

## Original Article

# The impact of different exercise intensities on working memory and BDNF protein expression in prefrontal cortex of sleep deprivation rat

Liewei Zhang<sup>1</sup>, Minglu Tang<sup>2</sup>, Fumin Wang<sup>2</sup>, Yongjun Zhao<sup>2</sup>, Zhenhua Liu<sup>2</sup>

<sup>1</sup>College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China; <sup>2</sup>Sleep Medicine Center of Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, China

Received March 2, 2017; Accepted April 11, 2017; Epub June 15, 2017; Published June 30, 2017

**Abstract:** Sleep deprivation (SD) seriously affects animal learning and memory ability. The appropriate intensity of exercise training can alleviate SD damage on brain function. This study observed the impact of different athletic intensities on working memory and BDNF protein expression in prefrontal cortex of SD rat to investigate the effect of exercise intervention on SD rats and potential mechanism. Male Sprague-Dawley rats were randomly divided into five groups, including group A as control, group B as SD model, and group C, D, and E as low, medium, and high intensity of exercise on SD model. The rats in group C, D, and E received platform exercise for 2, 4, and 8 weeks, respectively. The SD rat model was established using flower pot method. Y maze was applied to assess the ability of learning and memory. Colorimetric method was adopted to determine serum superoxide disproportionation enzyme (SOD), malondialdehyde (MDA), and cerebral cholinesterase alkali (AChE) and 5-HT levels. Western blot was selected to detect BDNF protein expression in prefrontal cortex. The correct response rate of Y maze test, serum SOD, and BDNF protein expression were reduced, while the electric shock time, serum MDA, cerebral AChE and 5-HT, and BDNF protein expression were increased in group B compared with group A ( $P < 0.05$ ). Compared with group B, Y maze performance, serum SOD and MDA content, cerebral AChE and 5-HT levels, and BDNF protein expression were obviously improved in group C, D, and E. The moderate intensity exercise group exhibited the strongest effect ( $P < 0.05$ ). SD rat demonstrated learning and memory ability slow down. Different intensities of exercise can improve the learning, memory, and brain function of SD rat. Moderate intensity exercise exhibited the strongest impact, which may be related to the upregulation of BDNF protein and regulation of central neurotransmitter.

**Keywords:** Sleep deprivation, exercise training, BDNF, neurotransmitter

## Introduction

Sleep plays an important role in maintaining the normal physiological and psychological activities, especially in the brain function. Sleep can promote and maintain synapse formation related to learning and memory, and enhance the elimination of nerve poison. Following the increase of social competition pressure, the modern population is susceptible to be lack of sleep, resulting in diseases associated with sleep deprivation (SD) [1, 2]. SD can cause the oxidative stress in the cardiovascular system and increase the risk of oxidative stress on multiple brain regions, such as prefrontal cortex and hippocampus, resulting in nerve cell dam-

age [3, 4]. Long term SD is prone to produce negative emotion and damage learning and memory ability. The degree of learning and memory ability damage is related to SD time, while learning and memory ability decrease after SD is associated with neurotransmitter changes in the brain [5, 6]. BDNF has various pharmacological activities, such as improving neuron pathological state, promoting neuron survival and differentiation, and preventing neuronal injury. It can affect nerve cell function through a variety of signaling pathways. BDNF and N-methyl-D-aspartate receptor protein may affect learning and memory function together. Brain-derived BDNF expression is associated with anxiety behavior after SD [7, 8]. It was

# The impact of exercise on sleep deprivation

showed that exercise training has positive impact on the brain functional recovery from multiple chronic diseases including dementia. Proper physical training can improve the stress adaptive capacity. Long-term aerobic exercise can improve the rat learning and memory ability, increase cortical thickness, promote neovascularization, and improve cognitive function in SD rat [9, 10]. At present, the mechanism of exercise training on SD rat has not been fully elucidated. Fast-paced modern life and the special profession inevitably lead to SD. Observing the rats with long-term moderate intensity exercise may provide reference to investigate whether long-term moderate intensity exercise can effectively alleviate the decline of executive function. This study observed the impact of different athletic intensities on working memory and BDNF protein expression in prefrontal cortex of SD rat to investigate the effect of exercise intervention on SD rats and potential mechanism from molecule and protein levels.

