Role of Persistent Sodium and Calcium Currents in Motoneuron Firing and Spasticity in Chronic Spinal Rats

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Li, Yunru, Monica A. Gorassini, and David J. Bennett. Role of persistent sodium and calcium currents in motoneuron firing and spasticity in chronic spinal rats. J Neurophysiol 91: 767–783, 2004; 10.1152/jn.00788.2003. After chronic spinal injury, motoneurons spontaneously develop two persistent inward currents (PICs): a TTX-sensitive persistent sodium current (sodium PIC) and a nimodipine-sensitive persistent calcium current (calcium PIC). In the present paper, we examined how these PICs contributed to motoneuron firing. Adult rats were spinalized at the S2 sacral level, and after 2 months intracellular recordings were made from sacrocaudal motoneurons in vitro. The PICs and repetitive firing were measured with slow triangular voltage and current ramps, respectively. The sodium PIC was examined after blocking the calcium PIC with nimodipine (20 μM; n = 12). It was always activated subthreshold, and during current ramps in nimodipine, it produced a sodium plateau that assisted in initiating and maintaining firing (self-sustained firing). The sodium PIC oscillated off and on during firing and helped initiate each spike, and near threshold this caused abnormally slow firing (2.82 ± 1.21 Hz). A low dose of TTX (0.5 μM) blocked the sodium PIC, sodium plateau, and very slow firing prior to affecting the spike itself. The calcium PIC was estimated as the current blocked by nimodipine or current remaining in TTX (2 μM; n = 13). In 59% of motoneurons, the calcium PIC was activated subthreshold to firing and produced a plateau that assisted in initiating and sustaining firing because nimodipine significantly increased the firing threshold current and decreased the self-sustained firing. In the remaining motoneurons (41%), the calcium PIC was activated suprathreshold to firing and during current ramps did not initially affect firing but eventually was activated and caused an acceleration in firing followed by self-sustained firing, which were blocked by nimodipine. The frequency-current (F-I) slope was 3.0 ± 1.0 Hz/nA before the calcium PIC activation (primary range), 6.3 ± 3.6 Hz/nA during the calcium PIC onset (secondary range; acceleration), and 2.1 ± 1.3 Hz/nA with the calcium PIC steadily activated (tertiary range). Nimodipine eliminated the secondary and tertiary ranges, leaving a linear F-I slope of 3.7 ± 1.0 Hz/nA. A single low-threshold shock to the dorsal root evoked a many-second-long discharge, the counterpart of a muscle spasm in the awake chronic spinal rat. This long-lasting reflex was caused by the motoneuron PICs because when the activation of the voltage-dependent PICs was prevented by hyperpolarization, the same dorsal root stimulation only produced a brief excitatory postsynaptic potential (<1 s). Both the calcium and sodium PIC were involved because nimodipine only partly reduced the reflex and there remained very slow firing mediated by the sodium PIC.

INTRODUCTION

In the months after a spinal cord injury, a spasticity syndrome develops that is characterized by hyperactive tendon jerks, increased muscle tone, clonus, and involuntary flexor withdrawal and extensor spasms. These symptoms are very debilitating as they can interfere with residual motor function, produce pain, disrupt sleep, and, at times, produce skin breakage (Little et al. 1989). Although studies have shown that exaggerated cutaneous/flexor reflexes (Bennett et al. 1999; Remy-Neris et al. 1999) and increased tonic stretch reflexes are involved in the production of this syndrome (Burke et al. 1970; Powers et al. 1989; Thilmann et al. 1991), the exact mechanisms underlying spasms and spasticity are still not fully understood. Increased excitatory spinal reflex pathways (Barbeau and Norman 2003; Schmit et al. 2002), decreased descending and segmental inhibition (Cavallari and Pettersson 1989; Crane et al. 2003; Mailis and Ashby 1990; Thompson et al. 1998), newly formed neuronal circuitry due to the sprouting after injury (Krenz and Weaver 1998), and increased neuronal excitability, including interneurons and motoneurons (Bennett et al. 2001c; Eken et al. 1989) may all contribute.

Our recent studies from spinal-cord-injured adult rats have shown that the development of muscle spasms is caused by large voltage-dependent persistent inward currents (PICs; e.g., calcium currents) that develop in motoneurons weeks to months after injury (chronic spinal) (Li and Bennett 2003). These PICs produce sustained depolarizations of the motoneuron plateau potentials; lasting seconds) in response to brief depolarizing stimuli, resulting in sustained firing that outlasts the stimulation (self-sustained firing). Thus the PICs amplify and prolong the response of motoneurons to transient sensory inputs and ultimately generate exaggerated and sustained reflex responses characteristic of the spastic syndrome. Further study indicates that the PICs in these motoneurons are mediated by a subthreshold TTX-sensitive persistent sodium current (sodium PIC) and a low-threshold nimodipine-sensitive persistent calcium current (calcium PIC; Cav1.3 L-type calcium channel) (Li and Bennett 2003). One goal of the present study was to examine how these two currents contribute to the long-lasting reflexes associated with muscle spasms.

Plateaus and PICs are also activated in motoneurons of animals with intact spinal cord and brain stem (Bennett et al. 1998; Gorassini et al. 1999a; Lee and Heckman 1998a) and uninjured humans (Gorassini et al. 1998, 2002; Kiehn and Eken 1997) and thus amplify and prolong synaptic inputs in normal behavior. Normally PICs depend critically on descending facilitation from brain-stem-derived serotonin (5-HT) or noradrenaline (Hounsgaard et al. 1988a; Hsiao et al. 1998; Lee and Heckman 1998a,b), and thus plateaus are eliminated with acute spinal cord injury. Surprisingly, the redevelopment of
PICs in motoneurons after chronic spinal cord injury occurs even though the monoamines that normally facilitate PICs, such as 5-HT, are greatly diminished below the injury site (Newton and Hamill 1988). The recovered PICs after chronic injury have amplitudes comparable to those recorded in spinal cord/brain stem intact animals (Bennett et al. 1998; Lee and Heckman 1998a,b) and normal awake humans (Gorassini et al. 2002). However, due to the loss of proper descending inhibitory control after spinal cord injury, even a brief stimulation is sufficient to trigger plateaus and produce very long-lasting reflexes and spasms (Bennett et al. 2001a).

The activation of the PICs may also contribute to the abnormally slow firing of motor units observed in humans with spasticity after chronic spinal cord injury. For example, the activated PICs may increase the conductance of the motoneurons (Bennett et al. 2001c), thus making it more difficult to increase the firing rate, which could contribute to the lower maximum firing rates reached during volitional contractions in humans after spinal cord injury (Zijdewind and Thomas 2003). Another interesting phenomenon seen in these injured humans is very slow clockwork-like motor unit firing, either occurring spontaneously or triggered after an innocuous stimulation (Zijdewind and Thomas 2001; M. A. Gorassini, unpublished data). Interspike intervals during this firing are up to half a second and are much longer than expected from normal motoneurons (Matthews 1996). A similar phenomenon has also been recorded directly in motoneurons of rats after chronic injury (Bennett et al. 2001c). It can be triggered by intracellular stimulation and thus is an intrinsic property of the motoneurons. Likely, it is mediated by repetitive re-activation of the voltage-dependent PICs with slow kinetics near firing threshold (as suggested by Bennett et al. 2001c; Carp et al. 1991; Hodgkin 1948; Kernell 1999). Thus the second goal of the present study was to specifically examine the role of PICs in this and other abnormal slow firing behaviors seen after injury.

Aside from abnormally slow firing, the firing behavior of motoneurons of chronic spinal rats is very much like that in normal motoneurons in the spinal-cord-intact state (i.e., in unanesthetized decerebrate cats, Bennett et al. 1998; Hounsgaard et al. 1988a; Lee and Heckman 1998b). Both exhibit self-sustained firing produced by large PICs (see preceding text), and both exhibit classic input-output properties with primary and secondary range firing responses to injected current (piecewise linear frequency-current, F-I, relations). However, with the chronic spinal rat preparation, recordings are made in vitro (Bennett et al. 2001c), making it possible to directly block the PICs pharmacologically and examine their role in the classic input-output firing properties of motoneurons. This was our third goal. We found that the activation voltage of the calcium PIC critically determines the firing behavior. When the calcium PIC is activated subthreshold to the initial firing level at recruitment (in about half the cells), then it assists in producing self-sustained firing, but this firing is linearly modulated with current, with a single F-I slope. However, when the calcium PIC is activated above the initial firing level, then it does not assist in low-frequency firing (in primary range) but causes a steep acceleration in firing when it is being activated (secondary range F-I slope). In all cells, after the calcium PIC is steadily activated, then it produces a paradoxically lower F-I slope (denoted as tertiary range), which we argue is due to the increased conductance provided by this calcium PIC. These results lead to the suggestion that steady-state firing in the primary, secondary and tertiary ranges can be directly defined in terms of the state of the calcium PIC (see DISCUSSION).

METHODS

Both normal adult female Sprague-Dawley rats (>60 days old, n = 5) and spastic rats with chronic spinal cord injury (>90 days old, n = 27) were included in the present study. For the spastic rat, a complete spinal cord transection was made at the S2 sacral level when the rat was 40–50 days old (Bennett et al. 1999, 2001a,c). Usually, within 30 days dramatic spasticity developed in the tail muscles, which are innervated by sacral motoneurons below the level of the injury. Only rats >50 days (also usually <120 days) post injury with clear spasticity were included in the present study. See Bennett et al. (1999) for details of the chronic spinal transection and spasticity assessment. All experimental procedures were approved by the University of Alberta animal welfare committee.

