons. We have thus estimated the synaptic input to a particular motor unit (test unit) by examining the firing rate of a similar, though slightly lower-threshold unit recorded from the same muscle (control unit). This paired-motor-unit method has been used before (Gorassini et al. 1998, 2001a), although in the present study, we have the unique advantage of knowing a priori that plateaus are present and knowing how they affect the input-output properties of the motoneurons (Bennett et al. 2001). In particular, for slowly graded low-amplitude current inputs (as in Fig. 2), the firing frequency usually tracks the injected current linearly in sacrocaudal motoneurons (Bennett et al. 2001) and accordingly produces linear $F-I$ plots as in Fig. 2C. Importantly, the $F-I$ plot is linear regardless of whether or not there is a plateau (in chronic vs. acute spinal rats), because the plateau (when present) is usually initiated at the time of recruitment and is fully activated immediately or at least within a second or so of recruitment (depending on the amplitude of the input) (Bennett et al. 2001). Thus presuming that the current provided by synaptic input acts like the intracellularly injected current (Granit et al. 1966; though see Lee and Heckman 2000), this synaptic input should be proportional to the firing rate.

Finally, because the control and test motor units are from the same muscle and have similar thresholds, they both likely receive the same synaptic input, and thus the firing rate of the control unit can be used to estimate the synaptic input to the test unit. This can be confirmed by examining how well the test and control units covary with each other (Fig. 4B) (Gorassini et al. 2001a,b) or with the net muscle force (Gorassini et al. 1998; common drive, DeLuca and Erim 1994). Interestingly, in cats considering the high incidence of plateaus in motoneurons in vitro (Bennett et al. 2001), other muscles innervated by the sacrocaudal cord likely also have plateaus in chronic spinal rats.
the recruitment order of gastrocnemius motor units is preserved for different types of synaptic input (stretch vs. cutaneous) (Sokoloff et al. 1999; though see Garnett and Stephens 1981), suggesting that variations in stimulation/synaptic input are seen equally by similar motor units.

Because we know the mean slope of the F-I relation (Bennett et al. 2001), we can approximately convert the control unit rate into estimated synaptic current (again assuming a rough equivalence of synaptic and injected current, see above). In this way, we have been able to show that after recruitment the estimated synaptic current can be reduced substantially without de-recruiting a unit. This reduction corresponds to an intrinsic current that assists in maintaining firing (presumably I_{IC}), which is \( \sim 1 \text{nA} \) (see results for details). This value corresponds reasonably well with the estimates of I_{IC} obtained directly during intracellular recording (0.8 nA) (Bennett et al. 2001). Further, the percent drop in estimated synaptic input at de-recruitment, compared with recruitment, is \( \sim 50\% \) (Bennett et al. 2001), indicating that the plateaus provide about half the net depolarization to maintain moderate contractions (at 10-30 Hz rates). Interestingly, similar results were obtained from motor-unit recordings in awake normal humans (Gorassini et al. 2001a), indicating that plateaus in chronic spinal animals are at least as large as normal although without the usual descending inhibitory control to turn them off.

In some pairs of motor units, the test unit was recruited well after the control unit (at a moderate control unit firing rate and thus estimated synaptic input; Fig. 3A), but de-recruitment only occurred when the synaptic input was reduced so much that the control unit stopped firing before the test unit (Fig. 3). We have seen similar de-recruitment reversals in human motor units before and have argued that they result from a larger plateau in the control unit compared with the test unit (see alternative explanations in Gorassini et al. 2001b). That is, while both units likely had plateaus, the current provided by the plateau (I_{IC}) in the initially higher-threshold test unit may have been at times slightly greater than in the control unit. Thus the test unit could sustain its firing longer when the common synaptic input was brought down even though it initially had a higher recruitment threshold. Likely, de-recruitment reversals are anomalies because they require a very gradual de-recruitment, so only one unit stops without the other. In practice, once both units stopped firing for a few seconds, the original recruitment order is restored (i.e., both have their presumed plateaus deactivated).

In anesthetized animals, the firing rate of a motoneuron can decline slowly over time during a steady intracellular activation. Such late spike frequency adaptation has been attributed to various mechanisms, including sodium inactivation and the accumulation of hyperpolarization from slow calcium-dependent potassium currents (Kernell 1999; Kernell and Monster 1982). In unanesthetized preparations, spike frequency adaptation also occurs, although to some extent it may be countered by the activation of persistent inward currents (I_{IC}) (Bennett et al. 2001; Lee and Heckman 1998a,b). Spike frequency adaptation might complicate the interpretation of the control unit firing rate in terms of synaptic input because over time the rate may drop even though the synaptic input does not. However, several arguments suggest this was not a major problem in the present study. 1) Most motoneurons in the sacrocaudal cord did not exhibit marked spike frequency adaptation for the low-amplitude slow current ramps employed in our companion in vitro study (Bennett et al. 2001). This may be partly because spike frequency adaptation is greater for higher firing rates and thus larger inputs (Kernell and Monster 1982). Thus we used low-amplitude slow synaptic inputs in the present motor-unit study to mimic the slow current inputs used in vitro. 2) We preactivated the control unit for many seconds before recruiting the test unit so that if any spike frequency adaptation was present, it would have to some degree taken place before the test unit was studied. 3) Most of the units studied were low- or moderate-threshold units, and thus the highest-threshold units that fire at the highest rates and are most likely to have spike frequency adaptation (Bennett et al. 2001; Kernell and Monster 1982) were not studied. And 4) if spike frequency adaptation did occur in the control unit, it likely also occurred to a greater extent in the higher-threshold test unit (with higher rates), and this would have caused an underestimate of the I_{IC} and plateau.

In conclusion, we have found that, after chronic spinal cord injury, the discharge of motor units is sustained by plateaus intrinsic to the motoneurons. Functionally, these plateaus and associated sustained discharges contribute to hypertonus and long-lasting exaggerated reflexes associated with spasticity following injury. We have used a paired-motor-unit method to detect the plateaus and found that there was a clear similarity between the plateaus estimated in this way and those measured directly with intracellular recording in the same population of motoneurons (Bennett et al. 2001). Also the method was verified by applying it to pairs of motoneurons, rather than motor units, where plateaus were independently tested with current injection (Fig. 2). These results provide strong support for the validity of the paired-motor-unit method and its previous use in human motor-unit studies (Gorassini et al. 1998, 1999b, 2001a,b). Thus those human studies, taken together with the present study, indicate that plateaus are important to normal behavior, disappear with acute spinal transection, and re-appear after chronic spinal cord injury and participate in generation of muscle spasms (see also Eken et al. 1989; Hounsgaard et al. 1988).

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