Plateau Potentials in Sacrocaudal Motoneurons of Chronic Spinal Rats, Recorded In Vitro

DAVID J. BENNETT, YUNRU LI, AND MEREK SIU
Division of Neuroscience, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Received 25 September 2000; accepted in final form 3 May 2001

Bennett, David J., Yunru Li, and Merek Siu. Plateau potentials in sacrocaudal motoneurons of chronic spinal rats, recorded in vitro. *J Neurophysiol* 86: 1955–1971, 2001. Intracellular recordings were made from sacrocaudal tail motoneurons of acute and chronic spinal rats to examine whether plateau potentials contribute to spasticity associated with chronic injury. The spinal cord was transected at the S_2 level, causing, over time, exaggerated long-lasting reflexes (hyperreflexia) associated with a general spasticity syndrome in the tail muscles of chronic spinal rats (1–5 mo postinjury). The whole sacrocaudal spinal cord of chronic or acute spinal rats was removed and maintained in vitro in normal artificial cerebral spinal fluid (ACSF). Hyperreflexia in chronic spinal rats was verified by recording the long-lasting ventral root responses to dorsal root stimulation in vitro. The intrinsic properties of sacrocaudal motoneurons were studied using intracellular injections of slow triangular current ramps or graded current pulses. In *chronic spinal* rats, the current injection triggered sustained firing and an associated sustained depolarization (*plateau potential;* 34/35 cells; mean, 5.5 mV; duration >5 s; normal ACSF). The threshold for plateau initiation was low and usually corresponded to an acceleration in the membrane potential just before recruitment. After recruitment and plateau activation, the firing rate changed linearly with current during the slow ramps [63% of cells had a linear frequency-current (*F-I*) relation] despite the presence of the plateau. The *persistent inward current* (I_{PIC}) producing the plateau and sustained firing was estimated to be on average 0.8 nA as determined by the reduction in injected current needed to stop the sustained firing [$\Delta I = -0.8 \pm 0.6$ (SD) nA], compared with the current needed to start firing ($I = 1.7 \pm 1.5$ nA; 47% reduction). In motoneurons of *acute spinal rats*, plateaus were rarely seen (3/22), although they could be made to occur with bath application of serotonin. In motoneurons of chronic spinal rats there were *no* significant changes in the mean passive input resistance, rheobase or amplitude of the spike afterhyperpolarization (AHP) as compared with acute spinal rats. However, there were significant increases in AHP duration and initial firing rate at recruitment and decreases in minimum firing rate and *F-I* slope. We suggest that the higher initial firing rate resulted from the plateau activation at recruitment and the lower *F-I* slope resulted from an increase in active conductance during firing, due to I_{PIC}. Brief dorsal root stimulation also triggered a plateau and sustained discharge (long-lasting reflexes; 2–5 s) in motoneurons of chronic (but not acute) spinal rats. When the plateau was eliminated by a hyperpolarizing current bias, the reflex response was significantly shortened (to 1 s). Thus plateaus contributed substantially to the long-lasting reflexes in vitro and therefore should contribute significantly to the corresponding exaggerated reflexes and spasticity in awake chronic spinal rats.

INTRODUCTION

Following spinal cord injury, exaggerated reflexes and muscle tone often emerge that contribute to a general spasticity syndrome in humans (Ashby and McCrea 1987; Kuhn and Macht 1948; Noth 1991; Young 1994) and animals (Ashby and McCrea 1987; Bennett et al. 1999a; Heckman 1994; Taylor et al. 1997). A central complaint of patients with spasticity involves intense muscle contractions, lasting for many seconds, that are triggered by numerous stimuli. These are related to various *long-lasting reflexes* that have been described experimentally in humans and animals with injury, including: spastic stretch reflexes (Burke et al. 1970; Powers et al. 1989; Thilmann et al. 1991), cutaneous/flexor-afferent reflexes (Bennett et al. 1999a; Kuhn and Macht 1948; Remy-Neris et al. 1999), general oligosynaptic reflexes (Hultborn and Malmsten 1983a,b; Mailis and Ashby 1990), and radiating muscle spasms (Kuhn and Macht 1948). Such long-lasting reflexes may have numerous causes including loss of inhibition from descending and segmental pathways (Cavallari and Pettersson 1989; Heckman 1994; Mailis and Ashby 1990; Thompson et al. 1998), neuronal sprouting (Krenz and Weaver 1998), and direct changes in intrinsic properties of spinal neurons (Eken et al. 1989).

The possibility that the intrinsic properties of spinal motoneurons change with spinal cord injury is consistent with data from motor unit firing in humans and animals after injury, including sustained poorly modulated discharges (Gorassini et al. 1999a; Thomas and Ross 1997), unusually low minimum firing rates (Carp et al. 1991; Powers and Rymer 1988; Thomas and Ross 1997), and generally inefficient control of firing rate in force production (Blaschak et al. 1988; Wiegner et al. 1993). The objective of the present paper was thus to investigate whether long-lasting reflexes that emerge after injury result, in part, from changes in intrinsic excitability of motoneurons, such that relatively uncontrolled firing can be triggered by a brief stimulation (e.g., due to plateau potentials) (Russo and Hounsgaard 1999).

Although motoneurons usually fire in proportion to the net excitatory input, their response can also be altered substantially

Address for reprint requests: D. J. Bennett, 513 HMRC, Div. of Neuroscience, University of Alberta, Edmonton, Alberta T6G 2S2, Canada (E-mail: david.bennett@ualberta.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

by numerous voltage-dependent currents intrinsic to the motoneuron membrane (Binder et al. 1996; Rekling et al. 2000; Russo and Hounsgaard 1999). For example, voltage-dependent persistent inward currents (I_{PIC}) can at times be activated by a brief stimulus and regeneratively cause sustained depolarizations (i.e., *plateau potentials;* abbreviated plateaus) and firing (Bennett et al. 1998a,b; Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988; Lee and Heckman 1998a,b; Schwindt and Crill 1984). Persistent inward currents are likely present in most motoneurons but are only manifested as plateaus when they are either directly facilitated or uncovered by reducing opposing outward currents (e.g., Ca^{2+}-dependent K^+ current), both of which may occur with extrinsically administered serotonin (5-HT) (Hultborn and Kiehn 1992; Russo and Hounsgaard 1999). The I_{PIC} responsible for plateaus is often mediated by L-type calcium channels (Hounsgaard and Kiehn 1989), although any relatively long-lasting voltage-gated inward current could also be involved, including persistent sodium currents (Schwindt and Crill 1995) and ligand-gated currents from *N*-methyl-D-aspartate (NMDA) receptors (Guertin and Hounsgaard 1998; Hochman et al. 1994; Kiehn et al. 1996).

The existence of plateaus in motoneurons appears to normally require monoaminergic facilitation from the brain stem [5-HT, norepinephrine (NE)]. That is, plateaus occur in brainstem-intact decerebrate cats, are largely eliminated by acute spinalization, and return with application of monoaminergic drugs (Conway et al. 1988; Hounsgaard et al. 1988). As there are few sources of 5-HT or NE within the spinal cord below a chronic spinal transection (except perhaps related to the autonomic system) (McNicholas et al. 1980; see also Newton and Hamill 1988), it is unlikely that plateaus mediated by monoamines could re-emerge with long-term complete spinal cord transection. However, because the inward currents (I_{PIC}) are ubiquitous, as discussed in the preceding text, and I_{PIC} and plateaus are facilitated by various other neuromodulators (acetylcholine, substance P and glutamate; via metabotropic receptors) (Russo and Hounsgaard 1999; Russo et al. 1997), plateaus may still play a role in chronic injury. Indeed, the similarity of the long-lasting reflexes in the chronic spinal cat to the tonic stretch reflex in the decerebrate cat led Hultborn's group to propose that both are mediated by plateaus on motoneurons (Eken et al. 1989; Nielsen and Hultborn 1993). Their preliminary evidence, from motoneurons recorded in two chronic spinal cats, supports this hypothesis.

In the present study, we have made intracellular recordings from motoneurons of chronic spinal rats to test for the presence of plateaus. The spinal cord transection was made at the sacral level, which, within a month, leads to pronounced spasticity in the tail muscles while sparing bladder and locomotor function (Bennett et al. 1999a). In these chronic spinal rats, there was often sustained tail motor unit firing, suggestive of the involvement of plateaus (Bennett et al. 2001). This preparation has the unique advantage that the affected adult sacrocaudal spinal cord is small enough to survive when acutely explanted and studied in vitro (whole adult cord) (Bennett et al. 1999b; Long et al. 1988; Bennett and Li, in preparation). Ventral root recordings were used to verify that the spastic reflex behavior persisted in vitro (Bennett et al. 1999b), and intracellular recordings were made from identified motoneurons. As anticipated, we found that with chronic spinal cord injury, the motoneurons recovered their ability to exhibit plateau behavior. These plateaus could be triggered by dorsal root stimulation, and they markedly prolonged reflex responses, thus playing a major role in the long-lasting spastic reflexes seen after chronic spinal cord injury.

METHODS

Intracellular recordings were made from sacrocaudal motoneurons of adult female Sprague-Dawley rats (age: 1.5–7 mo; 200–800 g) following sacral spinal cord injury. The spinal cord was transected at the S_2 sacral spinal level either acutely (*acute spinal* condition; $n = 29$ cells, $n = 14$ rats) or ≥1 mo prior to the experiment (*chronic spinal*, 1–5 mo post lesion, $n = 35$ cells, $n = 14$ rats), as described in Bennett et al. (1999a). In the latter group, only rats with clear spasticity symptoms were used (rating 3–5 in Table 1 of Bennett et al. 1999a). Recordings were made while the whole sacrocaudal spinal cord was maintained in vitro (Bennett et al. 1999b; Bennett and Li, in preparation). All procedures were approved by a local animal-welfare committee.

Surgery

The in vitro whole sacrocaudal adult rat preparation has been described previously (Bennett et al. 1999b; Long et al. 1988; Bennett and Li, in preparation) and is only briefly summarized here. Normal and chronic spinal rats were deeply anesthetized with urethan (0.18 mg/100 g; maximum of 0.45 mg per rat for rats 250 g), and the spinal cord caudal to the T_{12} vertebrae was transferred to a dissection dish containing oxygenated modified artificial cerebral spinal fluid (mACSF) at room temperature (20–21°C). In the dissection dish, a transection was made at the upper S_2 level with fine iridectomy scissors, just rostral to the original transection site in the chronic spinal rats. In normal rats, this S_2 transection was also made and served to provide an acute spinal lesion. Following a 1-h resting period in mACSF, the cord was transferred to a recording chamber, where it was submerged in normal ACSF flowing at 6 ml/min and maintained at 25°C. The cord was supported on a nappy paper mesh and secured by passing insect pins through lateral vasculature and connective tissue and into a silicone elastomer (Sylgard) base below the nappy paper.

Solutions

The normal ACSF had the following composition (in mM): 122 NaCl, 24 $NaHCO_3$, 3 KCl, 2.5 $CaCl_2$, 1 $MgSO_4$, and 12 glucose in distilled water, bubbled with 95% O_2-5% CO_2 and pH 7.4. mACSF was used during dissection and recovery to prevent excitotoxic injury. Initially mACSF consisted of ACSF with NaCl replaced by sucrose at equal osmolarity [0 NaCl, 213.6 mM sucrose (295 mOsm)] (Aghajanian and Rasmussen 1989). Later we noticed that sucrose tended to toughen the pia, making subsequent intracellular penetrations more difficult (e.g., dimpling occurred). We thus changed to a mACSF based on kynurenic acid, a broad spectrum antagonist of glutamate transmission. This mACSF composition was (in mM) 118 NaCl, 24 $NaHCO_3$, 3 KCl, 1.5 $CaCl_2$, 1.3 $MgSO_4$, 25 glucose, 1.4 NaH_2PO_4, 5 $MgCl_2$, and 1 kynurenic acid (McQuiston and Madison 1999). Regardless of the mACSF used, recordings were made in the same normal ACSF, and qualitatively similar results were obtained, although in general we noticed that the cords prepared with kynurenic-based mACSF were healthier, with larger reflexes and more pronounced plateaus. 5-HT (10–100 μM) was at times added to the ACSF in some acute spinal preparations after the main recordings in normal ACSF.

Intracellular and root recording

The long ventral and dorsal roots (at least sacral S_3, S_4, and caudal Ca_1) were mounted on silver-chloride wires supported above the recording chamber and covered in grease. Brief dorsal root stimulations were used to evoke long-lasting reflexes in spastic rats, and also to confirm the viability of the preparation (Bennett et al. 1999b). Reflex responses were recorded in the ventral roots and intracellularly on the motoneurons. Ventral roots were also stimulated to antidromically activate motoneurons for identification.

All segments of the in vitro sacrocaudal spinal cord could produce ventral root reflexes and thus had viable motoneurons (see also: Bennett and Li, in preparation). However, we focused on motoneurons in the caudal Ca_1 and sacral S_4 regions because this is the smallest, and presumably best oxygenated, portion of the cord in vitro, ventral root reflexes were largest and remained viable for the longest periods ($>$5 h and $\leq$18 h), and motoneurons in this region innervate only the tail muscles as opposed to bladder and pelvic regions served by higher sacral segments (Bennett et al. 1999a; Steers 1994).

