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Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats

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Summary

Spinal cord injury (SCI) induces incapacitating neuropathic pain in the form of allodynia—a painful response to normally non-noxious stimuli. Unfortunately, the underlying mechanisms of these sensory changes are not well understood, and effective treatments for allodynia have proven elusive. We examined whether physical exercise can improve sensory function after experimental SCI by promoting neurotrophin expression in the spinal cord and periphery, which modulates synaptic transmission and function. Female rats with moderate spinal cord contusion participated in treadmill training, swim training, stand training or were untrained. Exercise training began 4 days post surgery, lasted 20–25 min per day, 5 days a week for 7 weeks. Allodynia, as measured using von Frey hairs of different bending forces to the plantar hind paw, developed in the untrained group 3 weeks after SCI. Treadmill training ameliorated allodynia and restored normal sensation by 5 weeks. Swim training had a transient beneficial effect, but allodynia returned by 7 weeks. Stand training had no effect. Resolution of allodynia after treadmill training was associated with normal mRNA levels of brain-derived neurotrophic factor (BDNF) in both the lumbar spinal cord and soleus muscle. No other exercise paradigm restored BDNF centrally and peripherally. Greater recovery from allodynia correlated significantly with the degree of normalization of central and peripheral BDNF levels. These findings suggest that rhythmic, weight-bearing exercise may be an effective intervention to counter SCI-induced allodynia.

Keywords: neurotrophins; brain-derived neurotrophic factor (BDNF); allodynia; hyperalgesia; treadmill

Abbreviations: BDNF = brain-derived neurotrophic factor; dpo = days post-operative; HL = hind limb; LAM CTL = laminectomy control; NT-3 = neurotrophin 3; RT–PCR = reverse transcription–polymerase chain reaction; SCI = spinal cord injury; SCI No-Ex = spinal cord injury + no exercise training; SCI+ST = spinal cord injury + stand training; SCI+SW = spinal cord injury + swim training, SCI+TM = spinal cord injury + treadmill training; SOL = soleus muscle; ST = standing; SW = swimming; TM = treadmill; vFH = von Frey hair

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Introduction

Spinal cord injury (SCI) is often associated with sensory deficits including incapacitating neuropathic pain (Davidoff *et al*., 1987; Mariano, 1992; Siddall *et al*., 1999*a*) and hyperreflexia (Beric *et al*., 1988; Eide *et al*., 1996; Yezierski, 1996; Advokat and Duke, 1999; Schmit *et al*., 2000). These central sensory deficits, termed dysaesthesiae, take several forms including allodynia (painful responses to normally non-noxious stimuli) or hyperalgesia (exaggerated painful responses to noxious stimuli) (reviewed by Christensen and Hulsebosch, 1997). Unfortunately, treatments to restore normal sensory processing after SCI have had little success, making it imperative that new efficacious treatments and their

mechanisms of actions be identified. Physical activity improves motor function following neurological impairment in clinical and experimental settings. Interestingly, several studies suggest physical activity might be an effective treatment for improving sensory function (Hesse *et al*., 1995; Skinner *et al*., 1996; Edgerton *et al*., 1997; Harkema *et al*., 1997; Trimble *et al*., 1998).

A form of physical activity commonly investigated is treadmill training. Treadmill training can improve sensory function, given its effects on molecular systems involved with synaptic transmission and function. It has been shown to affect production of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) in the spinal cord and skeletal muscle (Gomez-Pinilla *et al*., 2002). These neurotrophins are found in motor neurons (for a review see Mendell *et al*., 2001), skeletal muscle (Griesbeck *et al*., 1995) and sensory neurons (Ernfors *et al*., 1990; Apfel *et al*., 1996). BDNF is localized to synaptic vesicles in the dorsal horn (Michael *et al*., 1997), modulates sensory input within the spinal cord (Kerr *et al*., 1999; Mendell *et al*., 1999), and is required for tactile discrimination by slow adapting mechanoreceptors (Carroll *et al*., 1998). However, the role that exercise and neurotrophins play on recovery of sensory function after SCI is unknown. Synapsin I is a vesicle-associated phosphoprotein, whose synthesis and phosphorylation are under the regulation of BDNF (Wang *et al*., 1995; Jovanovic *et al*., 1996) and NT-3. Synapsin I contributes to synaptic plasticity through modulation of neurotransmitter release (Baekelandt *et al*., 1994; Melloni *et al*., 1994; Wang *et al*., 1995), formation and maintenance of presynaptic structure (Takei *et al*., 1995) and axonal elongation (Akagi *et al*., 1996), thereby mediating BDNF-induced changes.

The beneficial effects of exercise and neurotrophins on functional recovery are likely to rely on activity-dependent events within select circuits activated by particular patterns of movements. The effects of treadmill locomotion on functional recovery can be attributed to load application on the affected limbs (Harkema *et al*., 1997). Alternatively, improvement in sensory processing after SCI occurred using passive rhythmic hind limb (HL) cycling with little or no load on the limbs (Skinner *et al*., 1996). Given the limited information regarding rhythmicity, load or a combination of both on sensory recovery after SCI, we tested the efficacy of three clinically feasible exercise paradigms—swimming, standing and treadmill training. Swimming (SW) requires rhythmic HL alternation with little load. Standing (ST) in an upright position places a static load across the HLs without rhythmicity, similar to standing frames used in neurorehabilitation of patients with SCI. Treadmill (TM) training incorporates both HL load and rhythmicity, and is being tested in a randomized clinical trial for patients with acute SCI. In the present study, we evaluated the differential effects of treadmill locomotion, swimming and standing on restoration of sensation following SCI and the role of neurotrophins in this recovery.

Material and methods

Subjects

The Ohio State University Institutional Laboratory Animal Care and Use Committee approved all procedures for these experiments. Forty-seven female Sprague Dawley rats weighing 250–300 g were randomly assigned to a laminectomy control (LAM CTL, $n = 7$) group or they received a moderate spinal cord contusion injury and were assigned to treadmill training (SCI+TM, $n = 7$), swim training (SCI+SW, $n = 10$), stand training (SCI+ST, $n = 9$) or untrained (SCI No-Ex, $n = 6$) groups. Seven rats were excluded based on inappropriate biomechanical injury parameters, intolerance of anaesthesia or an inability to swim with the hind limbs ($n = 1$). All outcome measures were collected and analysed in a blinded manner. Exercise training began at 4 days post-operative (dpo) lasting 20–25 min per day, 5 days per week for 7 weeks.

