Voluntary exercise ameliorates cognitive deficits in morphine dependent rats: The role of hippocampal brain-derived neurotrophic factor

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ABSTRACT

Chronic exposure to opiates impairs spatial learning and memory. Given the well-known beneficial effects of voluntary exercise on cognitive functions, we investigated whether voluntary exercise would ameliorate the cognitive deficits that are induced by morphine dependence. If an effect of exercise was observed, we aimed to investigate the possible role of hippocampal brain-derived neurotrophic factor (BDNF) in the exercise-induced enhancement of learning and memory in morphine-dependent rats. The rats were injected with bi-daily doses (10 mg/kg, at 12 h intervals) of morphine over a period of 10 days of voluntary exercise. Following these injections, a water maze task was performed twice a day for five consecutive days, followed by a probe trial 2 days later. A specific BDNF inhibitor (TrkB-IgG chimera) was used to block the hippocampal BDNF action during the 10 days of voluntary exercise. We found that voluntary exercise blocked the ability of chronic morphine to impair spatial memory retention. A blockade of the BDNF action blunted the exercise-induced improvement of spatial memory in the dependent rats. Moreover, the voluntary exercise diminished the severity of the rats’ dependency on morphine. This study demonstrates that voluntary exercise ameliorates, via a TrkB-mediated mechanism, the cognitive deficits that are induced by chronic morphine. Thus, voluntary exercise might be a potential method to ameliorate some of the deleterious behavioral consequences of the abuse of morphine and other opiates.

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1. Introduction

Opiate addiction has been considered to be a neuro-psychiatric disorder that is caused by continuous opiate intake (Robbins & Everitt, 1999; Stelten, Noblesse, Ackermans, Temel, & Vissers-Vandewalle, 2008). In spite of treatment and other attempts to control drug taking, addiction tends to persist (Hyman, Malenka, Vandewalle, 2008). In contrast, chronic exposure to drugs of abuse can hijack synaptic machinery that are dedicated to plastic changes in the excitability of principal hippocampus circuits in such a way that addiction is learned (Bao et al., 2007; Kauer & Malenka, 2007; Robbins & Everitt, 1999; Wolf, 2002). In contrast, chronic exposure to opiates can decrease spine density, neurogenesis, and alter synaptic transmission in the hippocampus (Bibb, 2003; Eisch, Barrot, Schad, Self, & Nestler, 2000; Eisch & Mandyam, 2004). It also reduces hippocampal long-term potentiation (LTP) (Bao et al., 2007; Pu, Bao, Xu, Ma, & Pei, 2002; Salmanzadeh, Fathollahi, Semnannian, & Shafizadeh, 2003a), a form of synaptic plasticity that may be a cellular substrate of learning and memory (Bliss & Collingridge, 1993). Chronic exposure to opiates also leads to the impairment of acquisition (Dougherty, Walsh, Bailey, Schlussman, & Grasing, 1996; Li, Wu, Pe, & Xu, 2001; McNamara & Skelton, 1992; Spain & Newsom, 1991) or retention of spatial memory (Alaei et al., 2006; Lu et al., 2010; Mendez, Montgomery, LaSarge, & Simon, 2008; Miladi Gorji, Rashidy-Pour, & Fathollahi, 2008). The reversal or prevention of the synaptic modifications that are induced by drugs of abuse could be a useful method for the treatment of relapse (Kauer & Malenka, 2007; Wolf, 2002).

Physical activity is one of the most important methods that can protect the central nervous system from the drug-related decline in cognitive functions. It is increasingly clear that physical exercise maintains brain health and the brain’s plasticity (Alaei et al., 2006; Pietropaolo et al., 2008). In human subjects and in rodents,
physical activity enhances cognition functions (Coles & Tomporowski, 2008; van Praag, Christie, Sejnowski, & Gage, 1999), delays the onset of Alzheimer’s disease (Cotman & Berchtold, 2007), improves some of the injurious, morphological, and behavioral consequences of aging (Cotman & Berchtold, 2007; Lambert, Fernandez, & Frick, 2005; van Praag, Shubert, Zhao, & Gage, 2005), speeds up recovery after brain damage (Gobbo & O’Mara, 2005; Luo et al., 2007), and promotes neurogenesis in the adult dentate gyrus (van Praag, Kempermann, & Gage, 1999). These findings demonstrate that physical activity can regulate neurogenesis, synaptic plasticity, and learning and memory in the brain. Although the mechanisms underlying the ability of exercise to improve neuronal and cognitive functions are not yet clear, accumulating evidence suggests that BDNF is an important modulator of physical activity’s effects on neural functions, such as synaptic plasticity, neurogenesis, and learning and memory (Eisenstein & Holmes, 2007; Farmer et al., 2004; Nestler, 2002; O’Callaghan, Ohle, & Kelly, 2007; Oliff, Berchtold, Isackson, & Gage, 1998; Stranahan, Zhou, Martin, & Maudsley, 2009; van Praag, 2008; van Praag et al., 2005). BDNF acts via the TrkB receptor, and blocking BDNF signaling prevents the enhancement in cognitive functions following physical exercise (Stranahan et al., 2009).

Given the well-known beneficial effects of physical exercise on learning and memory, voluntary exercise may be a potential method for treating learning and memory deficits in morphine addicts. Thus, the aim of the present study was to investigate whether voluntary exercise would ameliorate the cognitive deficits induced by morphine dependence. If an effect of voluntary exercise was observed, we aimed to identify the possible role of hippocampal BDNF in the voluntary exercise-induced enhancement of learning and memory in morphine-dependent rats.

