Spastic Long-Lasting Reflexes in the Awake Rat After Sacral Spinal Cord Injury

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Bennett, D. J., L. Sanelli, C. L. Cooke, P. J. Harvey, and M. A. Gorassini. Spastic long-lasting reflexes in the awake rat after sacral spinal cord injury. J Neurophysiol 91: 2247–2258, 2004; 10.1152/jn.00946.2003. Following chronic sacral spinal cord transection in rats the affected tail muscles exhibit marked spasticity, with characteristic long-lasting tail spasms evoked by mild stimulation. The purpose of the present paper was to characterize the long-lasting reflex seen in tail muscles in response to electrical stimulation of the tail nerves in the awake spastic rat, including its development with time and relation to spasticity. Before and after sacral spinal transection, surface electrodes were placed on the tail for electrical stimulation of the caudal nerve trunk (mixed nerve) and for recording EMG from segmental tail muscles. In normal and acute spinal rats caudal nerve trunk stimulation evoked little or no EMG reflex. By 2 wk after injury, the same stimulation evoked long-lasting reflexes that were 1) very low threshold, 2) evoked from rest without prior EMG activity, 3) of polysynaptic latency with >6 ms central delay, 4) about 2 s long, and 5) enhanced by repeated stimulation (windup). These reflexes produced powerful whole tail contractions (spasms) and developed gradually over the weeks after the injury (≤52 wk tested), in close parallel to the development of spasticity. Pure low-threshold cutaneous stimulation, from electrical stimulation of the tip of the tail, also evoked long-lasting spastic reflexes, not seen in acute spinal or normal rats. In acute spinal rats a strong C-fiber stimulation of the tip of the tail (20 × T) could evoke a weak EMG response lasting about 1 s. Interestingly, when this C-fiber stimulation was used as a conditioning stimulation to depolarize the motoneuron pool in acute spinal rats, a subsequent low-threshold stimulation of the caudal nerve trunk evoked a 300–500 ms long reflex, similar to the onset of the long-lasting reflex in chronic spinal rats. A similar conditioned reflex was not seen in normal rats. Thus there is an unusually long low-threshold polysynaptic input to the motoneurons (pEPSP) that is normally inhibited by descending control. This pEPSP is released from inhibition immediately after injury but does not produce a long-lasting reflex because of a lack of motoneuron excitability. With chronic injury the motoneuron excitability is increased markedly, and the pEPSP then triggers sustained motoneuron discharges associated with long-lasting reflexes and muscle spasms.

INTRODUCTION

The debilitating spasms that accompany spinal cord injury develop slowly are often not fully manifested for months after injury (Kuhn and Macht 1948). It is therefore likely that these spasms are a consequence of long-term plastic changes in spinal neuronal properties due to chronic injury. Acutely, hours or days after the injury, the spinal cord is nonexcitable. This lack of excitability is predictable from a number of studies of the intrinsic electrical properties of motoneurons, which have shown that motoneuron excitability is dependent on inputs descending from the brain stem and releasing the neuromodulators serotonin and norepinephrine, and this is lost after injury (Conway et al. 1988; Hounsgaard et al. 1988; Lee and Heckman 1999). Such neuromodulators allow the motoneuron to exhibit sustained depolarizations known as plateau potentials, which are due to slowly activating voltage-dependent persistent inward currents (PICs), in large part mediated by the low-voltage-activated Cav1.3 L-type calcium channels in the dendrites (Hounsgaard and Kiehn 1989; Perrier and Houns-gaard 2003; Powers and Binder 2001). Loss of the PICs after injury so greatly decreases motoneuron excitability that even strong reflex inputs have only weak effects (Bennett et al. 1999). However, recent studies in an in vitro sacral spinal rat model of chronic spinal injury (Bennett et al. 2001c; Li and Bennett 2003), and confirmed in awake rats and humans (Bennett et al. 2001a; Gorassini et al. 1999, 2004), show that motoneurons slowly recover their excitability over the weeks after injury by redeveloping the capacity to generate large PICs. These PICs enable motoneurons to produce vigorous discharges in response to brief inputs that, without the normal descending inhibitory control, ultimately produce the many second long spasms seen with chronic injury (Bennett et al. 2001a; Gorassini et al. 1999, 2003; Li et al. 2004a). In the in vitro sacral spinal rat model, these PICs cause long-lasting reflexes (seconds) in the tail motoneurons evoked by brief low-threshold afferent stimulation (Bennett et al. 2001c; Li et al. 2004a) and thus should ultimately produce whole tail spasms in awake chronic spinal rat (Bennett et al. 1999, 2001a). The present paper examines in the awake rat what sorts of synaptic inputs trigger these PIC-mediated long-lasting reflexes and how these inputs change with time after injury.

In contrast to the excitatory brain stem effects on motoneurons, descending brain stem inputs to the dorsal horn, especially monoaminergic, have a predominantly inhibitory affect on many afferents and interneurons, including those involved in flexor withdrawal reflexes (Baldissera et al. 1981; Clarke et al. 1996, 2002; Heckman 1994; Jankowska 1992; Jankowska et al. 2000; Lo et al. 2003). This descending inhibition is carried largely by the ipsilateral dorsal lateral funiculus (DLF), because injuries to the DLF cause major increases in reflex transmission (i.e., release of descending inhibition), whereas injuries that spare only the DLF do not cause this release of inhibition (see excellent review in Lundberg 1982). Thus without the massive loss of motoneuron excitability that occurs immediately after spinal transection, hyperexcitable reflexes...
should in principle develop immediately after injury. Indeed, with partial spinal cord lesions that only affect the descending innervation of the dorsal horn (DLF lesions), but not ventral horn (and thus spare the motoneurons PICs), spastic symptoms do develop acutely (Heckman 1994; Taylor et al. 1999), and segmental reflexes are in general augmented (Lundberg 1982). With complete spinal transections, low- and high-threshold afferent evoked inputs to the motoneurons [excitatory postsynaptic potentials (EPSPs)] are also enhanced immediately after injury (Baker and Chandler 1987; Cook and Woolf 1985; Crenna et al. 1982; Li et al. 2004a; Lundberg 1982). However, these EPSPs do not initially evoke large spastic reflexes because of the lack of motoneuron excitability (Bennett et al. 2001c; Li et al. 2004a). For example, in acute spinal rats and cats, there are unusually long polysynaptic EPSPs (~200–500 ms) in response to a low-threshold cutaneous stimulation pulse (Baker and Chandler 1987; Crenna et al. 1982; Li et al. 2004a) that are not seen in spinal cord intact preparations (Crenna et al. 1982; Heckman et al. 1994). However, these EPSPs do not trigger spasms immediately after injury. These low-threshold polysynaptic EPSPs are N-methyl-d-aspartate (NMDA)-dependent (Bennett et al. 2001b; Clarke et al. 2002) and particularly important in triggering spasms after chronic injury, because a single EPSP is sufficiently long (>200 ms) to evoke the slowly activating PICs in motoneurons that cause a many second long discharge (Bennett et al. 2001c; Li et al. 2004a). Likewise, the normal depression of mono- and polysynaptic reflexes with repeated stimulation is lost after injury (Mailis and Ashby 1990; Thompson et al. 1992), and the cutaneous polysynaptic reflexes are instead enhanced with repeated stimulation (windup of C-fiber and low threshold reflexes; Clarke et al. 2002; Gozariu et al. 1997; Li et al. 2004b). Thus repeated inputs can summate to produce sufficiently long EPSPs to trigger PICs and spasms in chronic injury (Li et al. 2004a).

