Spasticity in Rats With Sacral Spinal Cord Injury

D. J. BENNETT, M. GORASSINI, K. FOUAD, L. SANELLI, Y. HAN, and J. CHENG

ABSTRACT

We have investigated sacral spinal cord lesions in rats with the goal of developing a rat model of muscular spasticity that is minimally disruptive, not interfering with bladder, bowel, or hindlimb locomotor function. Spinal transections were made at the S2 sacral level and, thus, only affected the tail musculature. After spinal transection, the muscles of the tail were inactive for 2 weeks. Following this initial period, hypertonia, hyperreflexia, and clonus developed in the tail, and grew more pronounced with time. These changes were assessed in the awake rat, since the tail is readily accessible and easy to manipulate. Muscle stretch or cutaneous stimulation of the tail produced muscle spasms and marked increases in muscle tone, as measured with force and electromyographic recordings. When the tail was unconstrained, spontaneous or reflex induced flexor and extensor spasms coiled the tail. Movement during the spasms often triggered clonus in the end of the tail. The tail hair and skin were extremely hyperreflexive to light touch, withdrawing quickly at contact, and at times clonus could be entrained by repeated contact of the tail on a surface. Segmental tail muscle reflexes, e.g., Hoffman reflexes (H-reflexes), were measured before and after spinalization, and increased significantly 2 weeks after transection. These results suggest that sacral spinal rats develop symptoms of spasticity in tail muscles with similar characteristics to those seen in limb muscles of humans with spinal cord injury, and thus provide a convenient preparation for studying this condition.

Key words: awake rat; bladder; spasticity; tail

INTRODUCTION

While spasticity is a common problem that follows spinal cord injury in humans, it has been rather difficult to study experimentally, since spinal cord injury in animals produces only comparatively mild spasticity (Hultborn and Malmsten, 1983; Ashby and McCrea, 1987; Powers and Rymer, 1988; Noth, 1991; Taylor et al, 1997). Spasticity occurs in humans without complete spinal cord transections, whereas, in animals, such as rats and cats, incomplete spinal cord injury only leads to mild hyperreflexia. For example, after a few weeks of recovery, animals with T12 hemisections appear normal, and only detailed testing revealed differences in reflexes between the hindlimbs and gait abnormalities (Hultborn and Malmsten, 1983; and unpublished observations in rats). Complete spinal transections (e.g., T12 cut) in cats has, in some studies, led to spasticity (Naftchi et al., 1979; Ashby and McCrea, 1987; Sherrington, 1898). However, these cats require twice daily bladder and bowel expression, and bladder infection and pressure sores can be a chronic problem. Thus, the spinal cat is an impractical model, and many groups have used hemisections, partial transections, and contusions (Ashby and McCrea, 1987; Thompson et al., 1993) to study spinal cord injury, even though spasticity is not always prominent.

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Recently, Ritz's group has found that low sacrocaudal transections or contusions in cats causes pronounced spasticity in the axial musculature of the tail vertebrae, without disrupting bladder, bowel, or normal hindlimb locomotor function (Ritz et al., 1992; and Ritz, personal communication). Though the study of axial musculature has been relatively neglected in general, it is involved in spasticity in humans (cf. back muscles: Stauffer, 1974) and should therefore be considered relevant to the spastic syndrome. The tail is normally used in a well-coordinated fashion during locomotion, hovering off of the ground and bending to guide turning. Thus, straightforward observations of tail function can be used to assess motor recovery after spinal cord injury and following interventions that may promote recovery of function (Ritz et al., 1992). While the cat tail model of spasticity represents an important advance from a standpoint of practical animal care (cf. normal bladder function), it has not yet reached general use. This is perhaps because many investigators have moved to the use of rats, rather than cats, since rodents have become a standard in the development of techniques that promote recovery and possible regeneration following spinal cord injury (Jakeman and Reier, 1991; Schnell et al., 1994; Iwashita et al., 1994; Bregman et al., 1995; Skinner et al., 1997).

In this study, we have investigated sacral spinal cord lesions in rats and found that a spastic syndrome developed in rats similar to that in cats (Ritz et al., 1992). However, unlike in cats, we found that the awake rats tolerated prolonged examinations of the tail for spasticity assessment, including muscle stretching, surface electromyographic (EMG) recording, and Hoffman reflex (H-reflex) testing with tail nerve stimulation. Our chronic recording techniques were motivated by a remarkably simple awake rat preparation developed by Steg (1964), involving the small but accessible segmental muscles of the tail. Thus, in awake rats, we have been able to repeat many of the traditional measurements made in spinal cord injured humans. We have shown that a very realistic model of spasticity develops in the tail muscles, with many of the same characteristics found in the human spastic syndrome (Holmes, 1915; Kuhn and Macht, 1948; Dimitrijevic and Nathan, 1967; Lundau, 1974; Young, 1994; Katz et al., 1992) including (1) muscle hypertonus, (2) hyperreflexia both to muscle stretch and cutaneous stimulation, (3) clonus, (4) flexor spasms, (5) paraesthesia, and (6) clasp-knife responses.

