Voluntary exercise does not ameliorate spatial learning and memory deficits induced by chronic administration of nandrolone decanoate in rats

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ABSTRACT

Chronic exposure to the anabolic androgenic steroids (AAS) nandrolone decanoate (ND) in supra-physiological doses is associated with learning and memory impairments. Given the well-known beneficial effects of voluntary exercise on cognitive functions, we examined whether voluntary exercise would improve the cognitive deficits induced by chronic administration of ND. We also investigated the effects of ND and voluntary exercise on hippocampal BDNF levels. The rats were randomly distributed into 4 experimental groups: the vehicle-sedentary group, the ND-sedentary group, the vehicle-exercise group, and the ND-exercise group. The vehicle-exercise and the ND-exercise groups were allowed to freely exercise in a running wheel for 15 days. The vehicle-sedentary and the ND-sedentary groups were kept sedentary for the same period. Vehicle or ND injections were started 14 days prior to the voluntary exercise and continued throughout the 15 days of voluntary exercise.

Introduction

Anabolic steroids, which are technically known as anabolic androgenic steroids (AAS) are a large class of synthetic androgens that mimic the effects of the male sex hormones testosterone and dihydrotestosterone. They increase protein synthesis within cells, which results in the buildup of cellular tissue (anabolism), especially in muscles (Bhasin et al., 1996; Bhasin et al., 2003; Forbes, 1985). Although AAS were originally developed for therapeutic purposes (Basaria et al., 2001), they are now commonly used for illegal self-administration at supra-physiological doses to improve performance or body image (Kanayama et al., 2008; Trenton and Currier, 2005). Studies have shown that high doses of AAS can cause serious adverse effects, such as skeletal muscle injuries including an increased rate of muscle strains/ruptures, harmful changes in cholesterol levels, acne, high blood pressure, liver damage (mainly with oral steroids) and dangerous changes in the structure of the left ventricle of the heart (van Amsterdam et al., 2010).

Recent studies have suggested that the misuse of AAS in supersupraphysiological doses also affects several CNS-related behaviors, such as aggression, anxiety, depression and cognitive functions (Su et al., 1993; Trenton and Currier, 2005). Long-term administration of the AAS nandrolone decanoate (ND) leads to behavioral and neurochemical changes in the central nervous system in rodents (Clark and Henderson, 2003; Henderson et al., 2006; Kindlundh et al., 2004; Kurling et al., 2005; Penatti et al., 2009; Rossbach et al., 2007; Thiblin et al., 1999) which may underlie some of the behavioral changes that are observed in human AAS abusers. Two recent studies have shown that the chronic administration of ND to male rats impairs social and spatial memories via central androgen receptors
and dynorphinergic actions in the hippocampus (Kouvelas et al., 2008; Magnusson et al., 2009).

Ampel evidence from human and nonhuman animal studies has shown that exercise can improve cognitive functions in a variety of physiological and pathophysiological conditions (Anderson et al., 2000; Ang and Gomez-Pinilla, 2007; Ang et al., 2006; Baruch et al., 2004; Bekinschtein et al., 2011; Luo et al., 2007; Vaynman et al., 2004). Recent studies in animal models have investigated the biological mechanisms that underlie the beneficial effects of exercise. It is now clear that several neurotropic factors and neurotransmitters participate in exercise-induced cognitive benefits. Among various neurotropic factors, hippocampal BDNF plays a crucial role in learning and memory. Exercise enhances the level of hippocampal BDNF in mice or rats, and this effect is controlled by neuronal activity, neurotransmitters and interactions with peripheral factors, such as estrogen, corticosterone and possibly IGF-1 (Cotman and Berchtold, 2002). Inhibition of hippocampal BDNF has been shown to blunt the exercise-induced enhancement of learning and memory, which suggests a critical role for BDNF in mediating exercise effects on learning and memory (Vaynman et al., 2004).

Given the well-known beneficial effects of physical exercise on learning and memory, voluntary exercise may ameliorate learning and memory deficits induced by chronic ND. To address this issue, the present study was designed to examine the influence of exercise on the learning and memory impairing effects of chronic ND in rats. We also investigated effects of chronic ND and exercise on hippocampal levels of BDNF, a key molecule that links voluntary exercise with improvements in cognitive function (Vaynman et al., 2004).