Sharp intracellular electrodes were made from thick-walled glass capillary tubing (Warner, GC150F-10, 1.5 mm OD) with a Narishige puller (PE-2), filled with 2 M potassium acetate and bevelled with a rotary grinder (Sutter, BV-10) to give a final resistance of 50–100 MΩ. An Axoclamp2b intracellular amplifier (Axon Instruments) was used, either in bridge or discontinuous current-clamp modes (DCC; 4–5 kHz switching; all figures are from DCC recordings), with capacitance maximally compensated. The electrode was advanced with a stepper-motor micromanipulator (660, Kopf) while observing the electrode resistance changes and antidromic ventral root field potentials. Final cell penetration was achieved either by passing high-frequency current (buzz) or making a fast step with a piezoelectric element (WPI) mounted on the tip of the Kopf manipulator. On penetration, antidromic spike properties were measured. Only cells with $>$55 mV resting potential and $>$60 mV spike amplitude were accepted for analysis. For approximate classification, the injected current required to initiate firing during a slow current ramp (0.5 nA/s; see following text; recruitment current) was computed. All cells with a recruitment current less than the mean (1.75 nA, mean of all acute and chronic cells) were considered low recruitment threshold cells and the remainder high threshold. The intracellular current and membrane potential was low-pass filtered at 6 kHz and sampled at 16 kHz with an Axoscope system (Axon Instruments).

Usually the cord was placed horizontally in the recording chamber with the ventral side upward, and the intracellular electrode was advanced vertically, *perpendicular* to the cord, directly into the motor nucleus. The disadvantage of this approach was that dimpling of the pia during penetration could damage underlying neurons.

An alternate *longitudinal* approach through a transverse cut was used in some of the animals as follows. After removal of the cord from the animal, it was transferred to a vibratome in mACSF and a *transverse* cut was made at the S_4 level. Then following the usual incubation in mACSF, the remaining S_4 and caudal cord was transferred to the recording chamber and supported on a 30° ramp with the cut face pointing up the ramp. The cord was not submerged as usual but covered with nappy paper superfused with ACSF through a wick near the cut face of the cord (Long et al. 1988). The intracellular electrode was advanced into the cut face, longitudinally to the cord, directly into the motor nucleus near the visible interface of the white and gray matter (thus avoiding the pia). The main disadvantage of this approach is that the transverse cut is an additional injury and the reflexes recorded in the remaining S_4 and caudal Ca_1 roots were diminished. However, we did not notice differences in the intracellular plateau properties of motoneurons recorded with the perpendicular and longitudinal approaches, and cells from both methods have been included in the analysis.

Analysis of plateaus

Intracellular current pulses and slow triangular current ramps (0.5 nA/s standard, 0.4–3 nA/s range) were used to evoke voltage-dependent plateaus as described previously (Bennett et al. 1998a; Hounsgaard et al. 1988). During the current ramps, the I_{PIC} producing the plateau, and sustaining the firing, was estimated from the difference in injected current at recruitment, compared with de-recruitment (ΔI; see RESULTS). For computing the average ΔI for each cell, we only used responses from small, slow current ramps (0.5 nA/s as in Fig. 5*A*, unless there was a late, high-threshold plateau), optimized to avoid firing rate adaptation, as described in RESULTS. Also, to avoid any interactions between successive ramps (e.g., warmup) (Bennett et al. 1998b), we separated ramps by $\geq$10 s and removed any depolarizing current between ramps.

The size of the plateau was estimated from the afterpotential seen during current ramps (ΔV in Fig. 5*A*, see RESULTS). This was quantified by subtracting the potential at the end of firing (just before the last spike) from the potential at a *matched current* during the ascending ramp before the plateau and firing started. Because the potential can rise no higher than the firing level (ignoring the spike), this afterpotential measure, ΔV, underestimates the plateaus size that might be seen if spiking were not present. In cells that stopped firing early, and at higher currents than at recruitment (i.e., without plateau; acute spinal), the afterpotential was still computed but by comparing the potential just before recruitment to that at a matched current level after de-recruitment (see RESULTS). Some cells were recorded in bridge mode (not shown) instead of the usual DCC mode, and so the potential changes were corrupted by electrode rectification. However, by comparing the potential at matched currents on the ascending and descending current ramps, this electrode rectification problem was usually avoided (with a few exceptions, where rectification was sufficiently bad that we did not compute the afterpotential, thus missing the points in Fig. 4*C*).

To directly test the role of voltage-dependent plateaus on spastic reflexes, we also studied the amplitude and duration of excitatory postsynaptic potentials (EPSPs) evoked by dorsal root stimulation while changing the background depolarization of the cell with intracellular current bias (Bennett et al. 1998a).

The intracellular records were analyzed with Axoscope (Axon Instruments), Sigmaplot (Jandel Scientific) and a custom Linux-based program (G. R. Detillieux, Winnipeg). In the text and figures means $\pm$ SDs are shown. Statistical differences were assessed with a Student's *t*-test at the 95% confidence level ($P < 0.05$).

Histology

The spinal cords of additional rats (6) were sectioned and stained to examine the size and anatomy of the sacrocaudal cord. The animals were anesthetized and perfused with 4% paraformaldehyde as in Bennett et al. (1999a). The spinal cord was removed, serially dehydrated in ethanol, imbedded in paraffin for 7 days, and then sectioned at 10 μm on a microtome. Tissue was stained with silver nitrate (*Bielschowsky method*) and cresyl violet (Kiernan 1990).

RESULTS

Anatomy of the sacrocaudal ventral horn and motoneuron properties recorded in vitro

The small diameter of the sacrocaudal spinal cord (Fig. 1, *A* and *C*) was a major factor that enabled it to survive whole (unsliced) in vitro when it was acutely isolated from normal or chronic spinal adult rats because oxygen and nutrients only diffuse ~300 μm into tissue (Nicholson and Hounsgaard 1983). When intracellular recordings were made by passing the microelectrode into the cord directly through the ventral sur-

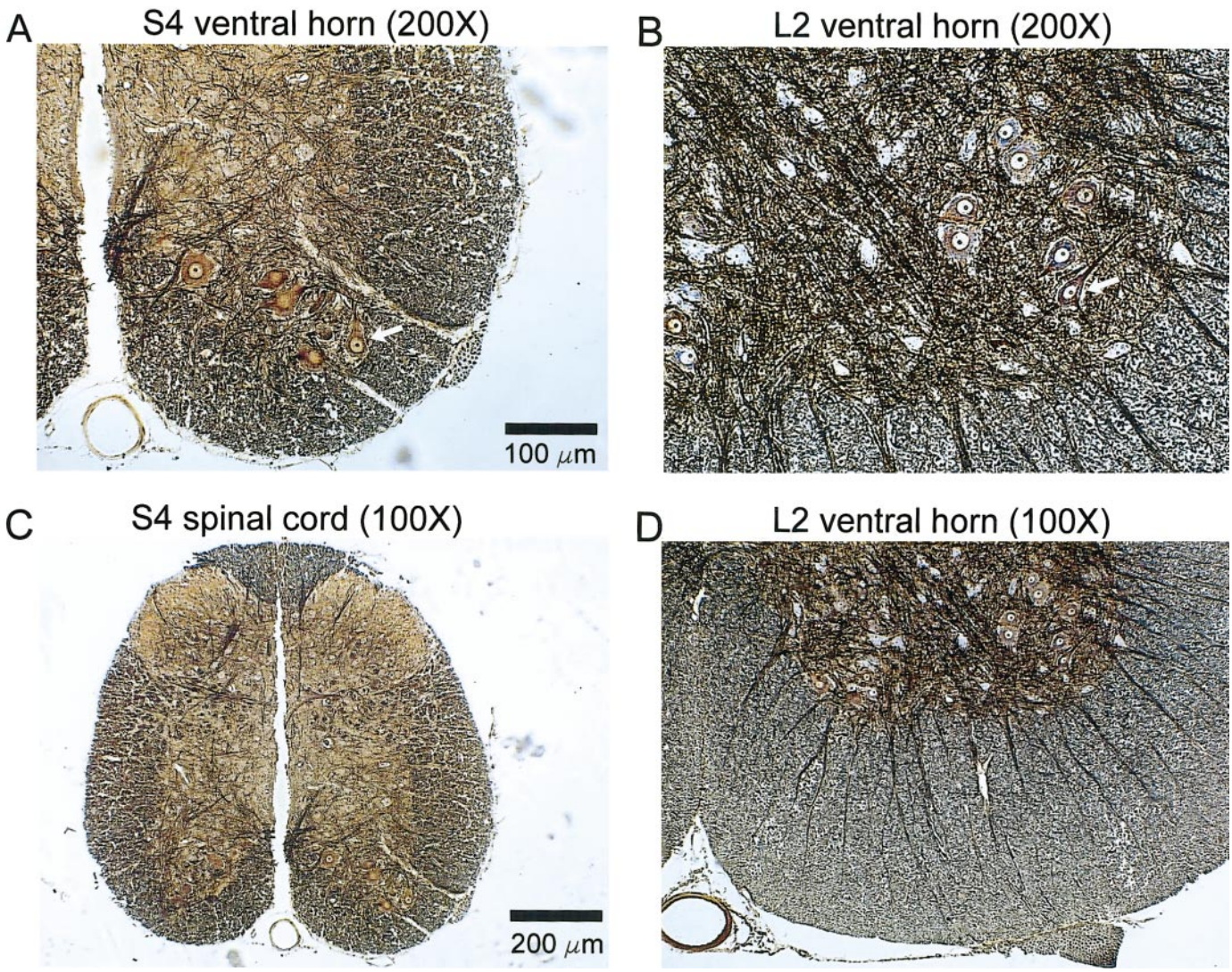


FIG. 1. Anatomy of sacrocaudal spinal cord. *A* and *C*: transverse section through the S_4 sacral spinal cord at 2 magnifications. Note the motoneurons within 100 μm of the surface (arrows). Caudal spinal cord sections are even smaller (200 μm radius at Ca_1; not shown). *B* and *D*: lumbar spinal cord sections are much larger although the lumbar and sacral motoneurons are similar in size (arrows). For orientation, note the midline ventral artery at bottom left corner in *A* and *D*. Cut axons in the white matter are seen as black dots/flecks. The S_4 section tore at midline during processing.

face (perpendicular approach; see METHODS), motoneurons were encountered at between 50 and 150 μm from the surface (>50 μm at Ca_1; >100 μm at S_4). Despite the small size of the sacrocaudal cord, the sacrocaudal motoneuron cell bodies were not significantly smaller than motoneurons in the lumbar enlargement (Fig. 1, *B* and *D*; average sacrocaudal motoneuron diameter: 35 ± 6 μm, n = 6 rats) and had similar basic electrical properties (Table 1).

In acute and chronic spinal rats, we recorded from motoneurons with a moderately wide range of input resistance (R_{in}; 9–25 MΩ), presumably relating to cell size (Binder et al. 1996). We found that R_{in} covaried with other cell properties, including afterhyperpolarization (AHP) duration, recruitment threshold, and rheobase (as with other motoneurons) (Binder et al. 1996). Despite the marked differences in the ability of cells to maintain plateaus in acute and chronic spinal rats (see following text), only slight (non-significant) differences were seen between these two populations in R_{in}, AHP amplitude, and rheobase (Table 1), consistent

TABLE 1. *Summary of passive input resistance (R_{in}), rheobase, and AHP in sacrocaudal motoneurons*

	n	R_{in}, MΩ	Rheobase, nA	AHP Amplitude, mV	AHP Duration, ms
Acute spinal	22	13.5 ± 7.8	2.4 ± 2.2	5.1 ± 1.8	73.5 ± 25.1
Chronic spinal	35	14.6 ± 10.5	1.7 ± 1.5	5.8 ± 3.4	102.1 ± 26.1*

Values are means ± SD. R_{in} measured with a hyperpolarizing current pulse or ramp (0.3 nA), starting from resting potential. After hyperpolarization (AHP) measured from an antidromically evoked spike. The AHP duration was significantly longer in chronic, compared to acute, spinal rats. R_{in}, rheobase, and AHP amplitude were not significantly different. *, significant difference in acute and chronic spinal rats.

with previous studies in spinal animals (Baker and Chandler 1987a; Cope et al. 1986; Gustafsson et al. 1982; Hochman and McCrea 1994). There was a significant increase in AHP duration in chronic spinal rat motoneurons (Table 1).

Motoneurons in acute spinal rats lack plateaus

We studied the firing behavior during intracellular current injection in 22 motoneurons from the acutely isolated sacrocaudal cord of normal rats (acute spinal condition) in normal ACSF. In most of these cells, there was no evidence of plateau activation (19/22). A brief current pulse did not trigger a sustained depolarization (Fig. 2*E*). Usually cells responded proportionally during slow triangular ramp current injections with the membrane potential and firing rate increasing linearly during the upward portion of the ramp and decreasing symmetrically during the downward portion of the ramp (see triangular reference lines drawn below potential in Fig. 2*A* and the overlapping frequency-current plots during the upward and downward ramps in Fig. 2*C*). Firing usually stopped at about the same current as where it started (Fig. 2, *A* and *C*). Overall, 59% of cells responded linearly like in Fig. 2*A*, and we have classified these cells as *type 1* cells (i.e., linear firing and no plateau).

In another 36% of acute spinal rat motoneurons, firing slowed substantially on the downward portion of the slow current ramps (0.5 nA/s standard speed; Fig. 3*A*), and the frequency-current plots showed a clockwise shape and an early de-recruitment, characteristic of cells with late firing rate adaptation (Fig. 3*C*) (Kernell and Monster 1982). We refer to these cells as *type 2* cells (rate adapting). Larger (or faster) ramps increased the incidence of rate adaptation, likely due to the higher firing rates achieved (not shown) (Kernell and Monster 1982).