These results were obtained mostly from reduced animal preparations (in vitro and decerebrate animals) (Bennett et al. 2001c; Li et al. 2004a), and it is not clear whether these low-threshold polysynaptic EPSPs are present prior to injury in the awake animal or whether they occur in the awake acute spinal animal. Furthermore, it is not clear how the above research on descending reflex control of hindlimb reflexes/EPSPs relates to tail muscle reflexes. Thus one purpose of the present experiments was to, in awake rats, indirectly test for the presence of a low-threshold polysynaptic EPSPs before, and at various times after, injury by examining the associated reflex EMG responses to tail nerve stimulation (Bennett et al. 1999). In the acute spinal state where reflexes are difficult to evoke (Bennett et al. 1999), a strong prior conditioning was used to evoke a weak tonic contraction (C-fiber reflex) and thus depolarize the motoneurons so that subsequent low threshold stimulation could produce an EMG reflex response (if present), even though it might otherwise only produce a subthreshold EPSP (Clarke et al. 2002). Furthermore, we tested in the awake rat whether this low-threshold reflex was amplified and prolonged with long-term injury, as would be expected from the emergence of PICs and the similar long-lasting reflexes seen with in vitro recordings from the same rats (Li et al. 2004). Finally, a related purpose was to characterize the long-lasting reflexes seen in the awake rat muscles with tail nerve stimulation, in terms of their threshold, latency, sensitivity to repeated stimulation, and time course of development, to ultimately confirm that these are the same reflexes that are recorded from ventral roots in response to dorsal root stimulation when the sacrocaudal spinal cord from the same rats is maintained in vitro (Li et al. 2004a,b). This is critical for validating the use of the in vitro sacrocaudal spinal cord technique for studying spasticity after spinal cord injury.

METHODS

Tail muscle reflex testing was performed in 19 awake adult Sprague-Dawley rats (initial weight, 250–500 g; age, 45–90 days old at transection) before, and at regular intervals after, sacral spinal transection. A further 22 adult rats were studied in acute experiments to establish reflex latencies and study the histology of the sacrocaudal spinal cord. All procedures were approved by the University of Alberta animal welfare committee.

Surgery

The S2 sacral spinal cord was transected in rats as described in Bennett et al. (1999). Briefly, under general anesthetic (sodium pentobarbital, 58.5 mg/kg) and sterile conditions, a laminectomy was performed on the L2 vertebral to expose the S2 spinal cord (see Fig. 9). The dura was slit transversely, and 0.1–0.3 ml Xylocaine (1%) was applied topically. Under a surgical microscope, the spinal cord was transected by holding the pia with fine forceps and sucking under the pia with a fine suction tip (made by heating and pulling a 1-ml syringe to a 0.1- to 0.2-ml tip). Extreme caution was needed to avoid damaging the anterior artery or posterior/dorsal vein, since the sacrocaudal spinal cord dies without this midline vasculature (Bennett et al. 1999). Even if the vasculature is occluded briefly, the spinal cord is damaged, because it leads to a less active preparation with much less spasticity. Also, we found that having the animal breathe pure oxygen during this period when the vessels are manipulated can improve the outcome. Usually, a vertical V-shaped 1- to 2-mm section of the cord was removed to provide good visibility and to assure that the transection was complete. The dura was closed with two 8-0 silk sutures, and the muscle layers and skin were tightly sutured over the cord.

Clinical assessment of spasticity

Following transection, rats were observed on a daily basis for the first week, and then on a weekly basis, to assess the development of spasticity in the tail. During all observations, the rats were housed in a plexiglas tube (Fig. 1), with the tail protruding out one end, free to hang vertically. The tail was stimulated manually with a standardized stretch/rub maneuver. This involved holding the base of the tail with the left hand, gripping nearby (caudally) with a damp piece of gauze held between the right thumb and fingers, and rapidly sliding this grip down the tail while maintaining firm contact for the length of the tail. This applied a lengthwise sliding stretch, similarly to how one would
very sensitive to light touch and withdrawal to even touch of a single coiling and clonus lasting tip of the tail and lasting 3–5 min after the tail was worked up by the initial three stretch/rub stimuli (as in Bennett et al. 1999). Hypertonus sensitivity and cutaneous sensitivity were assessed by gently bending the tail or light touch with von Frey hairs, respectively. To quantify the spasticity, a rating system (Table 1 of Bennett et al. 1999) was employed and is summarized as follows.

**RATING 0–1.** Very weak or nonexistent coiling of the tip of the tail in response to stretch/rub (<90° and lasting only a few seconds), little of no muscle tone, no clonus, no response to light touch of skin or hairs.

**RATING 2–3.** Strong flexor direction coiling spasms of whole tail in response to stretch/rub, with maximum excursions of 180–360° in the tip of the tail and lasting 3–10 s each. Repeated flexion spasms (coiling) and clonus lasting >10 min after stretch/rub; hypertonus; very sensitive to light touch and withdrawal to even touch of a single hair.

**RATING 4–5.** Similar to previous rating (2–3), but with extensor as well as flexor coiling spasms and responses to stretch/rub, so that the tail often obtained an S-shape with the proximal portion flexing under the rat, and the distal portion extending in the opposite direction; also, greater clonus, hypertonus, and side-to-side whipping.

To minimize variability, all rats were rated by the same experimenter, just prior to EMG recording of tail reflexes.

**Tail nerve trunk stimulation and EMG recording of segmental tail muscle reflexes**

The caudal nerve trunk was electrically stimulated near the base of the tail to provide a stimulation of all the afferents of the distal tail and evoke distal tail muscle reflexes (Bennett et al. 1999). Specifically, surface electrodes were placed on the tail for stimulation and EMG recording while the awake rat was in the plexiglas cylinder, as shown in Fig. 1. Custom-built cuff electrodes were used, made from silver wire sewn into short sections of tightly fitting Tygon tubing that were slit open on one side to allow them to be opened, filled with electrode gel, and snapped closed over the tail. Electrodes were placed relative to a standard reference point about halfway down the tail (C₈₉tail vertebrae; positive numbers are distal to this point): caudal nerve trunk stimulation anode, −4.5 cm; cathode, −2.5 cm; recording ground, −1 cm; segmental muscle EMG pair, +1 and +2.5 cm. Thorough cleaning of the tail with soap and alcohol (scrub off scales) was imperative to lower the electrode impedance sufficiently to obtain EMG recordings without large stimulus artifacts.