2. Materials and methods

2.1. Animals

Adult, male Wistar rats (220 ± 10 g) were individually housed in cages (50 × 26 × 25 cm) in a 12-h light/dark cycle at 22–24 °C, with food and water ad libitum. The experimental protocol was approved by research committees of Tarbiat Modares University (Tehran, Iran) and the Semnan University of Medical Sciences (Semnan, Iran). All of the experimental procedures were conducted in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Additionally, care was taken to use the minimum number of rats possible in each experiment.

2.2. Induction of morphine dependence

Morphine sulfate (Temad Company, Iran) was dissolved (10 mg/mL) in 0.9% saline and chronically administered via subcutaneous injections at a volume of 1 mL/kg. These injections were given twice per day at 12 h intervals for 10 days in the presence or absence of voluntary exercise (see below), as described (Bao et al., 2007; Pu et al., 2002). The treatment was continued for 7 days of learning and memory testing. Thus, the total duration of the morphine treatment was 10 and 17 days in Experiment 1 and Experiments 2 and 3, respectively. The control rats were treated similarly, with injections of saline replacing morphine.

2.3. Withdrawal rating scale

Naloxone hydrochloride (Sigma–Aldrich, Germany) was dissolved in 0.9% saline (2 mg/mL) in saline and administered via intraperitoneal injection in a volume of 1 mL/kg (Salmanzadeh, Fathollahi, Seymnnian, & Shafizadeh, 2003b). Immediately after the naloxone injection, the rats were taken to a quiet, isolated room with moderate illumination, and their behavior signs were monitored for 30 min according to a modified version of the Gellert–Holtzman scale (Gellert & Holtzman, 1978). On the Gellert and Holtzman scale graded signs, with the exception of weight loss, are assigned a weighting factor 1–4 based on frequency of appearance, and checked signs receive values of 2–3 depending upon the particular withdrawal sign noted, but regardless of frequency of appearance. Body weights were recorded immediately before and 24 h after naloxone injection, and the percentage of body weight changes was calculated. The weighting factor for weight was 1.0 for each 1.0% loss above the weight lost by control rats. Graded signs including jumps, wet dog shakes, and abdominal contractions were counted as the number of events occurring during the total test time. Checked signs including diarrhea, ptosis, erection or genital grooming, teeth chattering, writhing, and irritability were counted as positive if the sign occurred at any time during the observation period. After completion of the observation session, the overall withdrawal severity was calculated by summing the proper weighting factor of somatic signs. Additionally, the severity of any diarrhea was assessed using a four-point scale (Rasmussen, Hsu, & Vandergriff, 2004; Vandergriff & Rasmussen, 1999): 0 = absent; 1 = mild; 2 = moderate; and 3 = severe.

2.4. Voluntary exercise paradigm

Each of the exercising rats was given ad libitum access to a cage that was equipped with a running wheel (diameter = 34.5 cm, width = 9.5 cm) (Novidan, Tab, Iran) that was freely rotated against a resistance of 100 g. Each wheel was equipped with a magnetic switch that was connected to a separate counter, which was located outside of the animal house and monitored the revolutions per hour. The number of revolutions for each wheel was recorded every day at 6 a.m. The sedentary rats were confined to similar cages with no access to a wheel. The exercising groups were exposed to exercise during the development of dependence on morphine, which took 10 days before the start of the water maze experiments (Akhaban et al., 2008; Ebrahimi, Rashidy-Pour, Vafaee, & Akhaban, 2010; Vaynman, Ying, & Gomez-Pinilla, 2004). Exercising and sedentary rats were remained single housed throughout the entire experiment.

2.5. Testing learning and memory using the water maze

A detailed description of the apparatus and the tracking system has been given in our previous reports (Akhaban et al., 2008; Ebrahimi et al., 2010). In brief, the water maze (WM) was a black circular pool (140 cm in diameter and 60 cm high) that was filled to a 25 cm depth with 20 °C water.

To avoid an acute effect of morphine exposure on spatial learning, all of the rats were tested in the WM 2 h after receiving a morphine injection. At least 1 h before experiments, rats were individually carried in a cage to the testing room and allowed to adapt to the new environment. On day 11, the training started in the WM task. The WM protocol was a stringent protocol consisting of two trials per day for 5 days, which has been shown to be a good discriminative test for the effects of exercise on learning and memory (Ding, Vaynman, Akhaban, Ying, & Gomez-Pinilla, 2006; Vaynman, Ying, & Gomez-Pinilla, 2004). During each trial, the rat was placed into the water from one of the four cardinal points of the compass (N, E, S, and W), which varied from trial to trial in a quasi-random order. The rat had to swim until it climbed onto the escape platform. The rats were guided by hand to the platform if they failed to locate it within 60 s. The rat was allowed to stay on the platform for 20 s during the inter-trial interval. After the last
trial, the animal was towel dried and returned to its home cage with no access to a running wheel.

A spatial probe test was performed 2 days after the last acquisition trial, during which the platform was removed. The rats were allowed to swim for 60 s, during which, the latency to reach the platform location, the time spent swimming within a zone, which had a 20 cm radius that was centered either on the original training location (target zone) or on an equivalent location in other quadrants (opposite, left and right adjacent quadrants), and the proximity (the average distance from the center of the platform during the probe test) were recorded. The velocity of each animal was also calculated. The analysis of the time spent within a specified radius (zone) and the proximity measure are sensitive measures of the WM probe test performance, in terms of detecting group differences (Gallagher, Burwell, & Burchinal, 1993; Maei, Zaslavsky, Teixeira, & Frankland, 2009).