In vitro preparation

The experimental procedure has been previously described in detail (Li and Bennett 2003) and is only briefly summarized here. Normal and chronic spinal rats were deeply anesthetized with urethane (0.18 g/100 g; with a maximum of ~0.45 g for rats >250 g), and the whole sacral spinal cord was removed and placed in a dissection chamber filled with modified artificial cerebrospinal fluid (mACSF) maintained at 20°C. After an hour’s rest in the dissection chamber, the cord was transferred to the recording chamber where it was immersed in continuously flowing (5 ml/min) normal ACSF (nACSF), maintained at 25°C. The long ventral roots (usually sacral S4 and caudal Ca1) and caudal equina (which had attached dorsal roots) were mounted on silver-chloride wires above the nACSF and covered with high vacuum grease (Dow Corning). Sharp intracellular recording electrodes were made from thick-wall glass capillaries (Warner GC 150F-10, 1.5 mm OD) with a micropipette puller (Sutter P-87 puller), filled with a 1:1 mixture of 2 M KAcetate and 2 M KCl and beveled (2003).

Two kinds of ACSF were used in the experiments: nACSF in the recording chamber and mACSF in the dissection chamber prior to recording. The composition of nACSF was (in mM) 118 NaCl, 24 NaHCO3, 2.5 CaCl2, 3 KCl, 1 MgSO4, and 12 glucose. The composition of mACSF was (in mM) 118 NaCl, 24 NaHCO3, 1.5 CaCl2, 3 KCl, 5 MgCl2, 1.4 Na2HPO4, 1.3 MgSO4, 25 glucose, and 1 kynurenic acid. Both kinds of ACSF were saturated with 95% O2-5% CO2 and maintained at pH 7.4. Drugs added to the nACSF in the experiments included: 0.5–2 lM TTX (RBI), 3–20 lM nimodipine (Sigma), and 400 lM Cd2+ (Sigma) as detailed in Li and Bennett (2003).

Drugs and solution

Two kinds of ACSF were used in the experiments: nACSF in the recording chamber and mACSF in the dissection chamber prior to recording. The composition of nACSF was (in mM) 122 NaCl, 24 NaHCO3, 2.5 CaCl2, 3 KCl, 1 MgSO4, and 12 glucose. The composition of mACSF was (in mM) 118 NaCl, 24 NaHCO3, 1.5 CaCl2, 3 KCl, 5 MgCl2, 1.4 Na2HPO4, 1.3 MgSO4, 25 glucose, and 1 kynurenic acid. Both kinds of ACSF were saturated with 95% O2-5% CO2 and maintained at pH 7.4. Drugs added to the nACSF in the experiments included: 0.5–2 lM TTX (RBI), 3–20 lM nimodipine (Sigma), and 400 lM Cd2+ (Sigma) as detailed in Li and Bennett (2003).
Plateau and PIC activation in current- and voltage-clamp recording

Slow triangular current ramps (0.4 nA/s) and voltage ramps (standard speed: 3.5 mV/s, varied from 2 to 5 mV/s) were applied to the motoneurons to evoke the plateaus and the associated PIC. During the current ramps (in current-clamp), the PIC that contributed to a subthreshold plateau and sustained firing was estimated from the difference in injected current required to terminate a plateau (ΔI traveller), compared with the current required to start the plateau (ΔI = I end − I leak, see Fig. 1A) (see also Bennett et al. 2001c). Also, the subthreshold plateau was quantified by extrapolating the linear subthreshold voltage-current relation to just prior to the first spike (thin line in Fig. 1A) and subtracting this linear response from the actual depolarization (measured 5 ms prior to the 1st spike to avoid rapid upswing of the spike). The term plateau is used to denote any relatively sustained depolarization produced by a PIC. As discussed in Bennett et al. (1998), it does not imply a fixed depolarization. Instead, the depolarization produced by the PIC (plateau) can summate with other depolarizations, such as the passive depolarization during a current ramp, and thus a plateau can essentially ride on top of a passive current ramp response (Figs. 1A and Fig. 4B).

During the voltage ramps (in voltage-clamp), the PIC caused a negative-slope region in the I-V relation, and this negative slope region caused the plateau seen in current clamp (Li and Bennett 2003). To obtain an estimation of the passive leak current that summed with the PIC to give the total recorded current, a linear relation was fit to the subthreshold current response in the linear region 10 mV below the negative slope region onset and extrapolated to more positive voltages (leak current, thin triangular line overlaying current; Fig. 1B). The PIC amplitude was then estimated by subtracting this leak current from the recorded current, and this difference is indicated by the arrow in Fig. 1B (see details in Fig. 1 of Li and Bennett 2003). In a previous paper (Li and Bennett 2003), we defined the first zero slope point on the up ramp in the recorded current as the onset current (I onset) of the PIC and the corresponding voltage as the onset voltage (V onset); the second zero slope point of the recorded current in the up ramp is defined as the initial peak current (I peak) of the PIC. In the present paper, we measured the half-activation voltage (V half) of the PIC because this point corresponded well to when the plateau was activated in current-clamp (see Fig. 9 in Li and Bennett 2003) [V 1/2 is voltage at which half PIC activated, at (I leak − I leak − I peak)/2]. Also, the PIC was activated over a broad range, and the onset voltage V onset underestimates the voltage at which the PIC and plateau were functional, compared with V 1/2. The slope of the subthreshold leak current was denoted S 1. The slope of the I-V relation was also computed at suprathreshold voltages, above the negative slope region, and denoted S 2. The S 2 slope was measured over a small 1-mV range and at a common voltage when the PIC was fully activated (at V half in Fig. 1 of Li and Bennett 2003) to minimize time dependent activation effects. Thus the S 1 and S 2 approximate the membrane conductance.

Instantaneous firing frequency as a function of injected current (F-I) was computed using a custom Linux-based program (G. R. Detillieux, Winnipeg). The F-I slope was computed for piecewise linear regions of the F-I relation with a regression. To compare the difference in firing frequency before and after drug applications (nimodipine), only ramps with matched amplitude and speed were employed, and thus some cells were discarded because we did not have matched ramps. This was particularly important for cells with significant rate adaptation (Bennett et al. 2001c) that were very sensitive to ramp speed amplitude and speed. In some cells, there were significant changes in threshold input resistance after drug application, presumably due to leakage or electrode blockage, and such cells were also discarded from the F-I analysis. The initial firing level (spike threshold) for each cell was measured from the first spike elicited by the current ramp, at the potential where there first began a rapid acceleration in the rate of depolarization to >10 V/s (Li and Bennett 2003).

Dorsal root reflexes

A single electrical stimulation pulse was applied to the dorsal caudal root (Ca; 0.02–0.1 nA, 0.1 ms, 10-s minimum interval between stimuli) while the motoneuron membrane was held at different potentials with a bias current, and reflex responses were recorded in the motoneurons. This single shock was usually enough to elicit a long-lasting reflex.

Data analysis

Data were analyzed in Clampfit 8.0 (Axon Instruments). Data are shown as means ± SD. A Student’s t-test was used to test for statistical differences with a significance level of P < 0.05.
RESULTS

A total of 27 motoneurons from chronic spinal rats and 5 motoneurons from acutely transected normal rats were included in the present study. Previously, we have described the basic properties and persistent inward currents (PICs) in these same neurons (26 of them) (Li and Bennett 2003), and the purpose of the present paper was to determine how these PICs affected firing behavior. The PICs were measured with slow triangular voltage ramps, in voltage-clamp mode, as shown in Fig. 1B. During an upward voltage ramp, the current initially increased linearly due to the passive leak current (shown extrapolated with thin triangular line, Fig. 1B, middle), but after the onset of the PIC, the current deviated negatively and caused a negative-slope region in the I-V relation. The difference between the measured current and the extrapolated leak current was used as an estimate of the net PIC (vertical arrow in Fig. 1B; see METHODS). The half activation voltage of the PIC (V_{1/2}; vertical thin line in Fig. 1B) was used to quantify the location of the negative-slope region, which we have previously shown corresponds to the point at which a plateau is activated during a current ramp (see Fig. 9 in Li and Bennett 2003). In all motoneurons of chronic spinal rats (n = 27), the V_{1/2} was on average ±50.1 ± 4.72 mV, which was 3.79 ± 1.72 mV below the firing level (i.e., spike threshold, dashed line in Fig. 1B; significant difference), where the subthreshold plateau occurred (left arrow in Fig. 1A).

There are only two major currents that make up the PIC: a sodium PIC and a calcium PIC (Li and Bennett 2003). With the application of nimodipine, only the sodium PIC remains, and this current is shown in Fig. 1F (top), after leak current subtraction (see METHODS). The reduction in current with nimodipine represents the calcium PIC, and this is also shown in Fig. 1F (bottom). Nimodipine blocks the calcium PIC without affecting the fast sodium spike (Figs. 2 and 9, described later) and was thus particularly useful in studying the effects of PICs on firing as shown in the following text.