**MATERIALS AND METHODS**

**Surgery**

Adult Sprague Dawley rats, aged 50-90 days, of both sexes and weighing 250-450 g were used in this study with approval from the local animal welfare committee. Spinal cord lesions were made at the low sacral level (S2), since one of our primary objectives was to develop a model of spasticity that spared bladder and bowel functions, which are controlled through the L6-S1 spinal cord (Hebel and Stromberg, 1976; Schroder, 1980; Song et al., 1995). Animals were deeply anesthetized with sodium pentobarbital (50 mg/kg), and, under sterile conditions, a laminectomy was performed at the L2-L3 vertebrae (in later experiments, L2 sufficed). The dura was opened transversely, and the L4-S1 dorsal roots were gently pushed aside to gain access to the sacral cord, after 0.1-0.2 ml of Xylocaine (1%, lidocaine hydrochloride) was applied to avoid movements during root manipulations. From preliminary studies, we found that the S2 root could be reliably identified, since it usually entered at the level of the rostral half of the L2 vertebrae and was smaller than more rostral roots (Hebel and Stromberg, 1976). In 11 animals, the cord was transected at the S2 level, using fine forceps and suction (S2 spinalization). Care was taken to preserve the anterior (ventral) artery and dorsal vein, which supply the primary circulation, since the sacral cord contains no major radicular arteries, unlike in the lumbar region. Severing of the anterior artery results in a "dead tail syndrome," where the sacro-caudal cord dies (Ritz, personal communication), and this occurred in preliminary experiments not included in the present study. When possible we attempted to suture the dura with 8-0 silk after the lesion, though whether or not it was closed did not affect the final outcome. In six control animals, the same surgery was performed, except no transection was made (sham surgery). In two of these control animals, the S2 dorsal roots were cut, since these roots were normally damaged during the transection (S2 rhyzotomy). Animals were housed in pairs (except immediately after surgery), since spinal animals housed alone occasionally chewed on their tails. Otherwise, no special care was needed for animals, as they had normal locomotor, bladder, and bowel function immediately after surgery.

**Clinical Evaluation**

Clinical examinations were performed on the animals before and after surgery and included observations of (1) bladder and bowel function, (2) locomotion, (3) tail tone, determined by manual manipulations of the tail and observations of tail posture (cf. steady ventroflexed curling in Fig. 2 below), (4) cutaneous flexion reflexes in response to non-noxious stimuli involving light touch or rubbing of skin and hairs, (5) stretch reflex with manually imposed movements, (6) tail flick and flexion in response to brief pinch or radiant heat, (7) spontaneous tail movements not related to voluntary body movements, (8)
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clonus, a rapid tremor of the end of the tail during spasms, (9) yielding of muscle contractions in response to tail stretch (cf. clasp-knife-like response), and (1) paraesthesia, determined by skin stimulation. Video records of locomotion and tail posture were taken on a plexiglass platform with a mirror underneath, angled to see both top and bottom views simultaneously. Tail flick response latencies were taken as a measure of nociceptive threshold and measured by applying a standardized radiant heat source of 50°C to the distal tail, for a maximum of 20 sec, as is common in analgesia testing (Kim et al., 1995). A rating system similar to the Modified Ashworth scale (Katz et al., 1992) was used to rate the general severity of the spastic syndrome. As described in Table 1 of the Results below, we have included not only hypertonia in this clinical rating system, but also the degree of hyper-reflexia and clonus. For consistency, all ratings were done by a single experienced observer.

Tail manipulations were performed while the animals were in a plexiglass tube (in Fig. 2A below), with the tail hanging out the back and free to move over its full length. Rats usually needed no prompting to enter the tube and sat there quietly for long periods without distress, provided the tube was covered with a cloth to make it dark for the rat and good drainage of urine was provided by a gutter in the bottom of the tube. Between tests, the tail was elevated to a normal horizontal level, since we found the tails left hanging for more than 20 min had poor circulation and reflexes were diminished.

**Force and length measurements during imposed movements.** To quantify muscle tone and stretch reflexes we used the following apparatus to impose movements, while the rat was in the plexiglass tube. The tail was initially held horizontal, fixed at its middle (near C12 vertebrae) to a support rod, and at its end to a rotary manipulator. The manipulator had its axis of rotation aligned with the C12 vertebrae and perpendicular to the tail. To bend the tail, the manipulator was rotated (usually by 90 degrees, so the tip of the tail was vertical). This arrangement produced a smooth arcing of the distal half of the tail, which stretched either the extensor or flexor muscles, depending on whether the movement was downwards (dorsally) or upwards (ventrally), respectively. More selective stretching, of the extensor or flexor muscles on one side only, was achieved by orienting the tail so that the stretch instead occurred in the dorsolateral or ventrolateral directions. The resistance of the tail to this stretch was measured by a force sensor (Grass, Ft 03B) that coupled the end of the tail to the rotary shaft, and was aligned to measure force in the direction of the imposed movement. Tail movement was measured from the manipulator rotation.

**EMG measurements.** While the largest tail muscles are at the base of the tail and have long tendons that run to each segment, there are also small segmental muscles that run between segments of the tail (Steg, 1964; von Schumann, 1909). We found that surface EMG could be selectively recorded from these small segmental muscles in the distal half of the tail, since they are electrically isolated from other muscles (e.g., leg muscles). Standard surface electrodes were initially used (Beckman), but were hard to secure to the tail. We therefore developed a new cuff electrode (similar to a large nerve cuff), from silver wire sewn into 1-cm sections of flexible tubing (Tygon R 3603, area of exposed silver: 3 × 4 mm), with an internal diameter slightly smaller than the tail diameter. The tubing was slit lengthwise so that it could be stretched open to apply conductive electrode jell and then snapped closed onto the tail. The cuffs held snugly on the tail without any additional strapping, and were connected by multi-stranded flexible stainless steel wires (Cooner AS632) to the EMG amplifiers. To reduce the electrode impedance sufficiently (to <100 kΩ), it was necessary to first scrub the loose skin from the tail with soap and then alcohol.