2.6. Drugs

The recombinant human TrkB/Fc Chimera (TrkB/IgG) was acquired in powder form (R&D System, Inc., Minneapolis, MN, USA). This chimerical protein has been shown to be a highly potent and specific antagonist of BDNF action (Vaynman et al., 2004; Ying et al., 2008). We used cytochrome C (Cyt C), which was also obtained in powder form (Sigma, St. Louis, MO, USA), as the control drug because it has been successfully used as a standard control for microbead injections (Akhavan et al., 2008; Vaynman, Ying, & Gomez-Pinilla, 2003; Vaynman et al., 2004; Ying et al., 2008). The Cyt C was dissolved in sterile distilled water, with stock concentration of 100 ng/μL. Fluorescent latex microbeads (Lumafluor Corporation, Naples, FL, USA) were used as the vehicle for the drug delivery directly into the hippocampus. These microbeads have been used as an in vivo delivery system to examine the effects of neurotrophins on neurons (Riddle, Katz, & Lo, 1997). The sterile phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) was added to the vial to prepare a stock solution (100 μg/mL) (Vaynman et al., 2004). We prepared the microspheres using methods that have been previously described (Riddle et al., 1997; Vaynman et al., 2003, 2004; Ying et al., 2008).

2.7. Surgical and drug application procedures

For the drug injections, a stereotaxic apparatus was used to locate the injection site in the dorsal hippocampus (−3.8 mm from Bregma, 2.6 mm from the midline to the left or the right, and 3.7 mm ventral to the skull’s surface) in a similar manner to several previous studies (Ding et al., 2006; Vaynman et al., 2003, 2004). The microbeads were incubated overnight at 4°C in a 1:5 mixture of microbeads to a TrkB–IgG solution (5 μg/μL in PBS with BSA) or Cyt C; 100 ng/μL in sterile water) (Vaynman et al., 2004). The morning after coating the microbeads with the TrkB–IgG or Cyt C, the solution was centrifuged at 14,000 g for 30 min, and the microbeads were resuspended in cold sterile water to a final concentration of 10% microbeads. All rats were anesthetized for the

![Fig. 1. Timelines of experiments (see Section 2 for details).]
surgery 2 h after the morphine injection with an i.p. injection of a combination of 70 mg/kg ketamine hydrochloride and 10 mg/kg xylazine. Next, the animal’s head was secured in a stereotaxic frame, shaved, and scrubbed with betadine. Each rat was injected with 2 µL of TrkB-IgG or Cyt C, using a Hamilton syringe, over a period of 15 min. Each rat received two injections (at 5 days intervals) bilaterally into the right and left hippocampus during the 10-day running period (Fig. 1, Experiment 3). It has been shown that neurotrophins bind to the microspheres by passive absorption (Riddle et al., 1997) and that they gradually release over 5 days, allowing the drug to disperse to other areas of the hippocampus (Ding et al., 2006; Vaynman et al., 2004).

Immediately after the probe test (between 8.30 and 9 a.m. on day 17) in Experiment 3, all rats were killed by decapitation to minimize suffering. The decapitation methods used in the present study were similar to those in a previous study (Ding et al., 2006). Immediately after decapitation, each brain was rapidly removed, and the two hemispheres were separated along the midline. The right hippocampi were rapidly dissected out, immediately placed on dry ice, and stored at −70 °C until the amount of the BDNF protein in the tissue samples could be measured.

2.8. Protein measurements

The BDNF protein levels were assessed using E-Max ELISA kits (Promega, WI, USA), according to the manufacturer’s recommendations. The hippocampal extracts were prepared in lysis buffer (137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP-40, 1% glycerol, 1 mM phenylmethyl sulfon fluoride (PMSF), 10 µg/mL aprotinin, 1 µg/mL leupeptin, and 0.5 mM sodium orthovanadate). The homogenates were centrifuged to remove insoluble materials (12,500 g for 20 min at 4 °C), and the total protein concentration was determined according to the Micro BCA procedure (Pierce, Rockford, IL, USA). For the ELISA, 96 well flat-bottomed Immulon-2 plates were incubated overnight at 4 °C with carbonate coating buffer containing an anti-BDNF monoclonal antibody. The plates were blocked for 1 h with the block and the sample (B&S) buffer, followed by incubation with shaking of the samples and the BDNF standards for 2 h at room temperature. A standard curve was established using serial dilutions of known amounts of BDNF that ranged from 0 to 500 pg/mL and were diluted in B&S buffer. The plates were washed five times with TBST (20 mM Tris–HCl, 150 mM NaCl, 0.05% v/v Tween 20), followed by a 2 h incubation (at room temperature) with an anti-human BDNF polyclonal antibody, five washes with TBST, and a 1 h incubation (at room temperature) with horseradish peroxidase. The enzyme solution (TMB One) was brought to room temperature in advance and was subsequently incubated on the plate for 10 min (at room temperature). After the samples turned blue, the reaction was stopped with 1 N HCl, and the absorbance was measured at 450 nm using an automated plate reader (ELISA Reader) (Adlard & Cotman, 2004; Ding et al., 2006). According to the manufacturer’s information, this assay was designed to measure total free BDNF. It demonstrated very low cross-reactivity with related neurotrophic factors at concentrations as high as 100 ng/mL (Mandel, Ozdener, & Utermohlen, 2011).