Firing behavior classification (LLS and LAS type cells)

Previously, we have shown that motoneurons of chronic spinal rats can be divided into two distinct types based on their unique firing patterns (Bennett et al. 2001c), and these were again found in the present population of motoneurons, as shown in Figs. 1 and 2. In the first type of motoneuron (Fig. 1; n = 16/27 cells), there was initially a linear increase in potential during a current ramp, but ~5 mV below the firing level, the potential accelerated relatively steeply. This acceleration marked the onset of a subthreshold plateau from a PIC activation (left arrow in Fig. 1A) (Bennett et al. 2001c; Li and Bennett 2003) because if the current was reduced shortly afterward, there was a maintained depolarization and associated firing that continued even when the current was reduced far below the current that initiated firing (self-sustained firing). This self-sustained firing was quantified by ΔI = I_{start} - I_{end}, where I_{start} and I_{end} are the currents at the start and end of plateau and firing. The ΔI was on average ±1.13 ± 0.19 nA for this cell type. The plateau stopped just after firing ceased as seen by the after-potential at the end of the descending current ramp (right arrow in Fig. 1A). In this type of cell, the PIC was as fully activated as possible shortly after recruitment because larger ramps did not produce more self-sustained firing (not shown, though see Fig. 5 in Bennett et al. 2001c). Furthermore, shortly after recruitment there was no further evidence of PIC activation (no firing rate jumps), and the firing changed linearly with the current, even during repeated current ramps and during self-sustained firing (at currents below the plateau activation threshold; Fig. 1C). Thus we refer to this type of cell as low-threshold linear self-sustained firing cells (LLS type).

In the second type of motoneuron, shown in Fig. 2 (n = 11/27 cells), during a slow current ramp, there was also a subthreshold plateau and associated PIC activation like in LLS cells (left arrow in Fig. 2, A and B). However, this PIC was not fully activated at recruitment because only a small degree of self-sustained firing was produced when the ramp was turned around shortly after recruitment (ΔI = 0.6 in Fig. 2A; mean ΔI = 0.34 ± 0.15 nA for all cells). In contrast, larger ramps evoked a late acceleration in firing (labeled “s” in Fig. 2B) that marked the further onset of a PIC because this extra firing was sustained despite reduced current (F-I hysteresis), and there was significantly more self-sustained firing (ΔI = 1.2 in Fig. 2B; mean ΔI = 0.98 ± 0.15 nA). These cells we refer to a late-accelerating self-sustained firing cells (LAS type). LAS
type cells were on average lower rheobase cells (1.29 ± 1.53 nA) than LSS cells (2.53 ± 0.69 nA) (see Bennett et al. 2001c). The linear increase in firing with current prior to the late acceleration we refer to as primary range firing (p in Fig. 2B), and the firing during the late acceleration we refer to as ramp-evoked secondary range firing (s in Fig. 2B), similar to the classic steady-state firing responses (Bennett et al. 1998; Kernell 1965a; Schwindt and Crill 1982). The firing after the late acceleration we refer to as tertiary range firing (t in Fig. 2B). Classically, the steady-state primary and secondary range firing is obtained from an increasing sequence of 1-s current steps and measured in the last half second of each step (Kernell 1965a). We instead used very slowly increasing current ramps (lasting 10–20 s), but for the primary and tertiary ranges, the firing obtained at a particular current was similar to the steady-state firing during a classic current step (data not shown). In the secondary range during a current ramp, the firing was not quite in steady state, and thus we denote this the ramp-evoked secondary range. While the F-I slope in this range is steep compared with the primary slope, the firing still increases over a 2-s period (Fig. 2) and thus is approximately comparable to the classic secondary range response to two 1-s current steps.

**Subthreshold sodium and calcium PIC contributions to LLS-type cells**

In LLS type cells, the subthreshold plateau activation was caused by both sodium and calcium PICs because nimodipine significantly reduced it (from 9.49 ± 3.53 to 3.17 ± 2.09 mV), but blocking it required both nimodipine and TTX (see details in Li and Bennett 2003). Furthermore, direct measurements of these two PICs in voltage clamp (described in the preceding text and in Fig. 3 described in the following text) indicate that they were both activated subthreshold and thus should indeed produce a subthreshold plateau. That is, in LLS cells the half activation voltage \( V_{1/2}\) of the calcium PIC (~44.2 mV in Fig. 1F; mean of 47.4 ± 5.52 mV) and sodium PIC (~43.7 mV in Fig. 1F; mean of 46.8 ± 4.01 mV) were always lower than the firing level (~41.4 mV in Fig. 1F; mean of 44.6 ± 4.18 mV; significant differences; \( n = 7/7\) cells with nimodipine) and not significantly different from each other. Nimodipine significantly increased the current required to initiate firing (from 2.53 ± 0.69 to 3.26 ± 0.55 nA) and in most cells, lowered the initial firing rate (in 4/7 cells; lowered from 7.73 ± 1.34 to 6.38 ± 1.53 Hz, although not quite significantly; \( P = 0.13\)), so the calcium PIC was involved in initiating firing. Both the sodium and calcium PICs were nearly fully activated by the time the firing level at recruitment was reached (Fig. 1F), and this is consistent with the preceding conclusion that the PICs and plateau were as fully activated as possible at recruitment and subsequently did not contribute to accelerations in firing rate during the ascending current ramp (unlike LSS cells). Nimodipine also significantly reduced the afterpotential after de-recruitment (from 6.39 ± 1.33 to 3.01 ± 2.14 mV; right arrows in Fig. 1, A and D) and eliminated the brief accelerations in firing that are sometimes seen just prior to de-recruitment (* in Fig. 1, A and C, but not D) (also see Fig. 5 of Bennett et al. 2001c), and thus these were calcium PIC mediated.

The characteristic self-sustained firing evoked even by small current ramps in LLS cells was in large part caused by the low-threshold calcium PIC because this self-sustained firing was markedly reduced by nimodipine (\( \Delta I \) significantly lowered from 1.15 ± 0.22 to 0.26 ± 0.26 nA). A subthreshold calcium PIC occurred in all LLS cells and thus is a primary requirement for LLS type firing behavior. However, there did remain significant self-sustained firing in nimodipine, and this was due to slow firing caused by the sodium PIC, that under some conditions could last for very long periods (see Figs. 6–8 described in the following text).

**Calcium PIC causes the late acceleration in LAS-type cells**

In contrast, when nimodipine was applied to LAS-type cells \( (n = 5)\), there was no significant effect on the subthreshold plateau activation, the recruitment current (1.29 ± 1.53 nA before and 1.40 ± 2.25 nA after), or low-frequency firing (see details in Fig. 5B, described later), and thus only the sodium PIC was involved in subthreshold plateau behavior and low-frequency firing. However, when these cells were brought to fire at higher frequencies with a larger current ramp, there was a characteristic late acceleration in firing that was always blocked by nimodipine (5/5 cells), and thus this nonlinearity in the F-I relation (s range in Fig. 2B) was caused by the calcium PIC. In LAS-type cells, the calcium PIC (seen in Fig. 2) was activated at a potential (\( V_{1/2} = \) −44.8 ± 5.30 mV; right arrow in Fig. 2F) significantly higher (by 3.50 ± 1.67 mV) than the firing level at recruitment (−48.3 ± 4.48 mV; firing level at dashed line in Fig. 2F), and much higher than the subthreshold sodium PIC (\( V_{1/2} = \) −51.3 ± 4.56 mV; left arrow in Fig. 2F). Thus this higher-threshold calcium PIC in LAS-type cells was only activated after recruitment when the membrane potential was sufficiently depolarized during higher-frequency firing. At this time the calcium PIC activation produced the late acceleration in firing rate (firing level at this time indicated by * in Fig. 2F).

The self-sustained firing after the late acceleration was significantly reduced by nimodipine in LAS cells (from \( \Delta I = \) 0.97 ± 0.15 to 0.24 ± 0.31 nA), indicating that the calcium PIC played a major role in sustaining the firing, as for LSS cells. In summary, in LAS cells, the sodium and calcium PICs were distinctly separated in their activation voltages and, respectively, produced two distinct effects: an early subthreshold plateau and a late acceleration in firing rate, respectively, followed by self-sustained firing.

