Journal of Neuroscience Research 77:277–284 (2004)

Recurrent Paraplegia After Remyelination of the Spinal Cord

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We have conducted a long-term study of spinal cord morphology and motor function recovery in rats that have undergone lumbar spinal demyelination induced by the B-fragment of cholera toxin (CTB)-saporin. We found that, after the initial demyelination and paraplegia, motor function recovered and was stable for up to 9 months, after which there occurred a slow deterioration of motor function accompanied by loss of motoneurons and loss of spinal white matter. A striking morphological feature was the appearance of large spheroids of calcium in the ventral and dorsal horns and occasionally in the white matter. Motor performance deterioration occurred earlier and was more severe in rats that had been exercised on a treadmill, but the same morphological changes occurred in both exercise- and nonexercise-treated animals. Rats given treadmill exercise starting 3 weeks after toxin injection had a mean motor deficit score of 3.0 (i.e., paraplegia) at perfusion, whereas the nontreadmill-treated rats had a mean score of 1.8 (SD 0.38; n = 6; $P <$ .05). These findings suggest that, in addition to the acute effects of the toxin-induced demyelination from which there is recovery of motor function, there are chronic irreversible effects of the toxin, or the initial demyelination, that cause a slow progressive degeneration of the spinal cord. This model might therefore be useful in studying the long-term effects of spinal insult of the type associated with conditions such as postpolio syndrome.

Key words: CTB-saporin; neurotoxin; myelin; calcium deposits; motoneuron degeneration

We have previously described the behavioral and anatomical changes that occur following demyelination of the rat lumbar spinal cord by using a toxin prepared by conjugating saporin, a ribosome-inactivating protein, with the B-fragment of cholera toxin (CTB-Sap; Jasmin et al., 2000). Intrathecal application of CTB-Sap caused no mechanical trauma to the spinal cord but resulted in reversible paraplegia from a loss of oligodendrocytes. The recovery of function was mediated through the remyelination of white matter axons by both oligodendrocytes and Schwann cells. We wished to examine the long-term consequences of the remyelination and to determine the effect of exercise on motor recovery and whether functional changes could be correlated with histological changes.

Exercise has been advocated as a means to improve motor performance in humans and animals following spinal cord injury. Although the effects of exercise are generally positive, it is not known whether exercise is universally beneficial for all types of spinal cord dysfunction, particularly in rat models that are becoming more frequently used in spinal research. Exercise intended to improve motor function following spinal cord injury has been used most extensively in human (Dobkin et al., 1995; Dietz et al., 1997; Barbeau et al., 1998; Dietz, 2001; Drory et al., 2001; Wirz et al., 2001; McDonald et al., 2002) and cat (Lovely et al., 1986; Barbeau and Rossignol, 1987; Roy et al., 1998; De Leon et al., 1999) studies after transection or contusion injuries. Most reported results are positive, and, although the mechanisms underlying improvement have not been fully determined, the effects on the central pattern generator are thought to be important (Edgerton et al., 1997; De Leon et al., 1998; Lankhorst et al., 2001). Similar positive results have been reported in human nontraumatic conditions such as poliomyelitis (Birk, 1993), multiple sclerosis (MS; Kraft, 1999; Kilmer, 2002; Mostert and Kesselring, 2002; Nielsen and Norgaard, 2002; Patti et al., 2003), and amyotrophic lateral sclerosis (ALS; Drory et al., 2001).

Exercise strategies are now being tested in rodent models of traumatic spinal injury, and several studies have reported motor improvement following treadmill exercise (Merkler et al., 2001; Thota et al., 2001; Moshonkina et al., 2002; Multon et al., 2003) or enriched housing paradigms (Lankhorst et al., 2001). These studies contrast with an earlier study that found no improvement with training in a partial-transection-injury model (Thota et al., 2001).

Contract grant sponsor: NIH.

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Received 26 January 2004; Revised 18 March 2004; Accepted 19 March 2004

Published online 5 May 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.20143

For nontraumatic-injury models, there are fewer data. In one study on experimental autoimmune encephalomyelitis (EAE), the peak severity of the different types of induced EAE was not modified by exercise, but the onset of the chronic relapsing form was delayed (Le Page et al., 1994). Mixed results have been reported in mouse ALS models, with motor improvements varying between the sexes and ranging from none to modest (Kirkinezos et al., 2003; Veldink et al., 2003). In a rat denervation model of postpolio syndrome, it was found that exercise was not beneficial and that, after prolonged denervation, chronically enlarged motor units became exhausted and nerve terminals become unstable, resulting in terminal withdrawal (Tam et al., 2002).

