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NEUROPROTECTIVE AND NEUROREGENERATIVE EFFECTS OF LOW-INTENSITY AEROBIC EXERCISE ON SCIATIC NERVE CRUSH INJURY IN MICE

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Abstract—Here, we established a program of low-intensity aerobic exercise and compared the effects of exercise preoperative, postoperative, and a combination of both pre- and postoperative protocols on recovery from sciatic nerve crush injury in mice using behavioral, biochemical, and morphological assays. Sciatic nerve crush was performed in adult male mice. The animals were submitted to preoperative (for 2 weeks), postoperative (for 2 weeks), and a combination of preoperative-postoperative (for 4 weeks) training protocols. During the training period, functional recovery was monitored using the Sciatic Functional Index, the Sciatic Static Index, and mechanical and cold hypersensitivity analyses. Morphological and biochemical alterations were analyzed on the 14th day post-crushing. The functional recovery values of all of the exercised groups were significantly better than the nonexercised group. Biochemically, all of the exercise groups showed a reduction in the increase of interleukin-1β (IL-1β) in the sciatic nerve and in the IL-1β and interleukin-6 receptor (IL-6R) levels in the spinal cord. However, the levels of tumor necrosis factor alpha (TNF-α) decreased only in the postoperative group and in the combination exercise protocols. In the morphological analysis, the combination exercise subjects presented an increase in fiber and axon diameter, in the myelination degree and in the number of myelinated fibers. The present study showed that pre- and postoperative exercise achieved values for functional and morphological sciatic nerve regeneration that were significantly better than either the preoperative or postoperative protocols. This experimental study suggests that physical exercise can restore motor and nerve function to a substantial degree when performed using a prophylactic and therapeutic approach.

Key words: nerve regeneration, functional recovery, hypersensitivity, neuroprotection, physical exercise.

Peripheral nerve disorders are among the most common neurological problems that clinicians face, yet few therapies and interventions are available to arrest or reverse the damage associated with them (Zochodne, 2008). These disorders induce plastic changes in primary afferent fibers and on the spinal circuitry, which are related to the emergence of neuropathic pain (Casals-Díaz et al., 2009). Moreover, these injuries induce neuropathic pain through the upregulation of a complex network of molecules in the spinal cord and dorsal root ganglia (Xiao et al., 2002), including the proinflammatory cytokines (i.e. tumor necrosis factor alpha [TNF-α], interleukin-1β [IL-1β], and interleukin-6) (Watkins et al., 2001; Milligan et al., 2003).

Surgical repair, pharmaceutical intervention, and/or physical rehabilitation are current therapeutic strategies for the treatment of the peripheral nerve injury and promotion of regeneration (Marqueste et al., 2004; Roglio et al., 2008; Sabatier et al., 2008). Among the different strategies investigated for the promotion of axonal regeneration, physical exercise is a promising approach for the enhancement of axonal regeneration. Exercise training improves motor function after clinical and experimental peripheral nervous lesion. It is also an effective treatment for loss of sensory function (Herbison et al., 1983; Ilha et al., 2008), and its effects are related to the increased expression of brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine kinase B (trkB) (Gómez-Pinilla et al., 2001). However, other studies have shown deleterious effects of exercise on axonal regeneration and functional recovery (Herbison et al., 1974, 1980a,b). This controversy may be due to the different programs used because the training pattern and duration seem to affect nerve regeneration (van Meeteren et al., 1997, 1998; Sabatier et al., 2008). High-frequency or high-speed training can cause considerable muscle damage (Herbison et al., 1973, 1980a,b)

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Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; BDNF, brain-derived neurotrophic factor; Exer 1, exercise-preoperative for 2 weeks; Exer 2, combination of preoperative-postoperative training protocols for 4 weeks; Exer 3, exercise-postoperative for 2 weeks; GDNF, glial cell line-derived neurotrophic factor; IL-10, interleukin-10; IL-1β, interleukin-1β; IL-6R, interleukin-6 receptor; MAG, myelin-associated glycoprotein; MLSS, maximal blood lactate steady state; MNs, motor neurons; NGF, nerve growth factor; NMJ, neuromuscular junction; Non-exer, sciatic nerve crush control nonexercised; PBS, phosphate-buffered saline; PL, paw length; SFI, sciatic functional index; Sham, sham-operated; SSI, sciatic static index; TNF-α, tumor necrosis factor alpha; trkB, tyrosine kinase B; TS, toe spread; WHO, World Health Organization.

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and inhibit collateral and terminal axonal sprouting (Tam et al., 2001), but mild exercise, such as treadmill training at low speed, is an adequate program for the improvement of axonal regeneration and muscle reinnervation. In addition, exercise promotes neuroplasticity (Vega et al., 2006), neurogenesis (van Praag et al., 1999), and neuroprotection (Hayes et al., 2008) beyond learning and cognition (van Praag et al., 1999; Davranche and McMorris, 2009). A previous study demonstrated that when exercise is applied 7 days before a lesion, it conditions the neurons of the dorsal root ganglia and promotes axonal regeneration (Molteni et al., 2004). Although it is believed that physical exercise may be useful for neurorehabilitation (Sabatier et al., 2008), little information is available regarding the prophylactic role of physical exercise on the deleterious effects induced by sciatic nerve crush.

Here, we established a program of low-intensity aerobic exercise by measuring blood lactate concentration and compared the effects of physical exercise preoperative, postoperative, and a combination of both types of exercise protocols, in a sciatic nerve crush model in mice using behavioral, morphological, and biochemical analyses.

EXPERIMENTAL PROCEDURES

Animals and surgical procedures

Male Swiss mice (25–35 g, 8–9 weeks old) obtained from the Federal University of Santa Catarina (Florianópolis, SC, Brazil) were maintained at a constant room temperature of 22±2 °C under a 12-h light/dark cycle (lights on at 6:00 AM), with access to food and water *ad libitum*. The experiments were performed after the approval of the protocol by the Institutional Ethics Committee for Animal Research of the Federal University of Santa Catarina. All experiments were conducted in accordance with the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983). Surgical procedures were performed under deep anesthesia that was induced with a premixed solution containing ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). The sciatic nerve was exposed in the right thigh and crushed once for 30 s, 1 cm above its trifurcation, with 2-mm-wide forceps, as described previously, with minor modifications (Bridge et al., 1994). The Sham group was subjected to the same surgical procedures, but the sciatic nerve was not crushed.

Low-intensity aerobic treadmill running program

The exercise was performed on a treadmill designed for human use (Athletic Advanced 2) and modified for mice. The animals ran on the treadmill for 30 min at a speed of 10 m/min with no inclination, 5 days per week, as described previously by Sabatier et al. (2008), with minor modifications. Animals were subjected to a 1-week familiarization before experimental protocols began. Treadmill training began on the third postoperative day. The following groups were used: sham-operated (Sham); sciatic nerve crush control nonexercised (Non-exer); exercise-preoperative for 2 weeks (Exer 1); a combination of preoperative-postoperative training protocols for 4 weeks (Exer 2); and exercise-postoperative for 2 weeks (Exer 3) (Fig. 1). The animals in the Sham and Non-exer groups were handled and placed on a treadmill with no motion at the same times as the exercise groups.