Interestingly, the sodium PIC activation level (\( V_{1/2} \)) was ~4 mV higher in the LSS cells (~46.8 ± 4.01 mV) compared with LAS cells (~51.3 ± 4.56 mV, although not quite significant difference, \( P = 0.07\), because between cell comparisons of absolute potentials are variable). Also, the firing level was ~4 mV higher in LSS cells (~44.6 ± 4.18 mV) compared with LAS cells (~48.3 ± 4.48 mV, \( P = 0.09\)). In contrast, the calcium PIC activation was only ~2 mV lower in LSS cells (~47.4 ± 5.52 mV) compared with LAS cells (~44.8 ± 5.30 mV, \( P = 0.65\)). These differences between LSS and LAS cells in the sodium PIC and sodium spike activation were largest and closest to significance, suggesting that the differences in the firing behavior in LLS cells compared with LAS could be mostly attributed to higher sodium PIC and spike activation levels and to a lesser extent to a lower calcium PIC activation level.
Variations in calcium PIC determines firing behavior

In many cells (n = 13), we added TTX first, rather than nimodipine, and in these cells, we could not study the firing because the spikes were blocked together with the sodium PIC. However, in these cells, the calcium PIC could be observed directly as the PIC remaining in TTX (Fig. 3, C and D) and compared with the firing level at recruitment prior to TTX. The half activation potential of the calcium PIC (V_{1/2} calcium) clearly separated all these cells into two groups corresponding to the LLS and LAS classification of their firing behavior (prior to TTX application) as expected from the nimodipine experiments described in the preceding text. This is shown in Fig. 3E in which the degree of self-sustained firing (ΔI) is plotted as a function of the V_{1/2} calcium relative to the firing level. When the V_{1/2} calcium was below firing level (n = 7; Fig. 3C), pronounced sustained firing occurred (ΔI = 1.12 ± 0.16 nA) even for small ramps reaching just above the recruitment threshold (as in Fig. 3A; at left of vertical line in Fig. 3E), and these cells all corresponded to LLS type cells (n = 7/7) as described in the preceding text. When the V_{1/2} calcium was above the firing level (n = 6; Fig. 3D), significantly less self-sustained firing occurred (ΔI = 0.35 ± 0.16 nA) for the same small current ramps (Fig. 3B and • at right of vertical line in Fig. 3E), even though these cells had as large PICs (Fig. 3D) as the LLS cells (Fig. 3C). However, with larger ramps (like in Fig. 2B; ○ in Fig. 3E) pronounced self-sustained firing (ΔI = 1.02 ± 0.11 nA) could usually be evoked after a late acceleration in firing (n = 4/6), and these cells corresponded to LAS type cells (upper ○ at right of Fig. 3E).

There were, however, a few cells with the V_{1/2} calcium above the firing level that always had weak self-sustained firing and did not have a firing rate acceleration regardless of the ramp amplitude (lower ○ at right of Fig. 3E; n = 2/6 cells tested with TTX). These cells had large calcium PICs, but the calcium PIC threshold was too high to produce a late acceleration during firing evoked with a current ramp [due to accumulated afterhyperpolarization (AHP) effects] (see Li and Bennett 2003). Interestingly, in these cells the calcium PIC and firing rate acceleration could be activated by synaptic input (described in a later section), suggesting a difference due to dendritic location of the synaptic inputs and calcium PIC (Bennett et al. 1998).

Low F-I slope in chronic spinal rats results from Ca^{2+}-mediated conductance

As we have shown previously, motoneurons from chronic spinal rats fired with a significantly shallower F-I slope (mean: 4.56 Hz/nA, see following text) than the motoneurons of acute spinal rats (6.7 Hz/nA) (acute data from Bennett et al. 2001c) when tested with current ramps as in Fig. 2. We proposed that the main reason for this lower slope was the activation of the large PICs in chronic spinal rats that should have increased the conductance of the cells and thus increased the difficulty in depolarizing the cells with injected current and ultimately decreased the F-I slope (Bennett et al. 2001c).

To examine this issue, we first verified that the membrane conductance increased after the PIC activation by estimating the conductance from the slope of the I-V relation during the ramp voltage-clamp experiments (see METHODS). Indeed as shown in Fig. 4A (bottom trace), at voltages below the negative-slope region, prior to PIC activation, the conductance (I-V slope S_1) was much shallower than above the negative-slope region with the PIC activated (S_2, measured at common potential at vertical line). In motoneurons of chronic spinal rats, the ratio of these two slopes (S_2/S_1) was significantly >1 (S_2/S_1 = 5.28 ± 3.42, n = 13), whereas in motoneurons of acute spinal rats, the ratio was not significantly different from 1 (because there was usually a linear I-V relationship, with no PIC activation; S_2 slopes measured at same voltage as in chronic spinal rats) (see Li and Bennett 2003). This increased conductance in chronic spinal rats was mediated by both sodium and calcium PICs because the ratio of S_2/S_1 was significantly decreased when TTX or nimodipine were added to the bath (TTX shown in Fig. 4A, middle trace), and it was decreased to 1 after both drugs were applied (Fig. 4A, top linear trace). Importantly, the increased conductance after PIC activation was not caused by a voltage-dependent potassium conductance in the voltage range studied (< -30 mV) because TTX and nimodipine eliminated the increased conductance.
calcium PIC only activated after the late acceleration in firing, and the slope was only increased by nimodipine after this late acceleration, in the tertiary range, as described next.

Secondary and tertiary range firing caused by calcium PIC

In LAS-type cells (Fig. 5B), nimodipine had no significant effect on the F-I slope when the cell fired in the primary range subthreshold for late acceleration in firing (Fig. 5, B and C; mean slope: 3.04 ± 1.02 Hz/nA in the primary range before nimodipine, p, and 3.70 ± 1.02 Hz/nA after nimodipine, p′), as would be expected of the lack of calcium PIC in this range. In

**FIG. 4.** Increase in conductance mediated by activated PICs. A: current response of a motoneuron to a slow voltage ramp (as in Fig. 3B) plotted against the applied voltage, recorded in normal artificial cerebrospinal fluid (ACSF), after TTX, and after TTX and nimodipine application. Diagonal lines indicate conductances measured before (S₀) and after (S₁) PIC activation, and vertical lines indicate voltage where S₀ and S₁ were measured. Note the marked decreases in the conductance S₁ after TTX and nimodipine application. The star indicates a transient unclamped spike, which was removed for clarity. B: in TTX, voltage response from a motoneuron, plotted against the current applied during a standard slow upward ramp (upward arrow) and slow downward ramp (downward arrow). Note the resistance measured after plateau activation (R₃) was much smaller than before plateau activation (R₁).

when measured at a common voltage (see linear responses in top trace of Fig. 4A). Thus persistent sodium and calcium PICs mediated the increased conductance. The calcium-PIC-mediated increase in conductance (S₁) likely includes a Ca²⁺-activated K⁺ current (unpublished data).

The increased conductance after calcium PIC activation was also seen during current-clamp experiments where TTX was present (Fig. 4B), and a calcium-mediated plateau occurred without spiking. That is, on the plateau with the calcium PIC activated, the slope of the voltage curve (resistance, R₂ = 1/ conductance) was only 48% of the slope before the plateau started (R₀; significantly lower; R₀/R₂ = 2.10 ± 0.83; n = 12), suggesting that the conductance of the membrane was doubled by the activated calcium PIC.

Finally, to prove that the presence of the calcium PIC contributed to the shallow F-I slope seen in motoneurons of chronic spinal rats, we blocked this current with nimodipine (Fig. 5), which again, had no direct affect on the sodium spikes or AHPs. As expected, the F-I slope was found to be significantly steeper after nimodipine compared with before (7.37 ± 3.32 Hz/nA compared with 4.56 ± 1.19 Hz/nA, n = 9, Fig. 5A), provided that the slope before nimodipine application was measured in a region where the calcium PIC was known to be active (above calcium PIC threshold). The cell in Fig. 5A is an LLS cell with the calcium-PIC-activated subthreshold to firing, and the F-I slope was clearly affected throughout by nimodipine. Whereas, the cell in Fig. 5B is an LAS cell with the calcium PIC only activated after the late acceleration in firing, and the slope was only increased by nimodipine after this late acceleration, in the tertiary range, as described next.

**FIG. 5.** Effects of calcium PIC on F-I relation in motoneurons of chronic spinal rats. A: F-I plots of a LLS cell obtained from a slow triangular ramp (like Fig. 1C), in normal ACSF (○) and in nimodipine (●). Note simple linear F-I relation and self-sustained firing (Iₛ₋ₐ − Iₛₑ) prior to nimodipine. Nimodipine increased the F-I slope and threshold current (at start) and decreased the initial firing rate and self-sustained firing. B: F-I plot of a LAS cell in respond to a slow triangular ramp (same cell as in Fig. 2). Note on the upward ramp (↑), the initial linear F-I slope (primary range, p), then late acceleration to a steeper slope (ramp-evoked secondary range, s), followed by firing with a very shallow slope (tertiary range, t). On the downward ramp (↓), the shallow tertiary range slope continued, and there was marked self-sustained firing. C: same motoneuron in B after nimodipine. Note that the F-I slope was similar to the primary range prior to nimodipine. Thus the secondary and tertiary range firing resulted from the calcium PIC.
contrast, after the late acceleration in firing, and thus calcium PIC activation, the F-I slope (in tertiary range, t in Fig. 5B; mean: 2.10 ± 1.31 Hz/nA) was significantly less than the slope in nimodipine, even at matched frequency ranges (Fig. 5C; mean: 3.70 ± 1.02 Hz/nA), consistent with the activated calcium PIC reducing the F-I slope. Also consistent with this interpretation, the slope with the calcium PIC fully activated, in tertiary range (Fig. 5B; t; mean: 2.10 Hz/nA, as in the preceding text), was significantly less than the slope measured prior the calcium PIC activation, in the primary range (p in Fig. 5B; mean: 3.04 Hz/nA). Of course, during the late acceleration in firing (ramp-evoked secondary range), the F-I slope was very steep (s in Fig. 5B; mean: 6.32 ± 3.63 Hz/nA), and this steep secondary range firing was eliminated with nimodipine (as discussed in the preceding text), leaving only simple linear primary range firing for all currents (Fig. 5C; mean: 3.70 ± 1.02 Hz/nA). This is consistent with the idea that the secondary range firing is caused by the calcium PIC onset (Schwindt and Crill 1982).