SCI surgical procedures

Moderate SCI was produced using the Ohio State University injury device described previously (Bresnahan *et al*., 1987; Stokes *et al*., 1992; Hutchinson *et al*., 2001). Briefly, rats received antibiotic (gentocin 1 mg/kg) followed by ketamine/xylazine (80 mg/kg and 10 mg/kg, respectively). Removal of the T8 lamina exposed the meninges before suspending the rat in a spinal frame for stabilization. The impact probe was lowered onto the dura to a pressure of 3 kilodynes, then the surface of the cord was displaced 1.1 mm over a 20 ms epoch (Behrmann *et al*., 1992; Stokes *et al*., 1992) to produce a moderate contusion. Haemostasis was achieved before suturing the incision in layers. Subcutaneous lactated Ringer's solution (5 ml) and antibiotic spray were then administered. Bladders were manually expressed 2–3 times daily until spontaneous voiding returned (~2 weeks). Oral Vitamin C was given daily to all animals to prevent urinary tract infections. Rats survived 7 weeks and then received a lethal dose of ketamine/xylazine so that the spinal cord and muscle tissues could be collected.

Exercise training paradigms

Task acquisition

Prior to surgery, animals were acclimated to their respective tasks (TM, SW, ST) during daily sessions for 1 week.

Treadmill: Animals performed daily quadrupedal locomotion on a treadmill (Simplex II, Columbus Instruments, Columbus, OH, USA) until they could maintain a forward position on the belt moving at 11–13 m/min and continuously drink from a liquid dispenser containing sugar water. Negative reinforcement (tail shock) was not used.

Swimming: Rats learned to swim across a glass tank ($75 \times 48 \times 30$ cm) filled with tap water maintained at 35°C. After each pass, animals were removed from the end of the tank and given a short rest, the length of which depended on their past performance. These procedures facilitated a straight swimming trajectory across the tank and prevented escape behaviours. In addition, rats received intermittent positive reinforcement for successful trials (sugared cereal).

Standing: Rats were trained to stand in an upright position on their HLs in a small plexiglas container ($25 \times 14 \times 30$ cm) for elevated food reward. Animals were continually enticed to search/explore on extended HLs for food and sucrose water, using their forelimbs for balance on the walls of the container. The rat was replaced

immediately upright onto the HLs each time it attempted to place the forelimbs on the floor of the tank so that it completed ~20 min of continuous upright HL standing.

Animals not engaged in daily training (SCI No-Ex, LAM CTL) received sugared cereal rewards in their home cage 3–4 times per week. These animals were also handled for 10 min, twice a week for the duration of the study to minimize apprehension, which could impair performance during weekly collection of behavioural outcome measures.

Daily exercise training

Training began 4 dpo for all exercise groups and lasted for 20–25 min per day, 5 days per week for 7 weeks. We selected a 4-day delay in order to avoid over-stressing the rats and potentially impeding self-hydration. We have observed a predictable stress response evoked by the injury itself, which is marked by dark red porphyrin expression around the eyes and nose early after injury. This response tends to resolve within the first 4 days after injury. In addition, supplemental food and subcutaneous fluids to treat the dehydration that sometimes occurs following SCI were no longer necessary at this time point.

Treadmill: Trunk support was provided as needed by a custom-made Lycra® vest that had holes cut out for the forelimbs with a Velcro® closure on the back. A spring, suspended from a cross bar located ~25.4 cm above the forward part of the treadmill, was attached to the vest to prevent backward drifting on the treadmill, but did not unweight the rat. On some vests, an extended piece of Lycra could be unrolled down to the hip flexor region to support the lower trunk. Elastic supports at the ends of the extended vest were hand-held or attached to small hooks placed on the side walls of the treadmill to provide hindquarter support when necessary early after SCI. After ~3–4 weeks of training, rats stepped without lower trunk support. Tail pinching has been shown to elicit stepping in SCI animal models, but may compromise independent locomotion off the treadmill (Lovely *et al*., 1986; Roy *et al*., 1991; Edgerton *et al*., 1997) and, therefore, was not employed in this study. Rest periods and treadmill speeds were adjusted on a daily basis according to the tolerance of each rat. When we observed signs of stress (i.e. porphyrin response, increased respiratory rate, increased defecation rate), the speed was lowered or the rat was removed from the treadmill for a brief rest period. The amount of time actually spent stepping was recorded for each animal in order to document a training effect.

Swimming: Initially after SCI, rats required a vest with narrow strips of closed-cell foam on the back to assist with flotation, and they primarily used the forelimbs to swim. The foam was sufficient to keep the head above the water only when the rat swam. The foam was removed after 4–5 sessions when swimming movements of the HLs began to emerge and were sufficient to keep the rat afloat. Daily notation of HL swim performance included extent of movements and relative frequency of kick cycles. Time engaged in swimming was recorded for each animal to measure training effects. Rest periods of up to 2 min after each bout of swimming were provided early after SCI to avoid inducing marked stress and non-compliance of the rat. Rest times were gradually shortened to 30 s as the HL swimming movements increased.

Standing: Initially, rats could not stand on their HLs; therefore, we placed their forelimbs on a box (10 × 10 × 5 cm) in order to facilitate weight-bearing on the HLs. The box was removed by the fourth training session. As HL weight-bearing performance improved, the height of food reward was raised for the training session. As a measure of training effects, the highest height at which the animal successfully retrieved food rewards and the total time spent in HL weight-bearing postures was recorded for each animal.

Behavioural testing

Sensory function: innocuous stimulus

Rats were acclimated to the testing procedures (2 × 10 min session) prior to the onset of behavioural testing. Rats stood on an elevated $\frac{1}{4}$ inch wire mesh floor encased with an inverted plastic cage (20 × 10 × 10 cm) to confine their movement. They received sugared cereal rewards throughout testing to keep them from attending to the movements and procedures of the examiner. After a 10 min acclimation period in the apparatus, an 8.5 g von Frey hair (vFH) (Stoelting, Wood Dale, IL, USA; 2.5 to 125 g) was applied to the plantar surface of the foot, ~1 cm posterior to the footpad of the middle phalange, from underneath the elevated wire mesh floor. The vFH was applied with a pressure that caused a slight bend in the hair after which the stimulus was removed. If the rat retracted the hind paw, the next lower vFH in the series was applied. If the rat did not retract its hind paw in response to the stimulus, the next higher vFH in the series was applied. The series of vFH are calibrated to increase logarithmically. Any stimulus that lifted the paw, thereby producing proprioceptive rather than tactile input, was discarded and retested. After 20 stimuli presentations, the lowest gram force which produced a retraction at least 50% of the time determined the response threshold (psychometric threshold) (Dixon, 1948; Chaplan *et al*., 1994; Lindsey *et al*., 2000). We conducted the test unilaterally and arbitrarily selected the right HL for testing. The data were analysed with a repeated measures ANOVA (analysis of variance) and Scheffe's *post hoc* test. Thresholds were measured preoperatively and 7, 21, 28, 35, 42 and 49 dpo SCI. The 1-week values were discarded, however, as the rats did not have sufficient motor control at this point after SCI to lift their paw away from the stimulus. Response thresholds were determined on a subset of animals ($n = 20$, 4–6 rats per group).