Motoneurons of spastic chronic spinal rats have plateaus

Following the S_2 sacral spinal cord transection, exaggerated long-lasting reflexes associated with a general spasticity syndrome developed in the tail musculature within a month, as in Bennett et al. (1999a). Motoneurons ($n = 35$) were recorded from the isolated sacrocaudal cord of these spastic rats ≥1 mo after injury (chronic spinal; recordings in normal ACSF). Prior to each experiment, the hyperreflexia was verified in vitro by recording the associated long-lasting ventral root reflexes (Bennett et al. 1999b; Bennett and Li, in preparation).

When a brief intracellular current pulse was injected into motoneurons of chronic spinal rats, a sustained depolarization (afterpotential) and afterdischarge was produced (Fig. 2*F;* i.e., *plateau potential*), lasting many seconds, and not seen in acute spinal rats (Fig. 2*E*). Symmetrical triangular current ramps also triggered a sustained afterdischarge and afterpotential (ΔV) due to the plateau activation and a very asymmetrical response in relation to the current (Figs. 2*B* and 3*B;* 34/35 cells). That is, following recruitment of firing at a particular current on an upward current ramp (e.g., left vertical dashed line in Fig. 2*B*, 0.4 nA), de-recruitment only occurred when the current was reduced to a substantially lower level (right vertical dashed line; $\Delta I = -1$ nA). Thus firing stopped much later than expected (referred to as *self-sustained firing*; also see Fig. 3*B*) (Bennett et al. 1998a). The interpretation of this self-sustained firing is that the inward current I_{PIC}, associated with the plateau, was activated during the ascending current ramp, and then I_{PIC} effectively provided a depolarizing bias current, allowing the injected current to be substantially reduced before firing stopped (approximately: $\Delta I = -I_{PIC}$; see following text). The reduction in current at de-recruitment compared with recruitment, ΔI, thus provides a measure of the inward current I_{PIC} that helped to sustain the firing.

Note that the term "plateau" can be somewhat misleading during current ramps because the depolarizing inward current I_{PIC} combines with the injected current to produce the final response, and the potential is *not* locked at a fixed level (see details in Bennett et al. 1998a).

Quantification of plateau and comparison in acute and chronic spinal rats

As mentioned in the preceding text, in acute spinal rats there was little tendency for plateaus, and de-recruitment occurred at or above the current for recruitment, as summarized in Fig. 4*A* for all motoneurons ($I \geq 0$; ΔI not significantly different from 0, Fig. 4*B*). In three acute spinal rat motoneurons, there was a drop in injected current at de-recruitment ($\Delta I < 0$), indicating self-sustained firing and plateaus (3/22 cells). In contrast, in most chronic spinal motoneurons (34/35) the current dropped substantially at de-recruitment compared with recruitment (i.e., ΔI was significantly less than 0; $-I_{PIC} = \Delta I = -0.8 \pm 0.6$ nA). The average estimated I_{PIC} is summarized for acute and chronic spinal rats in Fig. 4*B*, which indicates a very significant (1.0 nA) increase in I_{PIC} with chronic injury. Note that in chronic spinal rats both neurons with a low (<1.75 nA) and high (>1.75 nA) recruitment threshold had plateaus, as indicated by ΔI. Further, there was no significant difference between the plateaus (ΔI) in low- and high-threshold neurons ($\Delta I = -0.7 \pm 0.5$ nA compared with -1.1 ± 0.9 nA), and there was considerable scatter in ΔI (regression value $r = 0.2$ in Fig. 4*A*).

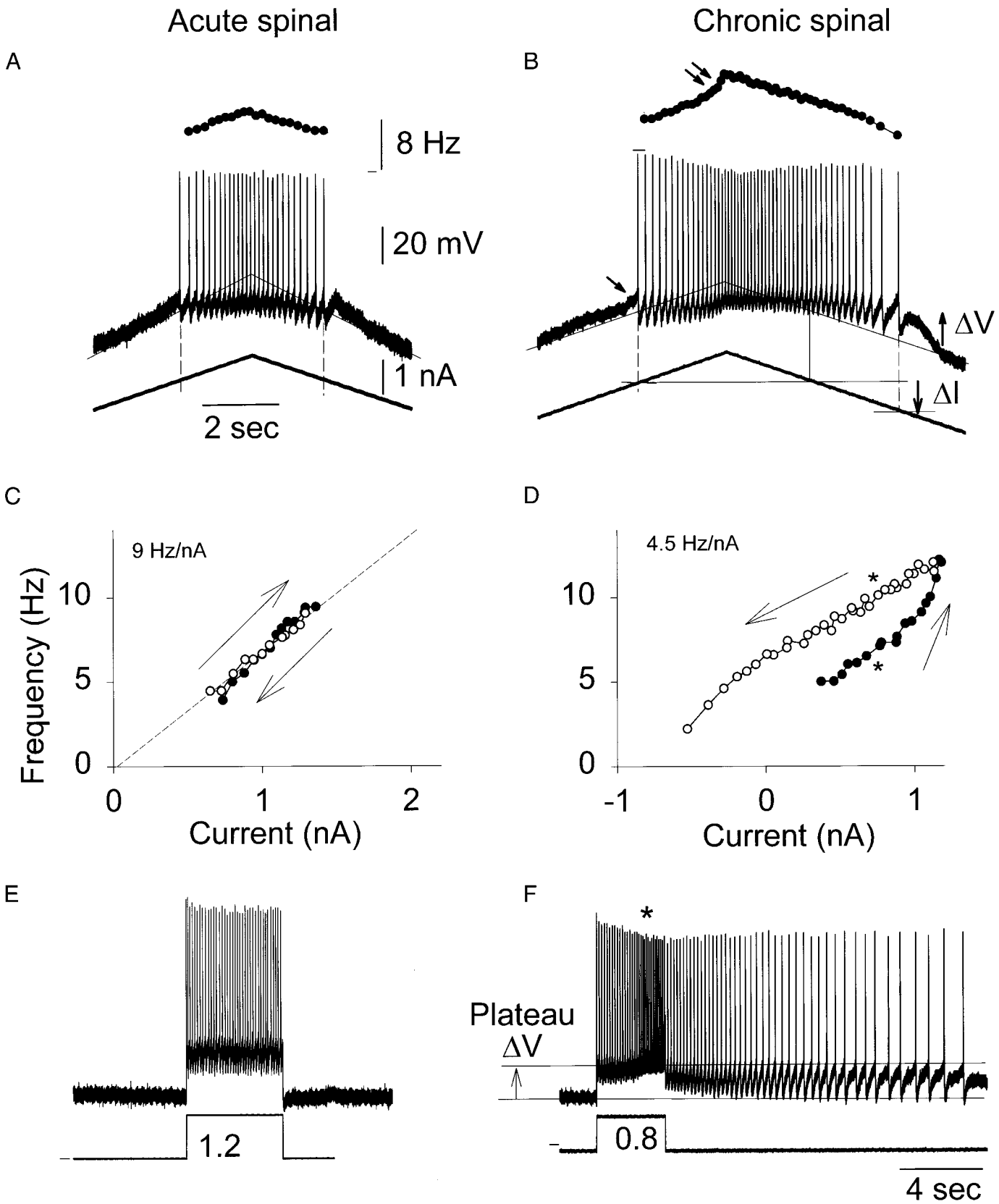
The depolarization produced by the plateau was most easily seen at the end of firing, and we have referred to this as the afterpotential (ΔV in Figs. 2*B* and 3*B;* see METHODS). In chronic spinal rats, the afterpotential, and thus plateau estimate, was 5–10 mV (5.1 ± 6.0 mV) and was significantly greater than in acute spinal rats (Fig. 4*D*). In acute spinal rats the afterpotential was not significantly different from zero.

In 12 motoneurons recorded in acute spinal rats, the ability of 5-HT to facilitate plateaus was studied (as in Hounsgaard and Kiehn 1989) (5 cells studied before and after 5-HT and 7 with 5-HT only). We used a concentration between 10 and 100 μM, which produced sustained activation of the ventral roots in response to dorsal root stimulation (not shown). The 5-HT usually depolarized cells, reduced the AHP and lowered their firing threshold current (by 2.2 nA, on average). Subsequent ramp current injections showed plateaus, although the estimated plateau current ($I_{PIC} = -\Delta I$; Fig. 4*B*) and afterpotential (ΔV; Fig. 4*D*) were not as large as in chronic spinal rats.

Characteristics of plateaus in chronic spinal rats

LOW-THRESHOLD PLATEAUS, INITIATED BEFORE RECRUITMENT (TYPE 3 CELLS). In the majority of chronic spinal rat motoneurons (63%, classified as *type 3* firing behavior), the plateau activation started before or simultaneous to recruitment during the current ramp, and firing rate acceleration was either not

seen (Fig. 3*D*) or only seen in the first few spikes (Figs. 5, *A* and *B*, left arrows; and Fig. 6*D*). In these cells, during the slow current ramp, the membrane potential increased linearly until it was within ~5 mV of the firing threshold, after which there was a gradual acceleration in the depolarization (lasting ~0.5–1.0 s; double arrow in Fig. 5*A*). This acceleration marked the onset of the plateau because the current could be reduced at any time afterward and there was still a sustained depolarization



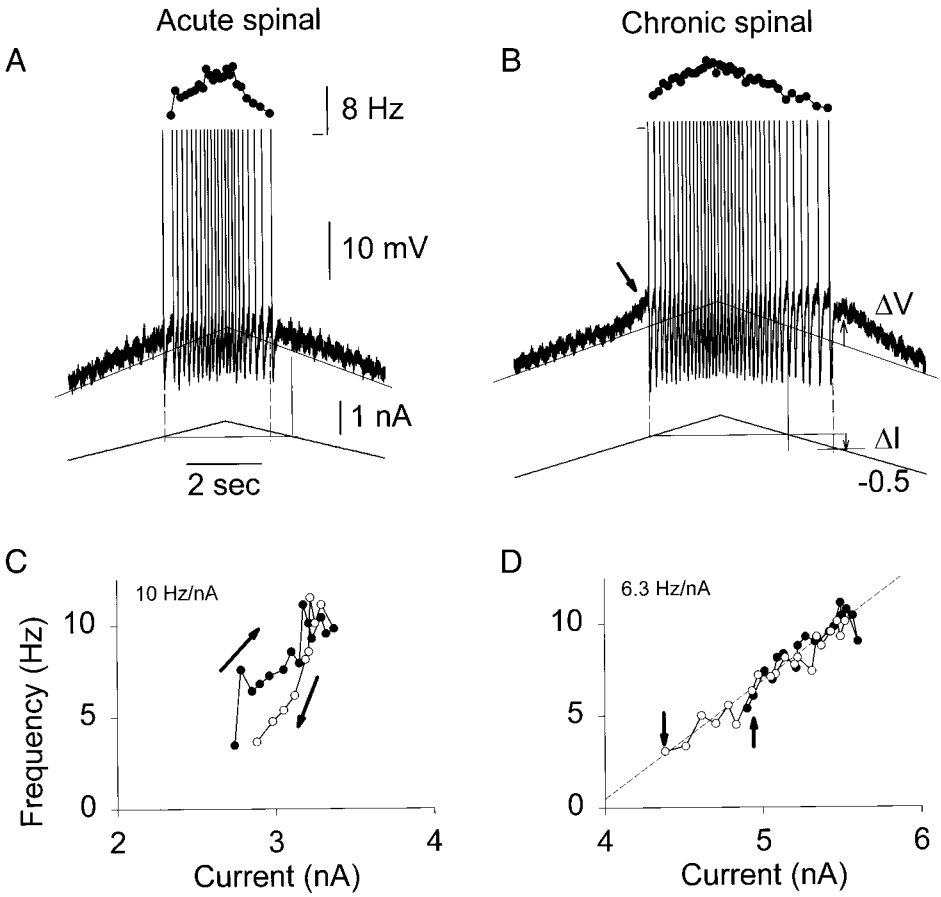


FIG. 3. Plateau in high-threshold motoneuron of chronic spinal rat. Same format as Fig. 2, *A–D* but with motoneurons with higher recruitment threshold. *A* and *C*: motoneuron from acute spinal rat that showed no plateau and had firing rate adaptation. *B*: motoneuron from chronic spinal rat that had a plateau (initiated at arrow) that continued until the current was reduced by $\Delta I = -0.5$ nA. *D*: note linear *F-I* plot for same cell with firing starting on the regression line at upward arrow.

and firing (self-sustained firing). The most dramatic examples of this occurred with small current ramps, where the upward current just activated the plateau (at acceleration in potential, Fig. 5*C*, arrow), and then immediately the downward current ramp started *just prior to recruitment*. In this case, the plateau continued to depolarize the cell and produce sustained firing even when the current was reduced by 1 nA [i.e., ≥1 nA sustained I_{PIC}; in acute spinal rats a comparable small current ramp did not evoke any firing or a plateau (not shown)].

In some of the motoneurons with a low threshold for plateau initiation, accelerated and irregular firing occurred for ~1 s after recruitment (Figs. 5, *A* and *B*, and 6*D*, mentioned in the preceding text), suggesting that the plateau was being further activated during this early period. Additional evidence for this comes from comparing the plateaus evoked with different amplitude ramps. Although a plateau could be activated with a very brief small ramp that just activated the cell (as described in the preceding text, Fig. 5*C*), a slightly larger ascending ramp that caused a few seconds of firing (Fig. 5*A*) had a larger effect ($\Delta I = -1.4$ nA, compared with -1 nA). Likely, this resulted from a more complete activation of the plateau with the larger ramp. Low-threshold plateau activation occurred in both low (Figs. 2*B* and 6*D*) and high (Figs. 3*B* and 5) recruitment threshold cells (range: -0.1– nA threshold).