2.9. Histological verification of microbead injection sites

At the conclusion of Experiment 3, the rats were decapitated, and the brains were removed. Then the hippocampus was rapidly dissected out. The left hippocampus was stored in 10% formalin. Coronal sections (5 µm thick) were on a cryostat and then stained using cresyl fast violet (Nissl) staining method. The microbead injection sites were verified by using a light microscope with a 40× objective lens.

2.10. Statistical analysis

The data expressed as the mean ± standard error of the mean (SEM). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post hoc analyses considered the Turkey’s test. A Student's t-test was used to compare the data from two groups. The two-tailed Mann–Whitney U non-parametric test was used to compare withdrawal scores between the groups. We used the Pearson correlation test to examine the association between running distances (Avg m/day) and acquisition performance (the average escape latency during the 5-days training period) or memory retention (percentage of time spent in the target zone and platform location latency during probe test) in the saline–exercise and dependent–exercise groups (see below). The statistical differences were considered to be significant at P < 0.05.

3. Results

3.1. Effects of voluntary exercise on the severity of naltrexone-precipitated morphine withdrawal signs in rats

To determine the effects of voluntary exercise on the severity of morphine dependence, the global severity of the morphine withdrawal responses was measured in the sedentary and exercising morphine-treated rats (n = 8 rats per group) after an acute injection of naltrexone, 2 h after the last injection of morphine on day 11 (Fig. 1, Experiment 1).

3.1.1. Running distances

The average distance run (m) at 10 days of voluntary exercise of the exercising-dependent rats was 10588.63 ± 1780.88. One-way ANOVA with repeated measure revealed significant effects of days (F9,63 = 6.39, P = 0.0001). In general, the running distance is increased significantly as exercise days progressed (data not shown).

3.1.2. Withdrawal signs

Analysis of the overall Gellert–Holtzman withdrawal scores revealed that the exercising-dependent rats displayed lower levels of withdrawal scores than the sedentary-dependent rats (P = 0.006) (Fig. 2A). With respect to the graded signs, the exercising-dependent rats made less abdominal contractions (P = 0.0005), made less wet dog shakes (P = 0.001) and displayed less weight loss (over 24 h) (P = 0.04) than the sedentary-dependent rats. With respect to the checked signs, the exercising-dependent rats displayed less diarrhea (degree 3) (P = 0.009), ptosis (P = 0.025), erection or genital grooming (P = 0.025), irritability (P = 0.025), and writhing (P = 0.04) than the sedentary rats (Fig. 2B). There was no statistically significant change in teeth chattering, and the number of jumps in two groups. Finally, injection of naltrexone to the saline sedentary rats did not produce any measurable withdrawal signs.

3.2. Effects of voluntary exercise on spatial memory deficits in morphine-dependent rats

To determine the effect of voluntary exercise on spatial memory deficits induced by chronic morphine, rats were divided into four equal groups (n = 10 rats per group): the saline–exercise group (Sal/Exc), the saline–sedentary group (Sal/Sed), the dependent–exercise group (D/Exc), and the dependent–sedentary group (D/Sed). The learning and memory capabilities of all groups were tested as previously described (Fig. 1, Experiment 2).

3.2.1. Running distances

Two-way ANOVA with repeated measure (day) for the average distance run (m) at 10 days of voluntary exercise revealed the
absence of a significant effect of groups ($F_{1,18} = 0.306, P = 0.587$), a significant effect of days ($F_{9,162} = 7.03, P = 0.0001$), and no significant interaction between both factors ($F_{9,162} = 0.89, P = 0.535$) (Fig. 3A). In general, the running distance is increased significantly in both groups as exercise days progressed. Meanwhile, the average distance covered during running (m) after 10 days of voluntary exercise did not differ significantly between the exercising groups: the Sal/Exc group ($12247 \pm 531$) and the D/Exc group ($10990 \pm 400$) ($t_{18} = 0.6, P = 0.557$).

3.2.2. Spatial learning

The acquisition data during the 5 days of training in the WM are illustrated in Fig. 3B. A three-way ANOVA (morphine treatment × exercise × training days) were used to analyze the escape latencies during training. All groups learned to locate the platform during the five successive days of training, as indicated by decreasing escape latencies as training progressed ($F_{4,144} = 29.186, P = 0.0001$). The effect of exercise was significant ($F_{1,144} = 7.575, P = 0.0001$): the exercising groups exhibited significantly shorter escape latencies only on day 5 of the WM training than those of the sedentary control groups (all, $P < 0.05$). The main effect of morphine treatment was not significant ($F_{1,36} = 0.361, P = 0.552$). There were no significant interaction between morphine treatment and exercise and days ($F_{4,144} = 0.702, P = 0.592$). These findings indicate that voluntary exercise enhanced the learning rate in both control and dependent groups.

Data related to the distance swum to reach the platform followed similar to the same pattern as the latency. All groups traveled shorter distances to reach the platform as training progressed ($F_{4,144} = 13.81, P = 0.0001$). The effect of exercise was significant ($F_{1,36} = 4.42, P = 0.042$): the exercising groups exhibited significantly shorter distances on day 5 of the WM training than those of the sedentary control groups (all, $P < 0.05$). The main effect

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Fig. 2. The effect of voluntary exercise on the signs of naloxone-precipitated morphine withdrawal. (A) The graded signs. Percentage of weight loss across 24 h and the number of abdominal contractions and wet dog shakes were counted during a 30 min observation. The Gellert–Holtzman score of the overall withdrawal severity was calculated using the proper weighting factor. (B) The checked signs. Each of the mentioned signs was checked if present in a given rat at any point during the 30 min observation period. Voluntary exercise significantly decreased both graded and checked signs of naloxone-precipitated morphine withdrawal. Data are expressed as the median ± inter-quartile range. In A: *$P = 0.005$, **$P = 0.001$ vs D/Sed group. In B: *$P = 0.04$, **$P = 0.025$, ***$P = 0.009$ vs D/Sed group.