The caudal nerve trunk (Fig. 1) originates from sacrocaudal spinal roots in the sacrum and travels the length of the tail (in 4 nerves: a dorsal and ventral nerve trunk on each side of the tail) to innervate the intrinsic muscles of the tail (segmental muscles), as well as joint and skin afferents (Bennett et al. 2001a; Steg 1964; Thompson 1970). The tail is controlled by both the segmental muscles that span each tail vertebrae and larger muscles that originate at the base of the tail and have long tendons that attach to each tail vertebrae (there are about 24 vertebrae/tail). Both of these muscle types are controlled by the spinal cord below the sacral S₂ lesion (see RESULTS). However, the above arrangement only records from the segmental muscles in the distal tail.

The stimulation parameters were 0.2-ms pulses usually delivered at 2- or 20-s intervals, with an amplitude varying from 0.5 to 10 mA. From separate acute experiments (described below), we found that 0.5–0.7 mA was consistently the afferent threshold (T; obtained from dorsal root recording), and 1–2 mA was the motor threshold. Stimuli were often expressed as multiples of motor threshold (MT, when direct activation could be seen in the EMG; M-wave in Fig. 2). EMG was recorded with a gain of 2,500, filtered with a first-order 100-Hz high-pass filter to remove movement artifact, low-pass filtered at 5 kHz, and sampled at 10 kHz. Average responses of the long-lasting reflexes were obtained from multiple trials by rectifying and smoothing the EMG (100-Hz low-pass filter). The reflex was quantified by measuring the peak-rectified smoothed EMG tonic response averaged over a 50- to 250-ms period after the stimulus and total duration (as shown in Fig. 3A). The tail skin temperature was maintained at 29°C (its usual temperature before the experiment) with a heating lamp and a temperature probe taped under the tail just rostral to the caudal nerve trunk stimulating electrodes.

**Cutaneous stimulation of tip of tail**

In addition to the above electrodes used to record the whole nerve trunk reflex, a pair of stimulation electrodes were placed 1 cm apart on the distal tip of the tail. Since this portion of the tail is highly sensitive to touch and contains very little muscle, this pair was used to provide a relatively pure cutaneous activation. Stimulation parameters were similar to above and expressed relative to afferent threshold (0.3–0.5 mA, T), which corresponded to the current where slight reflex movements could be seen (verified with dorsal root recordings in control experiments).

**EMG reflex testing procedure**

Before and at regular intervals after transection, EMG recordings were made from rats to track the changes that occurred in reflexes over time (daily for the first week and then weekly). Each recording session involved 1) testing the whole nerve trunk reflex with varying currents under resting conditions (2-s interval between stimuli); 2) repeat of 1, but with strong conditioning stimulation of the tip of the tail (20 × T) applied 300–900 ms prior to reflex testing to generate background EMG; 3) repeat of 1, but with varying intervals between stimuli (0.5–20 s) and current fixed at 1–2 × MT.

**Retrograde labeling of motoneurones involved in caudal nerve trunk reflex**

Since the S₂ transection was very low, we wanted to determine whether the lesion directly damaged the motoneurones involved in our caudal nerve trunk reflex testing. Thus separate control experiments were carried out in normal (n = 6) and chronically lesioned rats (n = 4). Under deep sodium pentobarbital anaesthesia (58.5 mg/kg), the caudal nerve trunk branches (both dorsal and ventral portions) were exposed at our standard reference point halfway down the tail (C₈₉tail), and retrograde tracers fluororuby (dextran tetramethylrhodamine, Molecular Probes) or fluorogold (4% in cacodylic acid, Fluorochrome) were applied to the freshly cut proximal stumps and given 2–3 h to absorb (Boyd and Gordon 2002). Following 1 wk for retrograde transport of the tracers to the motoneurones, the animal was killed and perfused with saline and 4% paraformaldehyde. After further overnight fixation of the whole spinal column, the spinal cord was removed, while making careful note of the location of the roots relative to the bone segments and marking the cord with a slit at the L₅ level as a reference point. The cord was dehydrated in a solution of 30% sucrose and 4% paraformaldehyde, frozen, and sliced longitudinally on a cryostat in 50-μm sections. Labeled motoneurones were counted with a fluorescence microscope at 1.25-mm intervals relative to the lumbar level to determine the longitudinal distribution of the tail motoneurone pools (described later in Fig. 9). In four additional normal rats without retrograde tracers, the cord was fixed and sliced in 50-μm

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sections as above and stained with cresyl violet and silver nitrate to label all neurons (Bennett et al. 1999). In these rats, the total number of putative motoneurons (large ventral horn cells, average 35 μm) were counted for comparison to the distributions of segmental motoneurons. All cell counts were corrected according to Abercrombie’s method (Abercrombie 1946) to compensate for multiple counting of cells.

Control experiments in pentobarbital-anesthetized rats

To identify the affereents involved in the spastic rat reflex and their peripheral conduction latencies, separate experiments were carried out on an additional four chronic spinal and four normal rats.

Each rat was anesthetized with sodium pentobarbital (58.5 mg/kg) and placed in a stereotaxic frame, an L2-L6 laminectomy was performed, and an oil pool was formed over the exposed spinal cord with the skin flaps. The dorsal or ventral roots were cut close to the spinal cord, and the distal ends were lifted onto pairs of bipolar recording electrodes (Bennett et al. 1996). The caudal nerve trunks were stimulated with surface electrodes, as above, and the incoming volley was recorded on the roots. Recordings were amplified with a gain of 10,000, low-pass filtered at 10 kHz, and sampled at 20 kHz. Dorsal root responses to manual tail skin stimulation were also recorded to determine the tail dermatomes. The spinal cord was kept at 36°C with a regulated heat lamp.

Means ± SD are shown in all text and figures. Statistical significance was tested at the 95% confidence level with the Student t-test.