The standard point of reference relative to which all electrodes were placed was the 12th caudal vertebra (C12, about the halfway point of the tail, or 15 vertebrae from the end). An EMG electrode pair was placed at 1 and 4.5 cm distal to C12 (in Fig. 2A below). A third electrode was placed 1 cm proximal to C12 for signal ground. Because of the small number of motor units in the segmental muscles (Steg, 1964), it was common to pick up single motor unit action potentials with the surface EMG. Intramuscular fine EMG wires (50-μm stainless steel) were used in some animals to verify the selectivity of the recordings. These were inserted percutaneously, through the shaft of hypodermic needle.

**Nerve stimulation and H-reflexes.** The cuff electrodes described above were also used for electrical stimulation of the tail nerves. The nerves for the distal segmental tail muscles recorded with EMG exit the vertebral canals at the base of the tail and travel distally in two nerve trunks, inferior and superior caudal nerve trunks (Steg, 1964). These nerve trunks were stimulated just proximal to the EMG electrodes in order activate tail reflexes (similar to H-reflex Hoffman reflex testing in humans). The stimulation electrode (−) was placed 2.5 cm proximal to C12 over the left inferior caudal nerve trunk (just ventral to the lateral vein), and the indifferent electrode (+) was placed 2 cm more proximal. A second pair of cuff electrodes was also placed on the distal 2–3 cm of the dorsal surface of the tail and was used to produce a relatively pure cutaneous stimulation, since this portion of the tail has very little muscle (Steg, 1964).

In a series of acute experiments, described in a sepa-
rate paper (Bennett et al., in preparation), we measured the afferent and efferent conduction velocities with dor-
sal and ventral root recordings and computed the ex-
etected latencies of the reflex (H-reflex) and direct mus-
cle activation (M-wave) components of the segmental tail
muscle EMG responses to nerve stimulation. The H-ref-
lex (short latency reflex component; Schieppati, 1987;
Thompson et al., 1992) occurred at the lowest thresh-
old, presumably because it was produced by the largest di-
ameter primary afferent fibers [Ia, activated at 0.6 times
the motor (M-wave) threshold (0.6 MT)]. Response am-
plitudes were measured from the averaged rectified sig-
als (in Fig. 4 below). The maximum H-reflex was de-
termined by testing with 0.2-msec constant current
stimuli ranging from 0.6 to 6 MT (usually about 0.6–6
mA, Isolator-11, Axon Instruments; 15 shocks of 0.2
msec repeated at 0.5 Hz at each current level) and usu-
ally occurred near motor threshold, since collisions with
the antidromic signals can occur above this level (Schiep-
patti, 1987). It is also important to note that the Ia affe-
rent and motor axons involved in this H-reflex testing
arrangement traveled only in sacral roots S3-S4 and cau-
dal roots, and thus the S2 spinal cord lesions did not af-
tect the H-reflex directly by interfering with the axons
involved (Bennett et al., unpublished results).

Signal analysis and statistics. EMG, force and length
signals were sampled at 10 kHz with a computer data
acquisition system (Axoscope, Axon Instruments, CA).
Before sampling, the EMG was highpass filtered at 100
Hz (first-order filter) to remove motion artifact. In
cases where quantification of EMG amplitude was
needed, raw EMG signals were rectified and smoothed
by a further analog circuit involving a 1-kHz highpass
filter (first-order filter), a rectifier, and then a 100-Hz
lowpass filter (fourth-order Bessel filter). Statistical
comparisons were carried out with a Student’s t test at
the 95% confidence level. Quoted values are means and
standard deviations.

Histology

Animals were deeply anesthetized with sodium pento-
barbital and perfused with saline and then 4% buffered
parafformaldehyde. The whole vertebral column was im-
mediately cut out and fixed overnight in paraformalde-
hyde. A laminectomy was then performed and the spinal
cord was removed and postfixed for 3–4 h, and then cry-
oprotected in 30% sucrose overnight. Longitudinal 30-
μm sections were cut on a cryostat and stained with the
silver nitrate physical developer method for axons (Kier-

![FIG. 1. Development of tail spasticity following sacral spinal transection. Drawings from video of rat tail before (normal), 1
week (acute, stage 1), 3 weeks (spastic, stage 2 and 3), and 8 months (spastic, stage 4) after transection. Normal: Walking with
tail elevated. Stage 1: Tail flaccid. Stage 2: Flexor muscle hypertonus; tail coiled and stiff. Stage 3: Hyperreflexia; eg, coiling
flexor spasm and clonus in response to light touch or stretch. Stage 4: Hypertonus in flexor and extensor muscles, clonus and
hyperflexia, the latter including positive curling reaction.

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RESULTS

Behavioral Tests of Normal Tail Function

Normal rats used their tails in a characteristic manner during locomotion and alert posture (11 rats studied preoperatively). During locomotion, the tail often hovered slightly off the ground over its entire length and was lined up with the axis of the body. At times, the tail bent actively with the body during turning, hovering just off of the walls of the cage, as if it were sensing or guiding the movements (Fig. 1). When the rat was resting, the tail had little or no muscle activity (EMG), and only in alert postural states or locomotion was there moderate activity during manual manipulations. The muscle activity was always well coordinated, keeping the tail straight or smoothly arced. The tail was never kinked or bent in an uncoordinated fashion, as was common in the spinal rats described below.