The CTB-Sap model used here is functionally more similar to demyelinating conditions such as MS, and we wished to see whether exercise had positive effects in a model in which there was no direct mechanical injury such as transection or contusion of the cord. Here we have studied the long-term outcome, i.e., up to 2 years, on motor function of previous spinal demyelination with and without exercise.

MATERIALS AND METHODS

Twenty-four female Sprague-Dawley rats (260–300 g; Harlan) were used in this study. All animals were exposed to light for 12 hr per day; food and water were available ad libitum for all animals at all stages of the disease. Procedures for the maintenance and use of the experimental animals were approved by the Animal Care and Use Advisory Committees at UCSF and were carried out in accordance with NIH regulations on animal use.

Drug Delivery

Intrathecal injections of CTB-Sap (3 μg; lot 5-145; Advanced Targeting Systems, San Diego, CA; n = 12), or sterile saline (n = 12) were made in the lumbosacral intrathecal space by lumbar puncture at the L5–L6 level.

Neurological Status

Motor and sensory functions were tested daily, and rats were videotaped walking on a treadmill every 3 days during the first 30 days postinjection and every week thereafter. The rats were habituated to the treadmill and to handling by the experimenter prior to any treatment. Videotaped recordings of the rats were captured on a computer, and the motor performance was assessed and scored by a frame-by-frame analysis of the videos. Control, nontreadmill animals, were placed on the treadmill once per week for 2 min for filming. Each animal was examined for proximal and distal tail tonus, maximum hindlimb extension, toe spread, and overall gait. The degree of motor impairment was quantified according to the parameters shown in Table I, adapted from a standard EAE scale (Reynolds et al., 1996). It should be noted that, for each score (Table I), the animal had the impairment listed for that score plus all the impairments of the lower scores, i.e., the impairment was additive and progressive. Since the most evident and reliable deficits were reduced hindlimb extension, we evaluated the capacity to extend the hindlimb and keep the hindquarters elevated when walking by measuring the end-of-stance angle, i.e., the angle between the dorsal aspect of the foot and the leg at the end of the stance, the point when the hindlimb reached its maximal extension (Jasmin et al., 2000). The animal was considered to show minor weakness when the end-of-stance angle was between 80° and 50° and the knee did not touch the floor (score 1.5). Moderate weakness was defined as the state when the end-of-stance angle was less than 50° and the knee touched the floor (score 2). Except for paraplegic animals (score = 3), six end-of-stance angles at each session were averaged to give the mean angle, and this value was used in determining the motor impairment score for the animals at each session.

TABLE I. Scale Used To Quantify the Degree of Motor Function

Motor impairment	Neurological score
None	0
Loss of distal tail tonus	0.5
Complete loss of tail tonus	1
Incomplete toe spread, minor weakness in hindlimb extension[a]	1.5
Toes do not spread apart, moderate weakness in hindlimb extension[b]	2
Marked proximal hindlimb paresis,[c] distal paralysis	2.5
Complete hindlimb paralysis	3
Light paresis of the distal forelimbs	3.5
Moderate proximal forelimb paresis	4

[a]End-of-stance angle between 80° and 50°; the knee never touches the floor; when the rat is held vertically in the air, the feet are elevated and the toes are directed upward.

[b]End-of-stance angle ≤50°; the knee touches or rests on the floor; when the rat is held vertically in the air, the paws are splayed outward to the front or sideways. The rat can stand on the hindlimbs only if leaning against a vertical support.

[c]On the walking surface, the hindlimbs are splayed outward and paws everted. The rat uses the forelimbs for forward movement; there is movement of the hips but no purposeful movement of distal hindlimbs. The rat is unable to raise the hindquarters against gravity (paws and toes remain downward when the rat is held vertically) and cannot stand up on the hindlimbs.