RESULTS

Normal and acute spinal reflexes with caudal nerve trunk stimulation: resting state

When the caudal nerve trunk was stimulated near the base of the tail (Fig. 1) in normal or acutely injured (<1 wk after injury) rats, little or no reflex was evoked in the segmental tail muscle EMG recordings (Fig. 2, A–C), regardless of the stimulation current intensity or rate of stimulation. Importantly, in both normal and acute spinal rats, there was never a long-lasting reflex evoked by this caudal nerve trunk stimulation (Fig. 2, A and B). When a small reflex did occur, it was very brief, had a relatively low threshold (0.9 × MT, as in Fig. 2A), and occurred with a short 18- to 24-ms latency (although later than the monosynaptic latency of 12–14 ms; see below). In normal rats, this reflex was variable (state-dependent), at times occurring when there was some ongoing EMG (Fig. 2A) and at other times not occurring at all. In some normal rats, the reflex inhibited the ongoing EMG activity (inhibitory reflex; see below). When the stimulus current was increased sufficiently, the motor axons were activated directly (>MT); thus a direct EMG response occurred at about a 2-ms delay (M-wave), and there was still little or no reflex (Fig. 2C, although a small F-wave sometimes occurred at high stimulation intensities at a 12-ms latency; data not shown; Hultborn and Nielsen 1995).

Chronic spinal reflexes: resting state

In contrast, stimulation of the caudal nerve trunk in chronic spinal rats (2–52 wk after injury) produced pronounced long-lasting segmental tail muscle reflexes (Fig. 2, D and E; note the long time scale shown in Fig. 2E). These reflexes occurred even when there was no prior EMG activity (as in Fig. 2, D and E; resting state). The average reflex in chronic spinal rats was very large, with its peak response approaching 30% of the maximal M-wave amplitude (Fig. 2D). Considering that the reflex was not a synchronous motor unit activation, in contrast to the M-wave, it is likely that the reflex activated substantially more than 30% of the motor units. There was often a 50-ms pause following the initial peak in the reflex, consistent with a substantial recruitment of the motoneurons and associated refractory period (Figs. 2D and 3A). Furthermore, when there was moderate background activity before the reflex, the reflexive change in EMG was often smaller than at rest, as if the EMG amplitude during the reflex was saturated (see Fig. 7F). Consistent with this, there was not a significant increase in reflex size with background EMG, contrary to that expected in normal reflexes and graded depolarization of the motoneuron pool (Matthews 1986).

REFLEX LATENCIES AND COMPONENTS. Usually (14/19 rats) the main reflex started at a polysynaptic latency of 18-–24 ms (about 6–12 ms of which was the central reflex delay; see quantification of expected central and peripheral latencies described below), although occasionally (5/19 rats) a small component was seen at 12–14 ms, the expected monosynaptic reflex latency (Fig. 2D, *). With the exception of the monosynaptic component, the reflex involved asynchronous firing of motor units (Fig. 2, D and E), and thus the average reflex, the various components could be discerned: an initial peak (at about 30 ms, nonmonosynaptic), followed by a 25- to 50-ms pause, and long-lasting tonic firing that lasted for 2.4 ± 1.1 s (tonic component, Fig. 3A; see also Fig. 2, D and E).

![Fig. 2. Reflexes in the awake rat before and after spinal cord injury. A: normal rat, segmental tail muscle EMG response to stimulation of the caudal nerve trunk (at dotted line). Single pulse at 0.9 × MT, repeated at 0.5 Hz (3 sweeps shown). B: same as A, but in acute spinal rat (1 day after S2 transection). C: response to stimulation just above motor threshold (1.5 × MT) in acute spinal rat. Note the M-wave and absence of reflex. D: reflex response in same rat as in A–C, but 4 wk after injury (chronic spinal, 1.5 × MT). Note the M-wave, as in C, but this is followed by a long-lasting reflex. *Small monosynaptic component. E: long-lasting reflex as in D, but shown on longer time scale for another chronic spinal rat (5 wk after lesion). Time scales are the same for A–D.](http://jn.physiology.org/doi/fig/10.1152/jn.00535.2003)
The decrease in the initial peak in the reflex with stimulus intensity (Fig. 3B, top) was in part related to antidromic activation of motor axons, because there was generally a 5- to 8-ms silent period that followed the M-wave (Fig. 2D; consistent with motor axon conduction time), and even ongoing/ background EMG was silenced in this period (data not shown). Furthermore, this silent period increased in duration, and the peak of the reflex was delayed (by 20–30 ms) as more motor axons were recruited (as M-wave increased; data not shown; presumably related to antidromic collision, motoneuron after-hyperpolarization activation, and Renshaw activation). To avoid this antidromic activation, we also stimulated the tail distally to the recording EMG electrodes. This produced similar long-lasting reflexes even at low threshold, but they grew progressively larger with increasing stimulation, indicating that high-threshold afferents were also involved in these spastic reflexes (including C-fibers, see Fig. 5).

**STIMULUS REPETITION AND REFLEX ENHANCEMENT.** In chronic spinal rats, repeated manual stimulation of the tail produced progressively larger reflexes (Bennett et al. 1999), similar to the windup phenomena described in the pain literature (Gozariu et al. 1997; Morisset and Nagy 2000). With electrical stimulation of the caudal nerve trunk, a similar windup occurred (Fig. 4A). This is quantified in Fig. 4B, where the average long-lasting reflex is plotted as a function of stimulation number (○, peak; ●, tonic component of reflex; Fig. 3). The reflexes increased dramatically with repeated stimulation, although there was some variability, with EMG dropping off spontaneously at times (e.g., drop off at end of Fig. 4A) and then returning. In chronic spinal rats, the tonic reflex to a single stimulus often lasted about 2 s, and thus successive reflex responses interacted as in Fig. 4, A and B, when the repetition interval was also 2 s (standard rate). With longer intervals, the interaction was less, and the reflexes were smaller. The effect of changing the stimulus rate is summarized in Fig. 4C, which shows that maximal caudal nerve trunk reflex amplitude occurred at about 0.5 Hz (2 s; standard rate). High-frequency stimulation trains (100 Hz, 0.5 s) applied to the caudal nerve trunk were no more effective in activating a long-lasting reflex than a single shock, and they sometimes produces inhibition, especially for high-threshold stimulation (data not shown).

**TABLE 1.** Characteristics of the polysynaptic tail muscle reflexes evoked by caudal nerve trunk stimulation before and after S2 sacral spinal transection.