Determination of training intensity

To establish the intensity of training, the test for measured the maximal blood lactate steady state (MLSS) was performed. The animals were submitted to six constant-load exercises, 30 min in duration, in which blood samples were obtained at 10 and 30 min of exercise for subsequent lactate concentration measurements. The tests were conducted on different days and in a randomized order, with the workload corresponding either to 10, 11.6, 13.3, 15, 16.6, or 18.3 m/min. The criteria for considering the MLSS intensity were a lactate concentration variation not higher than 1 mmol/L between the 10 and 30 min during the constant-load trials (Urhausen et al., 1993; Ferreira et al., 2007).

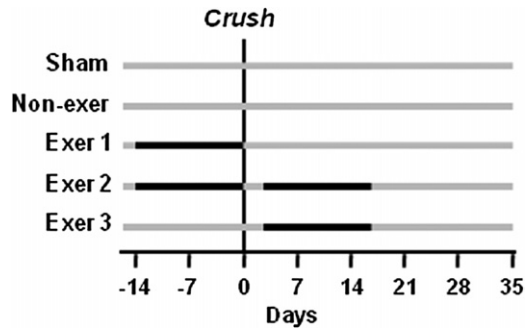

Fig. 1. Experimental protocols for the different groups of mice subjected to treadmill running. Horizontal, black and gray lines indicate time periods with or without treadmill running exercise, respectively. Day 0, vertical black line indicates the day of crushing surgery. Sham, sham-operated; Non-exer, sciatic nerve crush nonexercised; Exer 1, exercise before sciatic nerve crush; Exer 2, exercise before and after sciatic nerve crush; Exer 3, exercise after sciatic nerve crush.

After performing hygienic procedures with alcohol, the distal portion of the animal's tail was punctured with a small needle prick, and 25 μl of blood was collected in heparinized microcapillary tubes. The samples were deposited in 1.5 ml Eppendorf tubes that contained 50 μl of 1% sodium fluoride. The lactate concentrations were determined using a lactate analyzer (YSI Model 2700 SPORT; YSI, Yellow Springs, OH, USA). Before each test, the equipment was calibrated according to the manufacturer's instructions.

Mechanical hypersensitivity

To assess mechanical hypersensitivity using the von Frey test, mice were placed individually in clear plexiglass boxes (9×7×11 cm) on elevated wire mesh platforms to allow access to the ventral surface of the right hindpaw. The withdrawal response frequency was measured after 10 applications (duration of 1 s each) of 0.4 g von Frey filaments (VFH, Stoelting, Chicago, USA), as described previously by Bortalanza et al. (2002). The number of paw withdrawals was recorded and expressed as a percentage of the withdrawal response. For the mechanical and cold hypersensitivity (described later) tests, all of the groups were submitted to a preoperative evaluation and were re-evaluated at 3, 7, 10, 14, 21, 28, and 35 days after surgery, and 30-min and 24-h post-treatment.

Furthermore, mechanical hypersensitivity was also evaluated using the grip-force test (Kehl et al., 2000 with minor modifications), which was performed before the operation (day 0), and 7, 14, 21, 28, and 35 days after the operation, and 30-min and 24-h postexercise. Measurements of peak hind limb grip-force were conducted by recording the maximum compressive force exerted on the hind limb strain gauge setup, in a grip-force measurement system (Grip Strength Meters, Columbus Instruments, Columbus, OH, USA). During testing, each mouse was gently restrained and allowed to grasp the wire mesh frame (10×12 cm) attached to the strain gauge with their hind limbs. The experimenter moved the animal in a rostrocaudal direction until the grip was broken. Right and left limbs were evaluated separately, and grip-force was expressed as the difference in grams.

Cold hypersensitivity

Hypersensitivity to a cold stimulus was assessed using the cold plate test (Cold/Hot Plate Analgesia Meter, AVS Projetos, Campinas, São Paulo, Brazil) as designed by Bennett and Xie (1988) with minor modifications. The animals were placed on a cold stainless plate in a space surrounded by clear Plexiglass (12×20×10 cm). The temperature of the cold plate was continuously monitored and kept constant at 10±1 °C. The latencies to first paw lifting, licking, or shaking (i.e. pain-related behaviors) of the right hindpaw were recorded. The cutoff time of the latency was set at 120 s to prevent tissue damage.

Sciatic functional index (SFI) and sciatic static index (SSI)

SFI and SSI are standard methods for evaluating crush peripheral nerve injury. The SFI is a tool that evaluates the degree of functional loss (or recovery). It compares parameters from the normal and experimental footprints by a mathematical formula. SFI shows a high correlation with functional recovery (de Medinaceli et al., 1982; Bain et al., 1989). The SSI is another way of assessing recovery of function after sciatic injury in animal models. It uses the footprints acquired when the animal is on a static position, and minimizes bias related to gait velocity.

The animals were evaluated to obtain two footprint parameters: toe spread (TS), the distance between the first and fifth toes; and paw length (PL), the distance between the tip of the third toe and the most posterior part of the foot in contact with the ground.

The walking pattern was recorded with a video camera (Panasonic Camcorder Digital PV-GS19, Osaka, Japan) while the mice walked through the glass runway. To analyze the foot placings, 10 single frames were used, five of each foot. This image was loaded into a computer using a frame grabber as described previously (Baptista et al., 2007). The digital images were captured and analyzed using the image analysis software Image Pro Plus software 6.0 (Media Cybernetics, Bethesda, MD, USA). SFI was calculated as described previously by Inserra et al. (1998) according to the following equation: SFI $=118.9\times TSF-51.2\times PLF-7.5$.

Static footprints were obtained when the animal was in a resting position. SSI was calculated as described previously by Baptista et al. (2007) according to the following equation: $SSI=101.3\times TSF-54.03\times PLF-9.5$.

The SFI and SSI oscillate around 0 for normal nerve function, but indexes around −100 represent total dysfunction. All of the groups were evaluated before the operation (day 0), and 3, 7, 14, 21, 28, and 35 days after the operation.

Measurement of pro- and anti-inflammatory cytokine levels in the sciatic nerve and spinal cord

Twenty-four h after the last exercise session, mice were anesthetized with isoflurane and killed by decapitation. Both the sciatic nerve and the lumbar portion of the spinal cord (L1–L6) were removed and homogenized in a glass homogenizer (Dounce Tissue Grinders, Omni International, Kennesaw, GA, USA) with a PBS (phosphate-buffered saline) solution containing Tween 20 (0.05%), phenylmethylsulfonyl fluoride (PMSF) 0.1 mM, EDTA 10 mM, aprotinin 2 ng/ml, and benzethonium chloride 0.1 mM. The homogenates were transferred to 1.5 ml Eppendorfs tubes, centrifuged at 3000 *g* for 10 min at 4 °C, and the supernatant obtained was stored at −70 °C until further analyses. Total protein content was measured in the supernatant using the method of Bradford. Sample aliquots of 100 μl were used to measure the tumor necrosis factor alpha (TNF-α), IL-1β, interleukin-6 receptor (IL-6R), and interleukin-10 (IL-10) levels using mouse cytokine enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Minneapolis, MN) according to the manufacturer's instructions. The absorbance for all of the cytokines studied was measured using a microplate reader at 450 and 550 nm.