Light touch of the tail hairs often had little effect, though small withdrawal movements (of 1–2 cm) occurred when the animal was alert, both in the dorsal and ventral flexion directions. Flexion in response to dorsal touch of the end of the tail was most common. At times, during ventral contact only the portion of the tail that was touched withdrew, while above and below the contact point the tail bent in an opposite direction, resulting in the tail flexing around the experimenter’s finger. We refer to this as a positive curling reaction, in analogy to the positive supporting reaction produced by foot contact.

Since many of the reflexes occurred in the ventroflexion direction, we found it convenient to elevate the rat and to have its tail hang vertically so that it could flex freely. The rat was placed in a plexiglass tube, adjusted to its body length, so that the full length of the tail hung out the back of the tube (Fig. 2). Under these conditions, a brief pinch with forceps caused a rapid flick of the whole tail, usually about 2–5 cm away from the forceps. This lasted for 1–2 sec, and then the tail would resume its downward vertical position. Application of radiant heat gave a similar flick but with a delay, as is normal for this test and described further below.

The most effective method of initiating tail muscle spasms in spinal rats was to hold the base of the tail with one hand, and simultaneously stretch and rub the tail by
rapidly sliding the other hand down the length of the tail with a firm grip between the thumb and fingers. This stretch/rub maneuver took about 0.5 sec, with the tail being released as the fingers slid off the end of the tail in one motion. Normal rats either did not respond to this stimulation or responded with a slight flexion (20–30 degrees) bend of the distal half of the tail, which only lasted transiently (2–3 sec, compare to Fig. 2; see quantitative comparison to spinal rats below).

Sacral Spinal Rats: Locomotor and Bladder Function

On postoperative day 1 (PO1) after rats were spinalized at S2, they had full locomotor, bowel, and bladder function. One rat had a limp, which cleared up in a few days, and probably resulted from damage to the L3–L5 roots, which were moved to access the sacral cord. Bladder and bowel function continued to be normal for the duration of the study (up to 14 months) in all rats with successful S2 lesions (n = 11).

Acute Spinal: Stage 1 (PO1–14 Days)

The tail was flaccid for the first two weeks after spinalization (Fig. 1) in all rats. Responses to skin pinch could not be elicited anywhere on the tail except at the base, which is partly innervated by the S1 roots above the lesion site. However, the stretch/rub maneuver described above could elicit a slight bend of the distal tail (<10 degrees), indicating that the sacrocaudal cord was still alive.

Chronic-Spinal Hypertonus: Stage 2
(>PO14 days)

After 2 weeks, tonic muscle activity (recorded with EMG) that flexed the tail ventrally was often present (in all rats). This activity was greater following cutaneous stimulation or muscle stretch. With the tail hanging vertically the tip of the tail was often flexed steadily at more than 90 degrees from vertical and could remain that way for minutes at a time (in a coiled posture, as in Figs. 1 and 2). In contrast, in normal animals, the tail was rarely flexed more than 90 degrees from vertical, and any such flexion was transient (<3 sec). Judging from this abnormal flexion in the chronic spinal rats, we assume that the flexor muscles were abnormally activated and that this resulted in hypertonus. Indeed, the muscle tone, as judged from manual manipulations and measurements of stiffness, was markedly increased at these times. In 10 of 11 rats, this hypertonus occurred in the distal two-thirds of the tail, with the base remaining paralyzed. The remaining rat only developed tone in the distal one-third of the tail, and we presume that the lesion was made too low (at S4).

Chronic Spinal Hyperreflexia—Stage 3
(>PO14 Days)

Simultaneously with the development of hypertonus, the tail became hyperreflexive to muscle stretch and light touch (Fig. 1; 100% rats affected), and together these symptoms contribute to the spasticity syndrome seen in these rats. In all rats, the stretch/rub maneuver elicited an uncoordinated ventral flexion movement (Fig. 2), which we refer to as a flexor spasm. That is, the tail did not move in a smooth arc as in normals, and coiled with jerky movements with the tip often rotating more than 360 degrees (mean tip excursion 242 ± 51 degrees in chronic spinal rats, compared to 45 ± 34 degrees in normals, n = 11 rats). Such flexion reflexes were initiated with the same latency as normals, but the flexion that they produced lasted for minutes rather than seconds (mean flexor spasm duration 138 ± 64 sec, compared to 2.5 ± 0.4 sec in normals, as discussed above, n = 11 rats). Flexion spasms also occurred spontaneously without apparent stimulation (Fig. 1). Repeated stimulation (three stretch maneuvers applied in rapid succession) gave more muscle activity, which could last for >30 min, and included marked hypertonus (judged by steady flexion) interrupted by frequent flexion spasms (movements) and clonus. The clonus occurred in the tip of the tail (in all rats) and was characterized by rapid movements (5–10 Hz), which appeared to be triggered by the flick of the tail during a flexor spasm.

Manual manipulations of the tail indicated that during flexion spasms the muscle tone was much greater than in normal rats (n = 11 rats). Attempts to straighten the tail rapidly usually only increased the muscle tone and produced a coiling when the tail was released. However, at times, a sustained stretch that straightened the tail inhibited the muscle spasms, analogously to the clasp-knife response in limb musculature (occurred in n = 11 rats: Fig. 2B; Burke and Lance, 1973).

Flexor spasms could be elicited by whole tail stretch as described above or by bending a portion of the tail. It was difficult to dissociate the effects of muscle stretch from cutaneous stimulation, since the tail was very sensitive to even the slightest hair contact. However, the flexor spasms caused by holding onto the tail at one location usually subsided, and imposed bending movements could then be applied to demonstrate that muscle stretch increased tone and caused further flexor spasms (Fig. 3).