Treadmill

Rats in the exercise group were placed in an enclosure on a flat treadmill that was set at a constant speed of 6 feet/min for 20 min for 5 days per week. The enclosure consisted of four adjacent Plexiglas corridors 3.75 inches and wide 31 inches long, in which four rats were trained at the same time. One end of each corridor had a mesh that delivered a low intensity shock when touched by the rat and the other end had a darkened enclosure to induce the rats to walk to that end. The combination of the aversive and incentive stimuli ensured the rats walked on the treadmill for the duration of the training time. After two or three training sessions, the shock was not required and was turned off for subsequent sessions. Once per week, the rats were filmed walking on the treadmill and motor scores determined as described above.

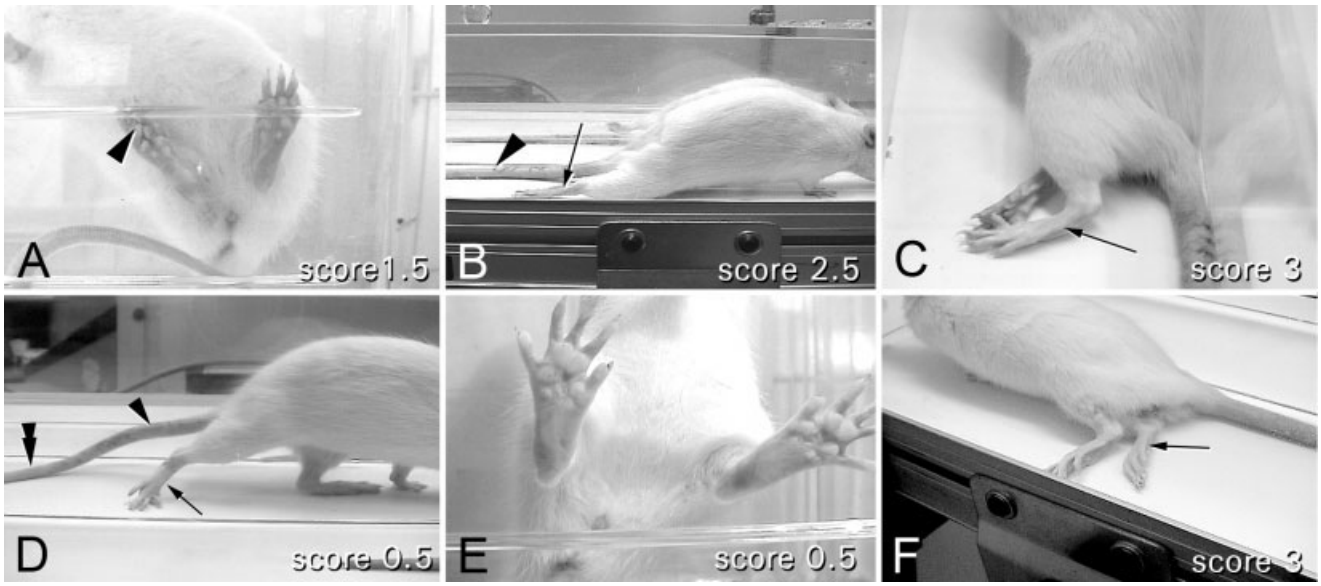

Fig. 1. Stage of progression of CTB-Sap-induced motor deficits. **A:** Initial motor symptoms include the inability to spread the toes (arrowhead) when standing. **B:** Twelve days postinjection, there is loss of proximal and distal tail tonus (arrowhead), and the rear limbs become progressively weaker (arrow). **C:** Complete paralysis of both rear limbs (arrow) 15 days postinjection. **D:** Seventy-five days postinjection, the rat has recovered from the paraplegia, is able to support its full body weight on the rear legs, and has a near normal gait. Note that there is return of proximal tail tonus (single arrowhead), but there is no recovery of distal tail tone (double arrowhead). **E:** At 75 days postinjection, the rats are able to spread the rear toes fully when supporting full weight. **F:** Three hundred fifty days postinjection, there is a recurrence of symptoms, and the rat has once again lost motor function of the rear limbs (arrow).