Morphometric analyses

At 2 weeks postoperatively, the animals were deeply anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and euthanized by transcardial perfusion with a fixative solution (1.5% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4). The sciatic nerve ipsilateral to the lesion was harvested, and a 3-mm segment immediately distal to the lesion site was dissected. The segments were postfixed in 1% osmium tetroxide, dehydrated in increasing concentrations of acetone (30–100%), infiltrated, Spurr-embedded, and prepared for the quantitative morphology of myelinated nerve fibers, as previously described (Raimondo et al., 2009).

Transverse sections of 0.5 μm (semithin) and 70 nm (ultrathin) were obtained using an ultramicrotome (RMC, Tucson, AZ, USA). The semithin sections were stained with 1% toluidine blue. Images were acquired on a light microscope (Olympus BX41, Center Valley, PA, USA) connected to a digital imaging system (Q-Color 3™, Olympus, Center Valley, PA, USA). For the ultrastructural analysis, 70-nm sections were obtained, contrasted with 2% uranyl acetate and 1% lead citrate, and observed through a transmission electron microscope, JEM-1011 (JEOL, Tokyo, Japan), equipped with an image acquisition system (MegaView II, Analysis-Imaging System).

A 1000× magnification was used to assess the general morphological condition of the nerves, the total number of myelinated fibers (N), and myelinated fiber densities (N/mm^2). A magnification of 8000× was used for the ultrastructural morphological measurement of myelinated fiber diameter (D), axon diameter (d), myelin sheath thickness (D-d), and G-ratio, which is a measurement of the degree of myelination obtained by dividing the inner circumference of the axon without myelin by the outer circumference of the entire fiber including myelin. In addition, was measured the percentage of each fiber size range, including myelinated and unmyelinated fibers. These parameters were quantified from randomly captured images of 10 fields (0.01284 mm^2/field) per tissue sample and statistically compared across groups (Raimondo et al., 2009; Geuna and Varejão, 2008).

Statistical analyses

Results are presented as the mean±SEM for each group. Functional analyses were compared using 2-way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's test when appropriate. Morphometric and biochemical assessments and the area under the curve (AUC) for behavioral tests were performed using a 1-way ANOVA followed by Student–Newman–Keuls test when appropriate. *P* values less than .05 were considered to be indicative of significance.

RESULTS

Animals performed a low-intensity aerobic treadmill running program

We observed an increase in blood lactate concentration of more than 1 mmol/L between 10 and 30 min during exercise with loads of 15, 16.6, and 18.3 m/min. The MLSS was found to be 13.3 m/min. The mice in our study performed the exercise at 10 m/min, which corresponded to 75% of MLSS (Table 1).

Table 1. Physical exercise intensity (blood lactate concentration)

Load exercise (m/min)	10 min of exercise	30 min of exercise
Rest	2.49±.12	2.49±.12
10	2.48±.20	2.63±.20
11.6	2.84±.15	3.26±.27
13.3*	3.15±.42	3.82±.67
15	3.78±.24	5.30±.42
16.6	4.01±.36	6.16±.75
18.3	4.29±.76	7.02±.87

Values indicate the blood lactate concentration (mmol/L) as the mean of 10 animals±SEM, assessments in the 10th and 30th minutes of exercise.
* MLSS.

Low-intensity exercise counteracted mechanical and cold hypersensitivity after sciatic nerve injury

The crush procedure induced marked and prolonged mechanical and cold hypersensitivity, with behavioral abnormalities still evident 28 days after injury compared with the Sham group ($P<.001$). A complete recovery of nociceptive responses was evident at 35 days postcrush injury (Figs. 2A, C). The results presented in Fig. 2A showed that the Exer 1 group had reduced mechanical hypersensitivity on days 7, 10 ($P<.001$), and 14 ($P<.01$) compared with the Non-exer group. The Exer 2 and Exer 3 groups had a significant decrease in mechanical hypersensitivity on days 7, 10, 14, 21, and 28 ($P<.001$) compared with the Non-exer group (Fig. 2A). The AUC analysis showed that mechanical hypersensitivity in the Exer 2 and Exer 3 groups was significantly lower than the Exer 1 group ($P<.05$) (Fig. 2B).

Similarly, exercise increased grip-force in all of the exercised groups on days 7, 14, 21, and 28 ($P<.001$; Fig. 2C) compared with the Non-exer group. However, the results presented in Fig. 2E showed that exercise in the Exer 1 group had no effect on cold hypersensitivity. The Exer 2 group showed a significant decrease in cold hypersensitivity evaluated 30 min postexercise compared with the Non-exer group ($P<.05$) from day 7 to day 28. Moreover, the Exer 3 group showed reduced cold hypersensitivity ($P<.05$) from day 7 to day 14 (Fig. 2E). The AUC analysis showed that exercise in the Exer 2 group produced a significant effect compared with the Exer 1 or Exer 3 groups on cold hypersensitivity ($P<.05$) (Fig. 2F).

Low-intensity exercise promoted motor functional recovery after sciatic nerve injury

The results showed that the SFI and SSI values (Figs. 3A, C) of the Non-exer group were significantly lower than the Sham group until the 21st ($P<.001$) and 28th ($P<.001$) postoperative day, respectively. The SFI values of the Exer 1 and Exer 3 groups were significantly better than the Non-exer group on the 14th and 21st days ($P<.001$). However, the start of motor functional recovery in the Exer 2 group occurred on day 7 ($P<.01$) and continued until the 21st postoperative day ($P<.001$) compared with the Non-exer group (Fig. 3A). The AUC analysis showed that exercise in the Exer 2 group produced a significant effect compared with the Exer 1 and Exer 3 groups in SFI ($P<.001$) (Fig. 3B). In addition, we evaluated motor impairment with SSI (Fig. 3C). A response pattern similar of the treated groups compared with the Non-exer group was observed, but no difference was found among the SSI values of the Exer 1, Exer 2, or Exer 3 groups using the AUC analysis (Fig. 3D).

Low-intensity exercise counteracted the increased of proinflammatory cytokines levels after sciatic nerve injury

The crush procedure increased the levels of proinflammatory cytokines (TNF-α, IL-1β, and IL-6R) in both the sciatic nerve (Figs. 4A–C) and the spinal cord (Figs. 4E–G) compared with the Sham group. The concentrations of IL-1β in both sites and the IL-6R in the spinal cord were reduced in all of the exercised groups ($P<.05$) compared with the Non-exer group. Nevertheless, a significant reduction in the concentration of TNF-α in the sciatic nerve ($P<.05$) and spinal cord ($P<.01$) was observed in the Exer 2 and Exer 3 groups compared with the Non-exer group.