Sensory function: noxious stimulus

The flexor withdrawal response, elicited by a noxious pinch applied with the fingernails between the second and third metatarsals of the right hind paw, was videotaped for kinematic analysis. Given the difficulty of determining the stimulus intensity of the pinch, we controlled as many variables as possible in order to yield reliable data. A single examiner, who was blind to the condition of the animal, randomly evaluated rats on each testing day. Testing occurred at the same time of the light/dark cycle on each testing day. The hindquarters of the rat were shaved and tattoos were placed over bony prominences of the HL under anaesthesia to ensure consistent marker placement throughout the study. Markers were positioned over the pelvic crest, greater trochanter, lateral femoral condyle, lateral malleolus and the head of the fifth metatarsal. A Panasonic wv-c1350 CCD video camera connected to a Panasonic VCR (image capture 60 fields/s) recorded the lateral view of the animal. Images were downloaded onto a personal computer and hand-digitized using the Peak Motus Motion Analysis System (Peak Performance Technologies, Inc., Englewood, CO, USA). Data were optimally smoothed using a Butterworth filter. Movement time (from the first frame when movement occurred until maximum flexion of all three HL joints was reached) and excursion of hip, knee and ankle movements were analysed using one-way ANOVAs and Scheffe's

post hoc test. Trials in which rats rotated out of the 2D plane of the camera were not included in analyses.

Lesion epicentre measurements

Unfixed spinal cord tissue containing the lesion centre or laminectomy site (1 cm block) was post-fixed in 10% neutral buffered formalin for several days before being embedded in paraffin. Every fifth transverse section (20 μm) was collected and stained with luxol fast blue. The section containing the largest central core lesion with the least myelin-stained tissue was identified as the lesion epicentre. The average location of the tissue section representing the lesion epicentre was used as a reference for identifying the 'epicentre' in LAM CTL rats. Tissue sections were digitized at 20× for computerized image analysis (MCID-M4, Imaging Research, Ontario, Canada) and the border of spared white matter was outlined manually. White matter was considered 'spared' if the myelin stain was dense, contiguous and grossly normal in appearance, with little or no gliosis and few swollen axons or vacuoles (Behrmann *et al*., 1992). Tissue sparing was expressed as the area occupied by spared white matter per total cross-sectional area of the cord measured at the lesion epicentre.

Neurotrophin expression: isolation of total RNA and real-time quantitative RT–PCR

Lumbar spinal cord tissue (L1-4) and soleus muscle (SOL) was dissected from the animal under deep anaesthesia and sterile conditions. The tissue was immediately frozen in liquid nitrogen and stored at –80°C for further mRNA processing.

Total RNA was isolated using RNA STAT-60 kit (TEL-TEST, Inc., Friendswood, TX, USA) per the manufacturer's protocol. The mRNAs for BDNF, synapsin I and NT-3 were measured by TaqMan real-time quantitative reverse transcription polymerase chain reaction (RT–PCR) using ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA). This system directly detects the RT–PCR product with no downstream processing. This is accomplished with the monitoring of the increase in fluorescence of a dye-labelled DNA probe specific for each factor under study plus a probe specific for the *glyceraldehyde-3-phosphate dehydrogenase (GADPH)* gene used as an endogenous control for the assay**.** Total RNA (100 ng) was converted into cDNA using TaqMan EZ RT–PCR Core reagents (Perkin-Elmer, Branchburg, NJ, USA). The sequences of probes, forward and reverse primers as designed by Integrated DNA Technologies (Coralville, IA, USA) were:

BDNF: (5′-AGTCATTTGCGCACAACTTTAAAAGTCTGC-ATT-3′), forward (5′-GGACATATCCATGACCAGAAAGAAA-3′), reverse (5′-GCAACAAACCACAACATTATCGAG-3′);

Synapsin I: (5′-CATGGCACGTAATGGAGACTACCGCA-3′), forward (5′-CCGCCAGCTGCCTTC-3^{I}), reverse (5′-TGCAGC-CCAATGACCAAA-′);

NT-3: (5′-TGACCGACAAGTCCTCAGCCATTGAC-3′), forward (5′-TGTGACAGTGAGAGCCTGTGG-3′), reverse (5′-TGTAACCTGGTGTCCCCGAA-3′).

An oligonucleotide probe (5′-CCGACTCTTGCCCTTCGAAC-3′) specific for the rat *GADPH* gene was used as an endogenous control to standardize the amount of sample RNA. The RT-reaction conditions were 2 min at 50°C as initial step to activate uracil glycosylase, followed by 30 min at 60°C as reverse transcription and completed by uracil glycosylase deactivation at 95°C for 5 min. The 40 cycles of two-step PCR-reaction conditions were 20 s at 94°C and 1 min at 62°C.

Statistical analysis

vFH data were analysed with a two way repeated measures ANOVA (group × time) and Tukey's *post hoc* tests. Neurotrophin levels were expressed as a percentage of LAM CTL for all groups and were analysed using one-way ANOVAs with Tukey's *post hoc* tests. Pearson Product Moment Correlation was used to determine the relationship between BDNF mRNA expression and vFH measures of sensation at 7 weeks. Tissue sparing at the lesion epicentre was analysed with a one-way ANOVA and Tukey's *post hoc* test. All data are shown as mean ± SEM.