Materials and methods

Animals and experimental groups

Adult male Wistar rats (210 ± 10 g) were individually housed in cages (50 × 26 × 25 cm) and kept on a 12-h light/dark cycle (6 am lights on–6 pm lights off) at 22–24 °C with food and water available ad libitum. All experiments were conducted between 8:30 and 12:00 h. The experimental protocol was approved by the research committee of Semnan University of Medical Sciences (Semnan, Iran). All of the experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. In addition, care was taken to minimize the number of animals that were used in each experiment.

Experimental groups

The rats were randomly distributed into 4 experimental groups, and each group contained 8 rats: the vehicle-sedentary group (VEH/SED), the ND-sedentary group (ND/SED), the vehicle-exercise group (VEH/EXC), and the ND-exercise group (ND/EXC). The rats in both of the sedentary groups were not submitted to any type of physical activity. The ND-treated rats received a subcutaneous (s.c.) injection of ND (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) every third day (15 mg/kg, which is a supra-physiological dose), and the vehicle-treated groups received an s.c. injection of the vehicle (propylene glycol). The ND dosage and the treatment schedule were selected based on previous reports (Kouvelas et al., 2008; Magnusson et al., 2009). Vehicle or ND injections were started 14 days prior to the voluntary exercise and continued throughout the 15 days of voluntary exercise (10 injections over 29 days) (Fig. 1). The rats were weighed four times during the study.

Exercise paradigm

Each of the exercising rats was given access to a cage that was equipped with a running wheel (the diameter = 34.5 cm, and the width = 9.5 cm) 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 (located outside the animal house) that monitored the number of revolutions per hour. The number of revolutions for each wheel was recorded every day at 6 am. The sedentary rats were confined to similar cages without access to a wheel. After a 15-day period of exercise, the running wheels were removed from the cages, and the rats were trained and tested on the water maze task.

BDNF measurements

The BDNF protein levels were assessed using Rat BDNF ELISA kits (Boster Biological Technology Co., Wuhan, China) according to the manufacturer’s recommendations. The hippocampal extracts were prepared in lysis buffer, and the homogenates were centrifuged to remove insoluble materials (12,500 × g for 20 min at 4 °C). 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 prior to incubation of the samples and the BDNF standards with shaking 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 (diluted in B&S buffer). The plates were washed 3 times with TBS (20 mM Tris HCl, 150 mM NaCl, 0.05% v/v Tween 20) prior to a 1 h incubation (at room temperature) with a biotinylated anti-rat BDNF antibody. After the antibody incubation, the plates were washed three times with TBS and incubated for 1 h (at room temperature) with avidin–bixin–peroxidase complex (ABC). After the incubation, an ABC working solution was added to each well, incubated at room temperature for 30 min, and washed 5 times with TBS. Then, TMB color developing agent was added to each well and incubated for 30 min at room temperature. After the samples turned blue, the reaction was stopped by the addition of TMP stop solution, and the absorbance was measured at 450 nm using an automated ELISA plate reader. The sensitivity of the assay was <2 pg/ml.

Fig. 1. Timeline of treatment, voluntary exercise and behavioral testing (see Materials and methods for details).
Testing learning and memory using a water maze (WM)

A detailed description of the apparatus and the tracking system (EthoVision, Noldus, The Netherlands) that we used to test learning and memory in a WM has been given in previous reports (Akhavan et al., 2008; Ebrahimi et al., 2010; Miladi-Gorji et al., 2011). The WM was a black circular pool (140 cm in diameter and 60 cm high) that was filled with 22 °C water to a depth of 25 cm.

The WM protocol was a stringent protocol that consisted of four trials per day for 5 consecutive days. During each trial, the rat was placed into the water at 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. If the rat failed to locate the platform within 60 s, it was guided by hand to the platform. The rat was allowed to stay on the platform for 20 s during the inter-trial interval. After the last trial, the rat was towel dried and returned to its home cage with no access to a running wheel.

The platform was removed during the spatial probe test, which was performed 2 days after the last acquisition trial. The rats were allowed to swim for 60 s, and we recorded the latency to reach the platform location, the time spent swimming within a zone (i.e., a 20 cm radius that was centered either on the original training location (target zone) or on an equivalent location in the opposite quadrant (opposite zone)), and the proximity (the average distance in centimeters of rats from the center of the platform location across the 60-s test). The velocity of each rat was also calculated. The analysis of the time spent within a specific radius (zone) and the proximity measure are consistently more sensitive to swim for 60 s, and we recorded the latency to reach the platform location. The velocity of each rat was also calculated. The analysis of the time spent within a specific radius (zone) and the proximity measure are consistently more sensitive measures of the WM probe test performance in terms of detecting group differences (de Hoz et al., 2004; Gallagher et al., 1993; Maei et al., 2009; Moser and Moser, 1998).