Low-intensity exercise promoted sciatic nerve morphological recovery after injury

Morphological changes were assessed using both light (Fig. 5) and electron microscopy (Fig. 6). The density and total number of myelinated fibers (Figs. 7A, B) were lower in the Non-exer group compared with the Sham group ($P<.05$). However, there were a greater number of myelinated fibers only when the treatment included postinjury exercise (Exer 2 and Exer 3 groups, $P<.05$).

The analysis of myelinated fiber sizes (Figs. 7C–F) showed a decrease in fiber diameter, axon diameter, and myelin sheath thickness, and an increase in the G-ratio ($P<.001$) in the Non-exer group compared with the Sham group. Exercise in the Exer 2 group completely restored all of these parameters ($P<.001$) compared with the Non-exer group. The analysis of the percentage of each fiber size range (Fig. 7G) revealed a normal pattern of distribution of fibers <3 μm among the groups. In the range of 3–7 μm, the Non-exer group showed a significant reduction compared with the Sham group ($P<.05$). Interestingly, exercise in the Exer 2 group increased the percentage of fibers >7 μm compared with the Non-exer group ($P<.01$).

DISCUSSION

Although the beneficial effects of exercise on axonal regeneration are well established (van Meeteren et al., 1997; Marqueste et al., 2004; Molteni et al., 2004; Sabatier et al., 2008), little information is available regarding the prophylactic role of it on the deleterious effects induced by sciatic nerve crush. In this study, we adopted the sciatic nerve crush model (Bridge et al., 1994) to examine the prophylactic and therapeutic effects of physical exercise on axonal deficits induced by sciatic nerve crushing. Here, we analyzed the effects of low-intensity exercise performed before nerve injury (i.e. prophylactic, Exer 1), after nerve

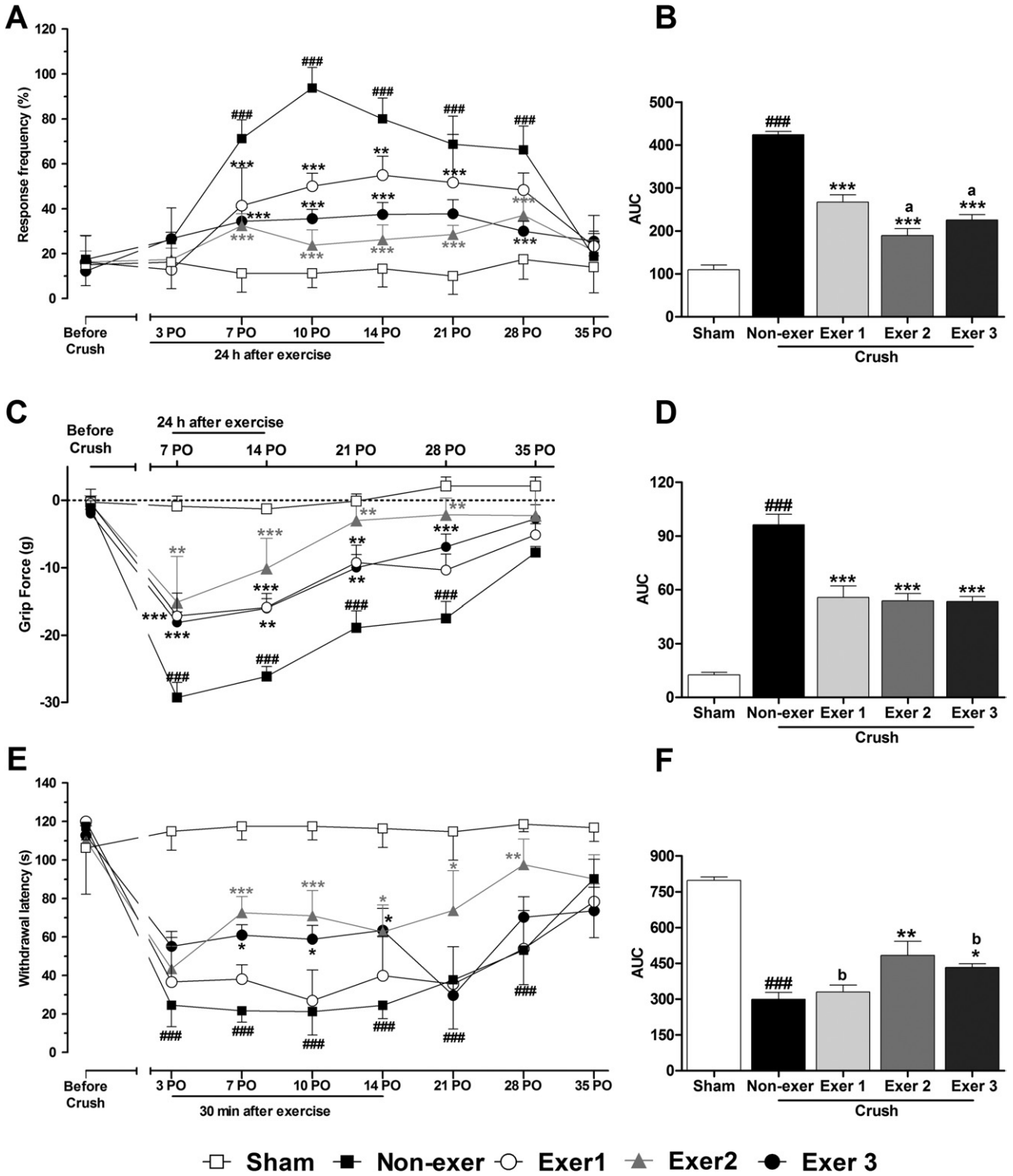

Fig. 2. Effects of low-intensity aerobic exercise on sensory recovery in mice after sciatic nerve crush. Mechanical hypersensitivity (von Frey and grip force tests, panel A and C, respectively) evaluated 24 h after exercise, and AUC (von Frey and grip force tests, panel B and D, respectively). Cold hypersensitivity evaluated 30 min after exercise (panel E), and AUC (panel F). Values represent the mean of eight animals; vertical lines indicate SEM. * $P<.05$, ** $P<.01$, *** $P<.001$, for comparisons of the Non-exer group vs. Exer 1 group, Exer 2 group, or Exer 3 group. ### $P<.001$, for comparisons of the Non-exer group vs. Sham group. Letter "a" corresponds to $P<.05$ when compared with Exer 1 group, and "b" to $P<.05$ when compared with Exer 2 group. Sham, sham-operated; Non-exer, sciatic nerve crush nonexercised; Exer 1, exercise-preoperative for 2 wk; Exer 2, combination of preoperative-postoperative exercise protocols for 4 wk; Exer 3, exercise-postoperative for 2 wk; PO, postoperative.