<table>
<thead>
<tr>
<th>Polysynaptic Reflex</th>
<th>Intact Rat</th>
<th>Acute Spinal Rat</th>
<th>Chronic Spinal Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak amplitude (%M&lt;sub&gt;max&lt;/sub&gt;)</td>
<td>3.0 ± 3.1</td>
<td>3.3 ± 2.7</td>
<td>27.3 ± 9.9*</td>
</tr>
<tr>
<td>Central latency (ms)</td>
<td>6–12</td>
<td>6–12</td>
<td>6–12</td>
</tr>
<tr>
<td>Threshold (×MT)</td>
<td>&lt;MT</td>
<td>&lt;MT</td>
<td>&lt;MT</td>
</tr>
<tr>
<td>Duration (s)</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>2.4 ± 1.1*</td>
</tr>
<tr>
<td>Conditioned reflex</td>
<td>Inhibitory</td>
<td>Excitatory</td>
<td>Excitatory</td>
</tr>
<tr>
<td>Spinal levels involved</td>
<td>S&lt;sub&gt;7&lt;/sub&gt;–Ca&lt;sub&gt;1&lt;/sub&gt;</td>
<td>S&lt;sub&gt;7&lt;/sub&gt;–Ca&lt;sub&gt;1&lt;/sub&gt;</td>
<td>S&lt;sub&gt;7&lt;/sub&gt;–Ca&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>No. of motoneuron involved</td>
<td>78 ± 19</td>
<td>78 ± 19</td>
<td>86 ± 42</td>
</tr>
</tbody>
</table>

Values are given as means ± SD. *Significant difference from 0 or intact state. M<sub>max</sub> is the maximum direct m-wave response of the muscle. MT indicates motor threshold. The polysynaptic reflex responses after cutaneous conditioning stimulation are indicated qualitatively (Fig. 7), with “excitatory” indicating that a long-lasting reflex was evoked and “inhibitory” indicating that the caudal nerve trunk stimulation produced a reduction in background EMG evoked by cutaneous conditioning.

**FIG. 3.** Quantification of the long-lasting reflexes. A: same caudal nerve trunk reflex response (stimulation 1.5 × MT) in chronic spinal rat shown in Fig. 2D, but rectified and filtered. Note that, following the M-wave, there is an initial peak in the reflex, a pause, and continued activity (tonic response). B: amplitude of the initial reflex peak and tonic reflex component (response in A averaged over 50–250 ms) at different stimulus intensities. M-wave shown for comparison, and dashed line indicates MT. C: summary of the maximum amplitude of the initial reflex (initial peak) for all rats. Chronic spinal rats > 2 wk after injury. Reflexes normalized relative to the maximum M-wave.

**AFFERENTS INVOLVED IN REFLEX.** Both the initial peak and the tonic component of the long-lasting reflex could be evoked at currents well below motor threshold (on average 0.6 × MT or −0.5–0.7 mA; Fig. 3B, left; tonic reflex is quantified in Fig. 3B as the average response over a 50- to 250-ms window after stimulus, see METHODS) and were thus in part mediated by the lowest threshold group I muscle afferents and/or Aβ cutaneous afferents (afferent threshold T is also about 0.5 mA). The group I effect likely involves IA afferents, because the segmental tail muscles do not have Ib afferents (Steg 1964). As the current was increased above the afferent threshold T, these reflexes quickly reached a maximum (at 0.9–1.0 × MT; Fig. 3B). When the current was increased further, so that an M-wave appeared (>MT), the reflexes began to decrease, and they continued to decrease as the M-wave increased (to 2–3 × MT; Fig. 3B). However, there remained significant long-lasting reflexes even at the highest stimulus intensities (10 × MT; Fig. 3B, right; initial peak and tonic components). The peak reflex was significantly larger than the peak reflexes seen in normal or acute spinal rats (Fig. 3C). A summary of this polysynaptic reflex before and after injury is shown in Table 1.
Cutaneous stimulation in chronic spinal rats

SINGLE SHOCKS. When the tip of the tail was electrically stimulated distally to the EMG electrodes, exaggerated long-lasting reflexes were again seen in the chronic spinal rat. These reflexes were of low threshold as in the caudal nerve trunk stimulation, but they grew progressively as the stimulus current was increased (see Fig. 5, A and C; also see Fig. 5 of Bennett et al. 1999). At times, full tail spasms (lasting 10–20 s) were triggered by a single stimulus at 10 × T (data not shown), and this was similar to the effect of a brief tail pinch (Bennett et al. 1999). Because the end of the tail has little muscle and is very sensitive to touch, this electrical stimulation provided a relatively pure stimulation of cutaneous afferents. Thus low-threshold cutaneous stimulation can evoke the long-lasting reflexes, and higher-threshold afferent stimulation (C-fiber) increased this reflex, indicating that both are likely effective in evoking long-lasting reflexes and spasms during natural stimulation.

STIMULATION TRAINS. When a brief high-frequency train (100 Hz for 0.5 s) of low-threshold electrical stimulation pulses was applied to the tip of the tail (2 × T, 0.2 ms; pure low-threshold cutaneous stimulation), very long-lasting reflexes were evoked (Fig. 5B; 10–20 s) significantly longer than the maximal responses evoked by single pulses (Fig. 5, A and C). These low-threshold stimulation trains were the most effective stimulation tested and mimicked the powerful spasm-inducing effect of manual rubbing of the tail, with very long-lasting rhythmic spasms evoked by a single brief stimulation train (Fig. 5B). This EMG activity is reminiscent of the rhythmic activity evoked in the neonatal rat tail in vitro by afferent stimulation (Lev-Tov et al. 2000). In Fig. 5, two spasms were induced, which were separated by a spontaneous pause. If the stimulation was applied for longer periods, the tail would flail from side-to-side in complex spiraling movements during the stimulation and continue to be active for minutes afterward (data not shown). Higher-intensity stimulation (10 × T, 0.5 s) evoked similar spasm-like behavior, with repeated spasms following the brief stimulation (Fig. 5D; 1 spasm indicated by EMG burst at arrow). However, habituation to this high-threshold stimulation occurred, making subsequent low-threshold stimulation less effective (data not shown).

Cutaneous/C-fiber stimulation in acute spinal rats

In acute spinal rats, long-lasting reflexes could not be evoked from low-threshold cutaneous stimulation of the tip of the tail, similar to the areflexia with stimulating the caudal nerve trunk. However, a strong electrical stimulation of the tip of the tail (single shock at 20 × T; C-fiber activation) could produce a weak tonic activation of the segmental muscles that lasted for ∼0.5–1 s (Fig. 6A). Why this nociceptive/cutaneous stimulation evoked activity, whereas even strong stimulation of the main caudal nerve trunk evoked nothing, is unclear, especially considering that the same afferents from the tip of the tail are stimulated in both cases. Presumably, the mixed nerve stimulation of the caudal nerve trunk is less natural and includes inhibitory effects that counter the excitation produced by cutaneous afferents.