Immediately after the probe test, the rats performed a 3-trial visible (cued) platform task to assess their motor ability. The platform extended 2 cm above the surface of the water, and it was moved to a novel quadrant in the pool on each trial (we followed the same procedure that was described for the learning and memory test).

Statistical analysis

The data expressed as the mean ± standard error of the mean (S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVAs). Post-hoc analyses consisted of Turkey’s S.E.M()).

Results

Running distances

Two-way ANOVA for the average distance that the rats ran (m) during the 15 days of voluntary exercise revealed significant group (F1,14 = 120.6, P < 0.001) and day effects (F4,196 = 17.95, P < 0.05) and a significant interaction between both factors (F4,196 = 9.13, P = 0.001) (Fig. 2). The difference between the VEH/EXC and ND/EXC groups was significant for all of the days except days 1, 5, and 6 (P < 0.05).

Acquisition

The acquisition data of the experimental groups during the 5 days of training are illustrated in Fig. 3. The data related to the distance that the rats swam to reach the platform followed a similar pattern as the latency. Thus, we only present the latency results. A two-way ANOVA for escape latency showed significant group (F1,28 = 28.04, P = 0.0001) and day effects (F4,112 = 38.62, P < 0.0001) and a significant interaction between both factors (F12,112 = 1.85, P = 0.05). Between-group comparisons indicated that the escape latencies of the VEH/EXC group were significantly shorter than those of the VEH/SED group on days 2–5 (P < 0.001). In contrast, the escape latencies of the ND/EXC group were significantly longer than those of the VEH/EXC group on days 2 (P < 0.001), 3 (P < 0.001), 4 (P < 0.03), and 5 (P < 0.009). The differences in escape latencies between the VEH/SED and ND/SED groups were significant on days 1 (P < 0.001) and 2 (P < 0.001). These findings indicate that voluntary exercise was unable to enhance learning acquisition in the ND-treated animals.

Retention

The data from the memory retention test are shown in Fig. 4. A three-way ANOVA for the time spent in zones (Fig. 4A) showed a significant interaction between groups × zones × treatment (F3,56 = 13.17, P < 0.001). Between-group comparisons indicated that the VEH/EXC group spent significantly more time in the target zone and less time in the opposite zone than the VEH/SED (both, P < 0.01) and ND/EXC (both, P < 0.01) groups. There was no significant difference in the time spent in the target zone between the ND/EXC and the ND/SED groups. Fig. 4B represents the average proximity to the platform. A two-way ANOVA showed a significant effect of groups (F1,28 = 6.78, P = 0.015) and treatment (F1,28 = 32.02, P = 0.001) and a significant effect of groups (Fig. 2).

Fig. 2. The effect of nandrolone decanoate administration on the total running distance in the exercising groups. The data are expressed as the mean ± SEM of the total running distance in kilometers per day for each exercise group. The running distance was monitored by counter devices attached to each cage. *P < 0.05 compared with the ND/EXC group. VEH: vehicle; ND: nandrolone decanoate; EXC: exercise.
interaction ($F_{1,28} = 17.06, P = 0.001$). The VEH/EXC group had a significantly smaller average proximity compared with the VEH/SED (P < 0.05), ND/EXC groups (P < 0.001), and ND/SED (P < 0.01). The ND/SED group had a significantly larger average proximity compared with the VEH/SED group (P < 0.05). These findings indicate that voluntary exercise was unable to ameliorate ND-induced impairment of memory retention.

To control for differences in the WM performance, we also recorded each rat’s swimming speed during probe test. We did not find any differences ($F_{3,28} = 1.78, P = 0.1$) in the swimming speeds between the four groups: VEH/SED (26.1 ± 1.32 cm/s), ND/SED (23.5 ± 1.78 cm/s), VEH/EXC (23.1 ± 0.92 cm/s) and ND/EXC (22.3 ± 0.90 cm/s).

**Latency to the visible platform**

One-way ANOVA of the visible platform latency results did not reveal any significant differences ($F_{3,28} = 2.1, P = 0.09$) between the four groups: VEH/SED (14.3 ± 0.25 s), ND/SED (15.3 ± 0.85 s), VEH/EXC (13.5 ± 0.20 s) and ND/EXC (14.7 ± 0.49 s).