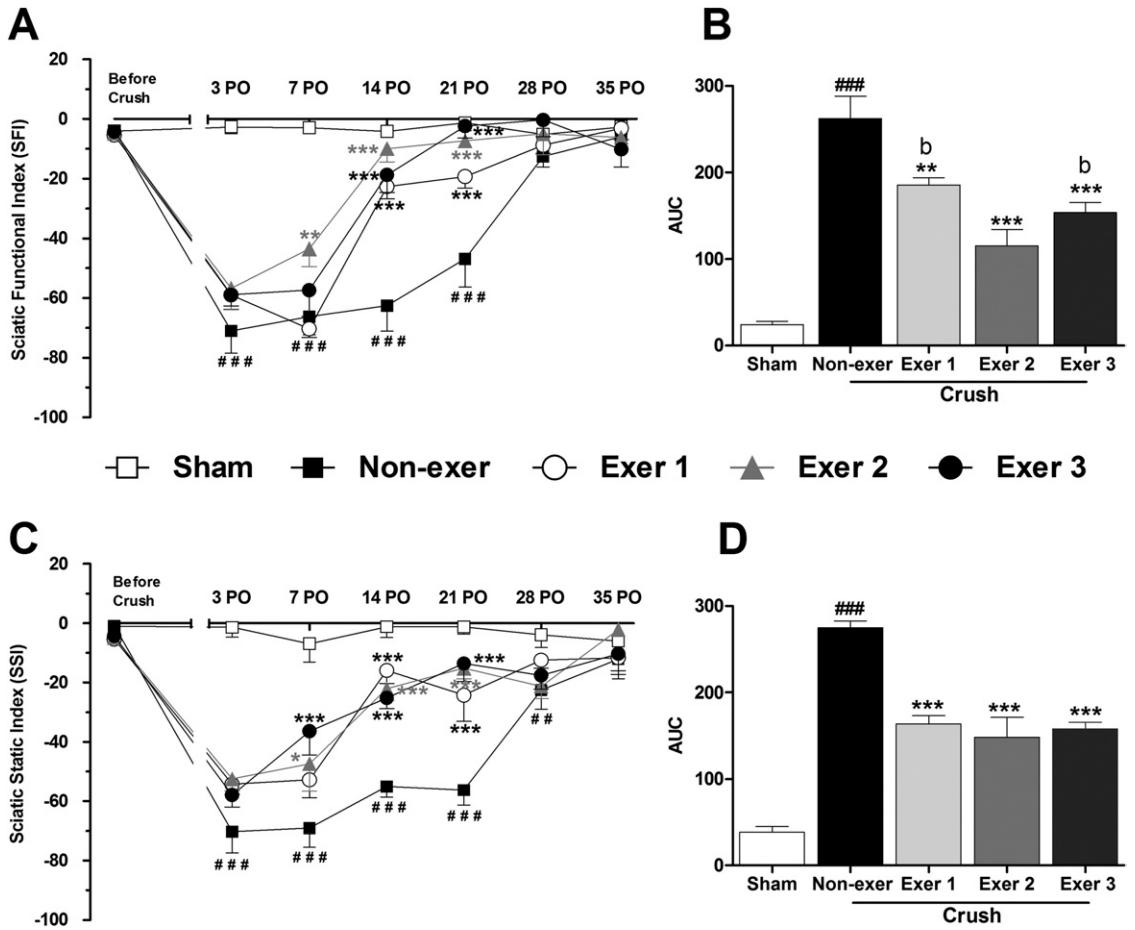

Fig. 3. Effects of low-intensity aerobic exercise on motor recovery in mice after sciatic nerve crush. SFI (panel A), and AUC (panel B). SSI (panel C), and AUC (panel D). Values represent the mean of eight animals; vertical lines indicate SEM. * $P<.05$, ** $P<.01$, *** $P<.001$, for comparisons of the Non-exer group vs. Exer 1 group, Exer 2 group or Exer 3 group. ## $P<.01$, ### $P<.001$, for comparisons of the Non-exer group vs. Sham group. Letter "b" corresponds to $P<.05$ when compared with Exer 2 group. Sham, sham-operated; Non-exer, sciatic nerve crush, nonexercised; Exer 1, exercise-preoperative for 2 wk; Exer 2, combination of preoperative-postoperative exercise protocols for 4 wk; Exer 3, exercise-postoperative for 2 wk; PO, postoperative.

injury (i.e. therapeutic, Exer 3), or the combination of both protocols (i.e. prophylactic plus therapeutic, Exer 2) on functional, morphological, and biochemical recovery of sciatic nerve injury. Our main findings were that low-intensity aerobic exercise reduced neuropathic pain and elicited neuroprotective and neuroregenerative effects in an experimental model of sciatic nerve crush injury.

It is now well known that, in the process of Wallerian degeneration distal to axonal injury, various cell types are activated and recruited to the injury site, including mast cells, macrophages, fibroblasts, neutrophils, and Schwann cells. These cells release adenosine triphosphate (ATP), chemokines, bradykinin, prostaglandins, nerve growth factor (NGF), and proinflammatory cytokines (Scholz and Woolf, 2007). The present study showed that sciatic nerve crushing in mice induced neuropathic pain as measured by mechanical and cold hypersensitivity. Furthermore, nerve injury caused a significant increase of proinflammatory and receptor cytokine levels (TNF-α, IL-1β, and IL-6R) in both the sciatic nerve and the spinal cord. In the current study, all protocols of exercise decreased mechanical hypersensitivity and proinflammatory cytokine levels. Glutamate release from nerve terminals in the spinal cord activates glial cells, which leads to the subsequent further release of proinflammatory cytokines, such as TNF-α and IL-1β (Wieseler-Frank et al., 2004). In this regard, it is well documented that spinal microglia and astrocytes are involved, respectively, in the onset and maintenance of neuropathic pain via the release of many modulators that change neuronal function (Saab et al., 2008). However, a recent study has demonstrated that short duration treadmill running reduces microglia and astrocyte activation (Cobianchi et al., 2010). Therefore, these previous findings and the present results allow us to speculate that low-intensity aerobic exercise can act by preventing the production and release of these proinflammatory cytokines in peripheral or central sites.