Very slow firing caused by persistent sodium currents

When a current ramp was applied to a motoneuron of a chronic spinal rat, the last few spikes of firing were usually very slow (mean: 2.28 ± 0.67 Hz; Figs. 1A, 2A, 3A, and 5B) and less than half the minimum repetitive firing rate that occurs in motoneurons of acute spinal rats (7.51 ± 3.53 Hz; significant difference) (Bennett et al. 2001c). We demonstrate in this section that this unusually slow firing is mediated by a sub-threshold oscillation of the large sodium PIC seen in motoneurons of chronic spinal rats. Very slow firing was most clearly studied when evoked by a brief current pulse in a cell held close to its firing threshold with a bias current (Fig. 6A). After the pulse, there was usually a pause in firing during which a plateau was slowly activated (at arrow in Fig. 6A, top), and then very slow self-sustained firing began. This self-sustained firing continued for many seconds, or even minutes, and then either stopped spontaneously or was terminated by a hyperpolarizing pulse (Fig. 6A). Typically, the firing rate was very low (mean: 2.82 ± 1.21 Hz) and extremely regular (SD in firing 0.26 ± 0.13 Hz), unlike the variable slow firing that can be driven by synaptic noise (Matthews 1996; Powers and Binder 2000). In some cells, we did see transient rate changes during the long periods of slow self-sustained firing (Fig. 7, asterisks, described later), but these were due to spontaneous synaptic events because excitatory postsynaptic potentials (EPSPs) of a similar duration could be seen prior to firing (not shown). The minimum firing rates corresponded to inter-spike intervals of 300–800 ms, which were much longer than the duration of the usual AHP (50–150 ms), measured in response to antidromic stimulation at rest (AHP indicated by length of box in the expanded section of data in Fig. 6A). Thus these cells fired like

FIG. 6. Very slow firing in motoneurons of chronic spinal rats. A, top: plateau and self-sustained firing in a chronic spinal motoneuron, induced by a brief current pulse and terminated by a hyperpolarizing pulse. Motoneuron held near threshold with a 1.7-nA bias current. Note the remarkably low firing rate (1.75 Hz) and the small variation (SD = 0.27 Hz). Bottom: amplification of part of the top. Note the plateau activation (in region of dotted line) and associated ramp&acceleration in potential before each spike (labels). Also note that the interspike interval is much longer than the afterhyperpolarization (AHP) duration (indicated by length of box, measured during antidromic spike, not shown). B, top: motoneuron of acute spinal rat without plateau. Firing only occurs during the current pulses and the minimum repetitive firing frequency (right pulse, 7.68 Hz) is much higher than in A. Also, the interspike interval is very close to the AHP duration (box in bottom).
clockwork at much lower rates than predicted by the AHP duration.

In contrast, in motoneurons of acute spinal rats the minimum repetitive firing rate during a depolarizing pulse was $7.51 \pm 3.53$ Hz (Bennett et al. 2001c) ($7.68$ Hz in Fig. 6B), which corresponds to an inter-spike interval of 133 ms, and this is close to the AHP duration (125 ms in Fig. 6B) as would be expected for motoneurons with small PICs that fire in the absence of synaptic noise (Kernell 1965b). Also, self-sustained firing could not be evoked in acute spinal rats (Fig. 6B) (Bennett et al. 2001c), consistent with the small PICs and lack of negative-slope region in the I-V relation in these cells (Li and Bennett 2003).

During slow firing in chronic spinal rats, the trajectory of the membrane potential between spikes was very much like the subthreshold onset of the plateau prior to the first spike after a brief current pulse (Fig. 6A, top, left arrow) or during a current ramp (left arrows in Figs. 1A–3A). That is, on the expanded time scale in Fig. 6A, after the current pulse, a plateau was activated (in the region of dotted line) that depolarized the cell above baseline (thin line). This plateau activation involved a slow ramp up and then a faster acceleration that ultimately triggered a spike (ramp&accelerate trajectory). After the 100-ms AHP from this first spike (region of box), the membrane potential rose again with the same ramp&accelerate trajectory (see ramp and acceleration labels in Fig. 6A) as though a plateau was again being activated (in region of dotted line in Fig. 6A), and this repeated with each spike. Thus a plateau was being activated prior to each spike and then deactivated by the AHP that followed that spike, and this process was repeated to cause slow firing.

Because the sodium PIC is rapidly deactivated by a hyperpolarization (in $<10–50$ ms) (Lee and Heckman 2001; Li and Bennett 2003), whereas the calcium PIC is not (remains on for $>500$ ms), it stands to reason that only the sodium portion of the plateau could be deactivated during each 100-ms AHP, and thus only the sodium PIC could participate in this slow firing. Indeed, in cells where the calcium PIC was activated far above the firing threshold and was only involved in high-frequency firing (LAS type; Fig. 6 shows such a cell), slow self-sustained firing was just as easily evoked as in other cells (LLS type).

Also, slow firing persisted when the calcium PIC was blocked with nimodipine ($n = 4$), with the same slow steady rate ($3.37 \pm 0.49$ Hz before and $2.60 \pm 0.67$ Hz after nimodipine; Fig. 7) and the same characteristic subthreshold plateau activation and associated ramp&accelerate interspike trajectory (in region of dotted line in Fig. 7B). Thus this very slow firing did not involve the calcium PIC. In contrast, in cells that had a poor persistent sodium PIC, which did not by itself produce a negative slope region (Fig. 10D; described later), very slow firing could not be evoked (Fig. 10A). Instead the minimum firing rate corresponded to an interspike interval similar in

![FIG. 7. Very slow firing does not depend on the calcium PIC. A: long-lasting slow firing caused by a brief current pulse in a chronic spinal motoneuron, as in Fig. 6A. Note again plateau onset, at left arrow, and very slow self-sustained firing with a characteristic interspike ramp and trajectory as a plateau is activated (in region of dotted line), seen in inset. B: after calcium PIC block with nimodipine, a plateau (sodium plateau) and very slow firing were again triggered by a brief current pulse. There were transit accelerations in firing frequency caused by synaptic noise (at asterisks) in this cell, before and after nimodipine.](image-url)
FIG. 8. Sodium PIC produces very slow firing and maintains normal rhythmic firing. A: voltage response of a chronic spinal motoneuron in current clamp. Note the characteristic ramp and acceleration in potential just before recruitment (plateau onset, left ↓) and slow firing prior to de-recruitment (the last spike, right ↑). B: ramp&acceleration in potential and slow firing remained in nimodipine (↓). Note nimodipine did not affect the spike threshold or amplitude (insets in A and B: spike from A overlaid with B as dotted line). C and D: when low-dose TTX was applied, the spike amplitude/threshold were unaffected (insets in C and D, with A overlaid again). However, the characteristic ramp and accelerate (plateau onset) prior to the first and last spike was abolished as was associated slow firing. Also, the cell was unable to generate rhythmic firing. Thus the sodium PIC and sodium plateau mediates very slow firing and helps maintain rhythmic firing.

duration to the AHP, as for motoneurons of acute spinal rats described in the preceding text. Thus a negative-slope region produced by the sodium PIC is the primary requirement for slow firing.

Finally to directly demonstrate that the sodium PIC caused slow firing and related subthreshold plateau activation, nimodipine was given together with a low dose of TTX (0.5 μM; Fig. 8). During low-dose TTX application, there was a brief window in time (5 mins) when the spike was unaffected (see Fig. 8, insets), but the sodium PIC was reduced (not shown). Because this window in time was only brief, we, in this case, studied slow firing with brief current ramps rather than pulses (pulse protocols are time consuming requiring the PIC threshold to be determined). Prior to drug applications, on the upward current ramp, there was the characteristic ramp and acceleration in potential as a plateau started (Fig. 8A, left ↓), and this as usual did not require the calcium PIC because it was not blocked by nimodipine (Fig. 8B; LAS cell described in the preceding text). Likewise, just prior to de-recruitment on the downward current ramp, there was the usual very slow firing (mean rate: 2.31 ± 1.04 Hz), with one very long interval, with a characteristic ramp&accelerate trajectory similar to the plateau onset (Fig. 8A, right ↓). This slow firing again did not depend on the calcium PIC because it was not significantly altered by nimodipine (Fig. 8B; mean rate: 2.28 ± 0.67 Hz).

When low-dose TTX was added, with nimodipine present, the characteristic subthreshold plateau onset was eliminated (no acceleration at left of Fig. 8, C and D), and likewise the slow firing on the downward ramp and the characteristic ramp&accelerate interspike trajectory were eliminated. Thus the subthreshold plateau onset and slow firing are both mediated by the TTX-sensitive sodium PIC. The firing became very erratic and failed on the downward current ramp in low-dose TTX, even when the spike was unaffected. Long irregular firing intervals were seen on the upward ramp, but with a very different interspike trajectory, without the ramp&accelerate shape (Fig. 8, C and D). Instead, long intervals occurred on the upward ramp simply because a spike failed to be produced by the end of the AHP and then was only produced as the cell was further depolarized by the increasing current. Furthermore, it appears that this sodium PIC is critical in enabling the cell to generate rhythmic firing at all because when this current was disrupted, firing became irregular (Fig. 8, C and D) as has been previously reported (Lee and Heckman 2001).

