Original Article
Effects of exercise of equal intensity on working memory and BDNF protein expression in the prefrontal cortex in rats with different degrees of sleep deprivation

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Abstract: Sleep deprivation (SD) is known to impair learning and memory function of animals. Physical exercise can alleviate SD-related functional defecit. This study observed the effect of physical exercise on memory or learning function plus expression of brain derived neurotrophic factor (BDNF) in rats under different SD paradigms, in order to investigate the potential effect of exercise on SD rats and related mechanisms. Male adult SD rats were assigned into control, 24 h SD, 36 h SD and 48 h SD groups, each of which was sub-divided into naïve or 6-week exercise groups (N = 10 each). SD model was generated by small platform localization. Learning score was evaluated by Y-maze. Serum superoxide peroxidase (SOD), malondialdehyde (MDA) levels, acetylcholine esterase (AchE) and 5-HT levels were measured by colorimetry. BDNF expression in the prefrontal cortex (PFC) was quantified by immunohistochemistry (IHC). All SD groups showed lower Y-maze scores, plus decreased SOD and BDNF levels, and higher MDA, AchE and 5-HT levels, all of which were correlated with time series (P < 0.05). Compared to naïve animals, exercised rats had improved Y-maze scores, higher SOD or BDNF expression, and lower MDA, AchE or 5-HT levels, with potentiated effects of elongated exercise time (P < 0.05). SD aggravated brain function and impaired memory or learning. Exercise improved SD effects of rat's learning and memory functions, possibly involving up-regulation of BDNF protein, anti-oxidation or neurotransmitter mediation. Such protective effects were facilitated with elongated treatment time.

Keywords: Sleep deprivation, physical exercise, BDNF, neurotransmitter

Introduction

Sleep is critical for maintaining normal body physiology and mental functions, especially brain functions, as sleep can facilitate and sustain synaptic formation which is related to memory and learning, and enhance the clearance of neurotoxic substances [1, 2]. Insufficient sleep can cause oxidative stress of the bodies cardiovascular system, enhance the oxidative risk in multiple brain regions including the prefrontal cortex (PFC) and hippocampus, leading to neuronal damage [3, 4]. Long-term sleep deprivation (SD) can lead to adverse mood disorders, and impair learning and memory functions, all of which is known to be related with duration of SD. Some studies showed the correlation between decreased memory or learning function after SD and alternations in brain neurotransmitter levels [5, 6]. Neurotransmitters play a critical role in the development of vascular dementia (VD), which is correlated with decreased levels of monoamine or acetylcholine neurotransmitters in the cortex. VD can disrupt the release of multiple neurotransmitters, which further affects neuronal functions. Brain derived neurotrophic factor (BDNF) has multiple pharmaceutical activities including improvement of neuronal pathological status, facilitating neuronal survival or differentiation, and preventing neural injury, as it modulates neuronal functions via multiple signal transduction pathways. BDNF has synergistic effects with N-methyl-D-aspartate (NMDA) receptors to modulate learning and memory

function, and is shown to be related with post-BD anxiety behavior [7, 8].

Previous studies showed that physical exercise had positive effects on functional recovery of chronic brain disorders such as dementia. Physical exercise at an appropriate load can improve body acclimation against stress. Chronic aerobic exercise can improve rat learning and memory function, increase the cerebral cortex thickness, and facilitates angiogenesis. Physical exercise can improve cognitive dysfunction in SD rats [9, 10]. Currently the functional mechanism of physical exercise on SD rats has not been fully illustrated. As SD can cause a series of body changes including cognition and mood status, this study observed the effect of fixed amounts of exercise on memory or learning functions in rats under different SD lengths, along with BDNF protein expression in the prefrontal cortex (PFC), plus neurotransmitter levels, in order to analyze possible mechanisms of exercise interventions in rat memory and learning from the perspective of molecular and protein levels.

Materials and methods

Animals and grouping

A total of 80 SPF grade healthy male Spargue-Dawley rats (6-month age, body weight 240-260 g) were provided by the Laboratory Animal Center of Kunming Medical University (Certificate No. SYXK-2013-0025) and provided food and water ad libitum. Animals were randomly assigned into control (0 h), 24 h SD, 36 h SD and 48 h SD groups, each of which was further sub-divided into naive and exercise groups (N = 10 each). All exercised groups received a 6-week physical exercise intervention.

Rats were used in all experiments, and all procedures were approved by the Animal Ethics Committee of Jiangsu Vocational College of Medicine (Yancheng, Jiangsu, China).

Drugs and reagents

Hydrate chloroform and paraformaldehyde were from Kemiou Chemical, China; Assay kits for SOD, MDA, AchE and 5-HT were purchased from Jiancheng Biotech, China; Rabbit anti-BDNF antibody, and horseradish peroxidase labelled goat anti-rabbit secondary antibody

are from CST, US. Y-maze is from Taimeng Tech, China.