In addition to the involvement of proinflammatory cytokines, an alternative mechanism of action to explain the exercise-induced analgesia may involve the activation of the serotonergic system (Korb et al., 2010). Several studies have shown that the activation of the bulbospinal se-

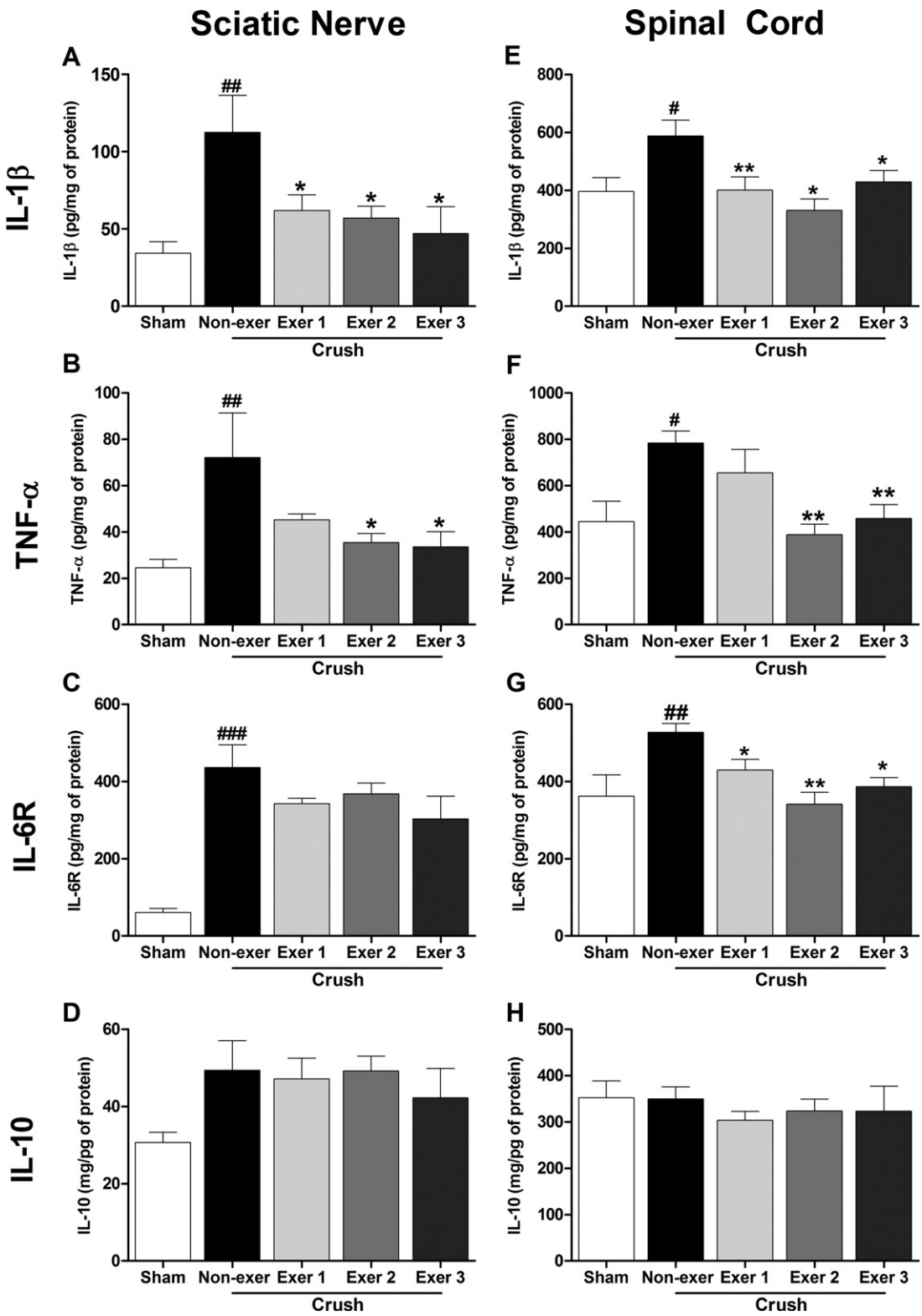

Fig. 4. Effects of low-intensity aerobic exercise on the production and release of pro and anti-inflammatory cytokines in mice subjected to sciatic nerve crush. Levels of IL-1β, TNF-α, IL-6R, and IL-10 in the right sciatic nerve (panels A–D) and spinal cord (panels E–H) 2 wk after crush. Values represent the mean of eight animals per group; vertical lines indicate SEM. * $P<.05$, ** $P<.01$, in comparisons of the Non-exer group vs. Exer 1 group, Exer 2 group, or Exer 3 group; $^{\#}$ $P<.05$, $^{\#\#}$ $P<.01$, and $^{\#\#\#}$ $P<.001$, for comparisons of the Non-exer group vs. Sham group. Sham, sham-operated; Non-exer, sciatic nerve crush nonexercised; Exer 1, exercise-preoperative for 2 wk; Exer 2, combination of preoperative-postoperative exercise protocols for 4 wk; Exer 3, exercise-postoperative for 2 wk.

