

Regular Exercise Reverses Sensory Hypersensitivity in a Rat Neuropathic Pain Model

Role of Endogenous Opioids

Nicola J. Stagg, Ph.D.,* Heriberto P. Mata, B.S.,† Mohab M. Ibrahim, Ph.D., M.D.,‡
Erik J. Henriksen, Ph.D.,§ Frank Porreca, Ph.D.,|| Todd W. Vanderah, Ph.D.,||
T. Philip Malan, Jr., Ph.D., M.D.#

ABSTRACT

Background: Exercise is often prescribed as a therapy for chronic pain. Short-term exercise briefly increases the production of endogenous analgesics, leading to transient antinociception. In limited studies, exercise produced sustained increases in endogenous opioids, sustained analgesia, or diminished measures of chronic pain. This study tests the hypothesis that regular aerobic exercise leads to sustained reversal of neuropathic pain by activating endogenous opioid-mediated pain modulatory systems.

Methods: After baseline measurements, the L5 and L6 spinal nerves of male Sprague–Dawley rats were tightly ligated. Animals were randomized to sedentary or 5-week treadmill exercise-trained groups. Thermal and tactile sensitivities were assessed 23 h after exercise, using paw withdrawal thresholds to von Frey filaments and withdrawal latencies to noxious heat. Opioid receptor antagonists were administered by subcutaneous, intrathecal, or intracerebroventricular injection. Opioid peptides were quantified using immunohistochemistry with densitometry.

Results: Exercise training ameliorated thermal and tactile hypersensitivity in spinal nerve–ligated animals within 3 weeks. Sensory hypersensitivity returned 5 days after discontinuation of exercise training. The effects of exercise were reversed by using systemically or intracerebroventricularly administered opioid receptor antagonists and prevented by

What We Already Know about This Topic

- Exercise is often used in conjunction with other modalities for acute and chronic pain management.
- Although opioids have been implicated in the immediate benefits of exercise against pain, whether this applies to chronic pain is unknown.

What This Article Tells Us That Is New

- In rats with neuropathic hypersensitivity, endogenous brainstem opioids contributed to a reduction in pain-related behaviors.

continuous infusion of naltrexone. Exercise increased β -endorphin and met-enkephalin content in the rostral ventromedial medulla and the mid-brain periaqueductal gray area.

Conclusions: Regular moderate aerobic exercise reversed signs of neuropathic pain and increased endogenous opioid content in brainstem regions important in pain modulation. Exercise effects were reversed by opioid receptor antagonists. These results suggest that exercise-induced reversal of neuropathic pain results from an up-regulation of endogenous opioids.

CLINICAL studies^{1–7} and clinical experience suggest that exercise decreases pain symptoms and improves function in patients with chronic pain, including those with pain syndromes thought to have a neuropathic component. However, there are few scientific studies of the mechanisms underlying the pain-relieving effects of exercise.

Extensive research⁸ has shown that a single episode of exercise increases the production of endogenous opioids, leading to transient antinociception in both animals and humans. More recent studies in experimental animals have shown that repeated exercise produces long-lasting antinociception in otherwise untreated animals^{9–12} and increases plasma and cerebrospinal fluid opioid concentrations.^{13,14} Furthermore, in animal models, exercise diminishes measures of inflammatory pain,¹⁵ chronic muscle pain,¹⁶ and

* Student, Graduate Interdisciplinary Program in Pharmacology and Toxicology, † Research Specialist, Department of Anesthesiology, ‡ Medical Student, § Professor of Physiology, || Professor of Pharmacology and Anesthesiology, # Professor of Anesthesiology and Pharmacology, The University of Arizona, Tucson, Arizona.

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Address correspondence to Dr. Malan: Department of Anesthesiology, The University of Arizona College of Medicine, 1501 N Campbell Ave, Tucson, Arizona 85724-5114. malan@u.arizona.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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chronic neuropathic pain.^{15,17,18} The mechanisms underlying exercise-induced reversal of chronic pain are incompletely understood. One study¹⁶ in a model of muscle pain showed exercise effects to be reversed by naloxone, suggesting the participation of endogenous opioids. The current study tests the hypothesis that regular repeated aerobic exercise will reverse neuropathic pain by enhancing endogenous opioid-mediated pain modulatory systems.

Materials and Methods

Animals

Approval was obtained from the University of Arizona Animal Care and Use Committee, Tucson, Arizona. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) weighed 250–380 g throughout testing. They were allowed water and food ad libitum and housed in a climate-controlled room. They were maintained under reverse light–dark conditions, with the room lit from 10 PM to 10 AM. They were handled twice daily in a stress-free environment and were allowed to equilibrate to the surroundings for 7 days before experimental manipulations began. All procedures conformed to the ethical guidelines for the care and use of laboratory animals, published by the International Association for the Study of Pain and the National Institutes of Health.

Spinal Nerve Ligation

L5–L6 spinal nerve ligation (SNL) was performed as described by Kim and Chung.¹⁹ Under isoflurane anesthesia, an incision was made lateral to the lumbar spine. The paraspinal muscles were separated from the vertebral processes at the L4 to the S2 levels. The left L6 transverse process was completely removed with a small rongeur, and the L4–L6 spinal nerves were exposed. The left L5 and L6 spinal nerves were isolated and tightly ligated with 4–0 silk sutures distal to the dorsal root ganglion. The incision was sutured closed, and each animal was allowed to recover for 7 days before exercise training. Sham–operated animals were prepared in an identical manner, but the spinal nerves were not ligated. Non–operated animals underwent no surgery but were otherwise treated identically to SNL or sham–operated animals. Animals demonstrating signs of sciatic nerve injury (*i.e.*, an inverted foot with markedly ventroflexed toes)¹⁹ or damage to the L4 spinal nerve (*i.e.*, hind paw paralysis)¹⁹ were removed from the study.