Physical exercise paradigm

According to previous methodology [11], the exercise group first received 1 week of acclimated treadmill exercise (10 m/min, 45 min/d, 15 min × 3, 5 days), followed by 6 weeks of accelerated aerobic treadmill exercise (no incline). A small platform within a water pool was used to construct the SD model. Aerobic exercise paradigm: 10 m/min initial velocity at 1st week, followed by gradient speeding (2 m/min increment per week). Velocity remained at 16 m/min and kept a constant speed until the end of exercise paradigm (5 d per week, 60 min per day, 15 min × 4).

Generation of SD model

The preparation of SD model was performed as previously recorded [12]. After acclimation in the water pool for 3 days (1 h daily), rats were put on a small platform (7 cm diameter, 8 cm height) on top of a SD chamber (30 × 30 × 30 cm). When rats reached rapid eye movement (REM) phase of sleep, they were disturbed and prevented from falling asleep due to the falling into water. Rats received 24 h (B), 36 h (C) or 48 h (D) of SD treatment, in parallel with control group without SD (A).

Y-maze task

The corrective response rate and time duration of electric shock were measured, in a Y-maze apparatus using 36 V working potential and 0.7 mA current. One single training session includes 10 trials, with 15 min intervals. A total of 40 trials were performed on each rat, each day for 3 consecutive days. Corrective response rate (in %) = number of correct responses/total trial number × 100%. Time duration of electrical shock = summation of electoral shock duration within 10 training trails.

Serum SOD and MDA assay

A total of 5 ml blood samples were collected from common carotid artery, and were centrifuged at 1500 g for 10 min, with the supernatant saved. Colorimetry was performed to quantify SOD and MDA levels following the manual instructions.
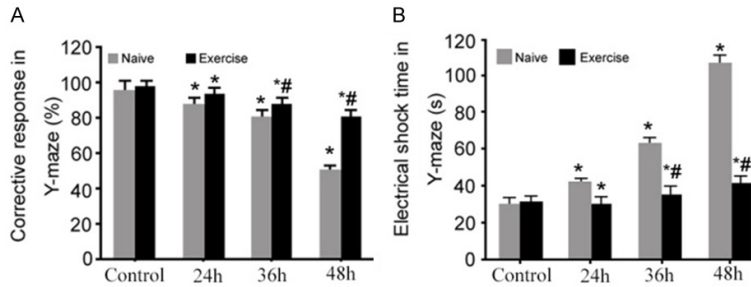
Figure 1. Effects of physical exercise on rat memory and learning function under differential SD paradigms. A. Effects of exercise with equal work loading on corrected response rate in Y-maze of SD rats; B. Effects of exercise with equal work loading on electric shock time duration in Y-maze on SD rats.
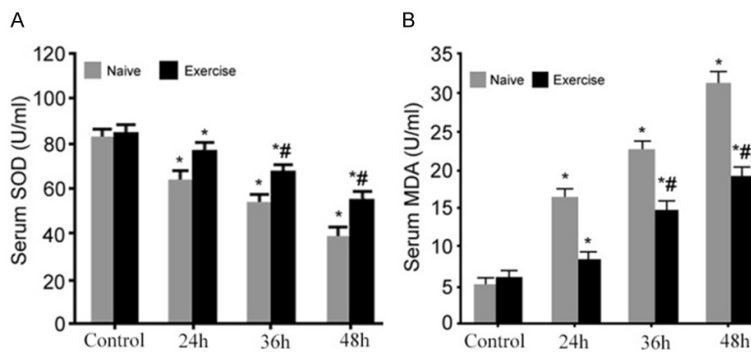

Figure 2. Effects of physical exercise on rat serum SOD and MDA levels under differential SD paradigms. A. Rat serum SOD level under SD and exercise; B. Exercise effects on serum MDA contents in SD rats. *, P < 0.05 compared to control group; #, P < 0.05 compared to naïve (un-exercised) rats.

Quantification of neurotransmitter in cerebral cortex

Rats were sacrificed and the whole brain was collected, with removal of cerebellum and olfactory bulb. The cerebral cortex was quickly removed and put on ice to prepare a 10% homogenate. Protein content was quantified by the Coomassie brilliant blue colorimetry approach. Levels of AchE and 5-HT were quantified following the manual instructions.