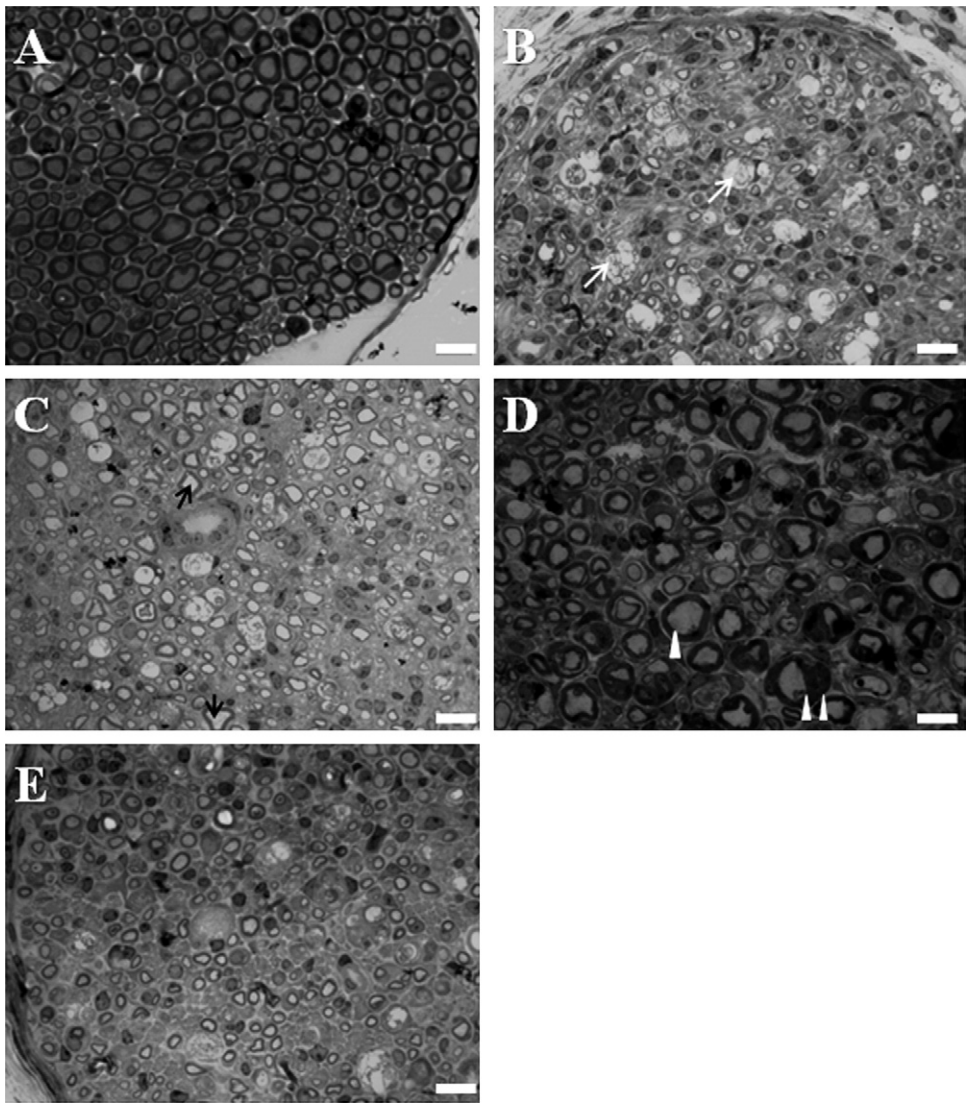

Fig. 5. Light micrographs of toluidine blue-stained semithin transverse sections of sciatic nerve, 2 wk after nerve crush in mice. (A) Sham showing a normal pattern of fiber morphology and distribution. (B) Non-exer showing the predominance of low-diameter myelinated fibers, increased endoneurial connective tissue between the nerve fibers and the presence of degeneration debris (arrows). (C) Exer 1 showing mainly low-diameter myelinated fibers (dark arrow), reduction of myelinated fiber numbers, increased endoneurial space, and degeneration debris. (D) Exer 2 showing increase of myelinated fiber numbers and enhanced myelin thickness (arrow head) that appeared similar to the Sham group. (E) Exer 3 was similar to the Exer 1 group, presenting low-diameter myelinated fibers and increased endoneurial space. All operated upon groups (Non-exer, Exer 1, Exer 2, and Exer 3) showed prominent Schwann cell nuclei (double arrowhead). Scale bar=10 μm.

rotonergic system inhibits noxious stimuli-induced responses (Veasey et al., 1997; Rahman et al., 2006). According to Jacobs et al. (2002), the analgesia caused by serotonin in the medulla might be stimulated by motor activity. However, Veasey et al. (1997) showed that motor activity induces an increase in serotonin in the ventral and dorsal horns of the spinal cord. Therefore, it is plausible that treadmill exercise performed in this study could have inhibited the release of algogenic agents from sensory neurons via a release of serotonin in the spinal cord.

Regarding the mechanism through which exercise exerts its antinociceptive action, an observation in the literature provides direct evidence of the involvement of endogenous opioids (Willow et al., 1980). It has been suggested that treadmill exercise releases endogenous opioids, like enkephalin, endorphin, and others, that are responsible for hyponociception (Willow et al., 1980; Koltyn, 2000). Although data are limited in studies of chronic pain, this release of endogenous opioids could partially explain the exercise-induced analgesia observed in this study.

Another finding of the present study is the demonstration that treadmill training at low speed accelerated the functional recovery of the sciatic nerve (shown here by SFI and SSI and as already reported in previous studies) (Cobianchi et al., 2010; Wang et al., 2010). This activity has been related to an increased expression of the BDNF and its receptor, trkB (Gómez-Pinilla et al., 2001). Nerve regeneration can be enhanced by various growth factors,

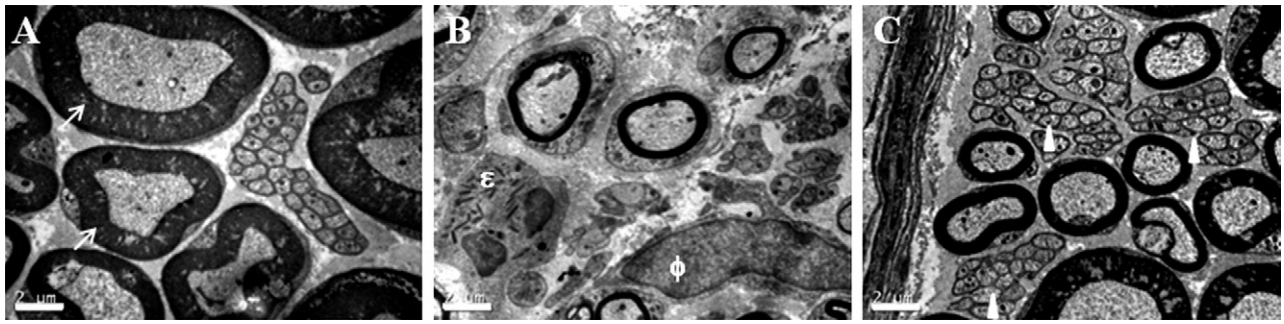

Fig. 6. Electron micrographs of ultrathin transverse sections of sciatic nerve, 2 wk after crush in mice.(A) Sham showing large-diameter fibers with thick myelin (arrows), unmyelinated fibers in well-organized clusters, and compact endoneurial space. (B) Non-exer showing mainly a decrease in the diameter of the myelinated fibers and myelin thickness compared with Sham; sprouting of unmyelinated fibers, larger endoneurial spaces, and the presence of cells such as macrophages (Φ) and neutrophils (ε). (C) Exer 2 showing myelinated fibers with various calibers and clusters of unmyelinated fibers (arrows heads) beyond enhanced myelin thickness compared with Non-exer. Scale bar=2 μm.

which help to sustain the growing nerve (Bregman et al., 1997). BDNF has a positive influence after neurorrhaphy when used alone or in combination with other factors (Lewin et al., 1997; Ho et al., 1998). *In vivo* studies have shown that the local continuous release of BDNF increases nerve fiber growth over time and induces remyelination and faster nerve regeneration in rats after sciatic nerve injury (Vögelin et al., 2006). BDNF enhances the expression of myelin-associated glycoprotein (MAG) and glycoprotein zero (P0) (Chan et al., 2001). Taken together, this evidence may explain the greater degree of fiber myelination in the Exer 2 group animals in our study.

In the CNS, exercise induces neuroprotection in animal models of stroke (Hayes et al., 2008), traumatic brain injury (Griesbach et al., 2009), and Parkinson's disease (Yoon et al., 2007). These experimental studies indicate the importance of the preventive effects of physical exercise. In addition, little information is available regarding the protective role of physical exercise on peripheral nervous system injuries. Interestingly, our study also showed that the combination of the two exercise protocols (i.e. the Exer 2 group) induced significant morphological improvement in sciatic nerve injury compared with the Non-exer, Exer 1, and Exer 3 groups. Although the effects of exercise are well documented for the treatment of sciatic nerve injury, the influence of exercise in prevention has not been thoroughly explored. One plausible explanation for our findings is that the increase in physical activity has been shown previously to alter the structure and function of the neuromuscular junction (NMJ). However, exercise increases the size and the degree of branching of motor nerve terminals at the NMJ (Andonian and Fahim, 1987), increases the total area of both pre- and postsynaptic elements (Deschenes et al., 1993), and increases the quantal content of acetylcholine release (Dorlochter et al., 1991). The dispersion of acetylcholine receptors, including both endplate perimeter length and area, is increased after resistance training (Deschenes et al., 2000).