Histology

Rats were deeply anesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. Fifty-micrometer-thick sections were cut on a freezing microtome and 5-μm-thick sections were cut on a cryostat. Sections were stained with 1) luxol fast blue, 2) cresyl violet, 3) luxol fast blue with cresyl violet (Klüver and Barrera stain), or 4) hematoxylin and eosin (H&E) according to standard protocols. Staining for calcium was carried out with Von Kossa's stain (Bancroft and Stevens, 1996). Immunocytochemistry was done according to standard protocols at antibody dilutions determined empirically by using the following primary antisera directed against: P0 (1:20,000; Schwann cell marker; courtesy of J. Archelos, Karl-Franzens-University, Graz, Austria), glial fibrillary acidic protein (GFAP; 1:8,000; Roche-Boehringer, Indianapolis, IN; clone G-A-5), oligodendrocytes (MAB1580; 1:200,000; Chemicon, Temecula, CA), OX-42 (1:4,000) or HIS-48 (1:1,000; PharMingen, San Diego, CA). For all antibodies, positive and negative controls were processed with the experimental tissue.

Quantification

Measurements were taken by using a computer-controlled microscope (Stereo Investigator; MicroBrightField, Colchester, VT). Luxol fast blue-stained sections were used to measure the area of gray matter, which was then subtracted from the area of the entire cord to give the total area of white matter. The area of myelinated white matter was measured and subtracted from the total area of white matter to give the area of demyelination,

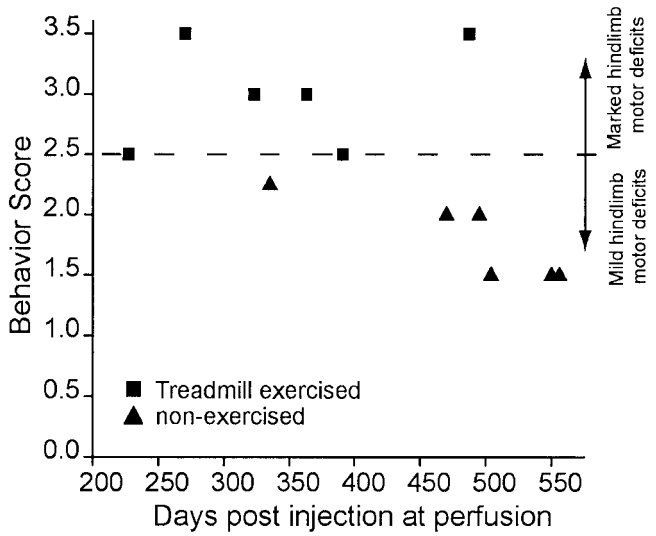

Fig. 2. Graph showing behavior score and time postinjection at perfusion. Each point represents one animal. The description of symptoms at each behavioral score is given in Table I, with a higher number indicating more severe deficits. The rats were 42 days old at the time of injection. Rats given treadmill exercise reached a higher score, and that score was reached earlier than in the nonexercised rats.