The dorsal surface of the distal 5 cm of the tail was most sensitive to light hair touch and would bend away when touched. Other skin surfaces also caused similar withdrawal responses (in dorsal and lateral directions), though they were smaller and decreased more rostrally.
There appeared to be an area of paraesthesia that extended over a 2–3-cm region at the base of the tail (tested by pinch responses) and corresponded to the skin innervation region of the S2 roots. The hairs/skin of the distal tail were often so sensitive that even light brushing or spraying with a fine mist of water caused a complete flexion spasm, clonus, etc., as for the stretching described above.

When the tip of the tail came into contact with a surface, as often happened during a flexor spasm when the tail coiled and reached the plexiglass cylinder in which the rat was supported (Fig. 2), there was a local rapid withdrawal, followed by a release, which caused a second contact and a second withdrawal, and so on (occurred in all rats, n = 11). This repeated reflex activation occurred rapidly (up to 10 Hz) and produced tremulous movements of the tail, which we refer to it as contact-clonus, to distinguish it from clonus that occurred without skin/hair contact (the latter presumably induced by stretch reflex).

**Long-Term Chronic Spinal: Stage 4 (2–14+ Months)**

The tail spasticity described above grew progressively more severe over the months after the injury, at least for as long as we have kept our animals (up to 14 months). After 2 months, excessive extensor muscle activity be-
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gan to occur in the distal third of the tail (in all rats, Fig.
3; n = 11). This extensor activity was coincident with
flexor activity in more proximal muscles, so the tail was
at times postured in an S-shape (Fig. 2B). Such combined
extensor and flexor activity led to marked increases in
muscle tone, as judged by manual manipulations and
stiffness measurements, and we have thus referred to this
condition as combined flexor and extensor hypertonus.
As before, muscle stretch and skin stimulation triggered
large tail movements, but in these older animals the
movements were both in the flexor and extensor direc-
tion (referred to as flexor and extensor spasms). At this
late stage, the positive curling reaction, which is
described above for normal rats, also reappeared (Fig. 1).
This is probably because combined extensor and flexor
reactions were more prominent, as is needed for this
effect. Combined flexor and extensor spasms at times pro-
duced such strong contractions that stimulation, such as
skin/hair contact, produced no further muscle activation.
In this stage, spontaneous muscle activity and clonus
were even more frequent than earlier. On average, the
clinical spasticity rating for rats in this late chronic spinal
stage was 4.3 ± 0.5 out of 5, compared with 2.6 ± 0.4
in the earlier chronic stage (n = 11 rats, stage 2 and 3;
for rating system, see Table 1 and Methods).

Hyperalgesia

Since low-threshold cutaneous responses were so
prominent in chronic sacral spinal rats, we thought that
they might also have a hypersensitivity to high-threshold
afferent stimulation (hyperalgesia), as is the case fol-
lowing peripheral nerve injury (Kim et al., 1995). We
thus examined nociceptive response latencies to stimula-
tion by a standardized radiant heat source in the chronic
spinal rats. Pinch of the tail usually evoked a rapid tail
flick and, at times, a full flexor spasm. Radiant heat
caused a similar tail flick, and, with this stimulation, it
was possible to measure the response latency, which was
significantly less than that in normals rats (11.4 ± 3.3 sec
compared to 14.6 ± 3.0 in normals, n = 11 rats, p <
0.05; Kim et al., 1995; Advokat, 1993). Thus, at least as
classified by the heat test, nociceptive thresholds were
slightly lower in sacral spinal rats, and this hyperalgesia
might contribute to the observed spasticity. Interestingly,
abnormal nociceptive responses also contribute to the
general spasticity syndrome seen in humans (Kuhn and
Macht, 1948).

Control Rats

Rats with sham surgeries involving only a laminecto-
yomy, without transections or root damage, behaved as
normal rats in all regards, with no hypertonus, muscle
spasms, or clonus (n = 3 rats). However, we noticed that,
in one rat, where we manipulated the sacral dorsal roots
and caused inadvertent damage during a sham surgery,
a condition of hypersensitivity to light touch developed (a
brief twitch occurred with light touch of the distal tail).
This rat did not have large amplitude, prolonged move-
ments (flexor spasms) that are characteristic of the spinal
rats (Table 1). However, we thought that the hypersensi-
tivity to light skin/hair touch seen in the spastic rats might
result in part from root damage that was invariably made
during the spinal transection surgery. Thus, we deliber-
ately cut the S2 dorsal roots in two additional control rats.

<table>
<thead>
<tr>
<th>Rat type</th>
<th>Flex tone</th>
<th>Ext. tone</th>
<th>Stretch reflex</th>
<th>Flex. spasm</th>
<th>Clonus</th>
<th>Cutan. reflex</th>
<th>Tail flick</th>
<th>Bladder and bowel</th>
<th>Locomotion</th>
<th>Rating</th>
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</table>

*0, minimal activity; +, normal or increased activity; ++, maximal activity; n/a, not applicable. Flexor (Flex) and Extensor (Ext)
tone tested by observations of steady posture and manually imposed movements. Stretch reflex taken as response to manual stretch.
Cutaneous reflexes were responses to light hair or skin touch. Flexion spasm was the folding movement in response to stretch/rub of
tail, quantified by amplitude and duration. Clonus was rated as + if it usually required skin contact (contact-clonus) or ++
otherwise (clonus without contact). Tail flick is response to deep pinch or radiant heat. Long-term (>60 days) spinal rats developed
extensor muscle activity and thus extensor tone. Tests done while rat resting in plexiglass tube with tail hanging vertically. The
rating scale shown was developed to quantify the severity of the spasticity taking into account muscle tone, reflexes, and clonus as
indicated in the first seven columns.
one of which also developed a similar condition of cutaneous hypersensitivity. While this issue warrants further study, we can at this point say that root damage may be involved, though it alone is not a sufficient condition for the development of spasticity. Interestingly, these control rats with root damage did not have hyperalgesia, as judged by their normal heat test response latencies (15.4 ± 2.3 sec).