## Materials and methods

### Materials

**Experiment grouping:** A total of 50 male Sprague-Dawley rats at 6-week old were provided by animal experiment center of Shandong University (SYXK-2013-0025). The rats were in SPF grade and weighted 240~260 g. The diet and drinking conformed to the experimental animal standard. The rats were randomly divided into five groups, including group A as control, group B as SD model, and group C, D, and E as low, medium, and high intensity of exercise on SD model.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University.

**Drugs and reagents:** Chloral hydrate and paraformaldehyde were got from Tianjin Kemio chemical reagent co., Ltd., SOD, MDA, AchE, and 5-HT were obtained from Nanjing Jiancheng Bioengineering Institute. Rabbit anti BDNF antibody and horseradish peroxidase labeled goat anti rabbit secondary antibody were purchased from CST. Y maze was provided by Chengdu Taimeng technology co., Ltd.

### Methods

**Exercise training:** According to the previous report [11], the rats in group C, D, and E received adaptive exercise training at 10 m/min and 45 min/d for 5 days. Next, they were treated by increasing load aerobic exercise training (gradient 0) for 2, 4, and 8 weeks, respectively. Then the SD rat model was established. The aerobic exercise plan started at 10 m/min for 1 week and followed by 2 m/min elevation every week. The speed kept when reached 16 m/min to the end of exercise. The exercise frequency was 5 d/w and 60 min/d.

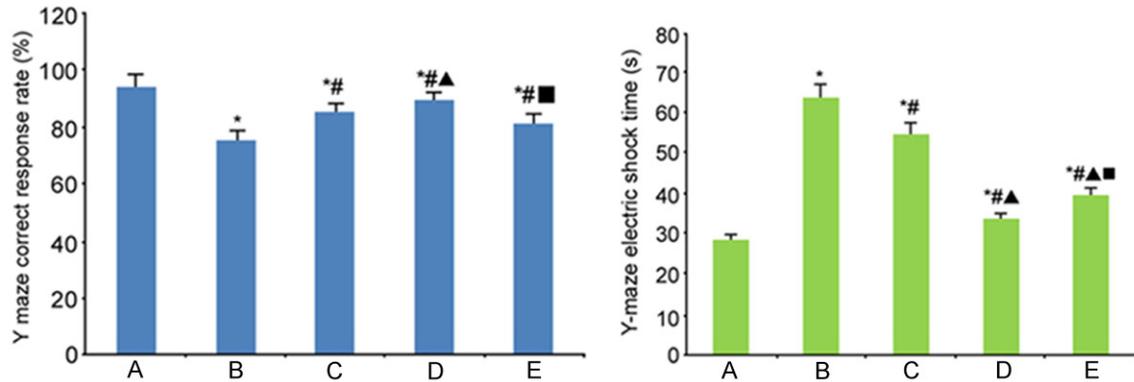
**SD model establishment:** According to the reference [12] and preliminary experiment, the rats in group B, C, D, and E received SD modeling. After adapted in water environment platform at 1 h/d for 3 days, the rat was put on the platform at 8 cm height and 7 cm diameter of the SD box at 30×30×30 cm. The rat was awaked when it entered the rapid-eye-movement sleep. The rat sleep was deprived for 48 h.

**Rat behavior test:** The correct response rate and electric shock time were observed. The working voltage was 36 V and the current was 0.7 mA. Ten times were considered as one training unit. The rat rested for 15 min and received 40 times/d for 3 days. Electric shock time = sum of 10 times of training electric shock time.

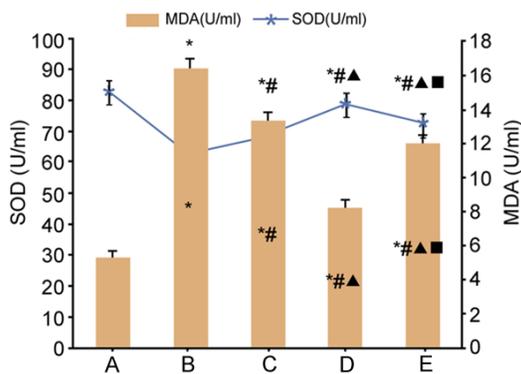
**Serum SOD and MDA measurement:** A total of 5 ml blood was extracted from the common carotid artery and centrifuged at 1500 g for 10 min to obtain the supernatant. SOD and MDA levels were tested by colorimetric method according to the manual.