In the chronic spinal rat motoneurons with a low plateau threshold (i.e., *type 3* cells; 63% of chronic spinal motoneurons), the firing rate profiles after plateau activation were remarkably linear (r^2 ranged between 0.85 and 0.95; Figs. 3*D*, 5*A*, and 6, *C* and *D*) during slow current ramps, and the *F-I* plots on the ascending current ramp overlapped the profiles on the descending ramp (Fig. 6, *C* and *D;* no counterclockwise hysteresis loop). Interestingly, the firing profile followed closely in proportion to the current even at currents well *below* the initial recruitment current on the descending ramp (Fig. 5*A*, *right*). A simple interpretation of this is that once the I_{PIC} was activated; it provided a steady depolarizing bias current and did not produce any further accelerations in membrane potential or firing (though see complication in the following text). Indeed once the plateau was activated, the injected current could be repeatedly increased and decreased above and below the current at plateau initiation (and recruitment) and the firing

FIG. 2. Plateau in low-threshold motoneuron of chronic spinal rat. *A*: membrane potential and firing rate response to slow current ramp in low-threshold motoneuron of *acute* spinal rat. Note symmetry of response in relation to current, and thus lack of plateau. Scaled current (thin line) plotted with potential for reference. Tick marks denote 0 Hz. *B*: response to same current ramp in low-threshold motoneuron of *chronic* spinal rat. Note acceleration in potential (single arrow) and firing (double arrow), indicating the onset of a plateau (i.e., I_{PIC}). The plateau caused a sustained depolarization (ΔV) and discharge that continued until the current was reduced by ΔI ($= -I_{PIC}$). *C* and *D*: frequency-current (*F-I*) plots for *A* and *B.* Black symbols, upward ramp; white, downward. Note the late acceleration in firing in *D* (chronic spinal), although overall lower *F-I* slope than in acute spinal rat. *E* and *F*: responses to current pulses in acute and chronic spinal rats. The plateau caused self-sustained firing in the chronic spinal rat (*F*, plateau onset at asterisk). Recordings in DCC mode. Normal artificial cerebrospinal fluid (ACSF). Same scales on *A, B, E,* and *F*.

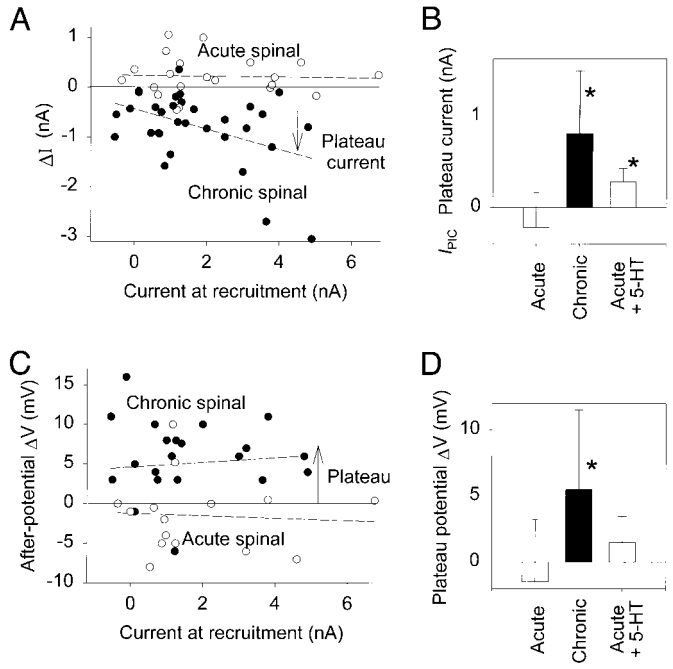


FIG. 4. Summary of plateau properties of all motoneurons. *A*: estimation of plateau current I_{PIC} from the average current at de-recruitment minus current at recruitment ($\Delta I = -I_{PIC}$). Mean value of ΔI for each acute (○) and chronic (●) spinal rat motoneuron is shown as a function of the recruitment current. - - -, respective linear regressions, although there is considerable scatter (r^2 = 0.2 and 0.2). *B*: summary of average I_{PIC} ($-\Delta I$) for acute spinal rats, chronic spinal rats and acute spinal rats with serotonin (5-HT). *, significant differences from 0. *C*: average afterpotential for each motoneuron plotted as a function of its recruitment current (same symbols as *A*). *D*: ensemble average of afterpotentials; same format as in *B*.

profile remained linear (on the same line in *F-I* plot; not shown).

FIRING RATE ADAPTATION (TYPE 2) AND EFFECT OF RAMP SPEED AND AMPLITUDE. In 17% of motoneurons of chronic spinal rats, there was firing rate adaption (slowing of firing; *type 2* cells), with clockwise hysteresis in the frequency-current plots, and in these cells, the self-sustained firing was weaker than average (i.e., plateau, ΔI, not as pronounced). As in acute spinal rats, this slowing of firing was accentuated with large or fast ramps. Indeed even cells with a clear plateau and little firing rate adaptation during slow ramps showed some slowing of firing, with less afterdischarge (ΔI) and less afterpotential (ΔV) during faster or larger ramps (Fig. 5, *B* compared with *A*).

LATE-ACTIVATED PLATEAUS (TYPE 4). In some motoneurons (20%; as in Figs. 6*E* and 2*B;* classified as *type 4* cells), although firing initially increased linearly with current, there was a late acceleration in firing, which was assumed to mark the main activation of I_{PIC} and the plateau (Fig. 2*B*, double arrows), as has been described in cat motoneurons (Bennett et al. 1998a; Hounsgaard et al. 1988). In these cells, there was a corresponding counterclockwise hysteresis loop in the *F-I* plot (Figs. 6*E* and 2*D*) and a marked drop in injected current at de-recruitment compared with recruitment ($\Delta I < 0$, i.e., large sustained I_{PIC}). All cells with this late plateau activation after recruitment were low recruitment threshold, presumably small, cells (7/35 cells; 0.12- to 2.5-nA recruitment thresholds).

Interestingly, the same late acceleration in firing could be seen during a steady pulse current injection (Fig. 2*F*, asterisk), and we supposed that this acceleration was caused by the cell spontaneously moving from the lower (black) to the upper (white) branch of the *F-I* relation as the plateau was activated (see asterisks in Fig. 2*D* at applied 0.8- nA current). Thus firing without full activation of the plateau was only *transiently* possible. Further, after the plateau was activated (when frequency acceleration complete, within a few seconds), the firing rate was modulated linearly with current ($r^2 > 0.85$).

PARTIAL DEACTIVATION OF PLATEAU DURING FIRING? In cells with a clear late acceleration in firing (type 4), which we have previously taken to mark the main activation of the plateau (Figs. 2*B* and 6*E*) (Bennett et al. 1998a), we were surprised to find that self-sustained firing (plateaus) could be evoked even when the current ramp was kept *below* the point where the frequency acceleration occurred [below main plateau threshold; compare Fig. 6, *E* and *F*, responses from the same cell; contrast to Fig. 2 of Bennett et al. (1998b)]. However, on closer inspection, we found that there was also an acceleration in potential just prior to recruitment (Fig. 2*B*, single arrow), and this was likely associated with an early plateau activation. Thus possibly there were two distinct plateau activations, one at recruitment and a second one later. We, however, favor an alternate interpretation based on two observations: *1*) during the current ramps, the accumulated effect of the AHPs tended to hyperpolarize the membrane following recruitment (i.e., mean potential between spikes; e.g., Fig. 5*B*) compared with the potential just before recruitment. *2*) Following the firing produced by a current pulse, there was, at times, a very slow hyperpolarization (sAHP), and pause in firing, before the afterdischarge (plateau) continued [Fig. 8*G* described later; see similar effects in Figs. 7 and 8 of Russo and Hounsgaard (1996)]. Thus while the plateau may be partly activated before recruitment, with moderate firing rates the accumulated hyperpolarization from AHPs may have prevented further activation or even caused partial deactivation of the plateau. Only when the cell was further depolarized by the increasing current ramp was the plateau fully activated (late acceleration, at high firing rate).

Even in cells with only a low-threshold plateau activation (without a late frequency acceleration; Fig. 5), this partial plateau deactivation by accumulated AHP hyperpolarization during firing may have occurred. Indeed, during the downward current ramp, and as the firing slowed, there was at times evidence for a *reactivation* of the plateau (29% of cells). That is, the mean membrane potential transiently increased, despite the deceasing current, and at times caused a brief acceleration in firing (Fig. 5*A*, right arrow). Likely this occurred because the firing slowed sufficiently to allow the accumulated effect of the AHPs to diminish, allowing a more complete activation of the plateau.

VERY SLOW FIRING. When the current was reduced during a plateau and firing slowed, surprisingly long intervals often occurred between spikes ($\leq$1 s), often many times the AHP duration and the related theoretical maximum interval (Figs. 5*A* and 8*H*) (Kernell 1999). In these cases, the plateau was just at threshold to deactivate, and thus perhaps each AHP transiently deactivated the plateau, and the plateau was then only slowly reactivated to produce a subsequent spike at a long interval. Indeed this slow rise before each spike was similar to

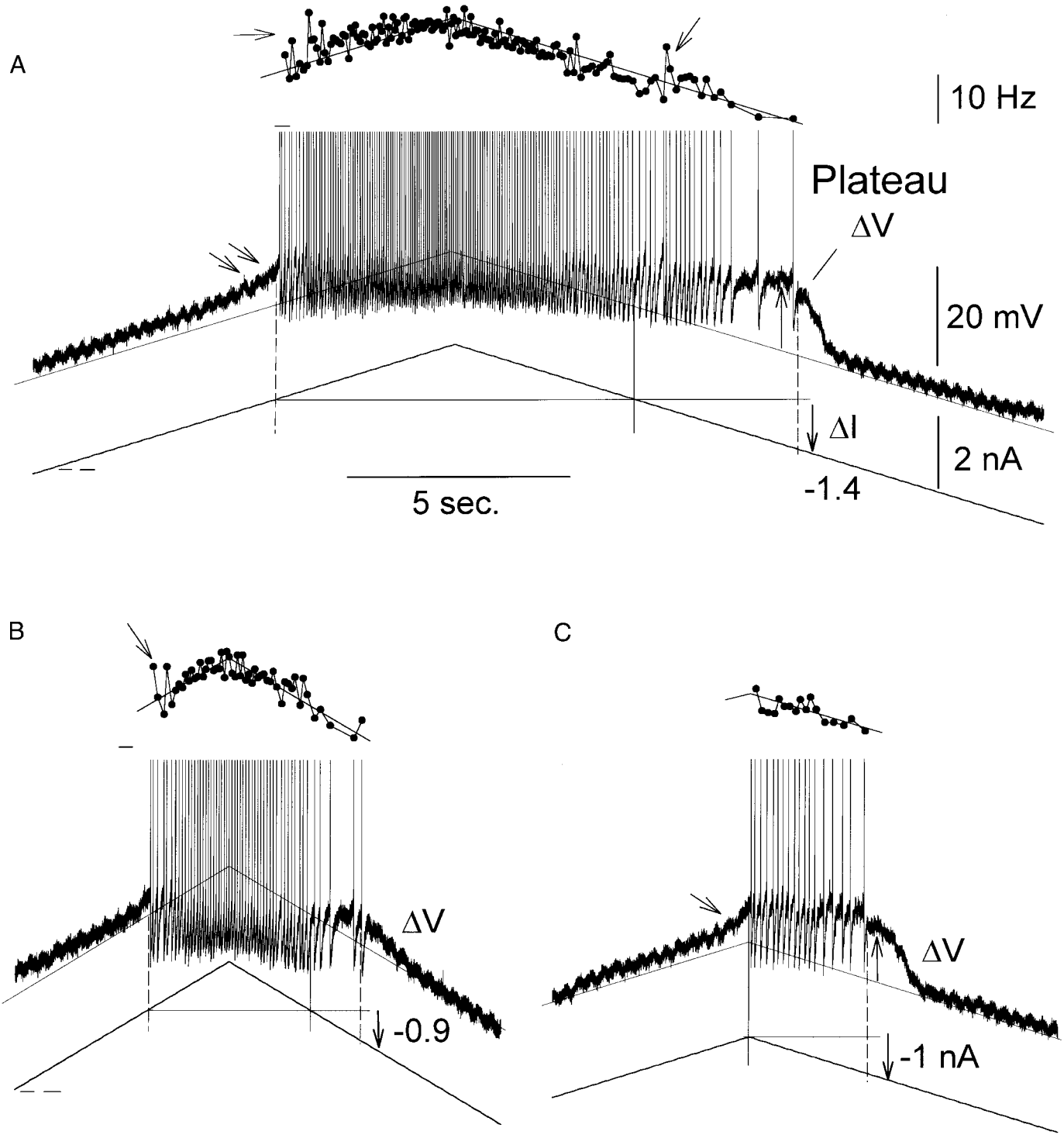


FIG. 5. Low-threshold plateau activation in chronic spinal rat motoneuron. Same format as Fig. 2. *A*: plateau and sustained firing evoked by slow ramp. Plateau was initiated before recruitment (at double arrow) and produced a large afterpotential (*V*) and an afterdischarge that continued until the current was reduced by $\Delta I = -1.4$ nA. *B*: same cell with faster current ramp. Note the smaller afterpotential and afterdischarge (related to ΔI). In *A* and *B*, there was accelerated firing just after recruitment (left arrows; plateau further activated), although afterward the firing rate varied proportionally to current (see thin reference line plotted with rate) except near de-recruitment (at arrow in *A*). *C*: verification of plateau initiation before recruitment (left at arrow), by using a small current ramp that was reversed just before recruitment. Same cell in *A–C*.

the slow rise in potential when the plateau was first activated just before recruitment (Fig. 5, *A* and *B*). This phenomenon was not transient because slow firing could continue for many seconds when pulses were used to evoke plateaus (Fig. 8*H*, described later). The possibility of slow firing generated by voltage-dependent inward currents with slow kinetics near firing threshold has been discussed by others (e.g., Carp et al. 1991; Hodgkin 1948; Kernell 1999).