Long-lasting spastic reflexes evoked by brief low-threshold afferent stimulation

In chronic spinal rats, a single low-threshold dorsal root stimulation shock produced a very long-lasting reflex response that we observed in most motoneurons during intracellular recording (stimulation 2× afferent threshold, T in Fig. 9B and typically 2–10 × T; n = 11/13), as described previously (Bennett et al. 2001c). This is consistent with the exaggerated reflexes recorded extracellularly from ventral roots of these chronic spinal rats with the same dorsal root stimulation (Li et al. 2004), and the associated tail spasms recorded with electromyography in awake chronic spinal rats (Bennett et al. 1999, 2001a).

To examine the role of plateaus/PICs in long-lasting reflexes, we have blocked the PICs with two separate methods: hyperpolarization and application of nimodipine. The first method relies on the voltage dependence of the PIC that underlies the plateaus (Bennett et al. 2001c) as demonstrated in a motoneuron of a chronic spinal rat in Fig. 9A. That is, although a brief depolarizing current pulse activated a large plateau and long-lasting self-sustained firing when the cell was held at rest, the same pulse could not activate a plateau when the cell was hyperpolarized (i.e., hyperpolarization abolished the plateau). Similarly, while a brief dorsal root stimulation triggered a long-lasting reflex in a motoneuron (Fig. 9B, top), no long-lasting reflex could be evoked when the motoneuron was held hyperpolarized (Fig. 9B). Therefore the long-lasting reflex was mediated by PICs/plateaus intrinsic to the motoneurons that were blocked by hyperpolarization (just as in Fig. 9A).

Hyperpolarization of the motoneuron did not block the synaptic input caused by the dorsal root stimulation, and this input produced a 0.5- to 1-s-long EPSP (Fig. 9B, bottom). It is this unusually long EPSP that triggered the slowly activating PICs and reflex when the cell was not hyperpolarized. This can be clearly seen in Fig. 9B in which the onset of spiking was delayed because the cell was very near threshold, and the EPSP could be observed first, followed by a PIC/plateau onset (at arrow) and then self-sustained firing. Also, cells without a clear long polysynaptic EPSP (n = 2/13) did not have long-lasting reflexes to a single low-threshold stimulation even though they had large PICs. They could, however, produce a long-lasting reflex (seconds) in response to repeated high-frequency stimulation (100 Hz, for 0.5 s) by the summed monosynaptic reflex (see following text) triggering a plateau/PIC.
In general, proving that only sodium and calcium PICs 
also be involved in the plateau and associated long-lasting 
[...i.e., voltage-dependent current facilitated by the dorsal root stimu-
lation [i.e., N-methyl-D-aspartate (NMDA) receptors] could 
also be involved in the plateau and associated long-lasting 
(reflux that was triggered by dorsal root stimulation, 
and this was blocked by hyperpolarization (Fig. 11A). How-
ever, this dorsal-root-evoked PIC and long-lasting reflex was 
not blocked by nimodipine. It was, however, reduced in am-
plitude, and there was slower firing and no acceleration in 
membrane, to block the 
plateau/PICs (as in 
A). abolished the long-lasting reflex on the same stimu-
lation, and thus the reflex was caused by the PICs. In this cell, an EPSP and then 
slow plateau onset (at top left arrow in upper trace) occurred prior to firing. The 
initial half-second of this EPSP remained after hyperpolarization; the rest was 
eliminated and must have resulted from the PICs. C: motoneuron from an acute 
spinal rat. Top: the same dorsal root stimulation produced a smaller, although 
similar long-duration EPSPs to that in B, but this did not trigger long-lasting 
reflex. Bottom: the EPSPs remained unchanged after hyperpolarization and thus 
were not amplified by PICs, unlike in B.

Calcium PIC alone produces the long-lasting reflex in some 
motoneurons

In addition to the sodium and calcium PICs, any other 
oltage-dependent current facilitated by the dorsal root stimu-
lation [i.e., N-methyl-D-aspartate (NMDA) receptors] could 
also be involved in the plateau and associated long-lasting 
reflex. In general, proving that only sodium and calcium PICs 
are involved is difficult because the sodium PIC cannot be 
easily blocked without blocking the synaptic input involved in 

triggering the long-lasting reflex (EPSP; TTX blocks the dorsal 
roots rapidly). Fortunately, there were some motoneurons (n = 
2/9) that had clear long-lasting reflexes (Fig. 10A) and only 
weak sodium PICs (Fig. 10D). In these motoneurons, 20 μM 
nimodipine blocked the long-lasting reflex regardless of the 
holding potential (Fig. 10C), and thus the calcium PIC medi-
ated these long-lasting reflexes. Furthermore, nimodipine did 
not block the EPSP evoked by the dorsal root stimulation (Fig. 
10C), and thus nimodipine’s primary effect was to block the 
L-type Ca^{2+} channels and associated self-sustained firing 
postsynaptically. In these cells, nimodipine also blocked the 
plateaus evoked with intracellular current injection (not 
shown), and this is consistent with the elimination of the 
calcium-mediated negative-slope regions in the I-V plot during 
voltage-clamp (compare Fig. 10, C and D). There was a small 
TTX-sensitive sodium PIC that remained in nimodipine (Fig. 
10D) although not large enough to produce a plateau, as 
mentioned in the preceding text (no negative slope region). In 
summary, in these cells with weak sodium PICs, the long-
lasting portion of the spastic reflexes were entirely due to 
calcium PICs on the motoneurons mediated by L-type calcium 
currents, and sustained postsynaptic NMDA-like currents were 
thus not involved in the long-lasting reflexes.

Sodium and calcium PICs produce long-lasting reflexes 
in other motoneurons

In other motoneurons (n = 7/9), persistent sodium currents 
were also involved in the long-lasting reflex. Again these cells 
had a long-lasting reflex mediated by a large PIC and self-
sustained firing that was triggered by dorsal root stimulation, 
and this was blocked by hyperpolarization (Fig. 11A). How-
ever, this dorsal-root-evoked PIC and long-lasting reflex was 
not blocked by nimodipine. It was, however, reduced in am-
plitude, and there was slower firing and no acceleration in 
firing, presumably due to the block of the calcium PIC (com-
pare Fig. 11, A and D). This PIC and long-lasting reflex that
remained in nimodipine was due to the sodium PIC currents because there remained a large PIC in voltage-clamp ramps (Fig. 11, F compared with C), there was characteristic very slow firing (Fig. 11D, top), and this sodium PIC was blocked by TTX (not shown). Thus in this cell both sodium and calcium PICs produced the long-lasting reflexes.

Dendritic origin of calcium PICs

Interestingly, reflex evoked calcium PICs and plateaus were more easily triggered (at a lower potentials) than PICs evoked by intracellular current injection, perhaps related to the dendritic location of the Ca$^{2+}$ channels and synaptic inputs (Bennet et al. 1998). That is, with intracellular current injection the calcium PIC was only activated in LAS-type cells with a substantial depolarization well above the initial firing level at recruitment (after frequency acceleration at left arrow in Fig. 9A, top; LAS-type cell), whereas with dorsal root stimulation, a subthreshold plateau and subsequent long-lasting reflex could be activated (e.g., left arrow in Fig. 9B, top). In the extreme case, there were cells that had high-threshold calcium PICs that could not be activated during firing evoked by intracellular current injection (described in the preceding text), but subthreshold calcium PICs and long-lasting firing could be evoked by dorsal root stimulation ($n = 3/3$).

Unusually long polysynaptic EPSPs in acute and chronic spinal rats

As mentioned in the preceding text, in chronic spinal rats, the low-threshold dorsal root stimulation evoked unusually long EPSPs that were clearly seen when the motoneurons were hyperpolarized to block the plateau and associated PICs (Figs. 11, A and B, 10, A and B, and 9B). These hyperpolarized EPSPs were on average $9.8 \pm 4.8$ mV in amplitude and lasted $960 \pm 270$ ms. Typically, the reflex response also had a monosynaptic component, and this was on average $14.4 \pm 8.3$ mV (Fig. 11B). The long EPSP followed in the tail of the monosynaptic EPSP and thus was of short latency. However, the long EPSP was definitely of polysynaptic origin because when the monosynaptic reflex was not present, then the long EPSP had a latency of 3.3 ms beyond the monosynaptic latency. Both the mono- and polysynaptic EPSPs were not blocked by nimodipine (Figs. 10 and 11) and thus did not depend on pre- or postsynaptic L-type calcium channels (calcium PICs).

Interestingly, in acute spinal rats, there were also very similar long polysynaptic EPSPs (Fig. 9C), and thus these must have emerged acutely with injury because indirect evidence from EMG recordings suggest that this EPSP is not present prior to injury in the awake rat (D. J. Bennett and C. L. Cooke, unpublished results). When these EPSPs were measured with the cell hyperpolarized (Fig. 9C, bottom), as in the preceding text, they were found to have a long duration (740 ± 190 ms) and short latency not significantly different from in chronic spinal rats. However, the amplitude of the long EPSP was significantly smaller (4.2 ± 1.6 mV) in acute compared with chronic spinal rats as was the monosynaptic reflex (3.2 ± 4.6 mV). Further, when the cell was held at rest, these long EPSPs never evoked firing (no reflex), although at times the monosynaptic EPSP evoked a spike (Fig. 9C). Further, there was never a plateau or long-lasting reflex triggered by these long EPSPs, and the EPSP was not amplified by depolarization (unlike in chronic, see following text), consistent with the lack of plateaus and the small PICs seen in acute spinal rats (Bennett et al. 2001c; Li and Bennett 2003).