Quantification of Stretch Reflex, Muscle Stiffness, and Hypertonia

In normal rats, when the flexor muscles were stretched by bending the tail dorsally and laterally to the contralateral side, usually no EMG was produced by the stretch reflex, and only a small passive resistance force occurred (low stiffness/tone; Fig. 3C). Occasionally, a phasic reflex was seen, but, usually by the time the tail was manipulated during the experimental setup, it was areflexive, as in limbs of human subjects instructed to relax. In spastic rats, the same stretch produced a much larger and more prolonged force response, caused by a phasic and tonic stretch reflex (Fig. 3B). When the extensor muscles were stretched (Fig. 3A), similar large stretch reflexes were seen in spastic rats, but not normal rats. The tonic component of the EMG reflex was analogous to the spastic stretch reflex (SSR) described by Thilmann et al. (1991) in spastic humans. At times, this reflex marked the onset of a muscle spasm, with irregular contractions (arrow in Fig. 3B) and clonus in the distal few centimeters of the tail that was not strapped to the manipulator. This SSR occurred in all spinal rats when

**FIG. 4.** Increased H-reflexes in spinal rats with spasticity. The inferior caudal trunk nerve was stimulated with single pulses, and EMG responses were recorded in the segmental tail muscles before, at 2 days after (acute) and 14 days after (chronic) S2 transaction (the latter taken the first day spasticity occurred after surgery; spasticity rating of 3.0). Measurements made in "resting condition," with background EMG low or absent before stimulation. (A) Stimulation above motor threshold (1.1 MT) produced an M-wave (motor response) and an H-reflex. (B) The H-reflex can be seen in isolation with a subthreshold stimulation (0.9 MT). Each panel shows seven responses and the average (thick line). EMG rectified and lowpass filtered at 100 Hz. Note that the H-reflex is markedly larger in spastic rats. Also note the pronounced tonic response, which never occurred in normals, and often had an oscillatory component (spastic condition, B).
tested after spasticity developed (mean, 0.16 ± 0.04 N) and was significantly different from that in normal rats where no SSR occurred \((p < 0.05; n = 11)\). Once a spasm was elicited by stretching the tail, subsequent stretches had less effect on the EMG, as if the motoneuron activity were saturated by tonic activation. Also, the stiffness was markedly larger once the spasm started because of the increased muscle activity (hypermotor).

Quantification of the Stretch Reflex: H-Reflex Testing

In order to assess the viability of the spinal cord after lesions, we developed a technique of stimulating the inferior caudal trunk nerve and recording the segmental tail muscle EMG with surface electrodes (Fig. 4). With this technique, we were able to accurately monitor changes in the excitability of the spinal cord, even when clinical and stretch related responses were barely perceptible in the acute stage of spinal cord injury. Since the tail muscles have Ia afferents and short latency reflexes (Steg, 1964), we first examined the associated H-reflex. In normal rats, the H-reflex was small or absent when tested with caudal trunk nerve stimulation (the ratio of the maximum H-reflex to the maximum M-wave × 100% was %Hmax/Mmax = 3.0 ± 2.7%, \(n = 11\) rats; Fig. 4). This response was small probably because of presynaptic, rather than postsynaptic, inhibition, since it remained small when the postsynaptic excitability was raised by increasing the background EMG activity by manual manipulations of the tail. In the acute stage following injury, when the tail was clinically flaccid (<14 days), the H-reflex was also small (%Hmax/Mmax = 3.2 ± 2.8%). With the clinical development of spasticity, the H-reflex increased by almost an order of magnitude, significantly above that in the normal and acute spinal states (%Hmax/Mmax = 20.7 ± 9.1%, \(n = 10\) rats, \(p < 0.05\)), and occurred at a remarkably low threshold (0.6 MT). Similar large H-reflexes could be seen with tonic background EMG, though the amplitude relative to the background EMG was reduced because the reflex seemed to be saturated at a maximal value. The large H-reflex at rest (Fig. 4) is functionally most relevant, since in this state the stretch reflexes are resisted minimally by the intrinsic stiffness of the muscles and are thus most likely to produce clonus (largest reflex gain; Bennett et al., 1996).

Long-Duration Muscle and Cutaneous Afferent Reflexes Following Nerve Stimulation

In terms of contributions to spasticity, perhaps at least as important as the H-reflex (short latency reflex) was the tonic reflex that followed the Ia afferent stimulation in spastic rats (Fig. 4). This reflex did not occur in normal rats, and, in chronic rats, it became large and significantly different from zero \((p < 0.05, n = 11\) rats). Likely, it is the electrical stimulation equivalent of the SSR reflex described for muscle stretch. This tonic reflex did not always occur with a single shock, but repeated stimulations (e.g., 10 shocks at 10-msec intervals) always elicited it in the spastic rat. It occurred at the lowest thresholds, indicating that it can be mediated by group I afferents. However, it got progressively larger as the stimulation was increased from 1 to 5 × MT, indicating that higher threshold afferents were also involved.