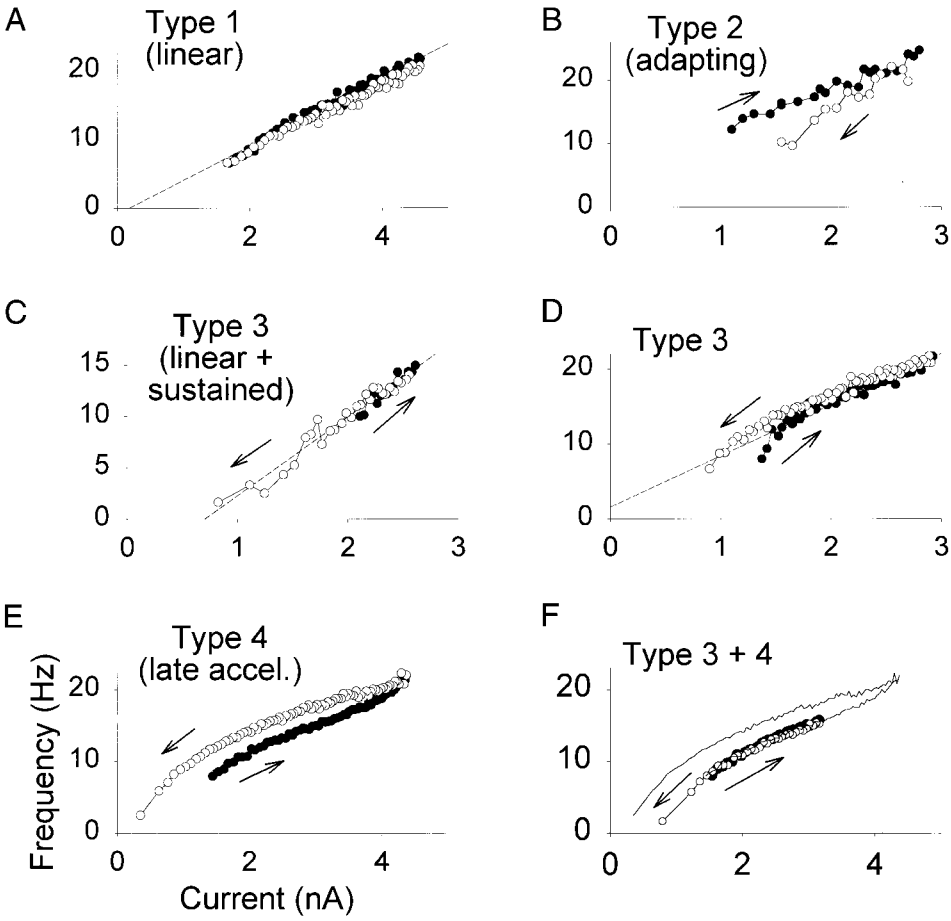


FIG. 6. Examples of 4 basic types of firing patterns. Instantaneous frequency-current (*F-I*) plots for upward (●) and downward (○) current ramps (0.5 nA/s). *A* and *B*: two neurons from acute spinal rats. *C–E*: three neurons from a chronic spinal rats. *F* shows the response in *E* (—) overlayed with the response of same neuron with a smaller ramp (● and ○). Regression lines shown in *A*, *C*, and *D*. See text for details.

Summary of firing behavior in acute and chronic spinal rat motoneurons

F-I TYPES. The distribution of cells between the four types of firing behaviors described in the preceding text (types 1–4) is summarized in Fig. 7*A;* the majority of the acute and chronic spinal cells behaved linearly, as type 1 and 3 cells, respectively. To summarize the type definitions: type 1 cells had a linear *F-I* relation, with overlapping frequency points for the ascending and descending current ramps, but no self-sustained firing (no plateau, all from acute spinal rats, Fig. 6*A*; see also Fig. 2*C*). Type 2 cells showed firing rate adaptation and usually no plateau (Fig. 6*B;* see also Fig. 3*C*). Some cells in chronic spinal rats were of this type, and they had only weak self-sustained firing with rate adaptation countering the plateau. Type 3 cells had linear characteristics as in type 1 but also showed self-sustained firing (plateau). Remarkably the firing rate remained on the linear *F-I* regression line even when the current was brought well below the recruitment current on the descending ramp (Figs. 6, *C* and *D*, and 5*A*). Some of Type 3 cells started firing directly on the *F-I* linear regression line (thin line in Fig. 6*C*), and we assume that the plateau was fully activated at recruitment. Others included in this type had a few accelerating spikes just after recruitment below the linear regression for the *F-I* relation (Fig. 6*D*), which we supposed indicated the early activation of the plateau, continuing for a second after recruitment (see preceding text). Type 4 cells had a late frequency acceleration, a few seconds after recruitment, followed by self-sustained firing (high-threshold plateau; Figs. 6*E* and 2*D*). As mentioned previously, type 4 cells behaved linearly, as with type 3 cells, when the current was kept below the level for a late frequency acceleration (Fig. 6*F*). With the exception of type 4 cells, which were purely small, low recruitment-threshold cells, the other three types included both cells of low and high recruitment threshold (see preceding text).

INITIAL AND FINAL RATES. In previous motor unit experiments, motoneurons with presumed plateaus were found to have significantly higher firing rates at recruitment, compared with at de-recruitment (Gorassini et al. 1998, 2001a), and this was thought to be due to an early plateau activation at recruitment that boosted the initial firing rate above the minimum rate. In chronic spinal rats with plateaus, this was also found to be the case, with recruitment at ~8 Hz, and de-recruitment at half that value (significant difference; Fig. 7*B*). In contrast, the firing rates at recruitment and de-recruitment were not significantly different in motoneurons of acute spinal rats (~8 Hz; Fig. 7*B*). The firing rate achieved at de-recruitment (*minimum rate*) in chronic spinal rats was significantly lower than in acute spinal rats, and, at times, as low as 1 Hz (see preceding text).

LOWER F-I SLOPE AND SLOWER STEADY FIRING IN CHRONIC SPINAL RATS. Overall, motoneurons of chronic spinal rats fired at lower rates than in acute spinal rats not just lower minimum rates. To further quantify this, we have fit a linear regression to the *F-I* ramp responses. The first instantaneous firing rate point that fell on the *F-I* regression line after recruit-

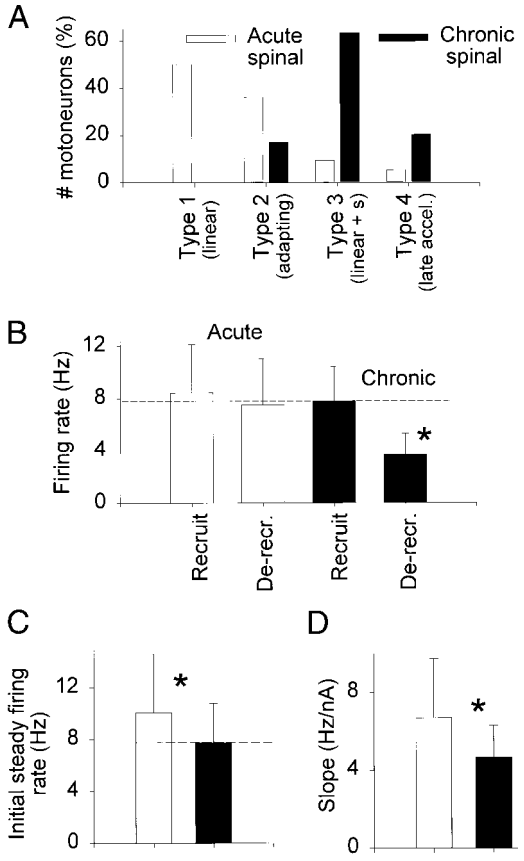


FIG. 7. Summary of motoneuron firing properties in acute and chronic spinal rats. *A*: distribution of motoneurons among the 4 types of firing profiles. Note that most cells had a linear *F-I* profile, both in acute (□) and chronic (■) spinal rats, although the latter also had self-sustained firing (type 3, denoted linear + self-sustained firing). Late acceleration in firing (type 4) and firing rate adaptation (type 2) were less common. *B*: mean firing rates at recruitment and de-recruitment. *C*: mean initial steady firing rates during slow ramps (see text). *D*: mean slopes of *F-I* plots (regression line) in acute and chronic spinal rats. *, significant difference.

ment was measured and referred to as the initial steady firing rate. This measure is not necessarily the instantaneous rate at recruitment because there were often overshoots (early adaptation) or undershoots in firing before the rate was modulated linearly with current (Fig. 6*D*). The initial steady firing rate was found to be significantly lower in chronic spinal rats than in acute spinal rats (Fig. 7*C*).

The slope of the *F-I* linear regressions was also significantly lower in chronic spinal rats (Fig. 7*D*). This lower slope may be explained by an increased conductance provided by I_{PIC} in chronic spinal rats (see DISCUSSION). Thus while the plateau may have enabled recruitment at twice the minimum rate, its associated conductance change may have made it more difficult to produce further increases in rate (i.e., lower *F-I* gain).

Voltage dependence of plateaus evoked with brief pulses

Although a brief current pulse in some chronic spinal cells could be readily used to evoke a plateau from rest (Fig. 2*F*), in others, a plateau could only be evoked by a pulse when there was an appropriate steady depolarizing bias current. We found that for a given cell, the parameters for producing a plateau from a pulse could be estimated from the ramp response as follows: first, the plateau threshold current was estimated, which was usually at the acceleration in potential just prior to firing (1.5 nA in Fig. 8, *A* and *B*, see arrow). Second, the plateau current was estimated ($I_{PIC} = -\Delta I$, which is 0.6 nA in Fig. 8*B*). Finally, a bias current was chosen that when added to the plateau current, exceeded the threshold current for plateau activation (e.g., bias $+0.6 > 1.5$ nA), thus allowing the plateau to remain activated after the pulse. By varying the bias level, we have been able to verify this recipe for plateau activation, as shown for a typical cell in Fig. 8, *C–H*. With no bias current (Fig. 8, *C–E*), a pulse could not produce a sustained afterdischarge, regardless of the pulse height, presumably because the plateau current, I_{PIC}, was only 0.6 nA, compared with the plateau threshold of 1.5 nA. [There was, however, evidence that the plateau was activated during the pulse because a delayed onset in firing and slow rise in potential could be seen when the plateau threshold current was reached (Fig. 8*D*, arrow; see details in Fig. 9).] When the bias current was increased to 0.7 nA (in Fig. 8*F*), a pulse evoked a sustained depolarization that outlasted the pulse (afterpotential; Fig. 8*F*, right arrow). This potential was slowly decrementing, suggest-

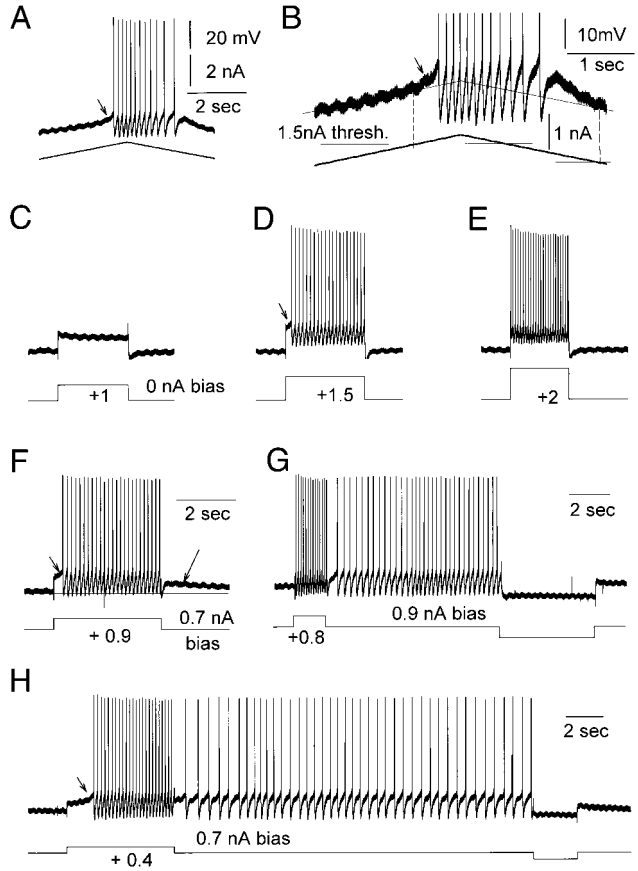


FIG. 8. Voltage dependence of plateau in motoneuron of chronic spinal rat. *A*: plateau initiated by slow current ramp. *B*: same data as in *A* but on different scale to showing threshold for plateau onset and estimation of I_{PIC}. *C–E*: responses to current pulses without a bias current. Note delay in onset of firing and slow rising potential at arrow in *D*. *F–H*: responses to current pulses with a depolarizing bias current. Note plateau activation with afterpotential (*F*) and afterdischarges (*G* and *H*). Also, note the pause in firing after the pulse (*G* and *H*). Small suprathreshold pulses (*H*) were more effective than larger pulses (*F*) in evoking a plateau at a given bias current. Plateau stopped by hyperpolarizing pulses in *G* and *H*. Same neuron for all panels. Same vertical scales in *A* and *C–H*. Same time scale in *A* and *C–F*.