Exercise Training

Forced treadmill running was used for exercise training because it is an established model in which exercise intensity and duration can be readily controlled.²⁰ Before surgery, animals underwent exercise pretesting on a rodent treadmill (18 m/min for 10 min, 2 days per week for 2 weeks). Animals unable to complete the pretest, whether from inability to run or from signs of stress (*e.g.*, porphyrin rings around the eyes or a red nose), were removed from the study. After surgery,

animals were randomized to a sedentary group or an exercise-trained group. Exercise training consisted of running on a 10-lane motor-driven rodent treadmill (in late afternoon, 5 days per week, for 5 weeks at a speed of 14–16 m/min and an 8% grade). The duration of exercise was incrementally increased to 30 min/day over 14 days. An electric grid behind the treadmill was activated with a weak current. To minimize stress, animals were allowed contact with the grid for a maximum of 2 min up to three times per session before being removed from the treadmill apparatus. Sedentary animals were treated identically to exercise-trained animals, except that they were placed in the treadmill lanes in acrylic glass boxes that did not allow contact with the moving treadmill. This exposed them to the same handling and environmental conditions as animals that exercised. Animals displaying signs of stress were removed from the study.

Assessment of Tactile Sensitivity

Tactile sensitivity was assessed by measuring the threshold for withdrawal of the hind paw from normally nonnoxious tactile stimuli. Rats were allowed to acclimate for 30 min within acrylic glass enclosures with wire mesh bottoms. Paw withdrawal thresholds were determined in response to probing of the left hind paw with a series of calibrated von Frey filaments, in logarithmically spaced increments, applied perpendicularly to the plantar surface of the paw. A maximal cutoff of 15 g was used because larger filaments lift the paw, preventing the assessment of active paw withdrawal. Data were analyzed by the up-and-down method of Dixon, as described by Chaplan *et al.*²¹ Tactile sensitivity was measured at baseline, 1 week after surgery, and at weekly increments during exercise training. Each point consisted of 1 day of testing. Where applicable, measurements were performed 23 h after exercise. Measurements were confirmed by an observer (H.P.M.) blinded to the treatment received.

Assessment of Thermal Sensitivity

Thermal sensitivity was assessed by measuring latency to paw withdrawal from a noxious heat source, as described by Hargreaves *et al.*²² Rats were allowed to acclimate within acrylic glass enclosures on a clear glass plate maintained at 30°C. A radiant heat source was focused through the glass plate onto the plantar surface of the left hind paw. Withdrawal latency was measured using a motion detector that shut off the heat stimulus and a timer on withdrawal of the paw. A maximal cutoff of 40 s was used to prevent tissue damage. Thermal sensitivity was measured at baseline, 1 week after surgery, and at weekly increments during exercise training. Each point consisted of 1 day of testing. Where applicable, measurements were conducted 23 h after exercise. Measurements were confirmed by an observer (H.P.M.) blinded to the treatment received.

Osmotic Minipumps

Osmotic minipumps were implanted subcutaneously under isoflurane anesthesia, 1 day before initiation of exercise train-

ing. After shaving of the surgical site, the skin was swabbed with povidone-iodine and alcohol, 70%. A small incision was made through the skin between the scapulae. A small pocket was formed using a hemostat to spread the subcutaneous connective tissue, and an osmotic minipump (Alzet model 2006; Alza, Mountain View, CA) was inserted into the pocket, with the flow moderator pointing away from the incision. Each pump was incubated in saline, 0.9%, at 37°C for 60 h before implantation. The incision was closed with wound clips or sutures.

Drug Administration

All drugs were purchased from Sigma-Aldrich (St Louis, MO) and dissolved in saline. For systemic administration, naloxone (1 mg/kg), naloxone methiodide (0.1 mg/kg), or vehicle (saline) was administered subcutaneously in the neck in a volume of 1 ml/kg. Single doses of opioid receptor antagonists were administered 23 h after completion of the final session of exercise training. For continuous administration of opioid receptor antagonist, subcutaneous minipumps delivered naltrexone (70 $\mu\text{g}/\text{h}$) or vehicle (saline) in a volume of 0.15 $\mu\text{l}/\text{h}$ for 1 day before and during 35 consecutive days of exercise training.

Intracerebroventricular Cannulation

Intracerebroventricular cannulas were inserted 5 days before the start of exercise training. Under isoflurane anesthesia, the scalp was shaved and the skin was swabbed with povidone-iodine and alcohol, 70%. The head was placed in a stereotaxic holder, and the skull was exposed. A 22-gauge guide cannula was directed to the right lateral ventricle (1.3 mm caudal to the bregma, 1.5 mm lateral to the sagittal suture, and 3.5 mm ventral to the dural surface). The cannula was secured in place by small stainless steel screws and dental cement. Drug was administered through a 28-gauge injection cannula inserted through the guide cannula. Naloxone methiodide (2 μg) was slowly injected in a total volume of 2.5 μl 23 h after completion of the final session of exercise training. Backflow was prevented by using an injection cannula 1 mm longer than the guide cannula.