Fig. 3. The effect of voluntary exercise on learning acquisition as measured by the WM task. (A) The average of running distance (expressed in meter per day) in the exercising groups. (B) Voluntary exercise improved the learning abilities of morphine-dependent rats in a similar manner to rats that were exercising daily and were given saline. The rats that were exercising took significantly less time to learn the location of the platform compared to the sedentary control groups on day 5. The data are expressed as the mean ± SEM; *$P = 0.039$ represents the significant difference between the D/Exc and D/Sed groups; **$P = 0.04$ represents the significant difference between the Sal/Exc and Sal/Sed groups.
of morphine treatment was not significant ($F_{1,36} = 0.909, P = 0.347$). There was no significant interaction between morphine treatment and exercise and days ($F_{4,144} = 1.49, P = 0.207$).

3.2.3. Spatial memory

The data for the memory retention test are shown in Fig. 4. A two-way ANOVA on the platform location latency (Fig. 4A) showed significant effects of exercise ($F_{1,36} = 32.64, P = 0.0001$), and of morphine treatment ($F_{1,36} = 4.22, P = 0.037$), and a significant interaction between both factors ($F_{1,36} = 4.44, P = 0.04$). The between-group comparisons indicated that the platform location latency of the D/Sed group was significantly longer than that of the Sal/Sed group ($P = 0.042$, Fig. 4A), indicating that a dependency on morphine impairs memory retention. The platform location latency of the D/Exc group was significantly shorter than that of the D/Sed group ($P = 0.0001$). A similar significant difference in the platform location latency was observed when the Sal/Exc and Sal/Sed groups were compared.

A three-way ANOVA with quadrant (Fig. 4B) as repeated measures showed a significant interaction between morphine treatment and exercise and zones ($F_{3,108} = 4.918, P = 0.003$). The between-group comparisons indicated that the Sal/Exc and D/Exc groups spent significantly more time in the target zone than the Sal/Sed and D/Sed groups ($P = 0.04$ and $P = 0.0001$, respectively). The D/Sed group spent less time in the target zone and more time in the opposite zone than the Sal/Sed group ($P = 0.048$ and $P = 0.032$, respectively).

Fig. 4C represents the average proximity to the platform. A two-way ANOVA revealed significant effects of exercise ($F_{1,36} = 62.9, P = 0.001$), and of morphine treatment ($F_{1,36} = 4.89, P = 0.034$), and a significant interaction between both factors ($F_{1,36} = 4.69, P = 0.037$). The D/Sed group had significantly larger average proximity value compared to the Sal/Sed group ($P = 0.019$). In addition, the Sal/Exc and D/Exc groups had significantly smaller average proximity values compared to the Sal/Sed and D/Sed groups ($P = 0.001$ and $P = 0.0001$, respectively).

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**Fig. 4.** The effect of voluntary exercise period on memory retention using the probe trial during the WM task. (A) The mean latency to reach the platform location. (B) The mean time spent in within a zone, which had a radius of 20 cm and was centered either on the original training location (the target zone) or on an equivalent location in other quadrants, was expressed as a percentage of the total time spent in both of the zones. (C) The proximity refers to the average distance of the animal from the platform location during the probe trial. Exercise improved memory retention, as evidenced by the fact that the exercising rats took significantly less time to reach the platform location (A), spent significantly more time in the target zone (B), and had significantly smaller proximity values compared to their sedentary control counterparts (C). In A: *, **, and *** represent the significant differences between the Sal/Exc and Sal/Sed groups, the D/Sed and Sal/Sed groups, and the D/Exc and D/Sed groups, respectively ($P = 0.046$, $P = 0.042$, and $P = 0.0001$, respectively). In B: *, **, ***, ***, +, $^+$, and $^+$ represent the significant differences between the Sal/Exc and Sal/Sed groups, the D/Sed and Sal/Sed groups, and the D/Exc and D/Sed groups, respectively ($P = 0.04$, $P = 0.048$, $P = 0.0001$, $P = 0.048$, $P = 0.032$, and $P = 0.0001$, respectively). In C: *, **, and *** represent the significant differences between the Sal/Exc and Sal/Sed groups, the D/Sed and Sal/Sed groups, and the D/Exc and D/Sed groups, respectively ($P = 0.001$, $P = 0.019$, and $P = 0.0001$, respectively).
To control for differences in WM performance, we also recorded each animal’s swimming speed. We found no difference ($F_{3,36} = 2.13, P = 0.114$) in the swimming speeds of all four of the groups: the Sal/Sed group (25.61 cm/s), the Sal/Exc group (24.49 cm/s), the D/Sed group (26.22 cm/s), and the D/Exc group (27.89 cm/s).

We found a significant negative correlation in individual rats, between the amount of voluntary exercise (m/day) and acquisition performance (the average escape latency during the 5-days training period) in both Sal/Exc ($r = 0.68, P = 0.03$), and D/Exc ($r = 0.74, P = 0.045$) groups (Fig. 5A and B). Also, a significant negative correlation was found between the amount of voluntary exercise and percentage of time spent in the target zone during the probe test in both Sal/Exc ($r = 0.63, P = 0.042$), and D/Exc ($r = 0.71, P = 0.022$) groups (Fig. 5C and D). Finally, we found the platform location latency in the probe test is negatively associated with the amount of exercise in both Sal/Exc ($r = 0.80, P = 0.005$), and D/Exc ($r = 0.67, P = 0.033$) groups (data not shown).