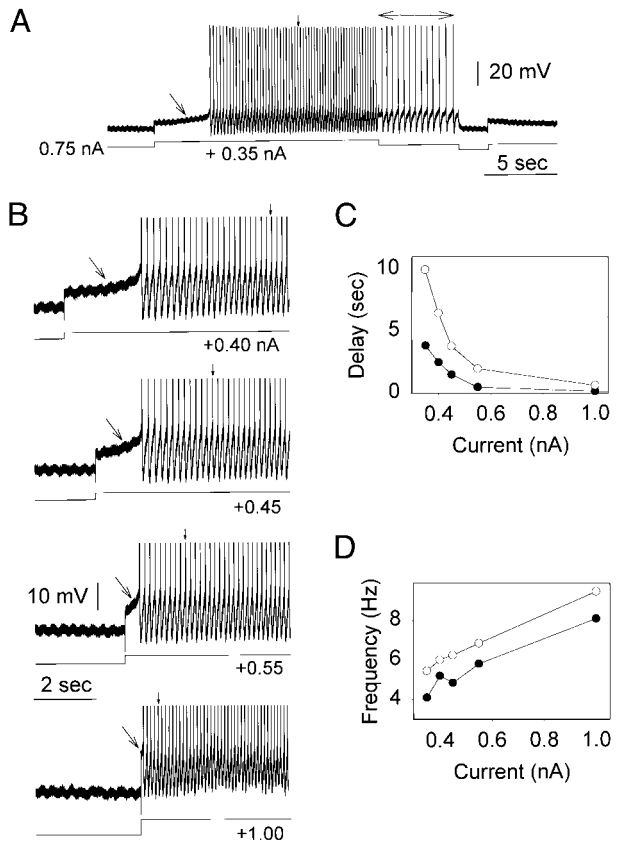


FIG. 9. Slow plateau activation in chronic spinal rat motoneuron. *A*: depolarizing current pulse just at threshold to initiate a plateau caused a very slow plateau activation (5-s delay, at left arrow) followed by an afterdischarge. *B*: progressively larger current pulses produced a faster plateau activation (arrows). Same bias current (0.75 nA) in *A* and *B*. Small arrows indicate time at which steady firing was achieved. Different time and voltage scales in *A* and *B*. *C*: delay in onset of firing (black symbols) and steady firing (white) as a function of current. Note that plateau activation was much faster for large pulses. *D*: initial (black) and steady state (white) firing frequency as a function of current.

ing that the bias current was set just below the plateau threshold ($0.7 + 0.6 < 1.5$ nA). A larger bias current (0.9 nA) produced more robust plateau activations ($0.9 + 0.6 = 1.5$ nA), with a greater afterpotential and afterdischarge (Fig. 8*G*).

Interestingly, following the pulse, a hyperpolarization and pause in firing often occurred (sAHP; Fig. 8, *G* and *H*), and this was followed by an acceleration in firing as the plateau continued. Probably this occurred because the accumulated effect of AHPs (sAHP) during the pulse produced a partial deactivation of the plateau, which in this case was only reversed once the pulse ended (see DISCUSSION). A related observation is that the plateau caused a greater afterdischarge when it was evoked by a lower amplitude pulse (compare Fig. 8, *F* and *H*). Thus smaller pulses, which produced less firing during the pulse, caused less plateau inactivation and a greater afterdischarge, presumably by producing less sAHP (as in Fig. 6 of Russo and Hounsgaard 1996).

Slow and fast plateau activation

The plateau activation speed increased systematically with the depolarizing pulse amplitude (Fig. 9, bias current fixed at 0.75 nA). With a minimum pulse size (0.34 nA in Fig. 9*A*), the membrane potential only very slowly depolarized as the plateau was being activated, and firing was substantially delayed (4 s in this case). Larger pulses produced faster plateau activation and less delay in recruitment (the latter summarized in Fig. 9*C*, solid circles; note that the time scale is faster in Fig. 10*B*, compared with 9*A*). With the largest pulses, the plateau was activated simultaneously with recruitment (Figs. 9*B*, *bottom*, and 8*G*). In these cases, the presence of the plateau had to be verified by looking for a discharge after the pulse (not shown in fast time scale in Fig. 9, but see Fig. 8*G*).

In some cells, there was a delayed acceleration in firing associated with the plateau activation during the pulse (mentioned in the preceding text in relation to Fig. 2*F*), and the delay for this acceleration also depended on the pulse amplitude. To quantify this, we have computed the time to reach steady-state firing during pulses of different amplitude (Fig. 9*B*, small arrows). For larger amplitude pulses, the firing increased to its steady state value faster, as summarized in Fig. 9*C*. The cell shown in Fig. 9 had its plateau primarily activated

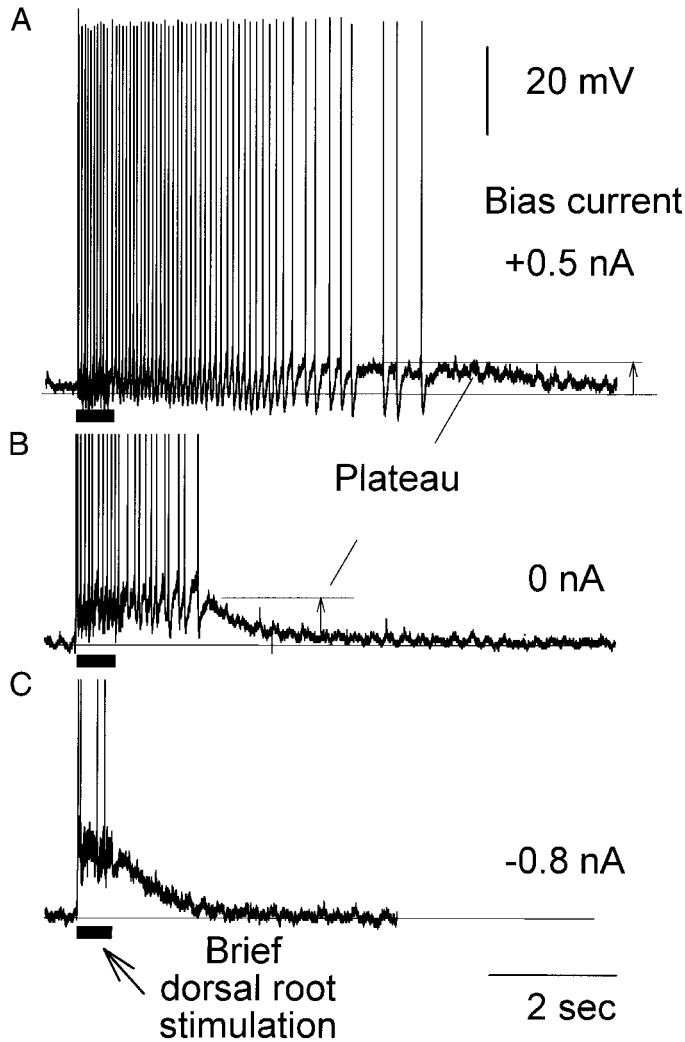


FIG. 10. Plateaus triggered by brief dorsal root stimulation in chronic spinal rat motoneuron. *A–C*: voltage responses of motoneuron to brief stimulation of the Ca_1 dorsal root (20 Hz, 5 × T) with various intracellular bias currents. Note the marked reduction in reflex duration as the cell was hyperpolarized and the effect of the voltage-dependent plateau was reduced. In *C*, a sufficient hyperpolarization was applied to eliminate the plateau, and there remained a 1-s response that outlasted the stimulation.

before recruitment and then had about a 1.5-Hz increase in rate that resulted from the further plateau activation during firing (Fig. 9*D*).

Voltage-dependent long-lasting reflexes

Because our ultimate goal was to relate the presence of plateaus to the exaggerated long-lasting reflexes seen with spasticity, we have examined the reflex activation of the motoneurons by dorsal root stimulation. In acute spinal rats, a brief dorsal root stimulation only triggers a brief ventral root response (Bennett et al. 1999a,b). In contrast, in chronic spinal rats, a brief dorsal root stimulation triggers a sustained response in the ventral roots with a duration similar to tail reflexes in awake spastic rats, of ~2 s (stimuli 1.5–10 times threshold) (Bennett et al. 1999a,b). Corresponding long-lasting reflex responses were seen during intracellular recording in chronic spinal rat motoneurons in response to the brief dorsal root stimulation, with an EPSP duration ranging from 2 to 5 s at rest (see Fig. 10*B;* no intracellular current bias, 2-s duration). The duration of this EPSP was always substantially reduced (to ~1 s in Fig. 10*C*) by hyperpolarizing the motoneuron with a steady bias current to eliminate any effect of the plateau. Conversely, a depolarizing current bias increased the duration of the reflex responses (EPSP; to 6 s in Fig. 10*A*) as might be expected from Fig. 8. The duration of the dorsal root stimulus could be as short as a single shock yet still evoke the long-lasting voltage-dependent reflexes as just described (not shown; see also ventral root reflexes) (Bennett et al. 1999b; Bennett and Li, unpublished data). This voltage-dependent behavior suggests that plateaus were triggered by the dorsal root stimulation and played a major role in amplifying and prolonging the reflex responses in chronic spinal rats. However, the plateau was not the only contributor to the sustained reflexes because when the plateau was eliminated by hyperpolarization, the stimulus still evoked a depolarization that outlasted the stimulus by ~1 s (Fig. 10*C*).

DISCUSSION

The results demonstrate that plateau potentials are prominent in sacrocaudal motoneurons of chronic spinal rats with spasticity. Further, the plateaus contribute substantially to the exaggerated long-lasting reflexes, prolonging the synaptic input by many seconds, and thus playing an important role in spasticity (Bennett et al. 1999a). The plateaus occurred spontaneously when recorded in normal ACSF in vitro, without exogenous neuromodulators added to the ACSF, and thus these motoneurons become somewhat like deep dorsal horn neurons that normally exhibit plateaus and related oscillations spontaneously (Jiang et al. 1995; Morrisset and Nagy 1999; Russo and Hounsgaard 1996). In contrast, plateau behavior was not usually seen in motoneurons of acute spinal rats although exogenously applied 5-HT could enable plateaus.

Because previous studies have provided evidence for plateaus in hindlimb/leg motoneurons of intact rats (Eken et al. 1989; Gorassini et al. 1999a), decerebrate cats (Hounsgaard et al. 1988), and awake humans (Gorassini et al. 1998, 2001a), it is reasonable to assume that plateaus were also present in sacrocaudal tail motoneurons of intact rats prior to injury. In addition, the finding that 5-HT facilitates plateaus in these sacrocaudal cells after acute injury is consistent with this assumption because such monoamines are thought to be a major facilitator of plateaus in normal animals (Eken et al. 1989). We can therefore conclude that motoneuron excitability provided by plateau behavior is acutely removed by spinal cord injury and is recovered in chronic spinal rats that develop spasticity, thus verifying the hypothesis and results of Eken et al. (1989) and Nielsen and Hultborn (1993), and the more recent inferences of plateau behavior from motor unit recordings in awake spinal-cord-injured rats (Bennett et al. 2001) and humans (Gorassini et al. 1999b).

We have found a remarkably high incidence of plateaus in motoneurons of chronic spinal rats (from intracellular and motor unit recordings) (Bennett et al. 2001). Nielsen and Hultborn (1993) found a lower incidence of plateaus in motoneurons of chronic spinal cats; however, we suggest that this was partly because of the criterion of hysteresis in firing that they used to identify plateaus. We found that, while most cells have plateaus, the plateaus are activated just before recruitment (low threshold), and thus clear counterclockwise hysteresis loops (*F-I* plot) with a late frequency acceleration are infrequent (type 4; 20%), compared with linear frequency plots with self-sustained firing and no open counterclockwise hysteresis loop (type 3; 63%).

The emergence of plateaus with chronic injury is very significant from a functional point of view because the normal descending inhibitory control is lacking, and uncontrolled long-lasting contractions may be triggered by brief stimuli (e.g., spasms and hypertonus) (Bennett et al. 1999a). The threshold, amplitude, and duration of the plateau is therefore important to quantify functionally as discussed in the following text. We suggest that spasticity associated with injury is not so much a condition related to motoneuron overexcitability (see discussion of plateau amplitude in the following text) but instead to a recovery of relatively normal excitability and plateau behavior *without the normal inhibitory control* to turn off plateaus and associated sustained firing.

Possible mechanisms for emergence of plateau in chronic spinal rats

The cause of plateaus after chronic spinal cord transection is unknown. The spinal cord has essentially no endogenous neurons that release monoamines (NE, 5-HT; only 1 5-HT spinal neuron per rat) (Newton and Hamill 1988), and peripherally derived monoamines [from sympathetic terminal sprouts (McNicholas et al. 1980); or other hormones] could not play a role in producing plateaus in the explanted in vitro spinal cord preparation that we have used. However, as mentioned in the INTRODUCTION, persistent inward currents are likely present in many neurons, and a number of neuromodulators, outside of monoamines, can enable them to dominate sufficiently over outward currents to enable plateaus (Russo and Hounsgaard 1999). Indeed, even in acute spinal animals a few motoneurons have plateaus (3/22 in our case; 1/20 in Hounsgaard et al. 1988), suggesting a latent endogenous capability for plateaus, perhaps controlled intrinsically or by interneuronal or afferent inputs (via substance P and glutamate) (Russo and Hounsgaard 1999; Russo et al. 1997).