Intrathecal Injection

Intrathecal injections were performed under isoflurane anesthesia. The lumbar region was shaved and prepared with ethanol, 70%. A 0.5-inch 30-gauge needle, connected to a 25- μl Hamilton syringe, was passed through the L5-L6 spinal interspace. Subarachnoid positioning of the tip of the needle was verified by a flicking motion of the tail or hind paw. Lidocaine, 4% (10–20 μl), was administered to a group of test animals, using temporary paralysis of the hind limbs as an end point to confirm the effectiveness of the injection technique. Naloxone methiodide (10 μg) was administered in a volume of 10 μl 23 h after completion of the final session of exercise training.

Immunohistochemistry

Rats were anesthetized with ketamine HCl-xylazine. The heart was surgically exposed, and the animals were perfused transcardially with 0.01 M sodium phosphate-buffered saline (PBS; pH, 7.4) until the exudate ran clear; in addition, for approximately 15 min, animals were perfused with PBS-buffered formalin, 10%. All harvested tissues were postfixed in PBS-buffered formalin, 10%, and transferred to sucrose, 20%, in 0.1 M PBS. Slide-mounted serial frozen sections of the periaqueductal gray area (PAG) and the rostral ventromedial medulla (RVM) were preblocked with normal goat serum, 10%, in PBS (for 1 h at room temperature), followed by incubation with normal goat serum, 2%, and Triton X-100, 0.3%/PBS/primary antibody for 24 h at 4°C. Secondary antibody was added for 2 h at room temperature. Primary anti-serum samples were rabbit anti- β endorphin (1:5,000; ImmunoStar Incorporated, Hudson, WI) and rabbit anti-met-enkephalin (1:10,000; Chemicon International, Temecula, CA). Secondary antiserum was goat anti-rabbit IgG (Alexa Fluor 568, 1:1,000; Invitrogen, Carlsbad, CA). After PBS washes, sections were dried and sealed with fluorescent mounting medium (Vector Laboratories, Burlingame, CA).

Image Analysis and Quantification

Fluorescence images of PAG and RVM brain sections were acquired with a fluorescence microscope (model E800; Nikon, Tokyo, Japan), outfitted with $\times 4$ numerical aperture 0.2, $\times 10$ numerical aperture 0.45, $\times 20$ numerical aperture 0.75, and $\times 40$ numerical aperture 0.75 objectives; a filter set for Cy3 (a dye of the cyanine family) (excitation, 540–580 nm; emission, 560–620 nm); and a C5810 color charge-coupled device camera and its proprietary image processor software (Hamamatsu Photonic Systems, Bridgewater, NJ). Digital images were produced using software (Adobe Photoshop 6.0; Adobe Systems Inc., San Jose, CA). Quantitative measurements in PAG were performed on 10 sections taken from five animals per group. Quantitative measurements in RVM were performed on 26 to 32 sections taken from five animals per group. Sections were selected for analysis based on the quality of the section (*i.e.*, lack of tears and bubbles). Images were of identical dimensions and were analyzed using a densitometry program (Scion Image 4.0.3.2; Scion Corp, Frederick, MD). Control and experimental tissues were processed and analyzed simultaneously.

Statistical Analysis

The data were assumed to meet the assumptions of parametric tests because the sample sizes used were not large enough to allow formal testing of these assumptions. When two groups were compared, a two-sample, two-sided, independent Student *t* test was used. Multiple groups were compared using one-way ANOVA. When the same group of animals was studied over time, a one-way repeated-measures ANOVA was applied. When multiple groups of animals

were studied over time, a two-way repeated-measures ANOVA (group \times time) was applied. *Post hoc* testing consisted of a pairwise comparison using the Bonferroni method for multiple comparisons. Where noted, only selected comparisons were performed, based on preanalysis planning, to minimize the statistical impact of multiple comparisons. All *P* values were calculated using two-tailed tests. Significance was defined as $P < 0.05$. All data are reported as mean (95% two-tailed CI). Statistical analysis and preparation of figures were performed with software (GraphPad Prism, Version 5 [GraphPad Software, La Jolla, CA]; and Stata, Version 11 [Stata Corp, College Station, TX]).

Results

Exercise Training Reversed SNL-induced Thermal and Tactile Hypersensitivity

There were no differences between animals assigned to the SNL and sham-operated on groups in preoperative withdrawal thresholds or latencies. By 1 week after surgery, SNL animals developed sensory hypersensitivity (decreased thermal withdrawal latencies and tactile withdrawal thresholds) compared with presurgical values (fig. 1, A and B), whereas sham-operated on or non-operated on animals did not. Statistical analysis using repeated-measures two-way ANOVA (group \times time) demonstrated a significant time-treatment interaction effect for both thermal withdrawal latencies ($P < 0.0001$) and paw withdrawal thresholds ($P < 0.0001$). *Post hoc* testing was performed using the Bonferroni method for multiple comparisons, specifically making the following comparisons. SNL reduced thermal withdrawal latency from 17.7 s (16.0–19.4 s) to 12.1 s (11.0–13.2 s) ($P < 0.0001$) and tactile withdrawal threshold from 14.7 s (14.0–15.4 s) to 1.8 s (1.6–2.1 s) ($P < 0.0001$). Regular exercise training ameliorated thermal and tactile hypersensitivity within 3 weeks. Thermal withdrawal latency increased after 3 weeks of exercise compared with sedentary animals ($P = 0.0064$); after 5 weeks, withdrawal latency had returned to 17.6 s (15.1–20.1 s) ($P = 0.0135$ vs. SNL sedentary animals) compared with 19.0 s (16.5–21.4 s) in sham-operated on animals (fig. 1A). Tactile withdrawal thresholds increased after 3 weeks of exercise compared with sedentary animals ($P = 0.0001$); after 5 weeks, paw withdrawal thresholds had returned to 13.9 s (11.2–16.7 s) ($P < 0.0001$ vs. SNL sedentary animals) compared with 13.9 s (9.2–18.6 s) in sham-operated on animals. Exercise did not alter withdrawal thresholds or latencies in sham-operated on or non-operated on animals. Statistical analysis used two-way repeated-measures ANOVA. For tactile sensitivity, $P = 0.26$ for the group \times time interaction; and $P = 0.33$ for the effects of treatment. For thermal sensitivity, $P = 0.51$ for the group \times time interaction; and $P = 0.027$ for the effects of treatment. However, *post hoc* testing using the Bonferroni method for multiple comparisons did not show an effect of exercise at any point in either non-operated on or sham-operated on animals.