Results

Spinal cord lesion volume

Moderate spinal cord contusion resulted in a complete loss of grey matter and spared a peripheral rim of white matter containing myelinated axons as well as swollen, collapsed and demyelinated axons. Forced physical activity exacerbates sensorimotor cortex lesions when initiated within the first week of injury (Kozlowski *et al*., 1996). Whether forced use of impaired limbs during the period of secondary lesion development after SCI (Bresnahan, 1978; Schwab and Bartholdi, 1996; Hutchinson *et al*., 2001) will exacerbate a spinal cord lesion had not been previously determined. A comparison of the percentage white matter sparing at the lesion epicentre between the exercise and SCI No-Ex groups revealed no significant differences in lesion size (SCI No-Ex: 15.4 ± 4.0; SCI+TM: 20.3 ± 2.2; SCI+SW: 24.4 ± 4.7; SCI+ST: 14.2 ± 1.5; $P > 0.05$; Fig. 1). Thus, engaging in rhythmic or load-bearing exercise failed to exacerbate the spinal cord lesion.

Diminished hyperalgesia with exercise training

We used a noxious pinch stimulus to the deep intrinsic muscles of the paw to elicit flexor withdrawal and determine whether hyperalgesia develops after SCI and is modulated by different exercise paradigms. Using kinematic analysis of flexor withdrawal movements of the HL (Basso, 2000), we quantified movement time (time to reach peak flexion) and the summative angular excursion of the hip, knee and ankle joints at pre-operative, 1 week and 7 weeks post-SCI. There were no differences in movement time or angular excursion between groups at pre-operative and 1-week post-operative time points (data not shown). However, hyperalgesia was evident at 7 weeks after SCI by the significant decrease in movement time for the SCI No-Ex group compared with LAM CTLs (SCI No-Ex: 0.12 ± 0.01 s; LAM CTL: 0.25 ± 0.02 s: $P < 0.01$; Fig. 2). Engaging in exercise training attenuated the faster movement response (SCI+TM: 0.17 ± 0.02 s, SCI+SW: 0.17 ± 0.01 s, SCI+ST: 0.17 ± 0.02 s) to intermediate levels such that movement times were no longer different from either LAM CTLs or SCI No-Ex groups

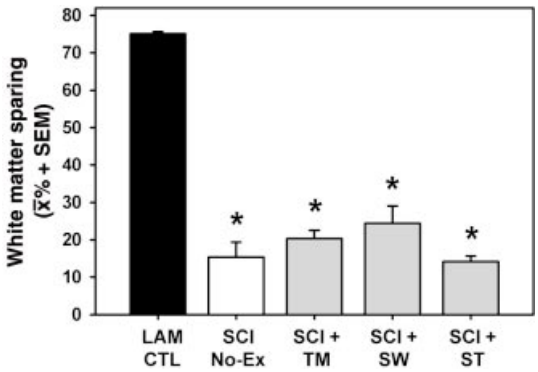

Fig. 1 Percentage spared white matter at the lesion epicentre (white matter per total cross sectional area of the cord) for LAM CTL, untrained (SCI No-Ex), treadmill trained (SCI+TM), swim trained (SCI+SW) and stand trained (SCI+ST) groups. Note that exercise training did not exacerbate lesion size. In the normal cord, 75% of the cross-sectional area is occupied by white matter; grey matter accounts for the other 25%. Therefore, sparing of all white matter as occurs in LAM CTLs is represented by a value of 75%. *$P < 0.05$ versus LAM CTL.

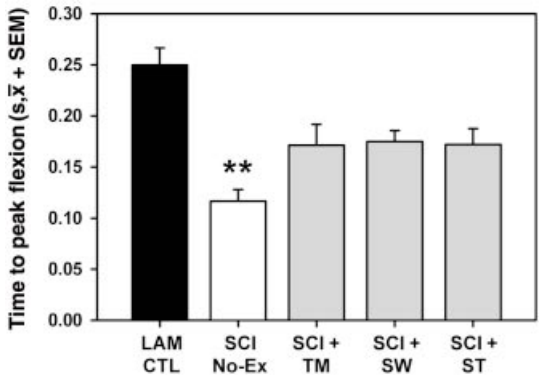

Fig. 2 Time (s) to peak flexion for flexor withdrawal at 7 weeks post-SCI for LAM CTL, untrained (SCI No-Ex), treadmill trained (SCI+TM), swim trained (SCI+SW) and stand trained (SCI+ST) groups. The hyperalgesia demonstrated by untrained rats was attenuated by all exercise paradigms. Flexor withdrawal was significantly faster for SCI No-Ex compared with LAM CTL. **$P < 0.01$.

(Fig. 2, $P > 0.05$). At 7 weeks post injury, angular excursion of the HL tended to be less than LAM CTLs, but this difference was only significant for the SCI+SW group ($P < 0.05$). We have previously shown that hyperalgesia does not develop until 2 weeks after moderate SCI (1.1 mm displacement) and is sustained for up to 4 weeks post injury (Basso, 2000).

Amelioration of allodynia with treadmill training

To evaluate whether different exercise paradigms would normalize sensory function below the level of the SCI, we measured allodynia of the hind paw using previously characterized von Frey monofilaments (Lindsey *et al*., 2000). We recorded the lowest force threshold that elicited retraction of the right hind paw in 50% of the stimulus applications pre-operatively and weekly from 21–49 dpo. After moderate spinal cord contusion, non-exercised rats demonstrated significantly lower thresholds than normal from 21–49 dpo (Fig. 3A), indicating pronounced allodynia of the hind paw (group means across time: SCI No-Ex: 34.58 ± 4.53 g; LAM CTL: 75.86 ± 0 g; main effect of group $P < 0.01$). Of all rats with contusion injury, 83% became hypersensitive, defined as a threshold response to a monofilament at least 1 level lighter than normal (75.86 g). Most rats (78%) responded to 15.14 g or less, which represents at least a 20% increase in tactile sensitivity. We found that treadmill training, but not stand training, had beneficial effects on sensory function. Treadmill training induced a complete recovery from the early onset of allodynia (Fig. 3A). The lower thresholds at 21 dpo (37.17 ± 13.29 g) returned to normal levels by 35 dpo for the SCI+TM group (75.86 ± 0) and were significantly different to the non-exercised group at 42 and 49 dpo (TM: 75.86 ± 0 g versus SCI at 42dpo: 29.36 ± 10.36 g; SCI at 49 dpo: 24.79 ± 10.80 g; $P < 0.05$). The time course of the resolution of allodynia after treadmill training was similar for all animals in the group. Significant allodynia developed in the swim-trained group by 28 dpo (29.95 ± 22.97 g, $P = 0.05$) demonstrating a brief resolution at 35 dpo that was not sustained by 49 dpo (39.94 ± 18.39 g, Fig. 3A). Allodynia returned for the majority of animals in the swim trained group by 42 dpo and became more severe by 49 dpo, which accounts for the wide variability in this group. Because the force increases logarithmically in the normal sensory range, a single animal having a normal threshold (75.86 g) will mask the allodynic thresholds (⩽15.14 g) of other animals in the group when averaged together (42 day time point). The stand-trained group also developed allodynia (28 dpo: 14.59 ± 3.89 g, $P < 0.01$), the severity of which varied considerably over time, and never recovered to normal levels (Fig. 3A).