Plateaus may have emerged as a result of unmasking the persistent inward currents by reducing voltage- and calcium-

gated outward K^+ currents, many of which normally participate in the AHP (Russo and Hounsgaard 1999). For example, 5-HT-mediated plateaus are associated with a reduction in the AHP (Hounsgaard and Kiehn 1989; Hultborn and Kiehn 1992). However, the AHP amplitude (or duration) in chronic spinal rats was *not* significantly smaller than in acute spinal rats, suggesting that the plateaus that emerged in chronic spinal rats were *not* facilitated by a reduction in these AHP-related K^+ currents (in contrast to how 5-HT works). Further, because there was a plateau *and* a large AHP, the accumulated hyperpolarization from the AHPs in chronic spinal rats produced peculiar effects not seen in motoneurons of plateaus mediated by 5-HT (Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988), such as partial plateau deactivation during firing. Similar plateau deactivation has been reported following high-frequency firing in plateau-generating turtle dorsal horn neurons and was associated with a large slow AHP (sAHP) that slowed or stopped repetitive firing (Russo and Hounsgaard 1996). Also, we found that, with low levels of injected current, the plateau was partially deactivated by each large AHP and then only slowly reactivated, enabling a further spike and AHP, etc, thus explaining the very slow steady firing rates seen in chronic spinal rats (1–4 Hz; see Fig. 8*H*) (see also similar discussions in Carp et al. 1991; Hodgkin 1948; Kernell 1999).

Alternatively, the plateaus may have emerged after chronic injury because of a direct facilitation of the persistent inward current (I_{PIC}) by metabotropic actions (e.g., mGluR1 or muscarine receptors) (Svirskis and Hounsgaard 1998) or even a permanent up-regulation of the associated channels or channel subunits (Ma et al. 1997). One prediction of a direct increase in I_{PIC} would be that the conductance should be increased in chronic spinal rats with plateaus compared with acute spinal rats, whereas if I_{PIC} was simply unmasked by reducing the opposing outward currents, the opposite might occur (Kernell 1999). Although we did not directly measure the conductance during the plateau (i.e., during firing), we did find that the *F-I* slope was lower in chronic spinal rats than in acute spinal rats. This finding is consistent with a greater conductance resulting from I_{PIC} in chronic spinal rats (i.e., more current needed to increase firing), especially considering that the basic cell properties in acute and chronic spinal rats were otherwise similar (similar passive R_{in}, rheobase, AHP amplitude; Table 1). Also, an increase in *F-I* slope has been associated with a decrease in the AHP and associated conductances in the presence of neuromodulators (Berger et al. 1992; see Fig. 1 of Kernell 1999).

The inward currents involved in the plateau after chronic injury remain uncertain and could, in principle, include persistent calcium (L-type), persistent sodium, NMDA and I_{CAN} currents (Russo and Hounsgaard 1999). Considering the plateau timing and broad activation range seen in chronic spinal rats, we suggest the involvement of L-type calcium channels, as in plateaus of other motoneurons and dorsal horn neurons in turtle and young rat (Morisset and Nagy 1999; Russo and Hounsgaard 1999). In particular, the very slow plateau onset during small current pulses (at threshold; Fig. 9*A*) is a characteristic property of plateaus mediated by L-type calcium channels of motoneurons (Hounsgaard et al. 1989; Svirskis and Hounsgaard 1997).

Low plateau threshold

Functionally, one important characteristic of plateaus is the threshold for activation. In decerebrate cats, the threshold varies widely depending on the cell type (Lee and Heckman 1998a) and mode of activation (intracellular vs. synaptic, somatic vs. dendritic) (Bennett et al. 1998a,b; see also turtle motoneurons, Svirskis and Hounsgaard 1997, 1998). That is, with intracellular current ramps, some cat motoneurons have plateaus activated near the recruitment level, but the majority have plateaus activated well after recruitment (at high firing rates). With synaptic activation, the threshold for plateau activation is lowered in cat motoneurons (Bennett et al. 1998a). In contrast, in chronic spinal rats the majority of motoneurons have plateaus that are initiated below the firing level, even during intracellular current injections (Figs. 5 and 7*A*).

Low plateau thresholds are normally characteristic only of slow, early recruited motoneurons in the ("normal") decerebrate cat (Lee and Heckman 1998a). Thus the reason why all motoneuron types have a low threshold after chronic injury is unclear, especially considering that the tail muscles have both fast and slow twitch muscles (Steg 1964). Interestingly, chronic spinal rats additionally have slower AHPs (Table 1), lower firing rates (Fig. 7) and longer muscle twitches (Stephens et al. 1999) than acute spinal rats, consistent with the whole motor unit behaving more slowly (see also Powers and Rymer 1988). This occurs even though the recruitment threshold (Fig. 4*A*) and input resistance (Table 1) are similar to that of acute spinal rats (as in Baker and Chandler 1987a; Hochman and McCrea 1994).

Plateau amplitude and I_{PIC}

How large the plateaus are in chronic spinal rats in relation to those in normals or brain-stem-intact animals is another important functional question. In chronic spinal rats, the plateau is ~5–10 mV (Figs. 4 and 5). Further, our estimates of the sustained current supplied by the plateau (I_{PIC}) ranged from ~0.5 to 1.5 nA, with an average of ~0.81 nA, which is substantial in relation to the current required to recruit the cell to steady firing (1.7 nA). That is, the plateaus provide about half the average current needed to recruit a motoneuron and maintain moderate firing rates (0.81/1.7). With 5-HT administration, the plateau effects are smaller (Fig. 4, *B* and *D*), suggesting that plateaus in the chronic spinal rat may be at least as large as in the normal brain-stem-intact rat. Motoneurons in brain-stem-intact decerebrate cats have very similar plateau amplitudes to the chronic spinal rat (~8 mV; Bennett et al. 1998a; see also: Hounsgaard et al. 1988; Lee and Heckman 1998a,b), and the sustained I_{PIC} is on average 6 nA (sustained peak in Lee and Heckman 1998b), which is again about half the average current to recruit the motoneurons (14 nA; cat motoneurons require 10 times more current to activate, compared with rat and turtle) (Lee and Heckman 1998a,b). Note, however, that the plateau can be further augmented in cats with additional monoaminergic agonists (I_{PIC} doubled with methoxamine) (Lee and Heckman 1998b; see also Svirskis and Hounsgaard 1998). Finally, in humans the plateau has a similar effect as in decerebrate cats, with the plateau again providing half the estimated input to maintain moderate firing rates (Gorassini et

al. 1998, 2001a). Thus the sustained depolarization and effective I_{PIC} current provided by the plateau in chronic spinal rats is comparable to that predicted in intact animals and humans.

Linear firing rate profiles with slow, low-amplitude current ramps

Considering the presence of plateau behavior in motoneurons, it is remarkable that the firing rate profiles were usually linear with the plateau prolonging the firing even though the firing rate remained on the *F-I* regression line (type 3 neurons, Fig. 6*C*). We suggest that this linearity occurred because the plateau was mostly activated at or before recruitment and thus did not markedly affect the linearity of firing afterward, other than to provide a depolarizing bias that brought the cell to a relatively high rate (possibly optimal rate), compared with its minimum rate. In some cells, there was evidence that the plateau was being further activated (or deactivated) during firing, and this produced some nonlinearities in firing (type 4 neurons). However, we have primarily studied the plateaus with intracellular current injection, which should raise the plateau threshold in comparison to the threshold seen with synaptic activation (see preceding text) (Bennett et al. 1998a) and exaggerate the firing rate nonlinearities. Importantly, the relative linearity of firing profiles implies that, even in motoneurons with plateaus, the firing rate profile should closely reflect the *input* to the motoneurons (Figs. 5*A* and 6, *C* and *D*), and this profile can be used to study the input-output properties of other higher threshold motoneurons (with motor unit recordings in the awake rat) (Bennett et al. 2001; Gorassini et al. 2001a,b).

The ramp profiles that we used were slow and small in amplitude, thus optimized to clearly see the sustained plateau. This avoided high firing rates that produced firing rate adaptation (Kernell and Monster 1982) and non-steady-state dynamics of the cells (ramp speed-related) and thus favored linear firing profiles. Nonlinear behaviors can be seen with faster and larger inputs where higher firing rates are achieved. Thus the stimulus parameters are very important to consider in designing experiments to study plateaus, especially when studying motor unit firing (Bennett et al. 2001). This is not to say that plateaus are not present with fast, large-amplitude inputs: only that they are harder to study. Finally, because more firing rate adaptation occurred in motoneurons without plateaus (acute spinal rats; Figs. 6*B* and 7*A*), it is possible that the plateaus themselves may have countered firing rate adaptation (in chronic spinal rats). Thus the presence of I_{PIC} may determine the degree of firing rate adaption and associated nonlinear firing, with the most firing rate adaptation occurring in pentobarbital anesthetized animals where I_{PIC} is blocked (Kernell and Monster 1982), less in the acute spinal unanesthetized case, and the least in the chronic spinal case where I_{PIC} is enhanced (see Lee and Heckman 1998a,b).

Role of plateaus in spasticity

When the dorsal roots were briefly stimulated in chronic spinal rats, a long-lasting reflex was seen in the motoneurons; this reflex is the counterpart of the long-lasting reflex seen in ventral roots and in the tail muscles during spastic behavior (Bennett et al. 1999a). The reflex was markedly reduced in duration by hyperpolarization (Fig. 10*C*), indicating that intrinsic voltage-dependent properties of the motoneurons contribute substantially to these spastic reflexes (i.e., plateaus amplify and prolong the reflexes). Further, spasticity in humans has been associated with tonic or poorly modulated motor unit discharge (Gorassini et al. 1999a; Thomas and Ross 1997), abnormally low firing rates (Powers and Rymer 1988; Thomas and Ross 1997), and impaired rate modulation (Heckman 1994; Wiegner et al. 1993); these findings are each consistent with the emergence of a plateau, as discussed in the preceding text (i.e., increased I_{PIC} and associated conductance, without lower AHP). The finding that the long-lasting reflexes associated with spasticity are mediated in large part by plateaus throws new light on the antispastic action of baclofen, which has recently been shown to inhibit L-type calcium currents and plateaus (Russo et al. 1998; Svirskis and Hounsgaard 1998).

Our results also indicate that the exaggerated reflexes following spinal cord injury are in part produced by a relatively protracted synaptic input (EPSP lasting ~1 s in Fig. 10*C*) (see also Baker and Chandler 1987b). A single low-threshold shock to the dorsal roots is enough to evoke this long EPSP, even at hyperpolarized levels. Considering that plateaus are slow activating, we suggest that this long EPSP serves to prolong the effect of a brief afferent stimulation sufficiently to trigger a plateau, which in turn produces many seconds of firing. A similar long EPSP is seen in motoneurons during flexor-reflex-afferent (FRA) stimulation in DOPA-treated acute spinal cats, which is likewise prolonged by plateau potentials intrinsic to the motoneurons (Conway et al. 1988).

As we have described in the preceding text, the plateau potential amplitude after chronic injury recovers to a level comparable to that estimated in normal intact animals, so the fact that we do *not* see spastic-like long-lasting reflexes in intact animals and humans likely involves major differences in inhibitory, as well as excitatory, control of motoneurons in intact and spinal states. Moderately long-lasting stimulation (1-s muscle vibration) can trigger self-sustained motor unit firing in normal humans (plateau) (Gorassini et al. 1998, 2001a), but this firing can be inhibited easily by descending inhibition (e.g., reduction in volitional effort). This descending inhibition is lacking following complete spinal cord injury.

In summary, following chronic injury, three factors combine in the production of spasticity: moderately long-lasting synaptic events emerge in response to brief, low-threshold afferent stimuli, plateau behavior is recovered and prolongs these synaptic events by many seconds, and the normal inhibitory control is lacking, enabling firing to continue and ultimately contribute to protracted muscle spasms and hypertonus associated with spasticity.

We thank L. Sanelli and Y. Han for expert technical assistance and P. Harvey, F. Giddes, and M. Gorassini for comments on the manuscript.

Funding was provided by the Medical Research Council, the National Sciences and Engineering Research Council, and Canadian Foundation for Innovation of Canada, and the Alberta Heritage Foundation for Medical Research.

REFERENCES

AGHAJANIAN GK AND RASMUSSEN K. Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices. *Synapse* 3: 331–338, 1989.

ASHBY P AND MCCREA DA. Neurophysiology of spinal spasticity. In: *Handbook of the Spinal Cord*, edited by Davidoff RA. New York: Dekker, 1987, p. 120–143.

BAKER LL AND CHANDLER SH. Characterization of hindlimb motoneuron membrane properties in acute and chronic spinal cats. *Brain Res* 420: 333–339, 1987a.

BAKER LL AND CHANDLER SH. Characterization of postsynaptic potentials evoked by sural nerve stimulation in hindlimb motoneurons from acute and chronic spinal cats. *Brain Res* 420: 340–350, 1987b.

BENNETT DJ, HULTBORN H, FEDIRCHUK B, AND GORASSINI M. Synaptic activation of plateaus in hindlimb motoneurons of decerebrate cats. *J Neurophysiol* 80: 2023–2037, 1998a.

BENNETT DJ, HULTBORN H, FEDIRCHUK B, AND GORASSINI M. Short-term plasticity in hindlimb motoneurons of decerebrate cats. *J Neurophysiol* 80: 2038–2045, 1998b.

BENNETT DJ, GORASSINI M, FOUAD K, SANELLI L, HAN Y, AND CHENG J. Spasticity in rats with sacral spinal cord injury. *J Neurotrauma* 16: 69–84, 1999a.

BENNETT DJ, GORASSINI MA, AND SIU M. *In vitro* preparation to study spasticity in chronic spinal rats. *Soc Neurosci Abstr* 25: 1394, 1999b.