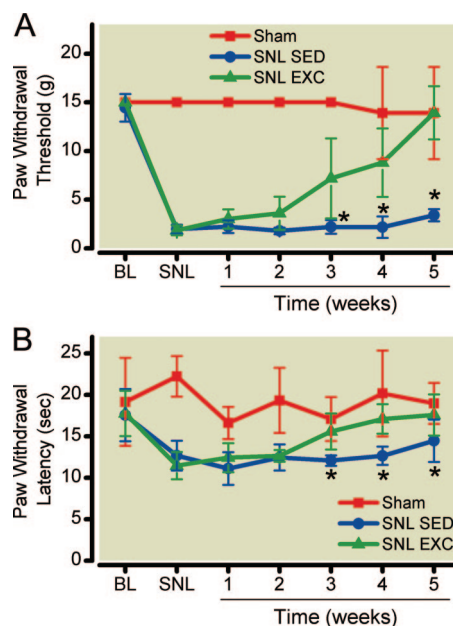


Fig. 1. Exercise training reverses nerve injury-induced sensory hypersensitivity. (A) Tactile paw withdrawal threshold in exercise-trained (EXC) versus sedentary animals (SED) that underwent spinal nerve ligation (SNL). Data from sham-operated on animals are included for comparison. (B) Thermal paw withdrawal latency to radiant heat in EXC versus SED animals that underwent SNL. Data from sham-operated on animals are included for comparison. Baseline measurements were taken 1 day before surgery. Further measurements were performed 1 day before and weekly after the initiation of exercise training. Measurements were made 23 h after exercise. Data are given as mean (95% two-tailed CI). * $P < 0.05$ compared with EXC (rats) that underwent SNL. $n = 6$ animals per group. BL = presurgical baseline.

Thermal sensitivity could not be studied for longer than 5 weeks after surgery, because it returns to baseline after several weeks in the SNL model (M.M.I. and T.P.M., unpublished data, 2000). In contrast, tactile hypersensitivity appears to last indefinitely. Because thermal hypersensitivity could not be studied in longer-term experiments, only tactile hypersensitivity was studied in the following experiments.

Exercise Effects Were Dependent on Exercise Intensity but not Frequency

Exercise training was conducted in SNL animals for either 3 or 5 days per week. Statistical analysis using a repeated-measures two-way ANOVA demonstrated that the effects of time-frequency interaction ($P = 0.112$) or frequency ($P = 0.091$) were not significant (fig. 2A).

Exercise training was conducted in SNL animals at speeds of either 10 m/min (lower intensity) or 16 m/min (higher intensity). Walking was required at the lower intensity and running at the higher intensity. Animals could not be trained at speeds of more than 16 m/min because animals that exercised at higher speeds exhibited signs of stress. The higher-

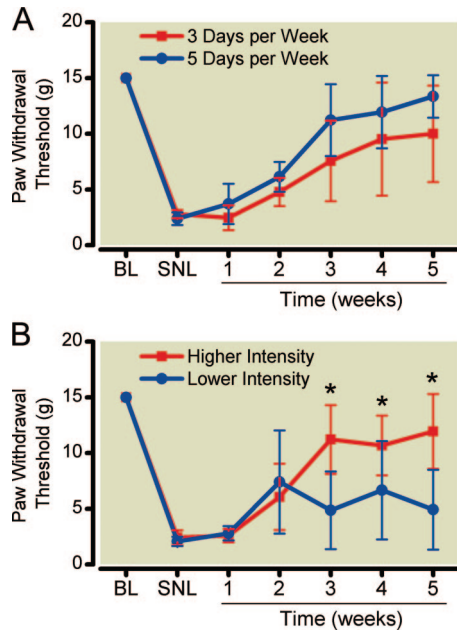


Fig. 2. More intense, but not more frequent, exercise training resulted in more complete reversal of sensory hypersensitivity. (A) Tactile paw withdrawal threshold after exercise training 3 or 5 days per week. (B) Tactile paw withdrawal threshold after exercise training at 10 m/min (lower intensity) or 16 m/min (higher intensity) 5 days per week. Data are given as mean (95% two-tailed CI). * $P < 0.05$ compared with lower-intensity exercise. $n = 6$ animals per group. BL = presurgical baseline; SNL = after spinal nerve ligation.

intensity group had a more complete reversal of tactile hypersensitivity than did the lower-intensity group (fig. 2B). Statistical analysis using repeated-measures two-way ANOVA demonstrated a significant intensity–time interaction effect ($P < 0.0001$) and an intensity effect ($P = 0.0064$). *Post hoc* testing was performed using the Bonferroni method for multiple comparisons, comparing paw withdrawal thresholds between higher- and lower-intensity training at each point. The tactile threshold was 12.3 g (9.3–15.3 g) after 5 weeks of higher-intensity exercise compared with 4.9 g (1.3–8.5 g) after 5 weeks of lower-intensity exercise ($P = 0.0003$). *Post hoc* analysis with repeated-measures one-way ANOVA also demonstrated that lower-intensity exercise did not significantly increase paw withdrawal threshold compared with preexercise values ($P = 0.068$). In contrast, *post hoc* analysis with repeated-measures one-way ANOVA demonstrated that higher-intensity exercise significantly increased paw withdrawal threshold compared with preexercise values ($P < 0.0001$).