As stated above, the cutaneous responses to light touch and pinch were larger and more prolonged in spastic rats than in normals. To quantify this, we found it best to electrically stimulate the distal tail to produce the electrical equivalent of such cutaneous stimulation and record the more proximal segmental tail muscle EMG (Fig. 5). In normal and acute spinal animals, such stimulation usually produced short latency inhibition that lasted 400–500 msec, followed by excitation, depending on the stimulation intensity (Fig. 5). On occasional trials, the excitatory response was large and associated with a brief tail flick. If the stimulation was repeated, any residual activity from the previous stimulation was silenced by the short latency inhibition. In chronic spinal rats, the same stimulation elicited a pure excitation that started with a short latency and was tonically activated (Fig. 5), and was significantly different from normals \((p < 0.05, n = 11\). Thus, the short latency component reversed from inhibitory to excitatory (reflex reversal). Repetition of the stimulation therefore produced larger and larger responses (Fig. 5), unlike in normals. The lack of inhibition to such stimulations is consistent with the clinical finding that repeated stimulation produced more and more muscle activity (e.g., flexor spasms and clonus).

Histology

During surgery, the S2 spinalization was verified to be complete by inspection under a surgical microscope. Thus, histology to verify the lesions has only been performed on three of nine spinal rats. In the remainder, the completeness of the transection was verified with a surgical microscope after sacrifice. A typical longitudinal section of the spinal cord is shown in Fig. 6B (14 months postlesion), which indicates a clear gap at the lesion site, and an intact spinal cord above and below the lesion (see large cells which are likely motoneurons in Cresyl violet-stained sections; Fig. 6C,D). No fiber tracts crossed the lesion (cf. silver stain), and only fibrosis and gliosis was seen at the lesion site. The white matter is also largely depleted below the lesion site (cf. Fig. 6A with 6B). Even though the original lesion was only 1-mm wide, the le-
FIG. 5. Reversal of cutaneous reflexes, from inhibitory to excitatory, in spinal rats with spasticity. Segmental tail muscle EMG responses to cutaneous and high threshold afferent stimulation applied to the tip of the tail (last 3 cm). Data recorded on same days and with same EMG configuration as in Fig. 4. (Left) Low threshold stimulation (first two stimuli in left in each plane, 2 T, 2 msec) produced short latency inhibition of ongoing EMG in normal and acute spinal rats, which lasted 500 msec. In spastic rats, only short latency excitation was produced by this stimulation. (Right) High-threshold stimulation (last four pulses in each plane, 5 T, 2 msec) produced an additional long-latency excitation. In spastic rats repeated stimulation produced a growing response (lower panel), which often resulted in a flexor spasm, since there was no inhibitory component to the reflex, as in the other conditions.
sion in this animal had spread to more than 5 mm, with cavitation on one side above the lesion. Comparison to a normal spinal cord (Fig. 6A) indicated that the rostral and caudal cord segments had pulled apart with time, since there was an unusually large separation between the S1 (above lesion) and S3 (below lesion) sacral roots.

**DISCUSSION**

Our results demonstrate that sacral spinal lesions in rats lead to a delayed onset of flexor and extensor hypertonus, hyperreflexia, flexor and extensor spasms, clonus, and paralysis in the tail muscles, without urinary tract and bowel complications (cf. daily bladder and bowel expression not necessary). A similar model of spasticity has been developed by Ritz et al. (1992) in cats. The hallmark of the chronic sacral spinal rats is a long-lasting (many minutes) tail muscle activity that follows stretch and cutaneous stimulation, and we suggest that the duration of this activity may provide a very simple measurement for assessing new treatments for spasticity (cf. antispastic drug testing, unpublished findings). We have found that the clinically observed symptoms in the rat tail can be investigated with straightforward surface stimulation and EMG recordings to examine associated changes in segmental reflexes, including the short latency and tonic (SSR; Thilmann et al., 1991) stretch reflexes and cutaneous reflexes. The changes seen in the short latency reflex (H-reflex) are consistent with recordings from hindlimb muscles in spinal cord injured rats (Thompson et al., 1992). One new finding was that a short

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**FIG. 6.** Longitudinal sections of sacral spinal cord after chronic transection. Tissue from a normal (A) and a spastic rat 14 months posttransection (B), stained with Cresyl violet and silver nitrate (axons in white matter light brown, roots dark brown). In both cases, all sacrococcygeal cord and half of L6 segment shown. For the spastic rat (B), the gray and white matter are shown completely transected at S2 and separated by a wider gap than the 1-mm lesion made at surgery. Dark brown staining at the lesion is from ventral roots that had adhered to the lesion by connective tissue. (C,D) Enlargements of grey matter above and below lesion, which shows large neurons stained with Cresyl violet (motoneurons) and depletion of white matter below the lesion.
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latency and long-lasting cutaneous reflex reversed from being inhibitory in normals to excitatory in chronic spinal rats (reflex reversal).