BENNETT DJ, LI Y, HARVEY PJ, AND GORASSINI M. Evidence for plateau potentials in tail motoneurons of awake chronic spinal rats with spasticity. *J Neurophysiol* 86: 1972–1982, 2001.

BERGER AJ, BAYLISS DA, AND VIANA F. Modulation of neonatal rat hypoglossal motoneuron excitability by serotonin. *Neurosci Lett* 143: 164–168, 1992.

BINDER MD, HECKMAN CJ, AND POWERS RK. The physiological control of motoneuron activity. In: *The Handbook of Physiology*. Oxford, NY: Am Physiol Soc, 1996, sect. 12, chapt.1, p. 3–53.

BLASCHAK MJ, POWERS RK, AND RYMER WZ. Disturbances of motor output in a cat hindlimb muscle after acute dorsal spinal hemisection. *Exp Brain Res* 71: 377–387, 1988.

BURKE D, GILLIES JD, AND LANCE JW. The quadriceps stretch reflex in human spasticity. *J Neurol Neurosurg Psychiatry* 33: 216–223, 1970.

CARP JS, POWERS RK, AND RYMER WZ. Alterations in motoneuron properties induced by acute dorsal spinal hemisection in the decerebrate cat. *Exp Brain Res* 83: 539–548, 1991.

CAVALLARI P AND PETTERSSON LG. Tonic suppression of reflex transmission in low spinal cats. *Exp Brain Res* 77: 201–212, 1989.

CONWAY BA, HULTBORN H, KIEHN O, AND MINTZ I. Plateau potentials in alpha-motoneurons induced by intravenous injection of L-dopa and clonidine in the spinal cat. *J Physiol (Lond)* 405: 369–384, 1988.

COPE TC, BODINE SC, FOURNIER M, AND EDGERTON VR. Soleus motor units in chronic spinal transected cats: physiological and morphological alterations. *J Neurophysiol* 55: 1202–1220, 1986.

EKEN T, HULTBORN H, AND KIEHN O. Possible functions of transmitter-controlled plateau potentials in alpha-motoneurones. In: *Progress in Brain Research*. Amsterdam: Elsevier, 1989, vol. 80, chapt. 21, p. 257–267.

GORASSINI MA, BENNETT DJ, EKEN T, KIEHN O, AND HULTBORN H. Activation patterns of hindlimb motoneurons in the awake rat and their relation to motoneuron intrinsic properties. *J Neurophysiol* 82: 709–717, 1999a.

GORASSINI MA, BENNETT DJ, AND YANG JF. Self-sustained firing of human motor units. *Neurosci Lett* 247: 13–16, 1998.

GORASSINI M, BENNETT DJ, AND YANG JF. Excitability of motor units in persons with spasticity from spinal cord injury. *Soc Neurosci Abstr* 25: 120, 1999b.

GORASSINI MA, YANG J, SIU M, AND BENNETT DJ. Possible role of plateau potentials in human motoneuron discharge: sustained voluntary contractions. *J Neurophysiol*. In press, 2001a.

GORASSINI MA, YANG J, SIU M, AND BENNETT DJ. Possible role of plateau potentials in human motoneuron discharge: short-term facilitation by repeated contractions. *J Neurophysiol*. In press, 2001b.

GUERTIN PA AND HOUNSGAARD J. NMDA-induced intrinsic voltage oscillations depend on L-type calcium channels in spinal motoneurons of adult turtles. *J Neurophysiol* 80: 3380–3382, 1998.

GUERTIN PA AND HOUNSGAARD J. Non-volatile general anaesthetics reduce spinal activity by suppressing plateau potentials. *Neuroscience* 88: 353–358, 1999.

GUSTAFSSON B, KATZ R, AND MALMSTEN J. Effects of chronic partial deafferentation on electrical properties of lumbar alpha-motoneurons in the cat. *Brain Res* 246: 23–33, 1982.

HECKMAN CJ. Alterations in synaptic input to motoneurons during partial spinal cord injury. *Med Sci Sports Exerc* 26: 1480–1490, 1994.

HOCHMAN S, JORDAN LM, AND SCHMIDT BJ. TTX-resistant NMDA receptor-mediated voltage oscillations in mammalian lumbar motoneurons. *J Neurophysiol* 72: 2559–2562, 1994.

HOCHMAN S AND MCCREA DA. Effects of chronic spinalization on ankle extensor motoneurons. II. Motoneuron electrical properties. *J Neurophysiol* 71: 1468–1479, 1994.

HODGKIN AL. The local electric changes associated with repetitive action in a non-medullated nerve. *J Physiol (Lond)* 107: 165–181, 1948.

HOUNSGAARD J, HULTBORN H, JESPERSEN B, AND KIEHN O. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J Physiol (Lond)* 405: 345–367, 1988.

HOUNSGAARD J AND KIEHN O. Serotonin-induced bistability of turtle motoneurones caused by a nifedipine-sensitive calcium plateau potential. *J Physiol (Lond)* 414: 265–282, 1989.

HULTBORN H AND KIEHN O. Neuromodulation of vertebrate motor neuron membrane properties. *Curr Opin Neurobiol* 2: 770–775, 1992.

HULTBORN H AND MALMSTEN J. Changes in segmental reflexes following chronic spinal cord hemisection in the cat. I. Increased monosynaptic and polysynaptic ventral root discharges. *Acta Physiol Scand* 119: 405–422, 1983a.

HULTBORN H AND MALMSTEN J. Changes in segmental reflexes following chronic spinal cord hemisection in the cat. II. Conditioned monosynaptic test reflexes. *Acta Physiol Scand* 119: 423–433, 1983b.

JANKOWSKA E, JUKES MGM, LUND S, AND LUNDBERT A. The effect of DOPA on the spinal cord. VI. Half-centre organization of interneurones transmitting effects from the flexor reflex afferents. *Acta Physiol Scand* 70: 389–402, 1967.

JIANG MC, CLELAND CL, AND GEBHART GF. Intrinsic properties of deep dorsal horn neurons in the L_6–S_1 spinal cord of the intact rat. *J Neurophysiol* 74: 1819–1827, 1995.

KERNELL D. Repetitive impulse firing in motoneurons: facts and perspectives. *Prog Brain Res* 123: 31–37, 1999.

KERNELL D AND MONSTER AW. Time course and properties of late adaptation in spinal motoneurones of the cat. *Exp Brain Res* 46: 191–196, 1982.

KIEHN O AND EKEN T. Prolonged firing in motor units—evidence of plateau potentials in human motoneurons? *J Neurophysiol* 78: 3061–3068, 1997.

KIEHN O, JOHNSON BR, AND RAASTAD M. Plateau properties in mammalian spinal interneurons during transmitter-induced locomotor activity. *Neuroscience* 75: 263–273, 1996.

KIERNAN JA. *Histological and Histochemical Methods Theory and Practice*. Elmsford, NY: Pergamon, 1990.

KRENZ NR AND WEAVER LC. Sprouting of primary afferent fibers after spinal cord transection in the rat. *Neuroscience* 85: 443–458, 1998.

KUHN RA AND MACHT MB. Some manifestations of reflex activity in spinal man with particular reference to the occurrence of extensor spasm. *Bull Johns Hopkins Hosp* 84: 43–75, 1948.

LEE RH AND HECKMAN CJ. Bistability in spinal motoneurons in vivo: systematic variations in rhythmic firing patterns. *J Neurophysiol* 80: 572–582, 1998a.

LEE RH AND HECKMAN CJ. Bistability in spinal motoneurons in vivo: systematic variations in persistent inward currents. *J Neurophysiol* 80: 583–593, 1998b.

LONG SK, EVANS RH, CULL L, KRIJZER F, AND BEVAN P. In vitro mature spinal cord preparation from the rat. *Neuropharmacology* 27: 541–546, 1988.

MA JY, CATTERALL WA, AND SCHEUER T. Persistent sodium currents through brain sodium channels induced by G protein betagamma subunits. *Neuron* 19: 443–452, 1997.

MAILIS A AND ASHBY P. Alterations in group Ia projections to motoneurons following spinal lesions in humans. *J Neurophysiol* 64: 637–647, 1990.

MCNICHOLAS LF, MARTIN WR, SLOAN JW, AND NOZAKI M. Innervation of the spinal cord by sympathetic fibers. *Exp Neurol* 69: 383–394, 1980.

MCQUISTON AR AND MADISON DV. Muscarinic receptor activity induces an afterdepolarization in a subpopulation of hippocampal CA1 interneurons. *J Neurosci* 19: 5703–5710, 1999.

MORISSET V AND NAGY F. Ionic basis for plateau potentials in deep dorsal horn neurons of the rat spinal cord. *J Neurosci* 19: 7309–7316, 1999.

NEWTON BW AND HAMILL RW. The morphology and distribution of rat serotoninergic intraspinal neurons: an immunohistochemical study. *Brain Res Bull* 20: 349–360, 1988.

NICHOLSON C AND HOUNSGAARD J. Diffusion in the slice microenvironment and implications for physiological studies. *Fed Proc* 42: 2865–2868, 1983.

NIELSEN J AND HULTBORN H. Regulated properties of motoneurons and primary afferents: new aspects on possible spinal mechanisms underlying spasticity. In: *Spasticity: Mechanisms and Management*, edited by Thilmann AF. Berlin: Springer-Verlag, 1993, p. 177–191.

NOTH J. Trends in the pathophysiology and pharmacotherapy of spasticity. *J Neurol* 238: 131–139, 1991.

POWERS RK, CAMPBELL DL, AND RYMER WZ. Stretch reflex dynamics in spastic elbow flexor muscles. *Ann Neurol* 25: 32–42, 1989.

POWERS RK AND RYMER WZ. Effects of acute dorsal spinal hemisection on motoneuron discharge in the medial gastrocnemius of the decerebrate cat. *J Neurophysiol* 59: 1540–1556, 1988.

REKLING JC, FUNK GD, BAYLISS DA, DONG XW, AND FELDMAN JL. Synaptic control of motoneuronal excitability. *Physiol Rev* 80: 767–852, 2000.

REMY-NERIS O, BARBEAU H, DANIEL O, BOITEAU F, AND BUSSEL B. Effects of intrathecal clonidine injection on spinal reflexes and human locomotion in incomplete parapalegic subjects. *Exp Brain Res* 129: 433–440, 1999.

RUSSO RE AND HOUNSGAARD J. Plateau-generating neurones in the dorsal horn in an in vitro preparation of the turtle spinal cord. *J Physiol (Lond)* 493: 39–54, 1996.

RUSSO RE AND HOUNSGAARD J. Dynamics of intrinsic electrophysiological properties in spinal cord neurones. *Prog Biophys Mol Biol* 72: 329–365, 1999.

RUSSO RE, NAGY F, AND HOUNSGAARD J. Modulation of plateau properties in dorsal horn neurones in a slice preparation of the turtle spinal cord. *J Physiol (Lond)* 499: 459–474, 1997.

RUSSO RE, NAGY F, AND HOUNSGAARD J. Inhibitory control of plateau properties in dorsal horn neurones in the turtle spinal cord *in vitro*. *J Physiol (Lond)* 506: 795–808, 1998.

SCHWINDT PC AND CRILL WE. Membrane properties of cat spinal motoneurons. In: *Handbook of the Spinal Cord*, edited by Davidoff RA. New York: Marcel Dekker, 1984, p. 199–242.

SCHWINDT PC AND CRILL WE. Amplification of synaptic current by persistent sodium conductance in apical dendrite of neocortical neurons. *J Neurophysiol* 74: 2220–2224, 1995.

STEG G. Efferent muscle innervation and rigidity. *Acta Physiol Scand* 61: 1–53, 1964.

STEERS WD. Rat: overview and innervation. *Neurourol Urodyn* 13: 97–118, 1994.

STEPHENS M, BOBET J, HAN L, SANELLI L, AND BENNETT DJ. Changes in muscle mechanical properties following chronic sacral spinal cord injury in rats. *Soc Neurosci Abstr* 25: 1145, 1999.

SVIRSKIS G AND HOUNSGAARD J. Depolarization-induced facilitation of a plateau-generating current in ventral horn neurons in the turtle spinal cord. *J Neurophysiol* 78: 1740–1742, 1997.

SVIRSKIS G AND HOUNSGAARD J. Transmitter regulation of plateau properties in turtle motoneurons. *J Neurophysiol* 79: 45–50, 1998.

TAYLOR JS, FRIEDMAN RF, MUNSON JB, AND VIERCK CJ JR. Stretch hyperreflexia of triceps surae muscles in the conscious cat after dorsolateral spinal lesions. *J Neurosci* 17: 5004–5015, 1997.

THILMANN AF, FELLOWS SJ, AND GARMS E. The mechanism of spastic muscle hypertonus. Variation in reflex gain over the time course of spasticity. *Brain* 114: 233–244, 1991.

THOMAS CK AND ROSS BH. Distinct patterns of motor unit behavior during muscle spasms in spinal cord injured subjects. *J Neurophysiol* 77: 2847–2850, 1997.

THOMPSON FJ, PARMER R, AND REIER PJ. Alteration in rate modulation of reflexes to lumbar motoneurons after midthoracic spinal cord injury in the rat. I. Contusion injury. *J Neurotrauma* 15: 495–508, 1998.

WIEGNER AW, WIERZBICKA MM, DAVIES L, AND YOUNG RR. Discharge properties of single motor units in patients with spinal cord injuries. *Musc Nerve* 16: 661–671, 1993.

YOUNG RR. Spasticity: a review. *Neurology* 44: S12–S20, 1994.