DISCUSSION
Motoneuron firing activated by injected current

Our results indicate that motoneuron firing is dramatically altered by the presence of large intrinsic sodium and calcium PICs in chronic spinal rats, and thus these currents play a major role in the input-output properties of motoneurons. When activated with intracellular current injection, the sodium PIC causes the activation of a subthreshold plateau potential (TTX-sensitive, and nimodipine resistant) that helps recruit the motoneuron and contributes to sustained firing that outlasts the
current injection (self-sustained firing; Figs. 1, 2, and 7). The sodium PIC is also critical in maintaining regular repeated firing (Fig. 8) (Lee and Heckman 2001) and can, by itself, produce very slow self-sustained firing (Fig. 7).

The calcium PIC also helps recruit and sustain firing in cells where it is activated subthreshold to firing, and these effects are clearly demonstrated with a nimodipine block of the calcium PIC (Fig. 1: LSS-type cells). However, in other cells where the calcium PIC has a higher threshold above the initial firing level, it does not participate in recruitment or low-frequency primary range firing (Figs. 2 and 5B; LAS). Instead, the calcium PIC causes a late acceleration in firing during its activation (causing the steep secondary range firing). After the late acceleration in firing, the calcium PIC helps sustain the firing (in tertiary range). The activation of the calcium PICs dramatically increases the conductance of the cell, likely through the activation of calcium-activated conductances (potassium) (Krnjevic et al. 1975; Viana et al. 1993) as well as directly through its own L-type calcium channel conductance (Li and Bennett 2003). This makes it harder to depolarize the cell and to increase the firing rate with further injected current. This causes a lower $F/I$ slope, or tertiary range, in all cells (LSS and LAS type); LSS-type cells are especially interesting, because they fire only in the tertiary range and have no primary or secondary range. Thus while PICs may help recruit and sustain the firing of a motoneuron, after they are activated they make it harder for subsequent inputs to influence the cell, ultimately lowering the input-output gain ($F/I$ slope) (see also Bennett et al. 1998).

Large PICs also occur in spinal cord intact animals (Hounsgaard et al. 1984; Lee and Heckman 1998a; Schwindt and Crill 1982), and thus some of the present results likely extend to normal motoneurons in the intact state or motoneurons modulated by transmitters such as 5-HT (Hounsgaard and Kiehn 1989). We know that during intracellular current injection, steady-state primary range firing occurs below the activation level of the calcium PIC or plateau (Bennett et al. 1998; Schwindt and Crill 1982), and thus the calcium PIC is likely not involved in this firing. Further, the steady-state secondary range firing in intact animals coincides with the onset of the calcium PIC (Schwindt and Crill 1982), and changes in the activation threshold of the PIC induces associated changes in the secondary range firing (Bennett et al. 1998). Thus the onset of the calcium PIC likely causes the secondary range firing in the intact state, like we have directly demonstrated for the chronic spinal rat using nimodipine (Fig. 5, ramp-evoked secondary range). With full PIC activation, the firing moves into what we call the tertiary range, above the secondary range, and the $F/I$ slope is often lower than the slope in the primary range (Fig. 2 in Bennett et al. 1998 with spinal-cord-intact animals), again like in the chronic spinal rat (Fig. 5). In some motoneurons, especially high-threshold cells, the calcium PIC slowly inactivates, and this further slows the firing, contributing to rate adaptation (Bennett et al. 1998, 2001c; Lee and Heckman 1998b).

**Motoneurons firing activated by synaptic inputs**

The calcium PIC arises largely from the dendrites (Carlin et al. 2000; Hounsgaard and Kiehn 1993). Thus, synaptic inputs that arrive at the dendrites more easily activate the calcium PIC compared with intracellular current injection, which is usually in the soma (see RESULTS) (see also Bennett et al. 1998). This leads to a dramatically altered input-output relation during synaptic input because the synaptic current can more readily activate the nearby calcium PIC and plateau compared with the somatic sodium spike. Thus the calcium PIC and plateau are always activated subthreshold to firing, even in cells where the opposite occurs during intracellular current injection (which is somatic) with firing prior to PIC activation (LAS type cells) (Bennett et al. 1998). That is, during graded synaptic excitation when cells begin firing, the calcium PIC is already being activated.

If we consider the present results that demonstrate that primary range firing occurs before the calcium PIC activation and secondary range firing occurs during the calcium PIC activation (Fig. 5), we are led to the conclusion that primary range firing cannot occur during synaptic excitation because the calcium PIC is activated prior to recruitment (Fig. 9B) (see also Bennett et al. 1998). Indeed if we re-define the secondary range as firing during the onset of the calcium PIC, then it is clear that secondary range firing can only occur just after recruitment, if at all, because the calcium PIC is mostly activated prior to the first spike and only continues to be activated during the first few spikes (like Fig. 9B) (see also Fig. 8 in Bennett et al. 1998). After this, the calcium PIC is tonically activated, which corresponds to the tertiary range. Thus during synaptic excitation, the firing moves quickly into the tertiary range, where the calcium PIC is tonically activated and the frequency increases slowly with current (corresponding to low $F/I$ slope). Indeed, in many motoneurons, the calcium PIC is fully activated prior to recruitment, even with current injection, and firing only occurs in the tertiary range (LSS cells; Fig. 1).

Interestingly, in this tertiary range, the firing increases linearly with current (Fig. 1C) (Bennett et al. 2001c), and likely synaptic currents summate linearly (Prather et al. 2001), and so the original concept of linear summation proposed by (Granit et al. 1966) still holds but functionally occurs in the tertiary not primary range. These conclusions are consistent with the relatively linear rate modulation and lack of firing rate accelerations seen in motor unit recordings of awake animals and humans (Gorassini et al. 1999a, 2002).

**Very slow firing in motoneurons after chronic spinal cord injury**

One special characteristic of firing in chronic spinal rats is very slow firing, and this also occurs in other animal models of spinal cord injury (Powers and Rymer 1988) and humans with spinal cord injury (M. A. Gorassini, unpublished data, Zijdewind and Thomas 2001). Slow firing can occur in noninjured humans briefly just prior to de-recruitment, but the long periods of low-frequency, low-variability firing seen with injury are not common (Gorassini et al. 2002). The reason for the prominence of very slow firing with injury is unclear but may relate to the particular combination of large sodium PICs and large AHPs seen with injury (Bennett et al. 2001c; Li and Bennett 2003). We have shown in RESULTS that a subthreshold oscillation of the sodium PIC and AHP mediates very slow firing. Although the concept of a subthreshold PIC oscillation has been suggested by others (Carp et al. 1991; Hodgkin 1948; Kernell 1999), it has not been directly demonstrated before for...
motoneurons. Similar oscillations of persistent currents underlie other important physiological functions with pacemaker activity of the heart being the classic example (Irisawa et al. 1993; Zhang et al. 2002).

In motoneurons of chronic spinal rats, the large sodium PIC is activated a few millivolts subthreshold to the spike (Figs. 1 and 2) as seen in other neurons (Elson and Selverston 1997; Sandler et al. 1998; Schwindt and Crill 1995). The sodium PIC by itself produces a TTX-sensitive plateau potential (at dotted lines in Fig. 6 and 7; provided that the sodium PIC has a negative-slope region) (Li and Bennett 2003), and this sodium plateau is very different from the classic calcium-mediated plateau described in motoneurons (Hounsgaard et al. 1984) because it is rapidly deactivated by a hyperpolarization and thus deactivated by a single brief (100 ms) AHP that follows a spike. This is because the sodium PIC is deactivated very rapidly (in <10–50 ms) (Lee and Heckman 2001; Li and Bennett 2003) in relation to the much slower calcium PIC, which can take a second to turn on or off (Li and Bennett 2003).

The sodium PIC can also activate rapidly with moderate depolarizations, but when the potential is near threshold, the sodium PIC activates very slowly (Li and Bennett 2003). Thus near threshold for the sodium PIC, a brief disturbance (current pulse or EPSP) causes the sodium plateau to be slowly activated (ramp-up at arrow in Fig. 7B, top). This plateau activation accelerates as the potential is more depolarized, presumably due to fast sodium PIC dynamics for larger depolarizations, and this ultimately triggers a spike (ramp&acceleration in Figs. 6 and 7). The AHP that follows the spike brings the potential far below the sodium PIC threshold for ~100 ms (expanded trace in Fig. 7B), which is adequate time to fully deactivate the sodium PIC (Fig. 6C in Li and Bennett 2003), and thus the sodium plateau is likely fully deactivated. However, when the AHP finishes (in 100 ms), the sodium plateau/PIC is reactivated by the same process, and this triggers a second spike and so on. In this way, a subthreshold oscillation of the sodium PIC, with repeated sodium plateau activation and deactivation, causes very slow firing.