Normalization of BDNF mRNA expression with treadmill training

Moderate spinal cord contusion injury without exercise resulted in a significant decrease in mRNA expression in the lumbar spinal cord relative to LAM CTLs for BDNF (SCI No-Ex: 57.17 ± 3.55, $P < 0.05$, Fig. 3C), while NT-3 (105.00 ± 10.49, $P > 0.05$, Fig. 5A) was unchanged. Cord levels of synapsin I (77.17 ± 6.71, $P > 0.05$, Fig. 4A) were decreased relative to LAM CTLs, but failed to reach significance. SCI No-Ex also showed a decrease in the mRNAs for BDNF (57.33 ± 4.31, $P < 0.01$, Fig. 3D) and synapsin I (69.00 ± 4.27, $P < 0.05$, Fig. 4B) in the SOL. All the exercise training paradigms increased BDNF mRNA levels in the injured spinal cord to the levels of

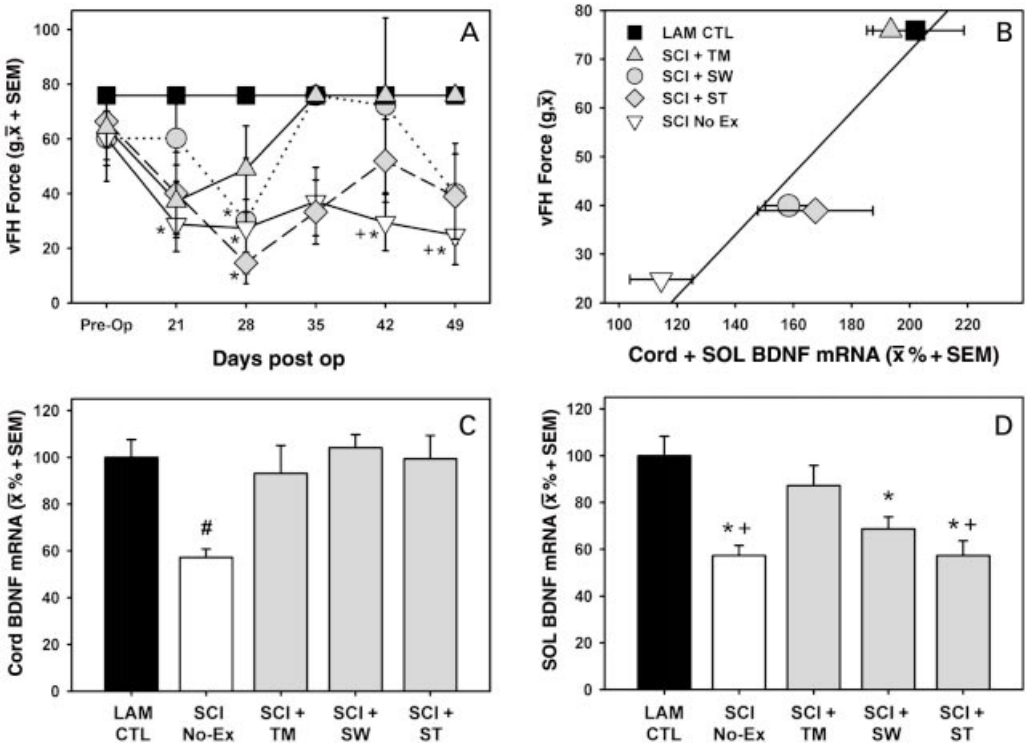

Fig. 3 Recovery of sensation after SCI and the relationship to central and peripheral levels of BDNF mRNA in exercised and untrained groups. (**A**) Allodynia as measured by 50% response thresholds for vFH stimulus (g) to the hind paw of rats in laminectomy control (LAM CTL, black square), untrained (SCI No-Ex, white triangle), treadmill trained (SCI+TM, grey triangle), swim trained (SCI+SW, grey circle) and stand trained (SCI+ST, grey diamond) groups across time. Note that treadmill training ameliorated the allodynia that develops in untrained and stand trained groups, while swimming produced only a transient improvement in sensory function. *Significantly lower than LAM CTL, $P = 0.05$. $^{+}$Significantly lower than SCI+TM, $P < 0.05$. (**B**) Normalization of BDNF mRNA levels centrally and peripherally as measured cumulatively in the lumbar cord and SOL correlated positively with recovery of sensory function based on vFH forces at 7 weeks post SCI ($r^2 = 0.86$; $P = 0.05$). (**C**) BDNF mRNA in the lumbar cord (L1-4) for SCI No-Ex, SCI+TM, SCI+SW and SCI+ST groups expressed as a percent of LAM CTL at 7 weeks post SCI. All exercise paradigms normalized BDNF levels from the significant decrease caused by SCI. $^{\#}$The levels for the untrained group were significantly lower than swimming, standing and laminectomy control groups ($P < 0.05$) and treadmill ($P = 0.06$). (**D**) BDNF mRNA in the SOL for SCI No-Ex, SCI+TM, SCI+SW and SCI+ST groups expressed as a percentage of LAM CTL. At 7 weeks post SCI, significant decreases in BDNF occurred in the untrained, swim trained and stand trained groups. Treadmill training was the only exercise paradigm to normalize BDNF in the SOL. Swim and stand training groups were not significantly different than the untrained SCI group. *Significantly lower than LAM CTL, $P < 0.05$. $^{+}$Significantly lower than SCI+TM, $P < 0.05$.

laminectomy controls (LAM CTL: 100.00 ± 7.58, SCI+TM: 93.14 ± 11.92; SCI+SW: 104.10 ± 5.57; SCI+ST: 99.33 ± 9.96, $P > 0.05$, Fig. 3C). However, TM but not SW or ST training resulted in normal levels of BDNF in the SOL (LAM CTL: 100.00 ± 8.30 versus SCI+TM: 87.29 ± 8.56, $P > 0.05$; SCI+SW: 68.70 ± 5.10, $P < 0.05$, SCI+ST: 57.33 ± 6.27, $P = 0.001$, Fig. 3D). A positive relationship between BDNF expression and recovery of sensation reached statistical significance ($P = 0.05$) when levels in the spinal cord and SOL were considered together (Fig. 3B) but not individually.