It is puzzling that sacral spinal cord lesions lead to these symptoms that resemble the human spasticity syndrome, even with partial lesions (Ritz et al., 1992), whereas higher lesions to the spinal cord do not reliably lead to spasticity in animals (rats, cats). Even in non-human primates, a syndrome like spasticity has been difficult to produce (for review, see Noth, 1991). A traditional rationale for the occurrence of spasticity in humans, but not animals, has been the predominance of supraspinal/cortical control in the former (Freund, 1987; Noth, 1991). Animals have clearly functional spinal circuitry that controls complex tasks such as scratching and locomotion (Dellagina et al., 1975; Grillner, 1981; Rossignol et al., 1989; Belanger, 1996), whereas humans have a much lower capacity for such spinal activities (Kuhn and Macht, 1948; Calancie et al., 1994). Plasticity that follows high spinal lesions in animals may involve increasing the excitability of such (lumbar) spinal locomotor circuits, rather than (or in addition to) those that regulate muscle tone (Burke et al., 1972). Analogously to the way the spinal cord is under strict supraspinal control in humans, the low sacral spinal cord in rats and cats receives strong propriospinal and supraspinal control (Masson et al., 1991; Wada et al., 1993), including input from hindlimb locomotor centres (Wada et al., 1996), and itself has only a limited repertoire of intrinsic motor circuits (e.g., tail flick). Thus, one suggestion is that spasticity develops in the rat tail after S2 transection since the sacrocaudal cord is deprived of its predominant control, as in the human situation.

An example that shows the differences that can be produced by low and high spinal lesions involves L2-L3 proprioceptive neurons, which inhibit transmission from flexor reflex afferents (FRA; cf. FRA reflexes) in more caudal segments in cats (Cavallari and Pettersson, 1989). Since these L2–L3 neurons are normally inhibited by the brainstem, a high spinalization (T10) increases their excitability and ultimately inhibits the FRA reflexes (Cavallari and Pettersson, 1989). In contrast, low spinal lesions that interrupt the descending axons of these L2–L3 proprioceptive neurons (in the dorsolateral funiculus) release the more caudal FRA reflexes from inhibition. A similar release from inhibition may in part explain the exaggerated high threshold muscle and cutaneous reflex transmission (i.e., FRA) with our low spinal lesions. There also appears to be a profound release from presynaptic inhibition on Ia afferent terminals in the short latency reflex, since the H-reflex was large compared to that in normal rats, regardless of the level of postsynaptic activity, as judged from the EMG (for relation of H-reflex to background EMG and presynaptic inhibition, see Stein, 1995).

The development of spasticity in the rat tail follows a similar time course to that in human limbs, suggesting similar underlying mechanisms, in spite of the species and anatomical differences. For example, significant reflexes are found in both only after a few weeks, and then initially flexor responses dominate. With time extensor tone and reflexes develop additionally in rats and humans (Kuhn and Macht, 1948; Ashby and McCrea, 1987). While some of these changes occur with high spinal transection animal models of spasticity, they are difficult to study and investigators have focused instead on the emergence of such behaviors as spinal locomotion on treadmills (Rossignol et al., 1989). Further, previous efforts to develop models that mimic specific symptoms of spinal spasticity, such as the clasp-knife reflex have failed from the point of view of timing. For example, while dorsolateral funiculus lesions do produce clasp-knife responses, they occur acutely (Burke et al., 1972; Rymer, 1979; Taylor et al., 1997). The decerebrate preparation also produces marked acute symptoms, such as tonic extensor rigidity (Liddell and Sherrington, 1924). Likely, for understanding the complex sequence of events that follow spinal cord injury in the spinal rat it will be useful to differentiate acute effects that simply change reflex gains (e.g., release of presynaptic inhibition), from long-term reorganization in spinal cord properties. In our rat tail model, the latter include (1) the emergence of tonic Ia afferent reflexes not seen in normals (SSR, Fig. 4), (2) reversal of cutaneous and high-threshold afferent reflexes to being strictly excitatory (Fig. 5), and (3) marked increases in tonic motoneuron activity (cf. hypertonia). Numerous mechanisms may explain these long-term changes (for review, see Ashby and McCrea, 1987; Woolf, 1988; Young, 1994).

Our objective has been descriptive, highlighting the changes in tail behavior that develop after chronic spinal cord injury, and where possible relating them to human spasticity. Obviously, spasticity in the rat tail muscles cannot be directly compared to spasticity seen in limbs of humans. However, since it does have important features of the human spastic syndrome (Table 1), it is worth considering its relevance. Possibly this rat tail model is closest to spasticity in the axial musculature of the back in humans with spinal cord injury (Stauffer, 1974), since these muscles have a similar anatomy to that of the rat tail. We have focused on complete transections initially, since the interpretation of behavioral changes is simpler. However, incomplete sacrocaudal lesions, such as hemisections or contusions also leads to spasticity in cats (Ritz
et al., 1992; and Ritz, personal communication) and rats (unpublished findings from two hemisected rats), thus providing a more realistic model of human injury.

By using sacral spinal cord lesions in rats, we have developed a model of spasticity that is convenient to study in the awake state. A number of simple methods have proven to be important, including (1) the use of a Plexiglas holding tube in which spinal rats will sit quietly for long examinations, (2) surface recording and stimulating electrode "cuffs," and (3) associated chronic reflex testing. For comparison, it should be noted that awake cats (as in the model of Ritz et al., 1992) will not tolerate being constrained for more than a few minutes, without extensive training. Further, surface electrodes or percutaneous electrodes that we used on the tail, are not tolerated by awake animals in other muscles (e.g., hindlimb), and chronic recording is thus much more involved, including implanting electrodes and associated headpiece connectors (Hiebert et al., 1994). An additional advantage of this preparation, that we have not yet mentioned, is that the associated adult rat sacrocudal spinal cord can survive in vitro whole or hemisected (Long et al., 1988; Bennett et al., unpublished), primarily because of its relatively small size (Fig. 6B). Thus, in principle it should be possible to study the chronically injured (spastic) rat spinal cord in vitro.

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