3.3. Effects of the BDNF action blockade on the exercise-induced improvement of spatial memory in the dependent rats

To determine the effects of a blockade of BDNF action on the exercise-attenuated memory impairment by chronic morphine, rats were randomly assigned to eight groups (n = 7–8 rats per group) as follows: a saline-treated, exercised group that received a cytochrome C injection (Sal–Exc/Cyt C); a saline-treated, exercised group that received a TrkB–IgG injection (Sal–Exc/IgG); a dependent, exercised that received a Cyt C injection (D–Exc/Cyt C); a dependent, exercised group that received a TrkB–IgG injection (D–Exc/IgG); a saline-treated, sedentary group that received a Cyt C injection (D–Sed/Cyt C), and a dependent, sedentary group that received a TrkB–IgG injection (D–Sed/IgG). All rats received injections of TrkB–IgG or Cyt C as previously described (Fig. 1, Experiment 3).

3.3.1. Microbead injection sites

The injection sites of the microbeads in the hippocampus were confirmed using tissue microscopy. Fig. 6 represents the tissue section in the coronal plane that contained the site of the injection in the hippocampi. The area (stratum lacunosum moleculare) of the microbead concentration is consistent with the previous studies that used the same method for the local delivery into the hippocampus of bioactive agents that were coupled to microbeads (Ding et al., 2006; Vaynman et al., 2004).

3.3.2. Running distances

Two-way ANOVA with repeated measure for the average distance run (m) at 10 days of voluntary exercise revealed lack of significant effects of groups ($F_{3,25} = 0.572, P = 0.638$), but a significant effect of days ($F_{9,225} = 58.90, P = 0.0001$), and no significant interaction between both factors ($F_{27,225} = 0.899, P = 0.614$) (Fig. 7A). In general, the running distance is enhanced significantly in all groups as exercise days progressed. Our results showed that the average distance ran (m) over 10 days of voluntary exercise did not differ significantly ($F_{3,25} = 0.57, P = 0.64$) between the exercising groups: the Sal–Exc/Cyt C group (11342 ± 230), the Sal–Exc/IgG group (10152 ± 550), the D–Exc/Cyt C group (11121 ± 188), and the D–Exc/IgG group (11160 ± 151).

3.3.3. Spatial learning

The acquisition data of the experimental groups during the 5 days of training in the WM are illustrated in Fig. 7B. Data related to the distance swam to reach the platform followed similar to the
same pattern as the latency. Thus, we only present results on the latency. A three-way ANOVA (treatment × exercise × training days) with repeated measure (day) were used to analyze the escape latencies during training. All groups learned to locate the target zone compared to the Exc/IgG rats. The Sal–Exc/Cyt C and D–Exc/Cyt C groups spent a significantly greater percentage of the time in the target zone than the Sal–Exc/IgG and D–Exc/IgG groups (both, \( P = 0.0001 \)). The Sal–Exc/Cyt C and D–Exc/Cyt C groups also spent a significantly greater percentage of the time in the target zone than the Sed/Cyt C or IgG groups (all, \( P < 0.05 \)).

The administration of TrkB–IgG fully prevented the exercise-induced preference for the target quadrant, such that there was no difference between the amount of time spent by the Sal–Exc/IgG and D–Exc/IgG groups in the target and opposite zones. The D–Sed/Cyt C and D–Sed/IgG groups spent significantly less time in the target zone than the Sal–Sed/Cyt C and Sal–Sed/IgG groups (\( P = 0.0447 \) and \( P = 0.027 \), respectively).

A two-way ANOVA on the average proximity data revealed a significant interaction between treatment and exercise (\( F_{3,51} = 9.58, P = 0.0001 \)) (Fig. 8C). The between-group comparisons revealed that the Exc/Cyt C groups (the Sal–Exc/Cyt C and D–Exc/Cyt C groups) had a smaller proximity value than the sedentary rats (the Sal–Sed/Cyt C and D–Sed/Cyt C groups) (\( P = 0.022 \) and \( P = 0.0001 \), respectively). The Sal–Exc/Cyt C and D–Exc/Cyt C groups had a smaller average proximity value than the Sal–Exc/IgG and D–Exc/IgG groups (both, \( P = 0.0001 \)).

We did not find any difference in the platform location latency, the time spent in the target zone, and the average proximity value between the Exc/IgG and Sed (IgG or Cyt C) groups. Lastly, we found no difference in the swimming speeds between groups (\( F_{7,51} = 0.59, P = 0.77 \)).

3.3.5. BDNF protein levels

The data on the effect of exercise and BDNF blockage on the BDNF protein levels in the hippocampus are shown in Fig. 9. Accordingly, we quantified the hippocampal BDNF levels for the different groups. BDNF data from one subject of the Sal–Exc/Cyt C group was excluded from the statistical analysis as an outlier. A three-way ANOVA (exercise × morphine × antibody) on the BDNF data showed significant main effects of exercise (\( F_{2,50} = 4.55, P = 0.02 \)), morphine (\( F_{1,50} = 23.8, P = 0.0001 \)), IgG (\( F_{1,50} = 4.55, P = 0.038 \)), and a significant interaction between exercise and IgG (\( F_{1,50} = 12.65, P = 0.001 \)). We found that exercise led to a significantly greater increase in the levels of BDNF in the hippocampus of the Sal–Exc/Cyt C and D–Exc/Cyt C groups compared to the Sal–Exc/IgG and D–Exc/IgG groups (\( P = 0.007 \) and \( P = 0.008 \), respectively). We found that blocking BDNF action with TrkB–IgG fully inhibited the exercise-induced effect on the levels of BDNF, but this blockade did not have an effect on the BDNF levels in the sedentary rats. Interestingly, the levels of BDNF in the D–Sed/Cyt C and D–Sed/IgG groups were significantly higher than those of the Sal–Sed/Cyt C and Sal–Sed/IgG groups (\( P = 0.012 \) and \( P = 0.006 \), respectively).