Onset of Exercise Effects Determined by Number of Weeks of Exercise Training

The observed timing of exercise effects might be because of the number of weeks of exercise training required to reverse sensory hypersensitivity. Alternatively, the timing of exercise effects might be because of maturation of the SNL model, in

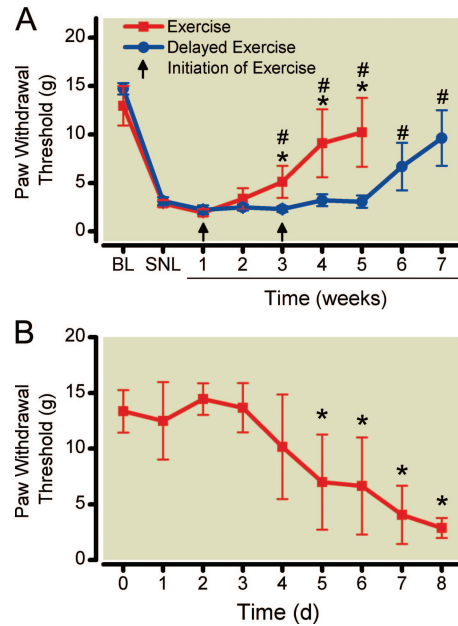


Fig. 3. (A) The onset of exercise-induced reversal of sensory hypersensitivity 3 weeks after the initiation of exercise training. Tactile sensitivity was measured in sedentary animals after spinal nerve ligation (SNL), exercise-trained animals after SNL, and delayed-exercise animals after SNL. Data are given as mean (95% two-tailed CI). # $P < 0.05$ compared with preexercise animals after SNL. * $P < 0.05$ compared with the delayed-exercise group. $n = 6$ animals per group. (B) Tactile hypersensitivity returned 5 days after the cessation of exercise training. Data are given as mean (95% two-tailed CI). $n = 6$ animals per group. BL = presurgical baseline.

which sensory hypersensitivity was not susceptible to exercise reversal until 3 weeks after surgery. To distinguish between these possibilities, in one group of animals, exercise was initiated 4 weeks after SNL as opposed to the 1-week recovery period used in other experiments. The reduction of sensory hypersensitivity occurred 3 weeks after the initiation of exercise, regardless of the interval after surgery (fig. 3A). Statistical analysis using repeated-measures two-way ANOVA (group \times time) demonstrated a significant group–time interaction effect ($P < 0.0001$) and a group effect ($P = 0.0009$). *Post hoc* testing was performed using the Bonferroni method for multiple comparisons, specifically comparing paw withdrawal threshold at each point after the initiation of exercise with paw withdrawal latency after surgery and before exercise testing. The results of this analysis are presented in figure 3A.

Sensory Hypersensitivity Returned Within 1 Week of Cessation of Exercise

Tactile hypersensitivity began to return 5 days after cessation of exercise training and returned to preexercise levels 8 days after discontinuing exercise (fig. 3B). Statistical analysis with a one-way repeated-measures ANOVA demonstrated a significant time effect ($P < 0.0001$). *Post hoc* analysis was performed using the Bonferroni method for multiple compar-

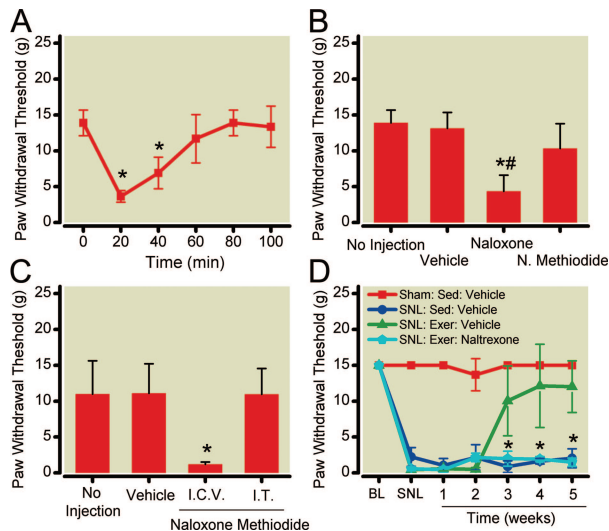


Fig. 4. Opioid receptor antagonists reverse the effects of exercise on sensory hypersensitivity. (A) Naloxone reversibly inhibited exercise effects. Exercise-trained animals (EXER) that underwent spinal nerve ligation (SNL) were administered naloxone (1 mg/kg) subcutaneously. $*P < 0.05$ compared with values before naloxone. (B) Subcutaneous naloxone methiodide (N. Methiodide) (0.1 mg/kg) had no effect on tactile hypersensitivity in EXER that underwent SNL. $*P < 0.05$ compared with EXER that underwent SNL and received vehicle. $\#P < 0.05$ compared with EXER that underwent SNL and received N. Methiodide. (C) Reversal of nerve injury-induced sensory hypersensitivity by exercise is mediated by endogenous opioids in the central nervous system. Intracerebroventricular (i.c.v.) injection of N. Methiodide (2 μ g) reversed exercise-training-mediated reversal of sensory hypersensitivity, whereas intrathecal (i.t.) injection of N. Methiodide (10 μ g) had no effect. $*P < 0.05$ compared with vehicle-treated EXER that underwent SNL. (D) Continuous naltrexone infusion (70 μ g/h) prevented exercise-induced reversal of tactile hypersensitivity. $*P < 0.05$ versus vehicle-infused animals. Data are given as mean (95% two-tailed CI). $n = 6$ animals per group. BL = presurgical baseline; SED = sedentary animals.

sons, specifically comparing paw withdrawal threshold at each point with paw withdrawal threshold before discontinuation of exercise training. The first statistically significant decrease in withdrawal threshold occurred on day 5 ($P = 0.0006$).