**Neurotransmitter level detection in cerebral cortex and hippocampus:** The rat was killed and the brain was extracted. After removing cerebellum and olfactory bulb, the brain was peeled to obtain cerebral cortex and hippocampus under the ice-bath. The tissue was homogenized and centrifuged for 10 min to obtain the supernatant. Protein content was determined by comassie brilliant blue colorimetric method. AchE and 5-HT levels were detected according to the manual.

## The impact of exercise on sleep deprivation



**Figure 1.** The impact of different exercise intensities on learning and memory abilities of SD rat. A: Control; B: SD model group; C: SD + low intensity exercise group; D: SD + moderate intensity exercise group; E: SD + high intensity exercise group. \*P < 0.05, compared with group A. #P < 0.05, compared with group B. ▲P < 0.05, compared with group C. ■P < 0.05, compared with group D.



**Figure 2.** The impact of different exercise intensities on serum SOD and MDA levels in SD rat. A: Control; B: SD model group; C: SD + low intensity exercise group; D: SD + moderate intensity exercise group; E: SD + high intensity exercise group. \*P < 0.05, compared with group A. #P < 0.05, compared with group B. ▲P < 0.05, compared with group C. ■P < 0.05, compared with group D.

### Western blot

The prefrontal cortex was lysed and centrifuged to collect the mixture. Protein level was evaluated by BCA method. The protein was separated by SDS-PAGE and transferred to PVDF membrane. After blocked for 1 h, the membrane was incubated in BDNF and internal reference primary antibodies (1:1000) at 4°C overnight. After washed by TBST, the membrane was incubated in goat anti rabbit IgG secondary antibody (1:1000) for 1 h. At last, the membrane was washed, developed, and analyzed using Quantity One software. Relative expression level was determined as absorbance value =

BDNF absorbance value/internal reference absorbance value.

### Statistical analysis

All data analyses were performed on SPSS20.0 software. The measurement data were tested by normality test and presented as mean ± standard deviation. The data were compared by one-way ANOVA or LSD test. P < 0.05 was depicted as statistical significance.

## Results

### SD rat learning ability comparison

Compared with control, SD model group exhibited significantly lower Y maze correct response rate and increased electric shock time (P < 0.05). Exercise training group demonstrated obviously elevated correct response rate and decreased electric shock time (P < 0.05). Moderate group showed the strongest effect (**Figure 1**).

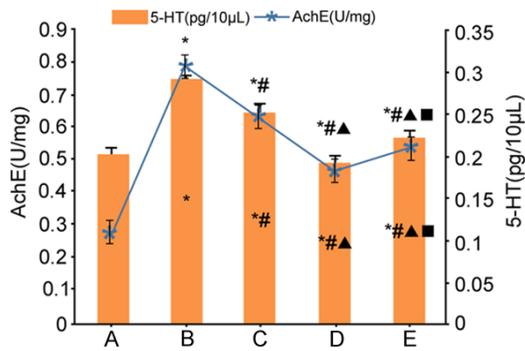
### The impact of different exercise intensities on serum SOD and MDA levels in SD rat

SOD activity was enhanced, while MDA content was declined in exercise group compared with group B (P < 0.05). Moderate group exhibited the strongest effect (**Figure 2**).

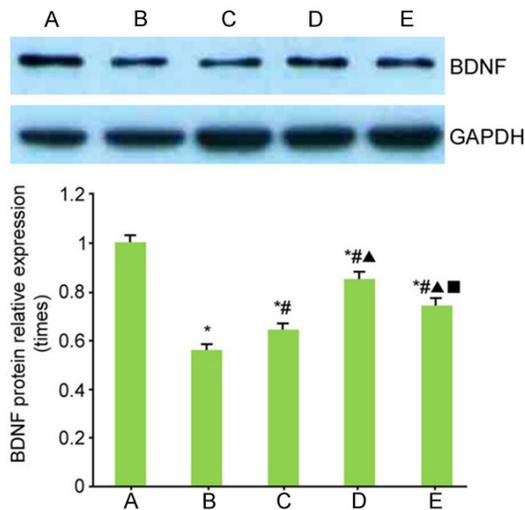
### The impact of different exercise intensities on AchE and 5-HT levels in brain tissue from SD rat