**BDNF data**

The data on the effect of exercise and ND administration on BDNF protein levels in the hippocampus are shown in Fig. 5. A two-way ANOVA for BDNF levels showed significant group ($F_{1,28} = 10.9, P = 0.003$) and treatment effects ($F_{1,28} = 9.6, P = 0.004$), but there was not a significant interaction between the factors ($F_{1,28} = 0.456, P = 0.5$). We found that exercise led to a significantly greater increase in the levels of BDNF in the hippocampus of the VEH/EXC group compared with the VEH/SED group (P = 0.006). In addition, we found that ND administration enhanced the levels of BDNF in the ND/SED group compared with the VEH/SED group (P = 0.006). Furthermore, the levels of BDNF in the ND/EXC group was significantly higher than the BDNF level in the VEH/EXC group (P < 0.01), indicating that ND administration enhanced the effect of exercise on BDNF levels.

**Discussion**

The present experiments examined whether voluntary exercise influenced the disruption of spatial learning and memory induced by chronic ND. The effects of chronic ND and exercise on hippocampal BDNF levels were also determined. Voluntary wheel running for 15 days prior to the onset of behavioral testing enhanced subsequent spatial learning and memory and hippocampal BDNF in rats. ND impaired spatial learning and memory, and this effect was not rescued by exercise. Moreover, ND further increased exercise-induced enhancement of hippocampal BDNF. These findings demonstrate that voluntary exercise cannot ameliorate the cognitive deficits induced by the chronic use of the AAS ND. Additionally, the enhanced hippocampal BDNF may play a role in ND-induced impairments in learning and memory.

To reproduce the effects of short exercise on cognitive functions, rats must run a minimum of 100 m each night (Ebrahimi et al., 2010; Shaw et al., 2003; Vaynman et al., 2004). We found that the running rates were initially slow in both the vehicle- and ND-treated exercising groups (a minimum of 296 m). They gradually increased over 15 days until the vehicle- and ND-treated exercising groups were running a minimum of 4 km and 6 km, respectively, on day 15. ND-treated rats, however, ran slower than vehicle-treated rats, indicating that the chronic injection of ND reduces wheel-running activity. These findings are consistent with several studies that have demonstrated that ND reduces wheel-running activities (Bronson et al., 1996; McGinnis et al., 2007); however, the underlying mechanisms that mediate the inhibitory effects of ND are not clear. A previous study showed that the testosterone-treated male rats displayed a significant increase in wheel-running activity, whereas the ND-treated rats displayed a significant decrease in running wheel activity. In addition, the ND-treated male rats, which showed a marked decrease in wheel-running activity, also had significantly lower serum testosterone levels compared with the controls, which suggests that reductions in wheel-running activity after exposure to AAS may result from decreased serum T levels (McGinnis et al., 2007). Although we did not measure serum T levels in the ND-treated rats, a reduction in serum T levels may also mediate the effects of ND on wheel-running activity.

Previous studies have demonstrated no significant changes in locomotor activity in male rats and mice following AAS administration (Clark and Henderson, 2003). These findings rule out the possibility that ND impairs general locomotor activity. In female mice, however, testes of running wheel activity revealed a dramatic suppression of spontaneous activity by AAS. It is suggested that the AAS suppression
Chronic administration of nandrolone decanoate enhanced the BDNF levels in the sedentary (ND/SED) and exercising (ND/EXC) groups. \( a P < 0.01 \) compared the VEH/EXC group with the VEH/SED group, \( b P < 0.01 \) compared the ND/VEH group with the VEH/EXC group, and \( c P < 0.05 \) compared the ND/SED with the VEH/SED. The data are expressed as the mean ± S.E.M. Legends are the same as Figs. 1 and 2.
of wheel running is due to disruption of the hypothalamic–pituitary–
gonadal axis (Bronson et al., 1996).

Another possibility that may explain slower running wheel activity in the ND–treated rats is the rewarding effects of AAS, and conse-
sequently the development of dependence to AAS. There is some evidence to indicate that AAS themselves may be rewarding and
may potentiate the rewarding effects of drug of abuse. For example, male rats develop a conditioned place preference to testosterone injec-
tions into the nucleus accumbens, an effect blocked by dopamine receptor antagonists (Wood, 2004). This finding suggests that androgen reinforcement is mediated by the mesolimbic dopamine sys-
tem, a common substrate for drugs of abuse. Clark et al. (1996) showed that AAS treatment enhanced the reinforcing effects of amphetamine in rats. There is also concern that dependence may de-
velop with chronic steroid use. Indeed, AAS share brain sites of action and neurotransmitter systems in common with other drugs of abuse (Wood, 2008).