Conditioning stimulation of C-fibers prior to low-threshold reflex testing

The finding that strong C-fiber/cutaneous stimulation of the tip of the tail could evoke some tonic motoneurone activity in...
acutely spinal rats enabled us to examine whether the lack of low-threshold reflexes simply resulted from a lack of excitability of the motoneuron pool immediately after injury, as described in the Introduction. That is, prior to low-threshold caudal nerve trunk stimulation, we gave a C-fiber conditioning stimulation to preactivate the motoneurons to assure that, subsequently, even a weak low-threshold EPSP would produce a detectable increase in EMG. Interestingly, when this C-fiber stimulation was followed within 1 s (during EMG activity) by low-threshold stimulation of the caudal nerve trunk (at 1.2 × MT), a moderately long-lasting, spastic-like reflex could be evoked in acute spinal rats (Fig. 6B, vertical arrow). The amplitude of the early part of the long-lasting reflex evoked by stimulation of the caudal nerve trunk following this conditioning stimulation (within 1 s) was comparable with that in the chronic spinal rat when averaged over a 50- to 250-ms window after the stimulus (Fig. 6D; see also Fig. 7 and compare with Fig. 3D). Furthermore, the activation of this conditioned reflex with increasing caudal trunk stimulation current was very similar to that in the chronic spinal rat (compare Figs. 6C and 3B). The reflex reached a maximum at a current below the motor threshold (mediated by low-threshold Aβ cutaneous and/or group I muscle afferents) and decreased at higher currents. However, the duration of this reflex was only 300–500 ms, significantly shorter than the long-lasting reflex in the chronic spinal rat (2 s; Fig. 2E). Thus there is a moderately long, low-threshold synaptic input to motoneurons in acute spinal rats (polysynaptic EPSP), which is very similar in duration to the synaptic input seen in acute and chronic spinal rats during intracellular recordings (0.5 s, see DISCUSSION) (Bennett et al. 2001c; Li et al. 2004a). This EPSP appears to normally be subthreshold in acute spinal rats (requires C-fiber conditioning to evoke reflex) and presumably produces a shorter response than in chronic spinal rats because of the lack of PICs/plateaus (see Introduction and Li et al. 2004a).

In normal rats, a similar weak preactivation of the motoneurons (tonic EMG) was produced spontaneously at times or could be triggered by various conditioning stimuli, including bending the tail or stimulation of the tip of the tail as in acute spinal rats. However, following these conditioning stimuli and during weak tonic EMG, the caudal nerve trunk stimulation
remained ineffective in evoking long-lasting excitatory reflexes (Fig. 7B), regardless of the conditioning stimuli or test stimulation intensity, just as without the conditioning stimulation (Fig. 7A). Occasionally, large responses were evoked, although these were not repeatable and were related to purposeful withdrawal movements of the tail in response to the stimulation or awkward postures (data not shown). Instead, it was common that the response to the caudal nerve trunk stimulation was inhibitory to the ongoing EMG evoked by the conditioning stimulation, and surprisingly, this inhibition could last for long periods (200 ms; Fig. 7B). Thus it appears that low-threshold stimulation of the caudal nerve trunk does not evoke long excitatory synaptic inputs to the motoneuron but does evoke inhibition. Together, these results indicate that, immediately following injury, there is a massive loss of inhibitory control over reflexes through lost descending or propriospinal control, and this acutely reveals long-duration low-threshold EPSPs that are capable of evoking spastic reflexes when the motoneurons become sufficiently excitable with chronic injury or conditioning stimulation.

In the chronic spinal rat, a conditioning stimulation of the tip of the tail that evoked background EMG (as in acute spinal rats) did not increase a subsequent reflex evoked by stimulation of the caudal nerve trunk during this background EMG. Instead, the long-lasting reflex was shorter and smaller than without the conditioning stimulation (significant decrease; compare Fig. 7, E and F; see also Fig. 8). Furthermore, the reflex amplitude was not significantly different from the same conditioned reflex evoked in acute spinal rats (Fig. 7, D and F, see also Fig. 6D).

**Time-course of development of tail reflexes in relation to spasticity**

After spinal cord injury, each rat was assessed regularly with manual tail manipulations, and a spasticity rating was given. The rating system took into account incidence of evoked spasms and associated hypertonus and clonus, as described in METHODS. As shown in Fig. 8A, the spasticity rating increased significantly by 2 wk after injury (marked by dashed line) and continued to increase until about 2 mo after injury, after which it remained high. The low-threshold long-lasting reflexes recorded with EMG in response to caudal nerve trunk stimulation increased in close correlation to the spasticity rating, with a significant increase in reflex by 2 wk (Fig. 8B, averaged maximum reflex shown in Fig. 8, B and C). In contrast, the low-threshold reflex that followed a high-threshold C-fiber conditioning stimulation was not as well correlated with the spasticity rating (Fig. 8C, peak reflex shown). As described above, this conditioned reflex was increased significantly immediately after injury. Also, note that the steady-state level reached by 2 mo after injury (Fig. 8C) was significantly lower than in the case without conditioning stimulation (Fig. 8B).

**Distribution of tail motoneurons and relation to lesion**

To investigate the tail motoneurons of the distal tail involved in the preceding reflex recordings, retrograde tracers were applied to the caudal nerve trunk just above where the EMG was recorded (see METHODS). The resulting back-labeled motoneurons were counted, and their distribution was plotted in relation to their longitudinal location along the spinal cord (Fig. 9). In normal rats, there were 78 ± 19 segmental tail motoneurons labeled per rat, and their distribution was centered at the S3–S4 level (n = 6 rats; Fig. 9C). In chronic spinal rats, there were 86 ± 42 motoneurons, which was not significantly different from normals (n = 4 rats; Fig. 9B; Table 1). The motoneurons labeled were well below the S2 spinal level where the lesion was made, and thus the lesion itself was unlikely to have directly damaged motoneurons involved in the reflex testing. However, the cord was significantly narrower and elongated below the lesion in chronic spinal rats (see Bennett et al. 1999). For comparison, the distribution of all sacral motoneurons in normal rats is shown in Fig. 9D, which was estimated from cresyl violet stain as described in METHODS. Note that the segmental tail motoneurons that are labeled retrogradely (Fig. 9, B and C; motoneurons of distal half of the tail) constitute less than one-third of the total motoneurons (Fig. 9D), even in the low sacral region where they are concentrated.

**Afferents involved in reflex testing and relation to lesion**

To determine the anatomical arrangement of the afferents involved in the tail reflex testing, we stimulated the intact caudal nerve trunk with surface electrodes as during reflex testing (Fig. 1) and recorded the afferent volley in the dorsal roots in pentobarbital anesthetized rats (n = 4 normal rats, see METHODS). The roots S3, S4, and Ca1 were activated by this stimulation, and the S2 dorsal root was not. Thus the S2 spinal cord injury did not directly damage the afferents involved in tail reflex testing. The afferent threshold in the relevant S3, S4,
and Ca1 roots was 0.5 × MT (<1 mA; Fig. 1), which was close to the long-lasting reflex threshold (0.6 × MT, Fig. 3B), and thus these reflexes were mediated in part by the lowest-threshold group I afferents.