4. Discussion

This study provides novel evidence that voluntary exercise can ameliorate the cognitive deficits that are induced by chronic morphine. Our findings demonstrate that BDNF plays an important role in mediating the beneficial effects of exercise on cognitive
functions in morphine-dependent rats. Application of the BDNF inhibitor TrkB–IgG during the voluntary exercise was sufficient to blunt the exercise-induced improvement of spatial memory in the dependent rats.

4.1. Voluntary exercise during morphine dependence development decreases dependence severity

We have shown that a period of 10 days of voluntary exercise through wheel running significantly decreases the severity of naloxone-precipitated morphine withdrawal signs in rats. Presently, the neurobiological mechanisms underlying the decreased withdrawal signs accompanying voluntary exercise are still unclear. Previous findings indicate that access to a running wheel reduces self-administration of amphetamine, ethanol (Cosgrove, Hunter, & Carroll, 2002; Ehringer, Hoft, & Zunhammer, 2009; Kanarek, Marks-Kaufman, D’Ani, & Przypek, 1995), morphine (Hosseini, Naderi, Sharifi, & Zahed, 2009), and cocaine (Cosgrove et al., 2002; Smith, Schmidt, Iordanou, & Mustroph, 2008). Access to a running wheel also reduces the craving for morphine in rats (Hosseini et al., 2009; Smith, McClean, & Bryant, 2004), the rewarding effects of cocaine (Lett, Grant, Koh, & Flynn, 2002; Smith & Yancey, 2003; Smith et al., 2008), and the potency of morphine (Smith & Lyle, 2006). Furthermore, it was shown that during chronic exercise (6 weeks), sensitivity to the antinociceptive effects of morphine and other mu opioids decreases, leading to the development of cross-tolerance to exogenously administered opioid agonists. These changes in sensitivity are positively correlated with exercise output, and mediated by the chronic release of endogenous opioid peptides and functional alterations in the opioid receptor system (Smith & Lyle, 2006; Smith & Yancey, 2003). Thus, these changes in potency and sensitivity of morphine by voluntary exercise may account for reduced withdrawal signs in our exercising rats. Finally, a very recent study has shown that voluntary wheel running produces a rewarding state, as measured by conditioned place preference, that persists following exercise cessation, and elicits plastic changes in the mesolimbic dopaminergic reward pathway (Greenwood et al., 2011). Taken together, these findings indicate that the rewarding properties of exercise, and consequently neuroplastic changes induced in the reward pathway could contribute to the benefits of exercise on brain health and functions.

4.2. Voluntary exercise ameliorates spatial memory deficits in morphine-dependent rats

In line with previous studies (Alaei et al., 2006; Lu et al., 2010; Miladi Gorji et al., 2008; Pu et al., 2002; Spain & Newsom, 1991), we found that morphine-dependent rats showed memory impairment 2 h after the last morphine injection. This result is unlikely...
because of spontaneous withdrawal, given that hyper-locomotion induced by morphine lasts longer than 2 h (Handal, Grung, Skurtveit, Ripel, & Mørland, 2002) and that the half-life of morphine (Handal et al., 2002; Kalvass, Olson, Cassidy, Selley, & Pollack, 2007) requires a longer duration before spontaneous withdrawal precipitates. Indeed, in rats, the behavioral effects of spontaneous withdrawal were observed only 8 h post-morphine (Schulteis, Yackey, Risbrough, & Koob, 1998). Thus, the cognitive deficits observed in the dependent rats could potentially be interpreted as an effect of residual chronic morphine and not a withdrawal effect. Although the mechanism that underlies the impairing effects of morphine remains unknown, it has been suggested that chronic morphine may abolish hippocampal CA1 LTP and spatial memory via the accumulation of hippocampal adenosine (Lu et al., 2010).

Findings of the present study confirm the results of a similar recent study showing that 10 days of force exercise is able to ameliorate passive avoidance memory deficits when done simultaneously with chronic morphine administration (Saadipour, Sarkaki, Alaei, Badavi, & Rahim, 2009). These findings indicate that both voluntary and forced exercise can have beneficial effects on the cognitive deficits-induced by chronic morphine, and suggest that common mechanisms may underlie the effects of both types of exercise on cognitive functions.

Our findings demonstrate that voluntary exercise enhanced learning acquisition and memory retention and rescued the...
cognitive deficits induced by chronic morphine treatment. In fact, we found that acquisition performance or the level of memory retention in both saline- and dependent-exercising rats was significantly associated with the amount of voluntary exercise, suggesting that exercise might be responsible for the cognitive recovery in morphine-dependent rats. We found that the voluntary exercise itself improved learning and memory performance in the saline-sedentary group. Thus, the observed effects of exercise in the morphine-treated group did not merely nullify the impact of chronic morphine. The possibility that two effects are independent and additive cannot be ignored.