**ND-induced impairment of learning and memory cannot be rescued with voluntary exercise**

In the first experiment, we found that ND impairs learning and memory and this effect does not ameliorate by voluntary exercise. These findings are consistent with previous studies that have shown the disrupting effects of a high dose of ND on spatial (Magnusson et al., 2009) and social memory in rats (Kouvelas et al., 2008). The underlying mechanisms that mediate ND-induced impairments of learning and memory are not clear. Previous studies have shown that ND–induced increases in BDNF, reduction of anxiety, memory impair-
ment, and enhancement of GABAergic transmission (Kouvelas et al., 2008; Penatti et al., 2009) are mediated by a direct activation of central androgen receptors. In addition, a 2-week administration of ND appears to upregulate androgen receptor immunoreactivity in the rat brain (Menard and Harlan, 1993). These results indicate that androgen receptors play a crucial role in mediating the effects of ND. Androgens specifically affect spatial working memory performance. Although some animal and human results have suggested a positive correlation between testosterone and spatial ability (Flood and Roberts, 1988; Janowsky et al., 1994), several reports have indicated that chronic treatment with androgenic compounds impairs spatial learning and the re-
tention of spatial information in young and middle-aged animals (Galea et al., 1995; Goudsmit et al., 1990) and humans (Gouchie and Kimura, 1991; Hampson, 1995). These findings indicate that the effects of androgens on cognitive functions depend upon the level of andro-
gen (i.e., the optimal level of androgens is associated with cognitive improvement, whereas supra-physiological levels may impair cognitive functions). According to these results, the observed learning and memory impairments following ND administration in the present work might be mediated by androgen receptors. Our future work aims to identify the mediating role of androgen receptors in cognitive deficits induced by chronic ND.

A recent study has shown that chronic ND increases prodynorphin mRNA in the male rat hippocampus and impairs spatial performance in the water maze (Magnusson et al., 2009). Previous studies have demonstrated a positive correlation between impaired memory and dynorphin levels (McDaniel et al., 1990; Sandin et al., 1998). Thus, increased dynorphnergic transmission could be one of the mechanisms that underlie the cognitive disrupting effects of long-term admin-
istration of supra-physiological doses of ND.

Chronic administration of ND also alters the glutamatergic (Rossbach et al., 2007), GABAergic (Henderson et al., 2006; Penatti et al., 2009), dopaminergic (Kindlundh et al., 2004) and serotonergic (Kurling et al., 2005; Thiblin et al., 1999) systems. Thus, an interaction of ND with these neurotransmitter systems may mediate the effects of ND on learn-
ing and memory.

**ND enhances BDNF levels in the sedentary and exercising groups**

BDNF, which is a member of the neurotropin family, is expressed in various brain regions that are involved in cognitive functions (e.g., hip-
campus) and plays an important role in learning, memory and synaptic plasticity (Lu et al., 2008; Vaynman et al., 2004). The actions of BDNF are transduced via the high-affinity tyrosine kinase receptor (TrkB). An-
tagonism of hippocampal TrkB fully abolishes the exercise–induced increase in BDNF and TrkB receptor mRNA (Gomez–Pinilla et al., 2008; Vaynman et al., 2003, 2004; Ying et al., 2008). The exercise–induced increase in hippocampal BDNF levels might underlie the ability of exercise to enhance cognitive functions (Miladi–Gorji et al., 2011; Vaynman et al., 2003).

In agreement with previous studies (Hopkins and Bucci, 2010; Miladi–Gorji et al., 2011; Vaynman et al., 2003, 2004), we found that voluntary exercise increased hippocampal BDNF levels in the exercising groups. In addition, chronic administration of ND enhances hippocam-
pal BDNF in the sedentary and exercising groups. Interestingly, ND further enhanced the exercise–induced increase in hippocampal BDNF (i.e., the BDNF increase in the ND–treated exercising rats was significantly higher than the exercising rats that received vehicle). This finding may suggest the existence of additive effects between chronic ND and exercise on hippocampal BDNF production.