Overexpression of NT-3 mRNA with exercise

All exercise paradigms produced significantly greater NT-3 expression in the SOL relative to the sedentary condition (SCI No-Ex: 88.33 ± 5.41 versus SCI+TM: 133.43 ± 10.66, $P < 0.01$; SCI+SW: 124.20 ± 4.84, $P < 0.05$; SCI+ST: 124.33 ± 8.56, $P < 0.05$, Fig. 5B). The NT-3 mRNA expression in the spinal cord was higher than normal (LAM CTL: 100.00 ± 5.92) in all exercise groups (SCI+TM: 151.86 ± 16.68; SCI+SW: 142.10 ± 6.94; SCI+ST: 129.62 ± 6.06), and reached statistical significance for treadmill and swimming groups ($P < 0.05$) (Fig. 5A).

Up regulation of synapsin I with swimming and standing

The ST (113.88 ± 10.08, $P < 0.05$) and SW (102.20 ± 6.00, $P = 0.09$) trained groups had greater synapsin I mRNA levels in the spinal cord than the SCI sedentary condition (Fig. 4A). synapsin I mRNA levels in the spinal cord after TM training (73.17 ± 2.72) remained similar to the SCI sedentary condition ($P > 0.05$). To determine whether synapsin I can

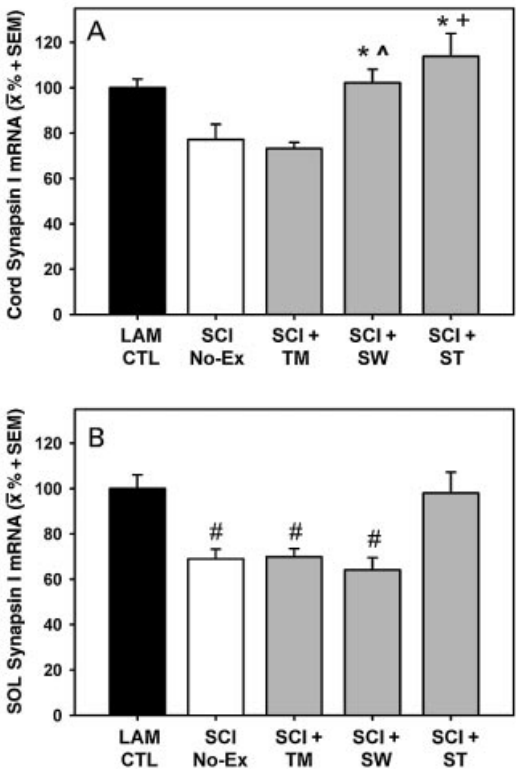

Fig. 4 Lumbar cord (**A**) and SOL (**B**) Synapsin I mRNA levels at 7 weeks post SCI for untrained (SCI No-Ex), treadmill trained (SCI+TM), swim trained (SCI+SW) and stand trained (SCI+ST) groups expressed as a percentage of LAM CTLs. Standing and swimming trained groups had higher levels of Synapsin I in the cord than the treadmill trained and untrained groups. A significant reduction in synapsin I occurred in the SOL for the untrained group, which was not improved by treadmill training or swim training. Standing training was the only exercise paradigm to normalize synapsin I in the SOL. *Significantly higher than SCI+TM, $P < 0.05$; +Significantly higher than SCI No-Ex, $P < 0.05$. ^ Higher than SCI No-Ex, $P = 0.09$. *Significantly lower than LAM CTL and SCI+ST, $P < 0.05$.

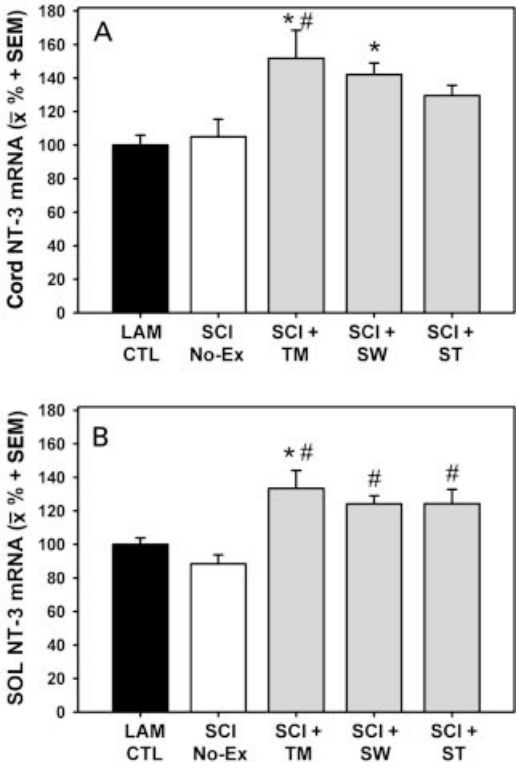

Fig. 5 Lumbar cord (**A**) and SOL (**B**) NT-3 mRNA levels at 7 weeks post-SCI for untrained (SCI No-Ex), treadmill trained (SCI+TM), swim trained (SCI+SW) and stand trained (SCI+ST) groups expressed as a percent of LAM CTLs. Treadmill and swim training produced overexpression of NT-3 in the lumbar cord above normal levels. In the SOL, all exercise paradigms resulted in higher levels of NT-3 than for the untrained group. *Significantly higher than LAM CTL, $P < 0.05$. #Significantly higher than SCI No-Ex, $P < 0.05$.

be synthesized by the muscle due to exercise, we examined the SOL. In the SOL, synapsin I mRNA in the TM (69.86 ± 3.62, $P < 0.05$) and SW (64.10 ± 5.41, $P < 0.01$) groups was as low as the SCI sedentary condition relative to LAM CTLs (100.00 ± 6.02) ($P < 0.05$, Fig. 4B). Synapsin I mRNA levels in the ST trained group (98.00 ± 9.25) were similar to those of the LAM CTLs.

Discussion

This study demonstrates that exercise training dramatically reduces aberrant sensory function that accompanies incomplete SCI. Physical activity incorporating both weight-bearing and rhythmicity ameliorates SCI-induced allodynia perhaps by normalizing BDNF mRNA levels in the cord and periphery. Exercise paradigms that increased BDNF levels in the cord alone were insufficient to mediate recovery from allodynia, but did attenuate hyperalgesia below the injury level. Exercise training, regardless of type, restored BDNF mRNA levels to normal in the lumbar cord, and normalized or produced over-expression of NT-3 in the soleus muscle and the cord. These general effects of exercise may be responsible for the attenuation of hyperalgesia below the level of injury we observed across exercise groups.