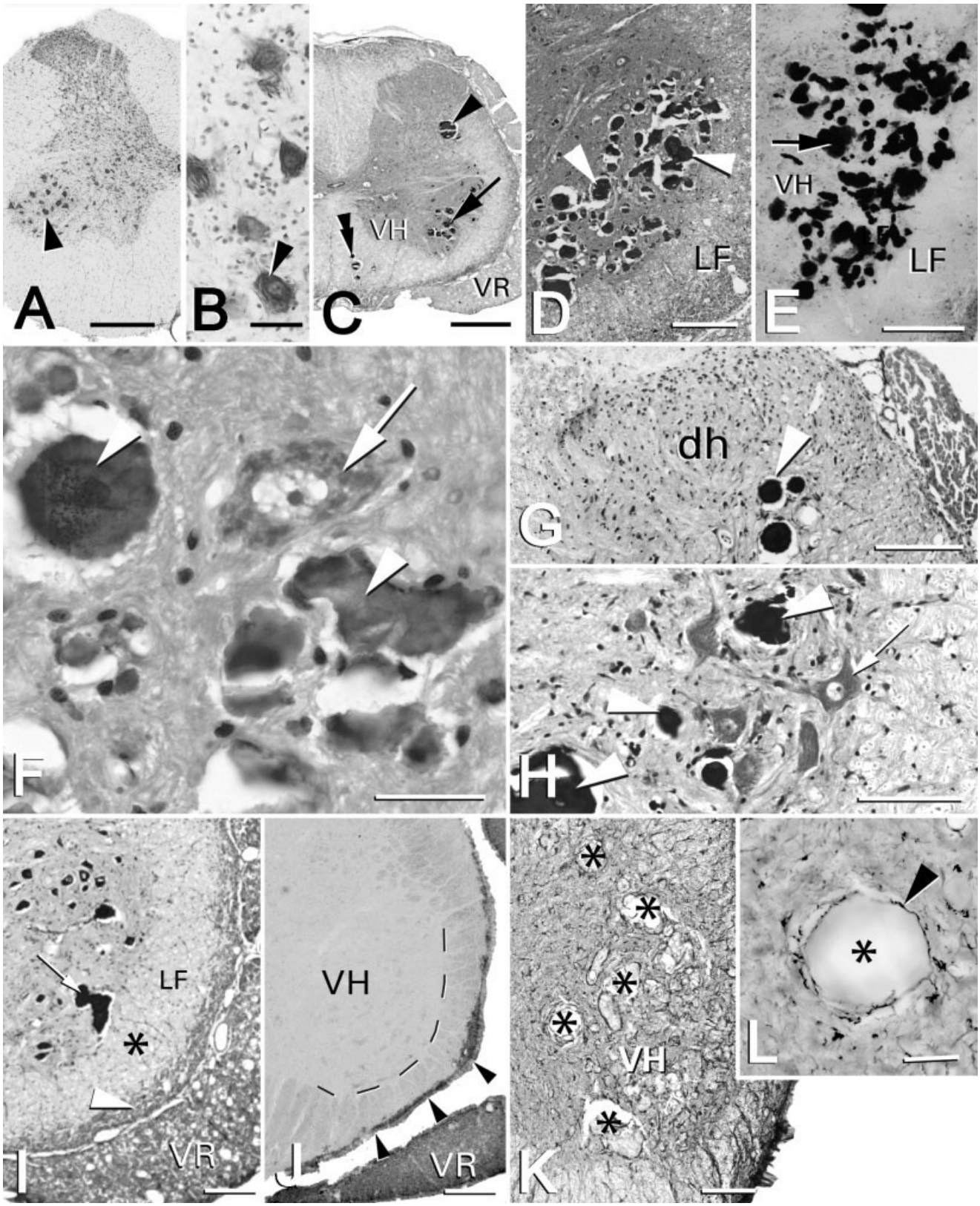

Figure 3.

which was then expressed as a percentage of the total white matter area in Results. All measurements were analyzed with Fisher's exact test and T Mann-Whitney test. $P < .05$ was considered significant.

RESULTS

Behavior

The progression of behavioral changes in the 3 months following lumbar intrathecal application of CTB-Sap has been described in detail previously (Jasmin et al., 2000). In brief, the rats underwent an ascending bilateral paraplegia that began with loss of tail tonus and progressed to complete paralysis of the hind limbs by 3 weeks postinjection (score 3; Fig. 1). There then occurred a progressive reversal of motor impairment, and, by approximately 10 weeks postinjection, the animals showed almost complete recovery of motor impairment (Fig. 1), loss of distal tail tonus being the most obvious remaining deficit. The rats at this stage were given a score of 0.5.

To examine the effects of exercise on recovery from the CTB-Sap-induced paraplegia, rats were divided into two groups; one group was given daily exercise on the treadmill, and the other group received no exercise. Thirty days after CTB-Sap injection, rats in the exercise group were placed on the treadmill for 20 min per day, 5 days per week. All the animals injected with CTB-Sap reached a maximum motor deficit score of 2.5–3.0 (n = 12), and, when the exercise group was first placed on the treadmill, they were showing a reduction of motor deficit and behavioral score (2.0–2.5; n = 12). At this stage, the animals showed some movement of the rear legs but could not fully support any weight on the rear legs, and initially the rats moved through use of the forelimbs. The nonexercise group (n = 6) was matched for progression of symptoms and behavior score with those animals in the exercise group. The exercise and nonexercise saline-injected groups showed no motor deficits (score 0) for the duration of the study.