AchE and 5-HT levels were significantly elevated in brain tissues from model group compared

## The impact of exercise on sleep deprivation



**Figure 3.** The impact of different exercise intensities on AchE and 5-HT levels in brain tissue from SD rat. A: Control; B: SD model group; C: SD + low intensity exercise group; D: SD + moderate intensity exercise group; E: SD + high intensity exercise group. \* $P < 0.05$ , compared with group A. # $P < 0.05$ , compared with group B.  $\Delta P < 0.05$ , compared with group C.  $\square P < 0.05$ , compared with group D.



**Figure 4.** The impact of different exercise intensities on BDNF protein expression in prefrontal cortex from SD rat. A: Control; B: SD model group; C: SD + low intensity exercise group; D: SD + moderate intensity exercise group; E: SD + high intensity exercise group. BDNF protein relative expression in prefrontal cortex. A: Control; B: SD model group; C: SD + low intensity exercise group; D: SD + moderate intensity exercise group; E: SD + high intensity exercise group. \* $P < 0.05$ , compared with group A. # $P < 0.05$ , compared with group B.  $\Delta P < 0.05$ , compared with group C.  $\square P < 0.05$ , compared with group D.

with control ( $P < 0.05$ ). AchE and 5-HT content markedly reduced in exercise group compared with SD model group ( $P < 0.05$ ). Moderate group demonstrated the strongest effect (Figure 3).

### The impact of different exercise intensities on BDNF protein expression in prefrontal cortex from SD rat

BDNF protein expression significantly declined in prefrontal cortex from model group compared with control ( $P < 0.05$ ). It obviously enhanced in exercise group compared with SD model group ( $P < 0.05$ ). Moderate group showed the strongest effect among three groups (Figure 4).

### Discussion

Long-term SD easily leads to various problems. It was considered that appropriate aerobic exercise can reduce the negative effect of SD, resulting in enhanced secretion of the neurotransmitter [13, 14]. However, the mechanism of exercise training on SD has not been fully elucidated [15, 16]. This study used different intensity of exercise training on SD rat to observe its impact on learning and memory, oxidative stress, neurotransmitter level, and BDNF protein expression. The results showed that the Y maze correct response score in SD model group decreased compared with control. Different intensities exercise improved the Y maze test score, suggesting that exercise can alleviate nerve function damage, improve learning and memory ability, and protect brain in SD rat.

The oxidation and anti-oxidation function was kept in dynamic equilibrium under physiological condition. Long-term SD increases oxidative stress and aggravates brain damage in the body. MDA level can reflect the lipid peroxidation and cell damage degree, while SOD can block the damage of oxygen free radical on cells [17]. Our results showed MDA elevated and SOD activity reduced in brain from SD group. Different intensities exercise decreased MDA level and enhanced SOD activity in the brain, indicating that exercise can alleviate brain function damage via inhibiting oxidative stress and elevating the activity of antioxidant enzyme. Its mechanism may be related to mitochondrial ATP sensitive potassium channel and the stability of the mitochondrial membrane [18].

Neurotransmitters play an important role in the process of learning and memory. Acetylcholine in the brain is maintained by choline acetyl-

# The impact of exercise on sleep deprivation

transferase and cholinesterase. AchE can indirectly reflect the Ach level [19]. AchE activity increased in SD rat, resulting in Ach hydrolysis and central cholinergic nerve system disorder. Different intensities exercise can reduce AchE activity in the brain and improve the cholinergic neurotransmitter metabolism. Monoamine neurotransmitter plays a critical role in memory formation and maintenance. 5-HT participates in cortex-hippocampus synapse connection. The regulation of temperature, pain, and sleep were related to 5-HT. 5-HT may be the potential regulatory substance in central nervous system fatigue. It was reported that 5-HT increased in SD rat, leading to cognitive disorder [20, 21]. Our study showed that 5-HT elevated in the brain tissue from SD rat, while it declined in exercise group. Moderate group exhibited the strongest effect, suggesting that exercise intervention may improve cognitive function through promoting 5-HT accumulation.