In humans, allodynia is a widely described phenomenon in which innocuous tactile stimulation elicits a painful response. This multifaceted reaction is composed of emotional, autonomic, endocrine, aversive and arousal components (Willis and Coggeshall, 1991; Christensen and Hulsebosch, 1997). No consensus exists on how many of these features must be demonstrated by animals for the response to be considered painful (Christensen and Hulsebosch, 1997; Lindsey *et al*., 2000). In some studies, an aversive response (i.e. withdrawal of the paw) to small calibre vFH filaments was considered a painful response (Bester *et al*., 2000; Chen and Chen, 2000; Deng *et al*., 2000), whereas other studies require that paw withdrawal be associated with evidence of supraspinal awareness such as vocalizations, grooming or orienting toward the stimulus (Siddall *et al*., 1995, 1999*b*; Christensen *et al*., 1996; Christensen and Hulsebosch, 1997;

Yezierski *et al*., 1998; Drew *et al*., 2001). In our study, the presence of an aversive response alone indicated allodynia. Orienting, grooming or vocalization behaviours were not included because our handling and testing procedures reduced the likelihood of their occurrence, even in normal rats. Specifically, our animals were extensively handled on a daily basis, which reduces or eliminates vocalization even to known noxious stimuli in normal rats (unpublished observations). Well-handled uninjured animals from this strain typically do not vocalize in response to pain. For example, strong pinch stimuli applied to the dorsum of the foot of an uninjured animal will evoke a vigorous withdrawal response, but the animal rarely vocalizes. Therefore, vocalization cannot be used as an indicator or evidence of a supraspinally mediated response to painful stimuli in the Sprague-Dawley strain using the methods employed in the current study. In addition, we presented the vFH filament while the rat was eating to prevent the rat from visualizing the stimulus application. This procedure likely precluded grooming, orienting and vocalization behaviours by the rat. Therefore, in this study, the lack of overt signs of supraspinal awareness cannot be taken as evidence of non-painful sensory perception. It is important to recognize that SCI animals in this study rapidly lifted the paw away from the vFH plantar stimulus, which is typically an indicator of pain. Normally, withdrawal of a limb providing weight support occurs only when pain is perceived.

Our finding that allodynia develops many segments caudal to the contusion injury is similar in time course and magnitude to data reported after grey matter excitotoxic lesions (Yezierski *et al*., 1998; Yezierski, 2000), spinal cord ischaemia (Hao *et al*., 1991; Xu *et al*., 1992), low thoracic hemisection (Christensen *et al*., 1996) and weight drop contusion (Siddall *et al*., 1995; Lindsey *et al*., 2000). Greater hind paw plantar surface tactile sensitivity to fine calibre vFH filaments with bending forces of ~28.84 gram-force (perceived as a faint touch by humans) occurred between 3–4 weeks after SCI across all groups, demonstrating a shift in sensation perception from touch to pain. The mechanisms responsible for this shift following SCI remain poorly understood, although a plethora of mechanistic-driven studies exist using peripheral inflammation or nerve injury models. Peripheral model evidence indicates that allodynia is related to centrally reorganized nociceptive pathways that become responsive to low threshold mechanoreceptors (Bester *et al*., 2000; Blomqvist and Craig, 2000). Nociceptive pathway activation by novel stimuli after SCI may be explained by changes at several points along the neuroaxis. First, Ab fibres, which respond to innocuous tactile input, may sprout from lamina III/IV into the nociceptive rich lamina (I/II) of the lumbar dorsal horn and form functional synaptic contacts as shown after peripheral injury (Willis and Coggeshall, 1991; Woolf *et al*., 1992, 1995; Koerber *et al*., 1999; Kohama *et al*., 2000; White, 2000). Secondly, a loss of descending modulatory inhibition in the cord produces nociceptive neuron hyperexcitability, which may facilitate central pain pathway responses (Yaksh, 1989; Hao *et al*., 1992; Yezierski and Park, 1993). Thirdly, SCI may induce neuroplasticity or changes in excitability of supraspinal regions, which integrate nociceptive and homeostatic functions so that innocuous tactile input elicits pathological recruitment of these higher centres. Supraspinal regions of interest include: the pontine parabrachial nucleus (Hermanson and Blomqvist, 1996; Bester *et al*., 2000), medullary reticular formation (Hubscher and Johnson, 1999), hypothalamus (Burstein, 1996; Pan *et al*., 1999), thalamus (Lenz *et al*., 1987; Craig *et al*., 1994) and cortex (Willis and Westlund, 1997), and may explain the motivational, emotional, and autonomic components of allodynia. Fourthly, SCI may alter the function of the mechanoreceptor itself since cutaneous mechanoreceptors produce faster conduction velocities when the afferent fibre central process is cut (Kolosova *et al*., 2000) and exhibit decreased p75 immunoreactivity following SCI in humans (Lopez *et al*., 1998). Given the limited understanding of mechanisms related to SCI-induced allodynia, it is not surprising that interventions to eliminate neuropathic pain have been unsuccessful (Balazy, 1992).

The present study is the first to examine exercise effects on neurotrophin expression in peripheral and spinal cord tissue after moderate contusion injury. Previous work in weight drop contusion found no significant levels of BDNF chronically in the lumbar cord despite up regulation of its receptor in descending white matter tracts (Widenfalk *et al*., 2001). We found that contusive SCI alone produced marked declines in central and peripheral BDNF mRNA levels, which were intrinsically up regulated through exercise. NT-3 mRNA levels in exercise trained animals were higher than levels in both untrained animals and normal animals. NT-3 induction was equally responsive to treadmill, swimming or standing training, suggesting that it is sensitive to general physical activity. This finding conforms to the fact that NT-3 and the TrkC receptor assist in maintaining proprioception (Ernfors *et al*., 1994; Farinas *et al*., 1994; Tessarollo *et al*., 1994; Liebl *et al*., 1997). While all types of exercise returned cord BDNF levels to normal levels, treadmill training alone normalized peripheral levels of BDNF (as measured in muscle), while swim-training produced intermediate levels of peripheral BDNF.