Both the exercise and the nonexercise groups of CTB-Sap-injected rats showed a reduction of neurologic signs during the following months, and both groups progressed to full weight bearing on the rear legs and normal gait (Fig. 1F). Although there were small differences in the scores between animals in the two groups, there was no obvious difference in the regression of signs between the two groups. However, with continued exercise on the treadmill, it was observed that the rats in the exercise group began to show progressive weakness of the rear limbs and motor performance deteriorated, as indicated by an increase in the behavioral scores. Animals in the exercise group were perfused when they were scored at 3.0 (paraplegic) on three consecutive testing sessions, although, in two cases, the deterioration progressed rapidly from 3 to 3.5 over three testing sessions (Fig. 2), and the rats were perfused with the latter score. In two cases, the rats were perfused at a score of 2.5 when they developed incontinence and progressive weight loss (Fig. 2). The mean time at which the treadmill-treated rats reached the maximal score was 343 days (±118 days) and the behavioral mean score at perfusion was 3.0 (SD 0.58; n = 6). The nonexercised group also showed deterioration of motor performance, but this was less severe and occurred over a longer period. One nonexercise animal that showed the most severe deficit was perfused at 335 days (score 2.25; Fig. 2) for comparison with animals in the exercise group. The other animals in the nonexercise group plateaued at the scores shown in Figure 1. The nontreadmill-treated rats had lower behavioral score (mean 1.8; SD 0.38; n = 6; $P < .05$) and longer postinjection time (mean 471 days, ±94 days) compared with the exercise-treated group, although the difference was not statistically significant ($P > .05$).

Histology of the Lumbar Spinal Cord

We have previously described the histological changes in the spinal cord that occur up to 75 days postinjection (Jasmin et al., 2000). Briefly, the CTB-Sap caused loss of oligodendrocyte and demyelination of the outer spinal white matter. Schwann cells rapidly entered the area of demyelination and remyelinated the demyeli-

Fig. 3. Histology of spinal cord from rats after treadmill exercise. **A:** Overview of Nissl-stained lumbar spinal cord from a 550 day postsaline-injected rat. There is no reduction of the white matter, a normal compliment of neurons (arrowhead), and no calcium deposits. **B:** High magnification of the ventral horn shown in A. Normal-appearing α-motoneurons are present (e.g., arrowhead), and there is no evidence of calcium deposits. **C:** H&E. Overview of lumbar spinal cord of a CTB-Sap injected rat. A small accumulation of calcium deposits is present in the ventral horn (arrow), and single large deposits are present in both dorsal horns (single arrowhead). Small deposits are present in the ventral white matter (double arrowhead). **D:** H&E. Detail of deposits in the ventral horn of a rat 16 months after injection. Note the large number of calcium deposits (arrowheads) that fill the entire ventral horn. **E:** High magnification of a section serial to the one shown in B, showing mineral deposits (arrow) from the lateral part of the ventral horn stained with Von Kossa's stain, indicating the presence of calcium in the deposits. **F:** H&E. High magnification of ventral horn calcium deposits (arrowheads) surrounding a single normal-appearing motoneuron (arrow). **G,H:** Cresyl violet. Dorsal and ventral horns, respectively, showing deposits (arrowheads) surrounded by normal appearing tissue. Arrow indicates a motoneuron. **I:** Cresyl violet. Low magnification of ventral lateral spinal cord showing thin rim of Schwann cell myelinated fibers within the spinal white matter (arrowhead). Normal, oligodendrocyte myelin is indicated (asterisk). **J:** Immunocytochemistry for P0 (myelin marker) showing Schwann cell infiltration (arrowheads). Note the overall reduction in the amount of white matter. Dashed line marks border between gray and white matter. **K:** GFAP immunostaining showing no change in staining intensity associated with calcium deposits (asterisks). **L:** High magnification of calcium deposit showing nonreactive astrocytes (arrowhead) surrounding calcium deposit (asterisk). dh, Dorsal horn; VH, ventral horn; LF, lateral funiculus; VR, ventral rootlet. Scale bars = 0.5 mm in A, 75 μm in B, 1 mm in C, 200 μm in D,E,G,H, 20 μm in F, 300 μm in I, 450 μm in J, 400 μm in K, 50 μm in L.

nated axons. At longer survival times (>75 days), the Schwann cell myelinated area was reduced and replaced by new oligodendrocyte myelin (Jasmin et al., 2000).