Opioid Receptor Antagonists Reversed Exercise Effects

Subcutaneous naloxone (1 mg/kg) decreased tactile withdrawal threshold in exercise-trained SNL animals, from 13.9 g (12.1–15.7 g) to 3.6 g (2.8–4.4 g) 20 min after injection (fig. 4A). Statistical analysis using a one-way repeated-measures ANOVA demonstrated a significant time effect ($P < 0.0001$). *Post hoc* testing using the Bonferroni method for multiple comparisons was used to specifically compare paw withdrawal threshold at each point with the value before naloxone. $P < 0.0001$ for paw withdrawal threshold 20 min after injection versus the preinjection value.

Subcutaneous naloxone methiodide (0.1 mg/kg) had no effect (fig. 4B). Statistical analysis using a one-way ANOVA demonstrated a significant between-group effect ($P < 0.0001$). *Post hoc* analysis using the Bonferroni method for multiple comparisons compared paw withdrawal thresholds in drug-treated animals with thresholds in vehicle-treated animals and withdrawal thresholds in naloxone-treated animals with thresholds in naloxone methiodide-treated animals. Systemic naloxone (1 mg/kg) reduced the paw withdrawal threshold in animals that exercised and underwent SNL compared with vehicle ($P = 0.0001$), whereas systemic naloxone methiodide did not ($P = 0.061$). Withdrawal thresholds were decreased after systemic naloxone administration versus systemic naloxone methiodide injection ($P = 0.002$). Neither naloxone nor naloxone methiodide altered withdrawal thresholds in sham-operated or SNL sedentary animals ($P = 0.886$, drug treatment effect from a two-way ANOVA).

Intracerebroventricular Injection of Naloxone Reversed Exercise Effects

When naloxone methiodide (2 μ g) was administered intracerebroventricularly after 5 weeks of exercise training, the paw withdrawal threshold compared with vehicle decreased from 11.1 g (6.98–15.22 g) to 1.2 g (0.9–1.52 g) (fig. 4C). Intrathecal administration of naloxone methiodide (10 μ g) had no effect. Statistical analysis performed using a one-way ANOVA yielded $P < 0.0001$ for between-group difference. *Post hoc* analysis using the Bonferroni method for multiple comparisons to compare naloxone methiodide- with vehicle-treated animals demonstrated that intracerebroventricular naloxone methiodide reduced paw withdrawal threshold in animals that exercised and underwent SNL compared with vehicle ($P = 0.0004$), whereas intrathecal naloxone methiodide did not ($P = 0.923$). Because intrathecal naloxone methiodide had no effect, we verified the effectiveness of this dose of intrathecal naloxone methiodide in reversing the analgesic effects of intrathecally administered morphine. Statistical analysis performed using a one-way ANOVA yielded $P = 0.0034$ for a between-group difference. *Post hoc* analysis was performed using the Bonferroni method for multiple comparisons. In otherwise untreated animals, morphine (30 μ g, intrathecal) increased paw withdrawal latency to radiant heat from 20.8 s (18.2–23.4 s) at baseline to 32.2 s (25.4–39.0 s) 30 min after drug administration ($P = 0.0024$). Preadministration of naloxone methiodide 15 min before morphine injection prevented morphine-induced analgesia, with a resulting paw withdrawal latency of 24.4 s (19.2–29.6 s) ($P = 0.002$ vs. morphine-treated animals). Intracerebroventricular naloxone methiodide-treated exercise-trained SNL animals showed signs (e.g., aggressive behavior and diarrhea) of opioid withdrawal within 20 min of naloxone methiodide injection. Naloxone methiodide did not alter tactile sensitivity or produce withdrawal in exercise-trained sham-operated on animals.

Continuous Infusion of Naltrexone Prevented Exercise Effects

One mechanism that would explain the observation that naloxone decreased withdrawal thresholds would be antagonist-precipitated withdrawal from the effects of endogenous opioids up-regulated by exercise training. To eliminate possible opioid withdrawal, and its confounding effects, we tested whether continuous infusion of naltrexone prevented exercise-associated reversal of sensory hypersensitivity. SNL animals were implanted with subcutaneous osmotic minipumps that delivered either naltrexone (70 $\mu\text{g}/\text{h}$) or vehicle for 5 weeks. Naltrexone infusion prevented exercise training-induced reversal of SNL-induced sensory hypersensitivity at all points tested (fig. 4D). Statistical analysis, performed using a two-way repeated-measures ANOVA (group \times time), yielded $P = 0.0001$ for treatment–time interaction and $P = 0.0001$ for treatment effect. A *post hoc* analysis was performed using the Bonferroni multiple comparison test to compare exercise-trained animals with exercise-trained animals receiving naltrexone infusion. Exercise-trained SNL animals treated with naltrexone exhibited paw withdrawal thresholds of 1.5 g (0.9–2.1 g) after 5 weeks of exercise training, lower than those of vehicle-treated animals (12.0 [8.4–15.6] g; $P < 0.0001$) and similar to those of sedentary SNL animals (2.0 [0.7–3.3] g).