It was revealed that BDNF and its receptor TrkB synergy may increase synaptic plasticity, promote the growth of axons and dendrites, and increase the density of the synaptic terminal [22]. BDNF participates in long-term memory formation and plays a key role in maintaining learning and memory and regulating neural synaptic plasticity. This study demonstrated that BDNF protein expression was obviously elevated in exercise group compared with SD model group, indicating that exercise can protect neuron, cognitive function, and synaptic transmission via BDNF mediated PI3K/Akt signaling pathway. The mechanism of endogenous neuroprotection in brain tissue damage is of great significance in brain tissue repair and regeneration. Endogenous neuroprotection activates slowly in the lack of exogenous intervention. Exercise intervention can promote synaptic plasticity and improve learning and memory abilities.

## Conclusion

In conclusion, learning and memory abilities declined in SD rat. Different intensities exercise improves learning, memory, and brain function in SD rat. Moderate exercise exhibited significant effect, which may be associated with the upregulation of BDNF protein and regulation of neurotransmitters.

## Acknowledgements

This work was supported by Shandong Natural Science Foundation of China (Y2006C133, Y2008SC27).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Zhenhua Liu, Sleep Medicine Center of Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, China. Tel: +86-0531-87938911; Fax: +86-0531-87938911; E-mail: zhenhualiuuu@sina.com

## References

- [1] Zhou X, Wu T, Yu J, Lei X. Sleep deprivation make the young's brain like elderly: a large-scale brain networks study. *Brain Connect* 2017; 7: 58-68.
- [2] Santos PD, Targa AD, Nosedá AC, Rodrigues LS, Fagotti J, Lima MM. Cholinergic oculomotor nucleus activity is induced by REM sleep deprivation negatively impacting on cognition. *Mol Neurobiol* 2016; [Epub ahead of print].
- [3] Chellappa SL, Gaggioni G, Ly JQ, Papachilleos S, Borsu C, Brzozowski A, Rosanova M, Sarasso S, Luxen A, Middleton B, Archer SN, Dijk DJ, Massimini M, Maquet P, Phillips C, Moran RJ, Vandewalle G. Circadian dynamics in measures of cortical excitation and inhibition balance. *Sci Rep* 2016; 6: 33661.
- [4] Kamphuis J, Baichel S, Lancel M, de Boer SF, Koolhaas JM, Meerlo P. Sleep restriction in rats leads to changes in operant behaviour indicative of reduced prefrontal cortex function. *J Sleep Res* 2017; 26: 5-13.
- [5] Legault G, Clement A, Kenny GP, Hardcastle S, Keller N. Cognitive consequences of sleep deprivation, shiftwork, and heat exposure for underground miners. *Appl Ergon* 2017; 58: 144-150.
- [6] Duan R, Liu X, Wang T, Wu L, Gao X, Zhang Z. Histone acetylation regulation in sleep deprivation-induced spatial memory impairment. *Neurochem Res* 2016; 41: 2223-2232.
- [7] Ward L, Pasinetti GM. Recommendations for development of botanical polyphenols as "natural drugs" for promotion of resilience against stress-induced depression and cognitive impairment. *Neuromolecular Med* 2016; 18: 487-495.
- [8] Baud MO, Parafita J, Nguyen A, Magistretti PJ, Petit JM. Sleep fragmentation alters brain energy metabolism without modifying hippocam-