During root recording, we also mapped out the dermatomes, which were widely overlapping and arranged as follows: the main caudal dorsal root (Ca1+) innervated the lower one-third of the tail, including the very sensitive tip; the S2–S3 dorsal roots innervated the long length of the tail except the tip; and the S2 dorsal root innervated the base of the tail and pelvic area.

It is also important to note that, even though the tail stimulation electrode was placed over the left side of the tail, dorsal root activation occurred in both left and right dorsal roots, even with relatively low threshold stimulation (>0.8 mA). Thus because of the small tail diameter, the stimulation current spread over the whole tail, probably getting to all four caudal trunk nerves equally (the pair of dorsal and ventral trunk nerves on each side; see METHODS).

Peripheral conduction times and expected monosynaptic reflex latency

We also measured root conduction times to estimate the peripheral components of the reflex latencies (n = 4 normal rats, 4 chronic spinal rats). The conduction velocities of the roots measured centrally at 36°C were 60 m/s for the fastest component of the dorsal roots and 55 m/s for the ventral roots (measured with direct stimulation of roots; see also Steg 1964). However, the conduction times from the caudal tail nerve stimulation (Fig. 1) to the spinal cord corresponded to much slower rates (~30 m/s), because the tail was cooler than the body (29°C; see METHODS). The mean dorsal and ventral conduction times from the tail stimuli to the cord were 4.5 and 5.5 ms, respectively. Thus, including the 2-ms delay from the stimulus to the production of an M-wave, we can predict the total peripheral delay in reflexes to be 12 ms (4.5 + 5.5 + 2), although this varied with tail length. This delay was used to estimate the latency for the monosynaptic reflex, which should be about 13 ms (assuming 1-ms synaptic delay; the time of occurrence of the F-wave was also used to estimate monosynaptic latency, with a similar result, unpublished observation). In the few rats that had a clear monosynaptic reflex volley (n = 5/19), it indeed occurred between 12 and 14 ms (Fig. 2D, *).

The main polysynaptic long-lasting reflex in spastic rats started at 18–24 ms, which was 6–12 ms beyond the predicted 12-ms peripheral delay and thus was not monosynaptic and had a central delay of 6–12 ms (Table 1). Cooling or heating the tail increased or decreased the peripheral conduction time significantly, but did not significantly affect the estimated central delay.

DISCUSSION

The results demonstrate that, in chronic spinal rats, long-lasting tail reflexes have the following characteristics: 1)they are very low threshold, triggered by the lowest-threshold afferents (group I muscle and/or Aβ cutaneous afferents), but also activated by higher-threshold afferents, including C-fibers; 2) they are evoked from rest, without prior EMG activity; 3) they are of polysynaptic latency; 4) they have a characteristic shape, with a large initial reflex peak,pause, and tonic activity lasting seconds; 5) they exhibit windup; 6) they are much larger and longer lasting that the reflexes in normal or acute spinal rats, and produce powerful whole tail contractions, at times involving a near maximal sustained activation; and 7) they develop gradually over time after injury, in close parallel to the development of the general spasticity in the tail. In normal and acute spinal rats, caudal trunk stimulation does not by itself evoke a long-lasting reflex, although in acute spinal rats, a weak tonic contraction can be evoked by strong C-fiber stimulation. Thus the long-lasting reflexes in chronic spinal rats are more excitatory and lower threshold, as in other animal models of spasticity (Taylor et al. 1999), and similar to the exaggerated flexor withdrawal reflexes seen in spastic humans after chronic spinal cord injury (Hornby et al. 2003; Kuhn and Macht 1948; Roby-Brami and Bussel 1987). Importantly, the above characteristics of the long-lasting tail muscle reflexes are identical to the reflexes measured from ventral root recordings of the same chronic spinal rats, but with the affected sacrocaudal spinal cord maintained in vitro (Li et al. 2004b). Thus in as much as the long-lasting reflex represents spastic/spasm behavior, these reflexes and spasticity in general can be studied with the in vitro sacrocaudal spinal cord preparation. Because the long-lasting tail EMG reflex to caudal

FIG. 9. Distribution of tail motoneurons involved in reflex testing. A: schematic of the sacrocaudal spinal cord from the chronic spinal rats in B, drawn to same scale as plots in B–D, showing segment levels, roots, and relation of cord to overlying vertebral bone segments. B: numbers of motoneurons retrogradely labeled from the caudal trunk nerves used in reflex testing (label applied on both ventral and dorsal left caudal trunk nerves at Ca12). Cells were counted in 1.25-mm bins, and averages are shown from n = 4 rats. C: motoneuron distribution for normal rats (n = 6, same format at B). D: total number of motoneurons in the normal sacrocaudal cord labeled with cresyl violet and estimated as described in METHODS (left, n = 4 rats). Distances measured caudal to the L6 segment.

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nerve trunk stimulation develops in close parallel to the general development of the spasticity syndrome, it indeed appears to be a good assay of spasticity. Furthermore, the characteristics of this reflex are consistent with triggered spasms in the tail. That is, the high reflex excitability (from rest), involvement of both low- and high-threshold afferents, and enhancement with repetition are similar to the characteristics of triggered spasms (Bennett et al. 1999; Hornby et al. 2003; Kuhn and Macht 1948).

Acute release of descending inhibition of low-threshold EPSPs with injury

Interestingly, low-threshold polysynaptic reflexes similar to the long-lasting reflex in the chronic spinal rat can be evoked in the acute spinal rat by stimulation of the caudal nerve trunk, provided that there is prior EMG activity from a strong C-fiber activation at the tip of the tail. A similar conditioned low-threshold reflex is not usually seen in normal rats. Thus it appears that there is a low-threshold reflex pathway that is normally inhibited and released from inhibition by acute injury, but not seen initially without a strong conditioning stimulation (similar to that in the spinal rabbit, Clarke et al. 2002). This acute release from inhibition has been extensively studied by Lundberg (1982).

The low-threshold polysynaptic reflex is likely not present without conditioning because of the massive loss of excitability of the motoneuron pool immediately following injury (loss of PICs; Bennett et al. 2001c; see Introduction). We know that there are unusually long low-threshold EPSPs that emerge immediately after injury (Baker and Chandler 1987; Li et al. 2004a), lasting 0.2–0.5 s, but normally the lack of motoneuron excitability prevents these EPSPs from producing large reflexes, if at all (Li et al. 2004a). However, with the strong conditioning stimulation that produces tonic EMG activity (for ∼0.5 s) and thus depolarizes the motoneuron pool, low-threshold EPSPs are likely capable of evoking a reflex and thus explaining the 0.3–0.5-s long polysynaptic synaptic reflexes seen with conditioning (Figs. 6 and 7). Long-duration cutaneous-evoked EPSPs are not seen in intact spinal cords of unanesthetized animals (Crenna et al. 1982; Heckman et al. 1994), and consistent with this, in normal awake rats, long-lasting reflexes cannot be evoked even with a conditioning stimulation that evokes EMG (Fig. 7). Thus the present results demonstrate that, prior to injury, there is a strong inhibition of low-threshold long polysynaptic EPSPs, and this inhibition is lost immediately after injury.