Exercise Training Increases Endogenous Opioid Concentrations in the PAG and RVM

The PAG and RVM displayed increased immunoreactivity, detected using immunohistochemical techniques, for β endorphin and met-enkephalin in exercise-trained compared with sedentary SNL animals. Measurements from sections from exercise-trained animals, obtained after 5 weeks of exercise training, were compared with those from sedentary animals using an unpaired two-tailed Student *t* test. Although additional points may have allowed temporal correlation of brainstem opioid content with exercise-induced reversal of sensory hypersensitivity, we limited this study to one measurement after the effects of exercise were established to minimize animal use. Exercise training increased PAG β -endorphin and met-enkephalin immunoreactivity by 304% and 114%, respectively ($P < 0.0001$ for both) (fig. 5). Exercise training increased RVM β -endorphin and met-enkephalin concentrations by 28% and 38%, respectively ($P = 0.0086$ and $P = 0.0009$, respectively) (fig. 6).

Discussion

Regular moderate aerobic exercise reversed the sensory hypersensitivity observed in the SNL model of neuropathic pain. Naloxone reversed the effects of exercise, suggesting that the activity of endogenous opioids is necessary for exercise training-mediated reversal of SNL-induced sensory hypersensitivity. The non–blood-brain barrier–permeable opioid receptor antagonist naloxone methiodide had no effect when administered subcutaneously, suggesting that endoge-

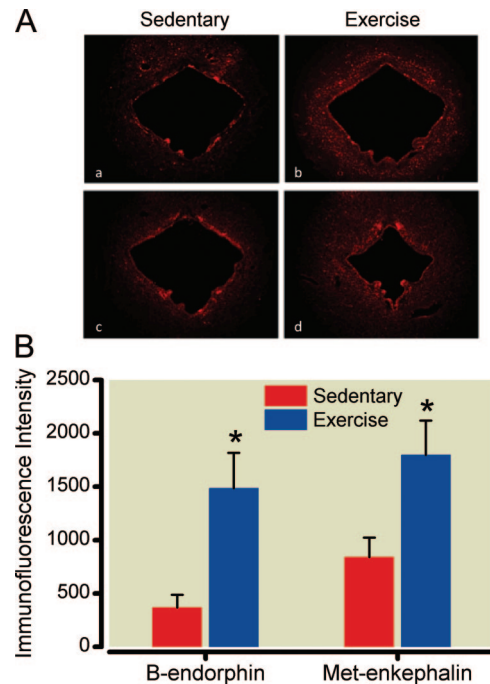


Fig. 5. Exercise training increases endogenous opioid content in the mid-brain periaqueductal gray area (PAG). (A) After 5 weeks of exercise training or sedentary conditions, PAG sections from nerve-injured animals were labeled with antibodies to β endorphin (a and b) or met-enkephalin (c and d). Images were magnified $\times 20$. (B) Densitometry was performed on 10 images from five animals per group. $*P < 0.05$ compared with sedentary animals that underwent spinal nerve ligation. Data are given as mean (95% two-tailed CI).

nous opioids mediate exercise effects by acting in the central nervous system. Thus, intracerebroventricular injection of naloxone methiodide reversed exercise effects, whereas intrathecal injection of naloxone methiodide did not, suggesting that the effects of exercise may be because of an increase in endogenous opioid content or release in the brain. This hypothesis was confirmed by the observation that the contents of β endorphin and met-enkephalin in the PAG and RVM were increased in exercise-trained SNL animals. Taken together with previous work^{23,24} demonstrating that supraspinal administration of morphine reverses sensory hypersensitivity in the SNL model, these findings support the hypothesis that regular exercise training reverses the signs of neuropathic pain by increasing the expression of endogenous brainstem opioids.

Our results confirm and extend previous findings that exercise produces antinociceptive effects. Extensive research in humans and animals demonstrates that a single episode of exercise increases nociceptive thresholds measured soon after exercise.⁸ Short-term forms of exercise have increased the release of endogenous opioids, suggesting that these endogenous analgesics may be responsible for the antinociceptive effects observed in short-term exercise models.

The antinociceptive effects of regular exercise have been less extensively studied. Shyu *et al.*⁹ demonstrated that sev-

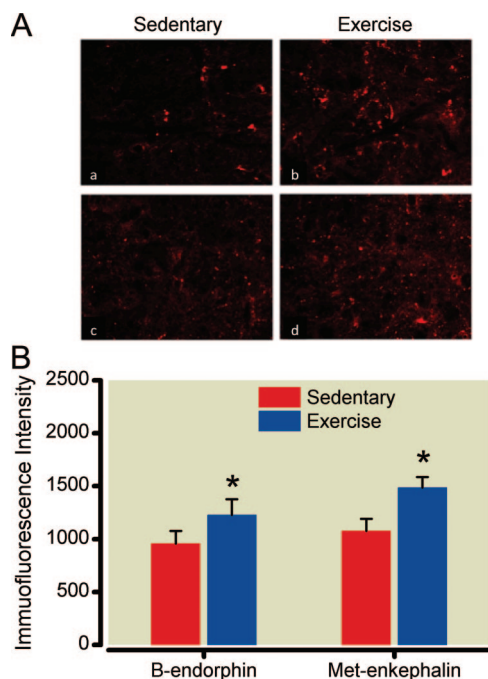


Fig. 6. Exercise training increases endogenous opioid content in the rostral ventromedial medulla (RVM). (A) After 5 weeks of exercise training or sedentary conditions, RVM sections from nerve-injured animals were labeled with antibodies to β endorphin (a and b) or met-enkephalin (c and d). Images were magnified $\times 40$. (B) Densitometry was performed on 26–32 images from five animals per group. * $P < 0.05$ compared with sedentary animals that underwent spinal nerve ligation. Data are given as mean (95% two-tailed CI).