The spinal cords of the saline-treated animals had a normal histological appearance (Fig. 3A,B), and all the following descriptions refer to CTB-Sap-treated animals. As noted above, the mean time of perfusion in the present study was far longer than that in our previous study. In the present study, examination of the lumbar spinal cord revealed changes in both the gray and the white matter in both treadmill- and nontreadmill-treated animals. The most severe disruption of the gray matter was the presence of large amorphous inclusions (Fig. 3C,D) that Von Kossa's staining revealed to be calcium deposits (Fig. 3E). The calcium deposits were located predominantly in the ventral horns, but, in four animals, small deposits were also present in the dorsal horn. The ventral horn deposits varied in number and size, and, in most instances, many deposits filled a large portion of the ventral horn (Fig. 3D). Histologically, normal motoneurons were occasionally present among the deposits (Fig. 3F), but, in sections with a large number of deposits, it was obvious that there was a loss of motoneurons, and this was not because calcium deposits had displaced the motoneurons to the periphery of the ventral horn. In some instances, the size and location or presence of internal structure gave the impression that some calcium deposits might have been motoneurons heavily stained for calcium. However, many of the mineral deposits were so large (Fig. 3) that they could not possibly represent individual, degenerate neurons. Occasionally, small calcium deposits were also present in the ventral white matter (Fig. 3C). Areas that contained no calcium deposits appeared histologically normal (Fig. 3G,H) even when they were adjacent or contralateral to sites with large calcium deposits.

In all cases, the amount of white matter was reduced when expressed as a ratio of total cord area or as a ratio of white matter to gray matter (treadmill, ratio gray/white = 0.87, SD = 0.026, n = 6; nontreadmill, ratio gray/white = 0.73, SD = 0.148, n = 6). There was no clear correlation between the amount of white matter reduction and the degree of motor impairment or the time course over which the changes occurred. As previously reported for shorter postinjection times, Schwann cells were present in the spinal white matter in all cases and formed a thin band at the periphery of the lateral white matter and were present in the dorsal part of the dorsal funiculus and the small parts of the ventral funiculus. The remainder of the white matter showed normal myelination (Fig. 3).

Sections of spinal cord, particularly the ventral horn where calcium deposits were present, were also examined by using immunocytochemical markers for inflammation and glia reaction. No OX-42 (macrophages) or His48 (granulocytes) immunostaining was seen, nor was there any reactive glia associated with the calcium deposits in the ventral horn or other parts of the spinal cord (Fig. 3I, J).

DISCUSSION

The purpose of this study was to examine the long-term effects of demyelination of the lumbar spinal cord and to see whether exercise would improve the recovery of function. We found that the spontaneous recovery of motor function we previously described for this model (Jasmin et al., 2000) was not stable and that, at very long survival times, there was a deterioration of motor function and structural changes in the ventral spinal gray matter. We found no beneficial effects of exercise on functional recovery, and, at very long survival times, exercise exacerbated the progression of recurrent motor deficits.

The long-term changes we describe here appear to be different from the short-term changes that cause the initial functional impairment. In the current model, it is likely that the initial demyelination causes paralysis by interrupting the input from descending long tracts and intersegmental connections possibly resulting in disruption of central pattern generator activity. There then occurs an intermediate state in which recovery of axonal function by Schwann cell and oligodendrocyte remyelination (Jasmin et al., 2000; Jasmin and Ohara, 2002) and possibly connectional reorganization brings about the restoration of motor function. At the much longer postinjection times reported here, there appears to be a second wave of changes probably resulting from secondary degeneration beginning to accumulate and cause irreparable damage to spinal motor systems. Although some of the motor impairment might result from deficiencies of oligodendrocyte remyelination, there is little histological evidence for such a possibility, and the most dramatic histological different in the long-term animals is the loss of motoneurons and disruption of the ventral horn by numerous calcium deposits. The loss of motoneurons is the most probable cause for the motor deficit, but the cause of this loss of MNs is not clear.

Although the data presented here are from rats that were older at perfusion (1.6 years) than the animals we described previously (Jasmin et al., 2000), we do not think that the changes we describe were age related. Rats have a life expectancy of 2.5–3.5 years (Sharp and LaRegina, 1998), so our animals were not near the end of their life span, and saline-injected rats perfused at the same time as the longest surviving CTB-Sap-injected rats showed no myelin or motoneuron loss and did not have calcium deposits.