Western blot for BDNF protein expression

Rat PFC tissues were lysed in lysis buffer, and were centrifuged to collect the supernatant. Protein content was quantified by BCA approach. Proteins were electrophoresed in SDS-PAGE and were transferred to a PVDF membrane, which was blocked for 1 h. Primary antibody against BDNF (1:1,000) or internal reference (1:1,000) was added at 4°C for over-night incubation, followed by TBST washing and goat anti-rabbit IgG secondary antibody staining (1:1,000) for 1 h incubation. After TBST washing, development, and exposure, protein bands were analyzed by Quantity One imaging software, and relative expression was presented as the ratio between absorbance value of BDNF against those of internal reference.

Statistical methods

SPSS 20.0 software was used for statistical analysis. Measurement data were tested for normality, and those that fitted a normal distribution were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) and LSD test were used for comparison of means among groups. Statistical significance was defined when P < 0.05.

Results

Elevated corrective response rate and lower electrical shock time in rats after exercise

Compared to the control group, all SD groups showed decreased corrective response rates in the Y-maze, accompanied with higher electrical shock time durations (P < 0.05), with certain correlations with SD duration. Compared to the naive group, exercised groups showed elevated corrective response rates and lower electrical shock times in the Y-maze (P < 0.05). Such ameliorating effects were elongated with longer SD duration (**Figure 1**).

Elevated SOD and decreased MDA levels after exercise

Compared to the control group, all SD groups showed decreased serum SOD activity and elevated MDA content (P < 0.05), both of which were related with SD time duration. Compared to naïve rats, exercised rats had elevated SOD
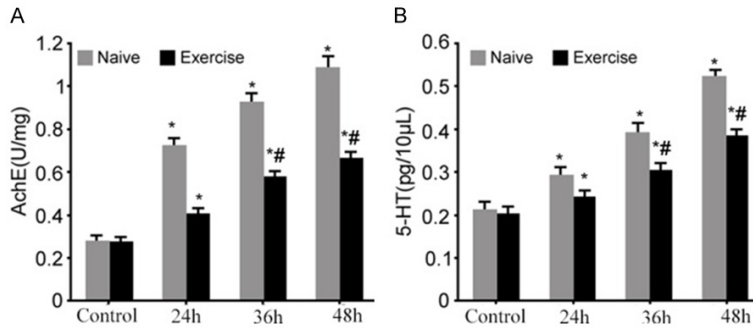
Figure 3. Effects of physical exercise on brain AchE and 5-HT levels in SD rats. A. Exercise and brain AchE levels in SD rats; B. Effects of exercise on 5-HT levels in SD rat brain tissues. *, P < 0.05 compared to control group; #, P < 0.05 compared to naïve (un-exercised) rats.
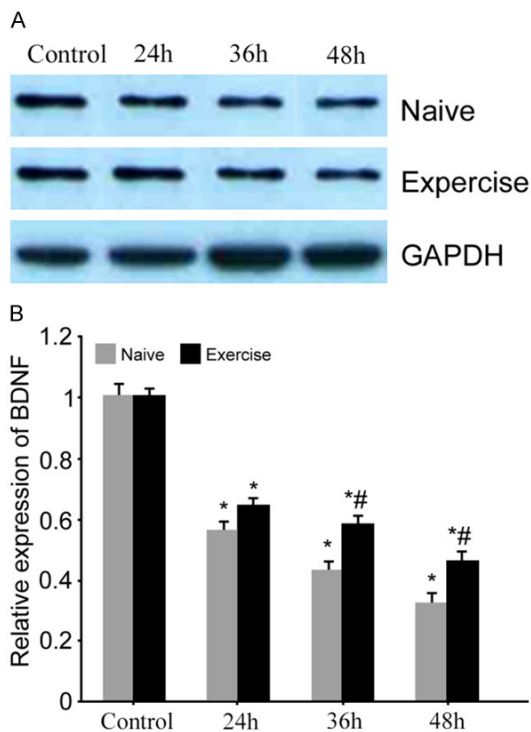

Figure 4. Effects of exercise on PFC BDNF expression in SD rats. A. BDNF protein expression by Western blot; B. Relative expression of BDNF protein in rat PFC. *, P < 0.05 compared to control group; #, P < 0.05 compared to naïve (un-exercised) rats.

activity and decreased MDA content (P < 0.05), with enhanced effects with elongated SD time duration (**Figure 2**).

Decreased AchE and 5-HT in SD rats after exercise

Compared to the control group, all SD rats showed elevated AchE and 5-HT levels in brain tissues (P < 0.05), both of which were related with SD time duration. Compared to naïve rats, exercised rats had decreased AchE or 5-HT expression (P < 0.05), with enhanced effects with elongated SD time duration (**Figure 3**).

Increased BDNF expression in PFC tissues of SD rat after exercise

Compared to the control group, all SD rats showed lower BDNF expression in PFC tissues (P < 0.05), which was correlated with SD time duration. Compared to naïve rats, exercised rats had elevated BDNF expression (P < 0.05), with enhanced effects with elongated SD time duration (**Figure 4**).