The fact that allodynia developed in the face of significantly lower levels of BDNF in the lumbar cord and SOL suggests that central mechanisms resulting from SCI outweigh the reduced sensitivity associated with BDNF deficiencies (Carroll *et al*., 1998; Watanabe *et al*., 2000). That is, greater excitability of peripheral sensory receptors combined with disinhibition of sensory pathways in the dorsal horn and presumptive sprouting of sensory fibres into novel sites in the spinal cord and brain appear to mask or negate the decreased modulation of mechanosensation resulting from BDNF reduction. However, low levels of BDNF might also produce allodynia in at least three ways: (i) the upregulation of TrkB receptors and/or N-methly-D-aspartate (NMDA) receptors in the lumbar cord could result in greater synaptic

responsiveness of dorsal horn neurons to lower levels of BDNF (Kerr *et al*., 1999; Widenfalk *et al*., 2001); (ii) the normal levels of NT-3 after SCI may have modulated synapsin I so that low levels of BDNF remained effective at stimulating neurotransmitter release (Wang *et al*., 1995; Jovanovic *et al*., 2000); and/or (iii) higher numbers of BDNF positive microglia after CNS injury (Batchelor *et al*., 1999; Dougherty *et al*., 2000) may in themselves induce allodynia. This was demonstrated by recent findings that allodynia is induced in normal rats when activated microglia expressing ATP receptors are infused into the spinal cord (Tsuda *et al*., 2003).

Normalization of BDNF levels in the cord and the periphery was associated with amelioration of tactile hypersensitivity after SCI. Correlational analysis showed that as exercise-induced BDNF mRNA expression approached normal levels in both the cord and periphery, greater recovery of normal tactile sensitivity occurred. Thus, the best predictor of tactile sensory recovery after contusive SCI was both central and peripheral levels of BDNF. Neither lumbar cord nor SOL BDNF levels alone predicted the sensory recovery. Only TM training ameliorated allodynia. One likely mechanism responsible was the normalization of BDNF expression peripherally and centrally. The exercise-induced increase in neurotrophins in the periphery may facilitate normalization of sensation by serving as an important source of trophic agents for neurons in the spinal cord and dorsal root ganglia since these agents are retrogradely transported from the muscle. Further support for BDNF promoting sensory recovery is our finding of intermediate effects with SW and ST training. These two groups had the same degree of hypersensitivity and similar low levels of cumulative BDNF. If tactile sensitivity is directly related to central and peripheral levels of BDNF, as we suspect, the transient improvement in allodynia after swim training may indicate an initial but unsustained rise in BDNF levels for this group. Future investigation into BDNF and synapsin I expression over time is necessary to establish that low BDNF causes tactile hypersensitivity after SCI.

Different neurotrophins are associated with specific sensory modalities. For example, nerve growth factor and NT-3 are involved with nociception and proprioception, respectively. In turn, slow adapting mechanoreceptors innervating Merkel cells within touch dome complexes of the skin require NT-3 for survival and require BDNF for transduction of tactile sensitivity to the spinal cord (Carroll *et al*., 1998). More importantly, administration of exogenous BDNF normalized tactile sensitivity in BDNF deficient mice using vFH monofilaments (Carroll *et al*., 1998). Therefore, we theorize that these BDNF-dependent, slow adapting mechanoreceptors are repetitively stimulated during rhythmic but not static loading of the HL, which produces normal levels of central and peripheral BDNF after locomotor training but did not do so after stand or swim training in our experiment. Since tactile sensitivity and mechanotransduction are dependent on normal BDNF levels, exercise-induced BDNF expression is likely to promote recovery of tactile sensitivity after traumatic SCI. BDNF may affect the dorsal root ganglia by regulating genes that encode proteins required for mechanosensation after retrograde transport from the muscle and receptors. BDNF is also likely to promote recovery of tactile sensitivity in the spinal cord by modulating synaptic interactions in lamina III/IV. It is important to note that the proposed mechanisms listed above may rely on central or peripheral sources of neurotrophins to mediate normalization of sensation after SCI. Our experiment implicates both central and peripheral levels of BDNF in sensory recovery, but it is unclear whether one source is more important than another.

Mechanisms for the action of BDNF on mechanosensation in normal or spinal cord injured animals remain elusive. Given the roles of BDNF on maintaining neuronal excitability, BDNF may affect transmission or transduction of sensory information. Accordingly, the synaptic vesicle associated molecule synapsin I—important for neurotransmitter release under the influence of BDNF—was decreased in the spinal cord after SCI, but increased to about control levels following SW and ST. Changes in synapsin I in response to SCI and subsequent training illustrate the synaptic effects of exercise and neurotrophins. BDNF and NT-3 have been shown to exert rapid, local effects on neuronal excitability (Kafitz *et al*., 1999) and synaptic efficacy (Poo, 2001). To evaluate a possible functional role for the increases in BDNF and NT-3 on synaptic plasticity, we measured the levels of synapsin I based on its involvement with the action of BDNF. It is known that BDNF stimulates neurotransmitter release through modulation of synapsin I (Jovanovic *et al*., 2000). We have been able to reduce the increase in synapsin I mRNA associated with exercise in the hippocampus by blocking BDNF action using the tyrosine kinase receptor blocker K252a (Vaynman *et al*., 2003). Therefore, it appears that the elevated expression of BDNF following training may affect synapsin I. The fact that synapsin I can be modulated by BDNF (Jovanovic *et al*., 2000) suggests that increases in BDNF as a result of selective training paradigms can impact synaptic growth and/or function. BDNF is known to facilitate synapses in the hippocampus, hypothalamus and spinal cord to an extent that alters spatial learning, locomotion and motor behaviours (Neeper *et al*., 1996; Houweling *et al*., 1998; Jakeman *et al*., 1998; Malenka and Nicoll, 1999; Kernie *et al*., 2000). Our results also indicate that synapsin I is synthesized in the muscle, probably by muscle fibres and intramuscular nerves. It is compelling to consider that neural and muscle activity during exercise might promote transport of synapsin I to the spinal cord and further facilitate synaptic plasticity. Further studies are required to determine specific mechanisms involved with these events.

Because neurotrophins do not cross the blood–brain barrier and are degraded by peptidases when injected peripherally (Barinaga, 1994), exercise may be an effective means to increase neurotrophic factor support in the CNS. The significant advantage of exercise to promote molecular changes using the intrinsic pharmacology of the spinal cord

and periphery is preservation of functional homeostasis. Thus, exercise can activate the whole molecular machinery required for a functional outcome (Molteni *et al*., 2002). By rescuing or maintaining normal levels of BDNF in the lumbar cord and periphery, exercise may normalize molecular events or strengthen neural connections required for normal sensation.

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