The very slow firing does not involve the calcium PIC and is exclusively produced by the sodium PIC because it is not affected by nimodipine (Fig. 7) and blocked by a low dose of TTX that reduces the sodium PIC without affecting the sodium spikes. The characteristic ramp&acceleration before the spike during slow firing (Figs. 7 and 8) is also mediated by the sodium PIC and not by the classic $I_A$ potassium current that can cause similar effects (Conner and Stevens 1971) because it is blocked by low-dose TTX (Fig. 8D) and is much slower than occurs with $I_A$. A depolarization after the AHP can be caused by other mechanisms than the sodium PIC, such as the hyperpolarization activated $I_h$ current (Russo and Hounsgaard 1999) and the classic late afterdepolarization after a single spike and AHP (Kernell 1965b). However, such late afterdepolarizations are very small in motoneurons (<1mV), only last ~100 ms after the AHP and are activated well below the sodium PIC threshold (Kernell 1965b; unpublished results) and thus cannot account for the much larger (many mV) and slower depolarizations between AHPs during slow firing.

Presumably, the sodium PIC is transiently activated prior to each spike even during moderately fast firing, but this is difficult to distinguish from the termination of the AHP from the prior spike. During very fast firing, the sodium PIC may not fully deactivate with each AHP and thus only provides a steady depolarization, similar to the calcium PIC. However, this issue and the relation of the sodium PIC to fast firing need further investigation.

Interspike intervals as long as 1 s can occur by the sodium-PIC-mediated slow firing (right of Fig. 7B), much longer than can be produced by normal motoneurons in acute spinal or anesthetized preparations (Bennett et al. 2001c; Hounsgaard et al. 1988b; Kernell 1965b). Theoretically, slow firing with interspike intervals somewhat longer than the 100-ms AHP duration can be obtained by synaptic noise superimposed on a subthreshold depolarization. However, this sort of firing is characterizedly variable (Matthews 1996) and decreases in variability as the cell is depolarized closer to threshold, where the AHP then determines firing rate. In contrast, the slow firing produced by the sodium PIC is extremely regular, and depolarizing synaptic excitation/noise only serves to increase its variability (Fig. 7; asterisks). Further, the slow firing does not require synaptic noise at all because it persists in drugs that produce a complete ionotropic synaptic blockade (P. J. Harvey and D. J. Bennett; unpublished results).

The sodium PIC is particularly prone to inactivation that occurs after a few seconds (Li and Bennett 2003), and thus a single sodium plateau also inactivates over a few seconds. However, when the sodium PIC is fully deactivated and then reactivated with each AHP, as occurs during slow firing, then the sodium plateau is essentially recharged after each AHP, and sodium inactivation does not play a major role in this slow firing. Thus slow self-sustained firing can continue for many minutes or hours, without stopping as we have seen during intracellular recordings (minutes) and in motor-unit recordings in the awake chronic spinal rats (hours) (Bennett et al. 2001a; unpublished findings). Long-lasting very slow firing is also seen in spontaneously active (involuntary) motor units in humans with spinal cord injury (Zijdewind and Thomas 2001; M. A. Gorassini et al., unpublished observations), and this is likely mediated by the same subthreshold sodium PIC oscillation, especially considering the lack of variability in this firing, which is a special characteristic of the slow sodium-PIC-mediated firing.

Long-lasting reflexes and spasms in chronic spinal rats

Both spinal cord intact and chronic spinal animals (but not acute spinal) have large PICs that produce self-sustained firing (as discussed in the preceding text), and evidence for this is even seen in intact and injured humans (Gorassini et al. 1998, 1999b, 2002; Kiehn and Eken 1997). However, uncontrolled muscle spasms only occur after chronic spinal cord injury. In the intact state, there must be numerous inhibitory control mechanisms that help to terminate PICs and avoid uncontrolled muscle contractions. These include control of volitional supraspinal inputs to motoneurons and segmental reflexes (Baldissera et al. 1981), control of brain stem monoaminergic systems that meduate motoneuron PICs (Hounsgaard et al. 1988a), and descending inhibition of segmental reflexes (Baker and Chandler 1987; Clarke et al. 2002; Jankowska 1992; Thompson et al. 1992). With chronic injury, this inhibitory control is lost: there is no volitional control, motoneurons exhibit plateaus/PICs spontaneously without the need for brain
stem monoamines (Bennett et al. 2001c), and segmental reflexes become disinhibited. Interestingly, the segmental reflexes become disinhibited immediately after injury, leading to unusually long low-threshold polysynaptic EPSPs (Fig. 9C) (see also Baker and Chandler 1987; D. J. Bennett and C. L. Cooke, unpublished data). However, these EPSPs do not trigger long-lasting reflexes until the motoneuron excitability is raised by the recovery of PICs with chronic injury (weeks after injury; Fig. 9B). Thus PICs must play a central role in the generation of long-lasting reflexes and associated spasms seen in the awake rat (Bennett et al. 1999), and this is discussed next.

Long-lasting spasms are caused by PICs on the motoneurons

Because PICs and plateaus are voltage dependent, their activation can be stopped by hyperpolarizing the motoneurons and the contribution of the PIC to long-lasting reflexes directly quantified. Interestingly, with hyperpolarization, the many second long portion of the reflex is completely blocked, leaving only a half-second-long EPSP (Figs. 9–11). This demonstrates that the long-lasting reflex results almost entirely from voltage-dependent PIC activation intrinsic to the motoneurons with only a half-second long contribution from synaptic input (EPSP). This leads to the surprising conclusion that spasms, which we know are closely associated with these long-lasting reflexes (Bennett et al. 1999), are caused by PICs intrinsic to the motoneurons and are not caused by many-second-long polysynaptic inputs to the motoneurons. The PICs and plateaus are triggered by polysynaptic EPSPs, which are exaggerated with injury (see following text), but the long-lasting discharge during spasms must result entirely from motoneuron PICs that far outlast these EPSPs.

In most motoneurons, nimodipine partly reduced, but did not eliminate, the long-lasting reflex response to a brief low-threshold dorsal root stimulation (slowed firing; Fig. 10), indicating that both the calcium and sodium PICs contributed to long-lasting reflexes and spasms. Nimodipine did not affect the EPSP that triggered the PICs/plateaus (seen with hyperpolarization) and thus acted primarily by blocking the postsynaptic calcium PIC. With nimodipine present, the sodium PIC alone caused a long-lasting reflex by producing slow self-sustained firing (see slow firing section and Fig. 10C). This slow reflex induced firing (2–3 Hz) likely produces slow unfused contractions in the awake rat (twitches are <100 ms for these tail muscles, unpublished data) and thus may contribute to clonus seen during muscle spasms (Bennett et al. 1999). With calcium PICs, together with the sodium PICs (prior to nimodipine), the firing rate accelerated during long-lasting reflexes, and thus the calcium PICs likely contribute to more powerful muscle contractions during spasms. In a few motoneurons, the sodium PIC was too weak to by itself produce a plateau, and nimodipine blocked the long-lasting reflex (Fig. 10). Thus in these motoneurons the calcium PIC alone produced the long-lasting reflex.

The hyperpolarization used to block the sodium and calcium PICs should theoretically also block any voltage-dependent component of the EPSP, such as that mediated by NMDA receptors. This component could contribute to the plateau and long-lasting reflex in addition to the sodium and calcium PICs. However, postsynaptic NMDA receptors are not likely involved in the many-second-long reflex response because in cells without large sodium PICs (just mentioned), nimodipine blocks the long-lasting reflex, and the EPSP that remains in nimodipine is not longer than the EPSP seen with hyperpolarization. Further, a block of the NMDA receptors with APV (Bennett et al. 2001b) or a complete block of all ionotropic transmission does not block the PICs or plateaus (Harvey and D. J. Bennett, unpublished results). Interestingly, APV does block the polysynaptic EPSP (Bennett et al. 2001b), and these EPSPs must therefore be mediated by NMDA receptors on interneurons.

EPSPs that trigger PICs and spasms

Any long depolarizing synaptic input, lasting about half a second, can activate the full PIC (Bennett et al. 2001c; Li and Bennett 2003), causing self-sustained firing and ultimately the long-lasting reflex associated with muscle spasms. Shorter depolarizations, such as from the monosynaptic EPSP, cannot evoke a plateau or long-lasting reflexes (see RESULTS). However, repeated monosynaptic stimulation or tendon vibration is effective in evoking PICs/plateaus (see RESULTS) (Lee and Heckman 1998b) and thus should trigger spasms. The reduction in the amount of presynaptic inhibition/depression of mono- and polysynaptic reflexes seen with spinal cord injury (Ashby et al. 1987) is thus functionally very important. In spinal cord injured humans (Dimitrijevic and Nathan 1968; Hornby et al. 2003; Kuhn and Macht 1948) and animals (Bennett et al. 2001a), cutaneous inputs are especially effective in evoking muscle spasms, and this is consistent with the emergence of unusually long EPSPs evoked by single low- or high-threshold cutaneous stimulation after spinal cord injury (Baker and Chandler 1987; Clarke et al. 2002). The long EPSPs in our sacral spinal cord preparation (Figs. 9–11) are also likely mediated by cutaneous afferents because they are best evoked by the most caudal dorsal roots that innervate the skin at the tip of the tail (Bennett et al. 1999).

In summary, muscle spasms after spinal cord injury are produced primarily by sodium and calcium PICs on the motoneurons triggered by any moderately long synaptic excitation. However, the increased afferent transmission and prolonged EPSPs, especially through cutaneous pathways, allow PICs and thus spasms to be triggered by brief nonnoxious afferent stimulation as is commonly seen in injured humans (Delwaide 1987; Kuhn and Macht 1948).

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