## The impact of exercise on sleep deprivation

- pal electrophysiological response to novelty exposure. *J Sleep Res* 2016; 25: 583-590.
- [9] Demos KE, Hart CN, Sweet LH, Mailloux KA, Trautvetter J, Williams SE, Wing RR, McCaffery JM. Partial sleep deprivation impacts impulsive action but not impulsive decision-making. *Physiol Behav* 2016; 164: 214-219.
- [10] Casse-Perrot C, Lanteaume L, Deguil J, Bordet R, Auffret A, Otten L, Blin O, Bartres-Faz D, Micallef J. Neurobehavioral and cognitive changes induced by sleep deprivation in healthy volunteers. *CNS Neurol Disord Drug Targets* 2016; 15: 777-801.
- [11] Zagaar M, Alhaider I, Dao A, Levine A, Alkarawi A, Alzubaidy M, Alkadhi K. The beneficial effects of regular exercise on cognition in REM sleep deprivation: behavioral, electrophysiological and molecular evidence. *Neurobiol Dis* 2012; 45: 1153-1162.
- [12] Saadati H, Sheibani V, Esmaeili-Mahani S, Darvishzadeh-Mahani F, Mazhari S. Prior regular exercise reverses the decreased effects of sleep deprivation on brain-derived neurotrophic factor levels in the hippocampus of ovariectomized female rats. *Regul Pept* 2014; 194-195: 11-15.
- [13] Tudor JC, Davis EJ, Peixoto L, Wimmer ME, van Tilborg E, Park AJ, Poplawski SG, Chung CW, Havekes R, Huang J, Gatti E, Pierre P, Abel T. Sleep deprivation impairs memory by attenuating mTORC1-dependent protein synthesis. *Sci Signal* 2016; 9: ra41.
- [14] Grassi D, Socci V, Tempesta D, Ferri C, De Gennaro L, Desideri G, Ferrara M. Flavanol-rich chocolate acutely improves arterial function and working memory performance counteracting the effects of sleep deprivation in healthy individuals. *J Hypertens* 2016; 34: 1298-1308.
- [15] Wang L, Chen Y, Yao Y, Pan Y, Sun Y. Sleep deprivation disturbed regional brain activity in healthy subjects: evidence from a functional magnetic resonance-imaging study. *Neuropsychiatr Dis Treat* 2016; 12: 801-807.
- [16] Twycross-Lewis R, Kilduff LP, Wang G, Pitsiladis YP. The effects of creatine supplementation on thermoregulation and physical (cognitive) performance: a review and future prospects. *Amino Acids* 2016; 48: 1843-1855.
- [17] Llombart V, Dominguez C, Bustamante A, Rodriguez-Sureda V, Martin-Gallan P, Vilches A, Garcia-Berrocoso T, Penalba A, Hernandez-Guillamon M, Rubiera M, Ribo M, Eschenfelder C, Giral D, Molina CA, Alvarez-Sabin J, Rosell A, Montaner J. Fluorescent molecular peroxidation products: a prognostic biomarker of early neurologic deterioration after thrombolysis. *Stroke* 2014; 45: 432-437.
- [18] Wakai T, Sakata H, Narasimhan P, Yoshioka H, Kinouchi H, Chan PH. Transplantation of neural stem cells that overexpress SOD1 enhances amelioration of intracerebral hemorrhage in mice. *J Cereb Blood Flow Metab* 2014; 34: 441-449.
- [19] de Bruin EJ, van Run C, Staaks J, Meijer AM. Effects of sleep manipulation on cognitive functioning of adolescents: a systematic review. *Sleep Med Rev* 2017; 32: 45-57.
- [20] Na JR, Oh DR, Han S, Kim YJ, Choi E, Bae D, Oh DH, Lee YH, Kim S, Jun W. Antistress effects of *Rosa rugosa* Thunb. on total sleep deprivation-induced anxiety-like behavior and cognitive dysfunction in rat: possible mechanism of action of 5-HT<sub>6</sub> receptor antagonist. *J Med Food* 2016; 19: 870-881.
- [21] Meyer C, Muto V, Jaspar M, Kusse C, Lambot E, Chellappa SL, Degueldre C, Baiteau E, Luxen A, Middleton B, Archer SN, Collette F, Dijk DJ, Phillips C, Maquet P, Vandewalle G. Seasonality in human cognitive brain responses. *Proc Natl Acad Sci U S A* 2016; 113: 3066-3071.
- [22] Zheng CX, Lu M, Guo YB, Zhang FX, Liu H, Guo F, Huang XL, Han XH. Electroacupuncture ameliorates learning and memory and improves synaptic plasticity via activation of the PKA/CREB signaling pathway in cerebral hypoperfusion. *Evid Based Complement Alternat Med* 2016; 2016: 7893710.