eral weeks of voluntary wheel running increased nociceptive thresholds in rats, as assessed using the threshold to vocalization in response to subcutaneous electrical stimulation. This increase in vocalization threshold was reversed by the opioid receptor antagonist naloxone. The increase in nociceptive threshold persisted until the day after exercise, as opposed to the antinociceptive effects of short-term exercise, which lasted only hours after the cessation of exercise. Other more recent studies^{9–12} have also demonstrated antinociceptive effects of regular exercise that are reversed by naloxone. These findings, together with observations that regular exercise produces tolerance to the effects of exogenously administered opioids,^{11,12} suggested that regular exercise may increase endogenous opioid expression in the central nervous system.

Regular running exercise increases serum and cerebrospinal fluid content of endogenous opioids.^{13,14} In these studies, cerebrospinal fluid¹³ and serum¹⁴ β -endorphin concentrations were increased after 5–6 and 8 weeks of exercise training, respectively (previous points were not tested), similar to our findings of naloxone-mediated reversal of sensory hypersensitivity and increased brainstem opioid content 5 weeks after the initiation of exercise training. Our finding that exercise effects on SNL-induced sensory hypersensitivity began to diminish 4 days after discontinuation of exercise is

consistent with previous work demonstrating a rapid decrease in endogenous opioid expression after the cessation of exercise. Hoffmann *et al.*¹³ found that cerebrospinal fluid concentrations of β endorphin remained increased for only 48 h after the cessation of regular exercise training.

The effects of exercise training in animals with preexisting pain conditions have not been thoroughly studied. Low-intensity treadmill training reversed mechanical hypersensitivity in animals with chronic muscle pain.¹⁶ Treadmill running completely reversed signs of neuropathic pain in animals with spinal cord injury.¹⁷ Extended swimming reduced inflammatory and peripheral neuropathic pain.¹⁵ Finally, 5 days of treadmill training reversed sensory hypersensitivity produced by chronic constriction injury of the sciatic nerve.¹⁶ Our study adds to this work by focusing on the role of endogenous opioids in exercise training-induced reversal of neuropathic pain.

Both the PAG and RVM are important sites in the regulation of descending pain-modulating pathways. They are the two principal areas of the brainstem responsible for the analgesia that occurs after opioid administration, with interactions between both structures producing the most effective analgesia.²⁵ These same sites are likely responsible for the reversal of nerve injury-induced sensory hypersensitivity by supraspinal actions of opioids. The microinjection of opioids into the PAG reversed sensory hypersensitivity in rats with injury of the tibial and sural nerves.²⁶

Previous studies in models of spinal cord or peripheral nerve injury-induced neuropathic pain have suggested that additional mechanisms may contribute to exercise training-induced reversal of sensory hypersensitivity. Hutchinson *et al.*¹⁷ found that treadmill training restored spinal cord and soleus muscle concentrations of brain-derived neurotrophic factor (BDNF), which had been diminished by spinal cord injury, to normal concentrations. Interestingly, BDNF administration in the brain or spinal cord produces naloxone-reversible analgesia,^{27–29} raising the possibility that an exercise-induced increase in BDNF concentrations may lead to increased concentrations of endogenous opioids. Although the effects of endogenous opioids reported herein appear to be localized in the brain and Hutchinson *et al.* observed increased BDNF expression in the spinal cord, Hutchinson *et al.* used a model of direct spinal cord injury and focused exclusively on spinal cord BDNF content. More recently, Cobia *et al.*¹⁸ demonstrated that 5 days of exercise training reduced sensory hypersensitivity in the chronic constriction injury model of neuropathic pain but that this effect dissipated with additional training. They found reduced spinal microglia expression after both short- and long-term treadmill running, reduced astrocyte expression after short-term treadmill running, and normalization of astrocyte expression after longer-term treadmill running. They also found that short-term treadmill running resulted in up-regulation of Cdc2 (cell division cycle 2 protein) and GAP-43 (growth associated protein-43) in the sciatic nerve. It is not

clear to what extent these processes in the spinal cord and peripheral nerve are responsible for the reversal of sensory hypersensitivity or how they may interact with the opioid-mediated mechanisms described herein. Furthermore, it is not clear why Cobianchi *et al.* observed a transient effect of exercise, whereas the changes in sensory sensitivity we observed persisted for at least 5 weeks, if exercise training was maintained.

In summary, regular moderate aerobic exercise reverses the sensory hypersensitivity observed in an animal model of neuropathic pain. Moderate aerobic exercise also increases endogenous opioid content in areas of the brainstem known to be important in pain modulation. Finally, the effects of exercise are reversed by opioid receptor antagonists. These results suggest that an increase in brainstem expression of endogenous opioids is necessary for exercise-induced reversal of neuropathic pain. Previous studies^{22,23} have suggested that the activation of brain opioid receptors is sufficient to reverse nerve injury-induced sensory hypersensitivity, supporting the feasibility of an endogenous opioid-mediated mechanism. Clinical studies¹⁻⁷ in humans have shown that exercise reduces chronic pain, including pain likely to have a neuropathic component. The current findings provide a mechanism that may contribute to the beneficial effects of exercise in the treatment of neuropathic pain.

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