Our finding is consistent with previous results showing that both voluntary wheel-running (van Praag, 2008) and forced treadmill training (Ang, Dawe, Wong, Moochhala, & Ng, 2006) enhance spatial learning in hippocampus-dependent tasks in rodents. Previous results have also shown that voluntary exercise reduces spatial learning deficits that are induced by kainic acid, prenatal ethanol exposure (O’Callaghan, Ohle, & Kelly, 2007), brain insults (Gobbo & O’Mara, 2005), and stroke (focal cerebral ischemia) (Luo et al., 2007). Moreover, physical activity has been shown to reverse some of the deleterious morphological and behavioral consequences of aging (van Praag et al., 2005) and to attenuate the harmful consequences of acute stress exposure (Pietropaolo et al., 2008). Physical activity in rodents also enhances many aspects of hippocampal physiology, such as LTP, neurogenesis, neurotrophin levels, dendritic spine growth (Gobbo & O’Mara, 2005; Lambert et al., 2005), the number of cholinergic neurons (Ang et al., 2006), and the consolidation of information into long-term memory (Coles & Tomporowski, 2008). These mechanisms might underlie the ability of exercise to enhance cognitive functioning in normal and dependent rats.

4.3. Voluntary exercise may ameliorate spatial memory deficits in morphine-dependent rats via a BDNF-mediated mechanism

BDNF is a member of the neurotrophin family that is expressed in various brain regions that are involved in spatial learning and memory and in LTP, such as the hippocampus (Goodman, 2008; Lu, Christian, & Lu, 2008; Vaynman et al., 2003). BDNF acts by binding to the tyrosine kinase B (TrkB) receptor with a high-affinity (Goodman, 2008). It has been reported that an injection of TrkB–IgGs directly to the hippocampus fully abolishes the exercise-induced increase in BDNF and TrkB receptor mRNA (Vaynman et al., 2003, 2004; Ying et al., 2008). It seems likely that BDNF regulates the production of itself and its receptors during exercise (Bramham & Messaoudi, 2005; Vaynman et al., 2003, 2004). Because of the importance of BDNF in synaptic plasticity and in learning and memory, it has been proposed that the exercise-induced increase in hippocampal BDNF levels might underlie the ability of exercise to enhance cognitive functions (Vaynman et al., 2003, 2004; Yamada, Mizuno, & Nabeshima, 2002).

We found that exercise was able to increase BDNF levels in both dependent and non-dependent groups. In addition, the amount of BDNF increase in the dependent-exercising rats was significantly higher than the sedentary counterparts. This finding may suggest an existence of some degree of additive affects between chronic morphine and exercise on BDNF production. We found that neutralizing the BDNF receptor by intra-hippocampal injections of TrkB–IgG during the exercise period abolished the benefit of exercise on the WM training performance, such that the exercising rats (the Sal–Exc/IgG and D–Exc/IgG groups) that received TrkB–IgG had similar acquisition latencies, platform location latencies, average proximity values, and preferences for the platform zone compared to their sedentary counterparts (the Sal–Sed/Cyc and D–Sed/Cyt C groups). Interestingly, blocking BDNF action with a TrkB–IgG injection did not have a significant effect on the sedentary morphine-dependent and non-dependent rats, suggesting that the blocking effect of BDNF is selective for exercise as previously reported (Vaynman et al., 2003; Ying et al., 2008). Consistent with previous findings, we also found that blocking BDNF action using antibodies reduces the exercise-induced BDNF protein levels in the hippocampus (Vaynman et al., 2003, 2004), suggesting that the change in BDNF induced by exercise was strongly dependent on TrkB-dependent mechanism. On the other hand, the slight increase in BDNF induced by TrkB–IgG in the sedentary groups might be expected as a result of compensatory mechanism. Thus it could be concluded that voluntary exercise ameliorates, via a TrkB-mediated mechanism, the cognitive deficits that are induced by chronic morphine.

Another interesting finding was that chronic exposure to morphine enhanced the BDNF protein levels in the hippocampus in the sedentary morphine-dependent rats, which were not inhibited by blocking the action of BDNF with TrkB–IgGs. This finding is consistent with those of previous studies that have demonstrated elevated BDNF levels in the brain and plasma of rodents and humans following the use of abuse drugs (Angelucci, Gruber, Khoury, Tonali, & Mathé, 2007; Cheng et al., 2005; Chul et al., 2009; Corominas, Roncero, Ribases, Castells, & Casas, 2007; Goodman, 2008; Joe et al., 2007; Kim et al., 2005; LE-Foll, Diaz, & Sokolo, 2005; Numan et al., 1998; Takayama & Ueda, 2005). However, the underlying mechanism of the elevated BDNF levels in the current study is not known. Previous studies have shown that chronic morphine treatment induced an increase in BDNF production in several brain areas by inducing BDNF gene expression (Hatami et al., 2007; Matsushita & Ueda, 2009; Takayama & Ueda, 2005). The elevated BDNF may contribute to synaptic plasticity (Angelucci et al., 2007; Kim et al., 2005), provide a basis for locomotion sensitization and reward (Kim et al., 2005), and induce long-term biochemical and morphological changes that facilitate drug-seeking behavior or craving and relapse in drug abusers (Angelucci et al., 2007; Corominas et al., 2007; Kim et al., 2005; LE-Foll et al., 2005; Numan et al., 1998). Although these studies suggest that increased central BDNF activity may be involved in the pathogenesis of drug addiction, but the determination of this phenomenon in the cognitive deficits in morphine dependent rats requires further research.

Presently, we have no explanation for the finding that blocking BDNF action did not reduce the morphine-induced BDNF protein levels in the sedentary morphine-dependent rats but blocked the exercise-induced enhancement of BDNF protein levels. One possible explanation is that chronic morphine may produce BDNF that...