One possible explanation for the loss of motoneurons is that destruction of descending or segmental input to the ventral horn results in anterograde transneuronal loss of motoneurons. Although in the short term there does not appear to be extensive loss of axons following the initial demyelination, and we could not detect any obvious motoneuron loss at shorter survivals (Jasmin et al., 2000), it is possible that it takes a long time for a small loss of axons to have an effect on motoneurons. It could also be that there occurs a continued loss of axons over time that is eventually significant enough to cause transneuronal motoneuron loss. We found a reduction in the amount of

white matter at the longest survival times, but we do not know whether this is a cause of the motoneuron loss or a result of the motoneuron loss. Based on previous studies, it seems unlikely that the motoneuron loss results from transynaptic degeneration because there is a consensus that even complete spinal cord transection appears to spare distal motoneurons (Stelzner et al., 1975; McBride and Feringa, 1992; Bjugn et al., 1997). Although this might suggest that, in our model, a minor loss of axons is unlikely to cause transneuronal degeneration, it should be kept in mind that demyelination of the entire lumbar cord involves both descending and intersegmental projections, whereas a high-level transection removes only descending input, only a percentage of which innervates motor neurons directly (Tracey, 1995; Kuchler et al., 2002; Raineteau et al., 2002). Furthermore, in contrast to the reports cited above, several studies do show motoneuronal loss following spinal transection (Kaelan et al., 1988; Eidelberg et al., 1989). Therefore, it is reasonable to suppose that the motoneuron loss might be a direct result of loss of afferents, but it is also likely that loss of descending input contributes to the loss of motoneurons but is not the primary cause of degeneration.

A second possible cause for the motoneuron damage is from direct effects of the toxin. When used as a toxin to target specific neuronal populations, Saporin brings about cell death within 2 weeks once it is internalized. If a large amount of CTB-Sap entered the motoneurons at the time of application, it would be expected to cause motoneuron loss within a few weeks, and this is not the case (Jasmin et al., 2000). On the other hand, if a small quantity of the toxin is taken up by the axons of motoneurons passing through the ventral white matter (Llewellyn-Smith et al., 2000), the large size of the cell body may buffer the effects of the Saporin so that it takes many months before the effects accumulate to a level that compromises cell function. Although there are no specific reports of long-term cell loss happening in such a manner, Saporin is know to be very resistant to enzymatic degradation within the cell (Santanche et al., 1997). The most immediate cytotoxic effect of Saporin involves its action on ribosome inactivation; however, it has recently been shown that an extremely low concentration of Saporin is able to induce DNA damage (Barbieri et al., 2003) leading to apoptosis. This latter mode of action is another possible explanation for the long-term destructive effects of Saporin. If the motoneuron loss does result from Saporin toxicity, this could also explain the different time course of the disease in exercise- and nonexercise-treated animals. The faster degeneration in the exercise group could result from the additional metabolic stress placed on the motoneurons by the increased motor activity.

The presence of the calcium deposits is unexplained. Although errors of calcium sequestration are associated with ALS-mediated neuronal degeneration (Krieger et al., 1994; Appel et al., 2001; Arakawa et al., 2002), this is usually a subcellular event and leads to the apoptotic death of the cell (Siklos et al., 1998; Alexianu et al., 2000). Calcium deposits similar to those described here have been shown in some brain areas following mercury poisoning (Mori et al., 2000), but they do not appear to be a common phenomenon of neural degeneration. Although some of the calcium deposits we describe are small enough to be motoneurons that are calcium filled, most of the deposits are far too large. A possible explanation for the large deposits is that they represent a site that serves as a nucleus for deposition of calcium that builds up to form the large deposits.

The most significant result is that, in the model we describe here, exercise is not beneficial and may in fact be detrimental to motor performance. This finding suggests that the efficacy of exercise is related to the type of motor deficit being treated. In the case of a complete or partial transection, exercise appears to be beneficial in retraining central pattern generators and maintaining muscle tone. In cases such as that described here and in similar conditions such as postpolio syndrome (Tam et al., 2002), the added stress placed on motoneurons by exercise is counterproductive. The long-term changes reported here also show that the CTB-Sap-induced demyelination model is useful for studying short-term effects of demyelination and long-term effects of axon and motoneuron loss.

ACKNOWLEDGMENTS

We thank Mr. Jack Tien and Ms. Lynn Huynh for technical assistance and Ms. Gabriella Janni for editorial assistance.

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