In addition, it has been previously shown that the glial cell line-derived neurotrophic factor protein (GDNF) helps to protect motor neurons (MNs) from chronic degeneration (Corse et al., 1999), which suggests an important role for GDNF in motor nervous system recovery. GDNF promotes the survival of somatic MNs from programmed cell death, rescues MNs from axotomy-induced cell death (Oppenheim et al., 1995), and slows the loss of MNs in mice exhibiting progressive motor neuropathy (Sagot et al., 1996). Moreover, GDNF protein content is increased in the skeletal muscles of animals trained for 2 weeks (Wehrwein et al., 2002). The overexpression of GDNF in skeletal muscle leads to hyperinnervation at the NMJ (Nguyen et al., 1998).

It is now known that sedentarism makes a big impact on public health resulting in the emergence of modern degenerative diseases in an ever-increasing number of people. However, the World Health Organization (WHO) has praised that regular moderate intensity physical activity such as walking, cycling, or participating in sports, has significant benefits for health. For instance, it can reduce the risk of cardiovascular diseases, diabetes, colon and breast cancer, as well as depression (WHO, 2011). Besides these benefits, in the present study, we have provided evidence that exercise is a preventative strategy to promote neuroprotection, and has great potential to be a valid treatment therapy after peripheral nerve injury.

However, additional studies are necessary to achieve a better understanding of the possible biochemical mechanisms involved in exercise-induced neuroprotection. Moreover, in the present study, we cannot discard the possibility of the acute effect of exercise, although the mice were killed 24 h after the last training session.

CONCLUSION

In summary, a prophylactic or therapeutic approach in which mice were trained for 2 weeks before or after nerve injury improved sensory motor functions but did not promote morphological regeneration. Interestingly, the combination of both exercise protocols (prophylactic plus therapeutic) accelerated the processes of motor recovery sciatic nerve and morphological regeneration beyond a reduction of hypersensitivity. This experimental study suggests that physical exercise can restore motor and nerve function to a substantial degree when performed using a

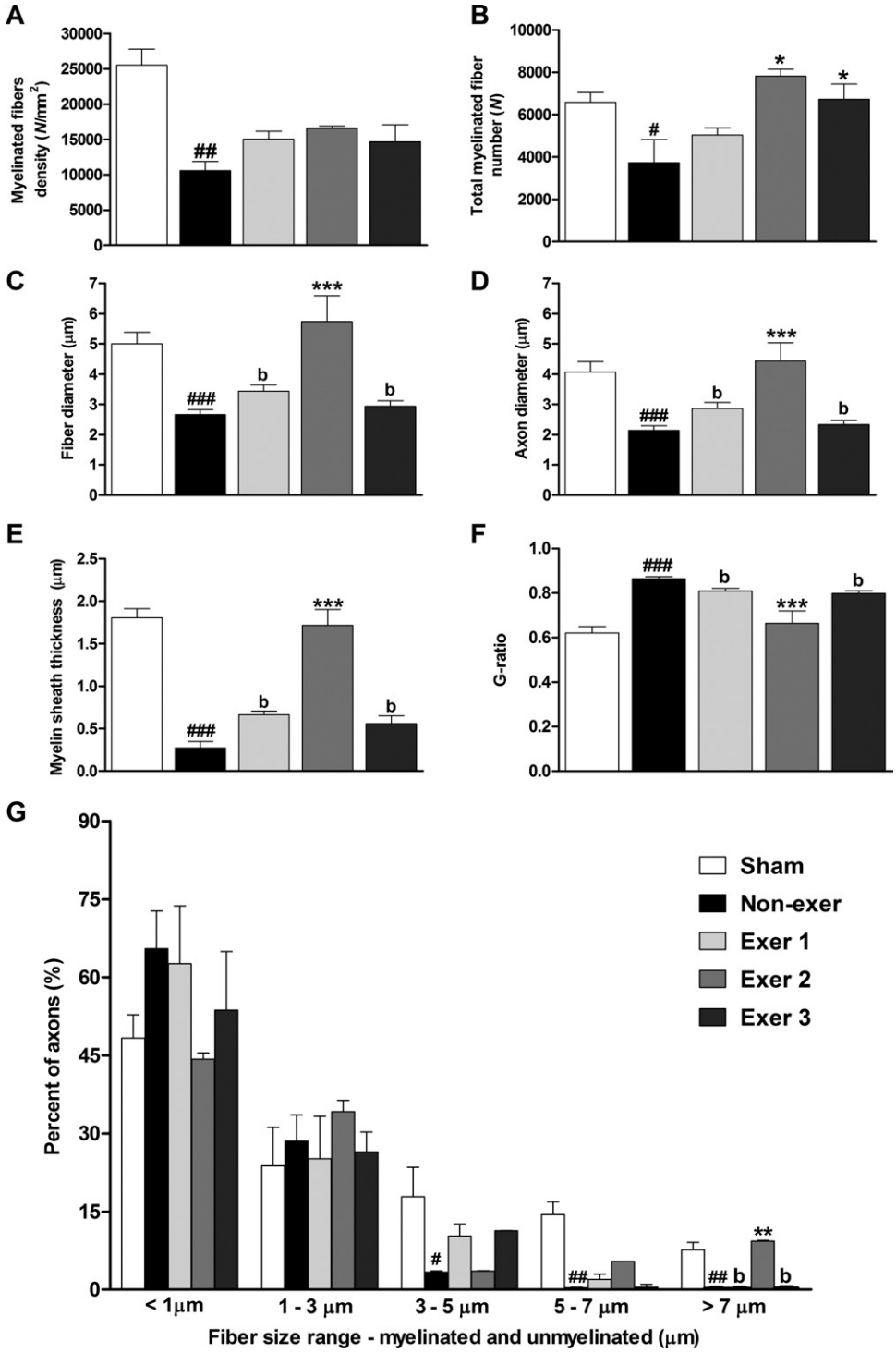

Fig. 7. Effects of low-intensity aerobic exercise on the morphological recovery in mice after sciatic nerve crush. Graphs show the quantitative morphology of myelinated nerve fibers: (A) myelinated fiber density, (B) myelinated fiber number, (C) myelinated fiber diameter, (D) axon diameter, (E) myelin sheath thickness, (F) G-ratio, and (G) the percentage for each fiber size range, the latter measurement included the unmyelinated and myelinated fibers. Values represent the mean of five animals per group; vertical lines indicate SEM * $P<.05$, ** $P<.01$, *** $P<.001$ for comparison of the Non-exer group vs. Exer 1 group, Exer 2 group, or Exer 3 group; $^{\#}$ $P<.05$, $^{\#\#}$ $P<.01$, and $^{\#\#\#}$ $P<.001$, for comparisons of the Non-exer group vs. Sham group. Letter "b" corresponds to $P<.05$ when compared with Exer 2 group. Sham, sham-operated; Non-exer, sciatic nerve crush nonexercised; Exer 1, exercise-preoperative for 2 wk; Exer 2, combination of preoperative-postoperative exercise protocols for 4 wk; Exer 3, exercise-postoperative for 2 wk.

prophylactic and therapeutic approach. Therefore, physical exercise may offer an inexpensive preventive strategy to prevent motor and sensorial incapacity and/or treat peripheral nerve injuries, as well as a large number of chronic diseases, as advocates the WHO.

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