The underlying mechanism of the elevated BDNF levels following chronic administration of ND in the current study is unknown. Previous studies have demonstrated that androgens regulate BDNF expression in several brain structures, autonomic neurons, peripheral tissues, and neuromuscular systems (Al–Shamma and Arnold, 1997; Verhovshek et al., 2010; Yang et al., 2004). Testosterone has been shown to increase BDNF levels in the high vocal center of the adult canary, and BDNF medi-
ates the effects of testosterone on the survival of new neurons in this region (Rasika et al., 1999). A recent study has shown that testosterone regulates the expression of BDNF and its receptor (i.e., TrkB) in spinal motor neurons and their target musculature (Verhovshek et al., 2010). BDNF expression is regulated through a calcium–dependent signaling pathway that involves the phosphorylation of the cAMP response element (CRE) and its binding protein CREB (Shieh et al., 1998; Tao et al., 2002). In addition, testosterone has been shown to activate both CRE and CREB (Aarnisalo et al., 1998; Auger et al., 2001). Taken together, previous studies have shown that androgens have a positive effect on BDNF production, which is in agreement with the enhanced hippocampal BDNF levels that were observed in the sedentary and exercising groups following chronic ND administration in the present study. How-
ever, our finding is in contrast with a recent study that showed chronic ND (5 mg/kg. s.c., once a day for 28 days) administration substantially reduced BDNF levels in the hippocampus and prefrontal cortex. Interest-
estingly, the reduction in BDNF was prevented by the androgen recep-
tor antagonist flutamide (Matrisciano et al., 2010). The reasons for the contrasting results may result from differences in the experimental pro-
cedures. One important difference between the present study and that of Matrisciano et al. (2010) is the number of drug injections (28 times vs. 10 times), which could influence injection–related stress. Another difference is the time of the BDNF measurement. We measured BDNF 8 days after the last injection (Fig. 1), whereas Matrisciano et al. (2010) measured BDNF 1 day after the last injection.

Traditionally, the actions of BDNF in CNS have been thought of as fa-
cilitative, survival promoting and neuro-protective. More recently, however, detrimental actions of BDNF on neuron survival and morphol-
ogy have been reported. For example, BDNF can enhance the sensitivity of spinal cord motor neurons in vitro to excitotoxic insults through acti-
vation of the TrkB receptor (Hu and Kalb, 2003; Mojsilovic–Petrovic et al., 2006), and have deleterious effects on dendritic morphology (Lom and Cohen–Cory, 1999; McAllister et al., 1997). These findings suggest that BDNF under certain circumstances can have inhibitory effects on neurons. The possible role of the elevated BDNF in ND–induced learning and memory impairments is not clear. One possibility is that the higher
levels of BDNF observed in the hippocampus of the ND-treated animals may contribute to cognitive impairments. This hypothesis may be supported by findings from two recent studies showing that overexpression of BDNF resulted in cognitive deficits in mice. In an interesting study in mice, a widespread overexpression of BDNF in the forebrain resulted in learning impairments in a passive avoidance task, increased neuronal excitability, and susceptibility to seizures (Croll et al., 1999). Similarly, another study has shown that chronic BDNF over-expression in the forebrain impaired learning in both instrumental and spatial memory tasks (Cunha et al., 2009). These findings suggest that high levels of BDNF might have potentially detrimental effects on learning, plasticity and excitability. Our findings are to some extent in agreement with these findings. We found the highest levels of impairment in the ND/EXC group, who coincidentally had the highest BDNF levels, suggesting a role for BDNF in the resulting impairment. Future studies, however, are required to confirm the role of BDNF in ND-induced learning and memory impairment.

Our findings suggest that chronic use of AAS disrupts learning abilities of young student athletes, who may be abusing AAS and are learning new concepts in school. More importantly, a recent study found that exercise (20 or 40 min of aerobic exercise) can enhance students’ cognitive functions (Davis et al., 2011). The study demonstrated that the more exercise the students got, the more their brain activity increased in the prefrontal cortex, a region of the brain associated with complex cognitive functions (Teffer and Semendeferi, 2012). Hence, another important implication of our findings is that AAS abuse may interfere with such beneficial effects of exercise. Alternatively, exercise may not be able to improve cognitive abilities in AAS abusers.

In conclusion, the results of the present study suggest that chronic administration of high doses of ND impairs spatial learning and memory and enhances hippocampal BDNF. Voluntary exercise does not ameliorate learning and memory deficits induced by ND. Presently, the role of elevated BDNF levels following chronic ND remains unknown. Elevated BDNF may contribute to the understanding of the underlying biological pathways that lead to AAS-related cognitive and mood disorders in humans.

Conflict of interest statement

We attest that we have herein disclosed any and all financial or other relationships that could be construed as a conflict of interest and that all sources of financial support for this study have been disclosed.

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