Amplification and prolongation of reflexes with chronic injury

In the chronic spinal rat, these low-threshold EPSPs are amplified and prolonged by many seconds because they trigger voltage-dependent PICs on the motoneurons (including persistent calcium and sodium currents) that produce plateau potentials and self-sustained firing that are not present in acute spinal rats (see Introduction and Bennett et al. 2001a,c; Li and Bennett 2003; Li et al. 2004a). This explains why the reflexes last much longer in chronic (seconds) compared with acute spinal rats (Figs. 2E, 4A, 5) (Bennett et al. 2001). When PICs are blocked (by drug application or hyperpolarization) or are not present (acute spinal state), a low-threshold stimulation only evokes a 0.2- to 0.5-s-long EPSP, corresponding to the synaptic input without amplification from PICs (Li et al. 2004a). PICs are somewhat slow to fully activate, requiring at least a 0.2-s depolarization stimulation (Li and Bennett 2003), and thus this 0.2- to 0.5-s-long synaptic input evoked by a single low-threshold nerve shock (Li et al. 2004a) is of ideal duration for triggering PICs (when present) and associated self-sustained firing lasting many seconds (Bennett et al. 2001c; Li and Bennett 2003). Repeated PIC activation results in a warmup phenomena, where the PICs/plateaus get larger with repetition (Bennett et al. 1998; Svirskis and Hounsgaard 1997), and this likely contributes to the windup and enhanced reflex responses to stimulation trains in chronic spinal rats (Figs. 4 and 5).

Prior to spinal cord injury, PICs are present and prominently amplify normal motoneuron output (Hounsgaard et al. 1988; Lee and Heckman 1999); however, low-threshold long EPSPs do not appear to be prominent, and there is strong descending and segmental inhibitory control that can terminate motoneuron firing, even though it is maintained by intrinsic PICs (Fig. 7, long inhibitory reflex). Thus involuntary muscle spasms do not occur prior to injury.

In chronic spinal rats, amplification of synaptic inputs by intrinsic motoneuron PICs occurs just subthreshold to firing (Bennett et al. 2001c; Li et al. 2004a). Thus when the motoneurons are recruited from rest, the PICs maximally amplify the synaptic input (as in Fig. 7E). However, when there is a preactivation of the motoneurons, as with the conditioning stimulation in Fig. 7F, the PICs in these motoneurons are likely fully activated (Bennett et al. 2001c), and thus subsequent synaptic inputs are not amplified and prolonged by the PICs in these preactivated motoneurons (although synaptic inputs may be amplified by PICs in other motoneurons newly recruited by the reflex). This may in part explain the smaller and shorter reflexes seen after a conditioning stimulation (Fig. 7F) compared with at rest. If this is the case, the reflex response in Fig. 7F approximates the synaptic input unamplified by PICs, and this is indeed remarkably similar in duration to the conditioned reflex response in acute spinal rats (Fig. 7D) and the low-threshold EPSPs measured when the PICs are blocked in acute and chronic spinal rats (0.5 s; Li et al. 2004a). This is consistent with the idea that there is a low-threshold polysynaptic input to the motoneurons that appears acutely after injury and persists with chronic injury.

Interneurons in the long-lasting reflex pathway may also increase their excitability by gaining the ability to exhibit PICs/plateau potentials (unpublished observations), and this possibility clearly needs to be explored. However, the acute emergence of the low-threshold polysynaptic reflex (EPSP) is not likely mediated by PICs, as opposed to loss of descending inhibition, given that large PICs are not seen even in motoneurons acutely. Also, there are likely many other slow changes in afferent input following spinal cord injury, including synaptic plasticity/sprouting (Krenz and Weaver 1998), but these have yet to be studied in the sacral cord. We know from this study that the motoneurons and afferents involved in the long-lasting reflex are not directly injured during the spinal transection (see Results and Fig. 9), and thus any plasticity in these neuronal elements must be indirectly induced by the injury.
Summary of causes of spasticity

The present results from the awake sacral spinal rat, taken together with associated in vitro recordings in the sacrocaudal spinal cord, lead to the following interpretation of what causes the general spasticity syndrome after spinal cord injury. Immediately after injury, motoneurons receive unusually long polysynaptic EPSPs (200–500 ms) in response to low- or high-threshold afferent stimulation, especially cutaneous stimulation (measured directly in Li et al. 2004a and inferred from conditioning reflex experiments in Fig. 7), consistent with the acute loss of descending brain stem innervation of the dorsal horn (see Introduction). These EPSPs do not easily cause reflexes immediately after injury (Fig. 2) because of the profound loss of motoneuron excitability that occurs with acute injury (Bennett et al. 2001c), primarily due to loss of intrinsic PICs that normally amplify and prolong synaptic inputs several-fold (Lee and Heckman 1999). This loss of motoneuron excitability is likely the main reason for spinal shock that occurs in the first days after injury, considering that the excitatory reflex pathways (EPSPs) are enhanced rather than reduced in this acute phase of injury.

After about 1 month, the motoneurons recover their excitability, and the same low-threshold polysynaptic inputs to the motoneurons are profoundly amplified and prolonged by PICs that regeneratively activate, cause plateau potentials, and ultimately produce many second long discharges (Bennett et al. 2001a; Li et al. 2004a). Thus normally innocuous stimulation, such as gently rubbing the skin, evokes these polysynaptic EPSPs, which in turn trigger PICs and plateaus that ultimately cause many second long reflex responses and whole limb spasms. Even passive movement of the limb should trigger such low-threshold long-lasting reflexes and thus trigger spontaneous spasms. Also, movements caused by the long-lasting reflexes reactivate low-threshold afferents and trigger further polysynaptic EPSPs, which likely get larger with repetition due to the windup of the polysynaptic reflex. This should ultimately lead to low-frequency clonus, with a period of oscillation longer than the 200-ms EPSP duration (<5-Hz clonus). The classic exaggerated stretch reflexes after injury are also seen in the tail (Bennett et al. 1999), although as in spinal humans (Kuhn and Macht 1948), exaggerated cutaneous reflexes play a more major role in spinal spasticity (as opposed to in spasticity following stroke). These results from the sacral spinal rat model explain many of the characteristic features of the spinal spasticity syndrome, including spasms, clonus, hyperreflexia, and hypersensitivity to normally innocuous stimulation (Kuhn and Macht 1948; Young 2004). Similar cellular mechanisms likely underlie spasticity in humans after spinal cord injury (Gorassini et al. 1999, 2003).

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