Discussion

Previous studies showed that a small platform in a water pool could efficiently deprive all rats of REM sleep, these rats showed impaired learning potency or enhanced excitability [13, 14]. Sleep has certain anti-oxidative effects, and chronic sleep deprivation leads to various issues. Some studies believed that aerobic exercise alleviated adverse effects of insufficient sleep, and enhanced neurotransmitter release. Currently functional mechanisms of exercise on the intervention of SD has not been fully illustrated [15, 16]. This study observed the effect of exercise on memory and learning functions in SD rats with different SD lengths, along with oxidative stress, neurotransmitter levels and BDNF protein expression, to investigate the effect of exercise intervention on SD-related brain injury. Results showed decreased Y-maze score in SD rats, and decreased learning ability with elongated SD duration. Exercise training significantly improved Y-maze performance, indicating improved neuronal performance in SD rats after exercise intervention, and improved learning and memory functions. Exercise training has certain brain protective effect in SD rats, with enhanced effects with elongated SD duration. Sleep can facilitate GABA release, and it plays an important role in initiating cyclic anti-

oxidation. Under physiological conditions, the body maintains a dynamic balance between oxidation and anti-oxidation. Long-term SD elevates body oxidative stress levels, with surmounting internal oxidation potency over anti-oxidation buffering, thus aggravating brain damage. MDA levels can reflect body lipid peroxidation and cellular damage conditions, and internal SOD can block tissue injury effects by oxidative free radicals [17]. This study found elevated MDA levels in SD rat brain tissues, accompanied with higher SOD activity, which can help to elevate anti-oxidase activity and alleviate rat neural dysfunction via suppressing body oxidative stress levels, which may be related with mitochondrial ATP sensitive potassium channel and mitochondrial membrane stability [18], suggesting that exercise could improve anti-oxidation potency of SD rats, with enhanced anti-oxidation effects with elongated SD duration.

Neurotransmitter plays important roles in memory and learning function of the brain, in which Ach level was maintained by Ach transferase and cholinesterase, and AchE can indirectly reflect Ach level [19]. SD rats showed enhanced AchE activity, which facilitates Ach hydrolysis, and leads to central cholinergic system dysfunction. Exercise of different loads suppresses AchE activity in brain tissues, and improved cholinergic neurotransmitter metabolism. Monoamine neurotransmitter plays crucial roles in memory formation and maintenance. 5-HT participates in synaptic connections between the cortex and hippocampus, and is related with body temperature modulation, pain sensation and sleeping, and is one potential modulator for central fatigue regulation. Previous studies demonstrated elevated 5-HT level in SD rat brains, and consequent cognitive dysfunction [20, 21]. In this study, SD rats showed elevated 5-HT levels, which were correlated with the duration of SD treatment. Exercised rats showed decreased 5-HT level, with potentiated effects with longer SD, indicating that exercise could improve SD rat cognitive function via deposition of 5-HT, as consistent with Na et al, who reported disrupted monoamine neurotransmitter by SD [22]. A previous study showed that co-activation of BDNF and its receptor TrkB could enhance synaptic plasticity, facilitate axonal and dendritic growth, and increase synaptic terminal density [23]. BDNF participates in the formation of

long-term memories, and plays important roles in maintaining learning/memory and regulating synaptic plasticity. This study showed significantly higher BDNF protein expression in the PFC region from exercised rats, compared to naïve SD rats, as consistent with Barnes et al, who showed that SD decreased BDNF expression of anti-oxidation potency in the rat amygdala, and physical exercise could enhance BDNF expression in SD rats [23]. These results suggested that physical exercise might exert neuroprotective functions via up-regulating BDNF expression in SD rat brain tissues, and the downstream PI3K/Akt pathway which is activated by BDNF, thus exerting certain protective functions for neural cognitive functions and synaptic transmission. Endogenous neuroprotective mechanisms in brain tissue injury are critical for brain tissue repair and regeneration. Endogenous neuroprotection showed slow initiation without exogenous intervention, and exercise intervention facilitates synaptic plasticity to improve rat memory and learning functions. SD rats showed impaired memory/learning function. This study, however, only observed the effect of fixed strength on rat memory/learning function and BDNF expression in SD rats with different SD paradigms, leaving other mechanisms to be explored.

Conclusion

SD accelerates brain damage and weakens memory or learning functions. Exercise improves memory/learning functions in SD rats, probably related with up-regulation of BDNF protein expression, anti-oxidation and regulation of central neurotransmitters. Within certain ranges, exercise has potentiated neuroprotective effects with elongated SD duration.

Disclosure of conflict of interest

None.

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