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Research report

Paternal treadmill exercise enhances spatial learning and memory related to hippocampus among male offspring

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HIGHLIGHTS

- Paternal treadmill exercise improved the spatial learning and memory capability of male pups.
- Paternal treadmill exercise increased BDNF and reelin expression in the hippocampus of male pups.
- Paternal treadmill exercise can enhance the brain functions of their male offspring.

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ABSTRACT

Both epidemiologic and laboratory studies suggest that parents can shape their offspring's development. Recently, it has been shown that maternal exercise during pregnancy benefits the progeny's brain function. However, little is known regarding the influence of paternal exercise on their offspring's phenotype. In this study we attempt to determine the effects of 6 weeks paternal treadmill exercise on spatial learning and memory and the expression of brain-derived neurotrophic factor (BDNF) and reelin in their male offspring. Sibling males were divided into two groups: the control (C) and the exercise group (E). The mice in the E group were exercised on a motor-driven rodent treadmill for 5 days per week for 6 weeks. After 6 weeks of exercise, the male mouse was mated with its sibling female. After weaning, male pups underwent behavioral assessment (Open field and Morris water maze tests). Immunohistochemistry staining, real time-PCR and western blot were performed to determine hippocampal BDNF and reelin expression of the male pups after behavior tasks. Our results showed that paternal treadmill exercise improved the spatial learning and memory capability of male pups, which was accompanied by significantly increased expression of BDNF and reelin, as compared to those of C group. Our results provide novel evidence that paternal treadmill exercise can enhance the brain functions of their F1 male offspring.

1. Introduction

Regular physical exercise has been demonstrated to beneficially effect neural health and function and can protect neurons from various brain insults. Studies in humans have shown that exercise can decrease cognitive decay associated with aging [1] and reduce the risk of various neurological diseases [2]. Consistent with the human research, rodent studies demonstrate that exercise improved spatial learning and memory co-occurring with changes in hippocampal plasticity, including increased neurogenesis, enhanced long-term potentiation (LTP) [3,4], and elevated expression of brain-derived neurotrophic factors (BDNF) [5,6]. BDNF, a member of the neurotrophin family of neurotrophic factors, are mainly synthesized by hippocampal neurons and play a prominent role in the survival, growth, and maintenance of neurons during development [7] and modulation of synaptic-plasticity in the adult brain [8]. Reelin, an extracellular glycoprotein, is also crucially important in the developmental organization of neurons in the brain [9,10]. In the adult hippocampus, reelin expression occurs in interneurons residing primarily in the hilar region of dentate gyrus, and the stratum lacunosum-moleculare layer of the hippocampus proper [11]. In recent years, it has become clear that reelin not only controls neuronal migration during embryogenesis, but also promotes neuronal maturation and functions at postnatal ages. Reelin functions in the postnatal developing and adult hippocampus

Abbreviations: BDNF, brain-derived neurotrophic factor; CA, cornus ammonis; CM, paternal sedentary male pups; DG, dentate gyrus; EM, paternal exercise male pups; HFD, high-fat diet; LTP, long-term potentiation; MWM, Morris water maze.

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by affecting synaptic strength and plasticity [12,13]. Studies have shown that reelin signaling plays an essential role in both the maintenance of synaptic integrity and the behavioral expression of learning and memory in a spatial task [14–16].

Environmental factors (e.g., drugs, diet, and stress) experienced by parents can alter the development of future generations. Results from epidemiological and animal studies demonstrate that the nutritional factors during pregnancy alter development of cardiovascular, metabolic, and endocrine systems in the offspring [17–19]. A proper maternal diet is vital for the development of the central nervous system in offspring [20]. Metabolic conditions during pregnancy (diabetes, hypertension, and obesity) are associated with autism spectrum disorder, developmental delays, or impairments cognition and behavior in the offspring [21–23]. In addition, researchers found that maternal smoking or stress during pregnancy had been associated with long-term neurobehavioral and cognitive deficits in offspring [24–26]. It has been reported that regular physical activity during pregnancy is beneficial to both mother [27] and developing fetus [28]. Kim et al. suggested that swimming during pregnancy alleviates pregnancy-associated decrease in memory function of mothers through an increase in cell proliferation in the hippocampus [29]. Furthermore, exercise during pregnancy also has positive effects on the offspring. LeMoyne et al. demonstrated that short-term memory and learning ability were enhanced in rats born to mothers who exercised during gestation, and this result was seen with increased hippocampal neurogenesis in pups [28]. Akhavan et al. found that both voluntary running and forced swimming during gestation increased the quantity of cells in the cornus ammonis 1 (CA1) and dentate gyrus (DG) regions of the hippocampus of the rat pups and facilitated learning in the MWM test [30]. Few human studies, however, have investigated the relationship between physical activity during pregnancy and the offspring's cognitive functioning. Clapp et al. compared the children of women who continued to be active during their pregnancy to those of closely matched women who voluntarily stopped exercising during their pregnancy. Results demonstrated that the children of the mothers who were active during their pregnancy scored higher on the orientation and state regulation subscales of the Brazelton Neonatal Behavioral Assessment Scales [31]. LeMoyne et al. recently extended the work of Clapp by comparing the neurophysiological brain potentials of babies born to women who were active during their pregnancy to those of babies born to sedentary women. They reported that children born to mothers who exercised during their pregnancy presented shorter latencies and larger mismatch negativity amplitudes, indicating more efficient auditory memory process [32]. Jukic et al. used data from the Avon Longitudinal Study of Parents and Children (born in 1991–92) to investigate maternal physical activity during pregnancy and the offspring's language development. The most robust finding was a transient increase in offspring vocabulary score at young ages associated with maternal leisure activity [33].

Mothers are considered to be primary influencer of child neurodevelopment, with fathers influencing offspring only through modification of maternal characteristics. Previously there has been limited examination of instances whereby fathers directly influence the neurodevelopment of offspring. Despite emerging evidence that paternal age, nutrition, and drug use, may place offspring at risk of developmental issues [34,35], there is increasing evidence that paternal experiences on also influences offspring developmental outcomes. Studies show that social experiences lead to divergent phenotypes in males, and that these phenotypes have implications for the level of postnatal reproductive investment of their female mates toward offspring, with consequences for offspring development [36,37]. Studies have demonstrated the transmissibility of both depressive- and anxiety-like phenotypes to the F1 generation in male, and to a lesser extent in female offspring of socially defeated male mice [38,39]. However, little information exists about the effects of paternal exercise on cognitive brain function in offspring. It is therefore of great interest to define the transgenerational effects of paternal environmental conditions on mammals and the underlying mechanisms that may mediate these effects. We investigated whether paternal exercise positively influences learning and memory abilities of the brain of the offspring. Thus, we studied the effects of paternal treadmill exercise on spatial learning and memory by assessing performance in the open field and MWM tests. Our results showed that paternal exercise had significant effects on the spatial learning and memory of male pups, along with increased expression of hippocampus BDNF and reelin compared with the control group. Our results provide new insights into the relationship between parental experience and the brain development of offspring.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (8 weeks old, $n = 10$) and female C57BL/6 mice (8 weeks old, $n = 10$) were obtained from Beijing HFK Bioscience Limited Liability Company. Mice were housed in vented cages in a temperature-controlled room (20–23 °C; 35–55% humidity) with a 12-h light/dark cycle and free access to food and water. At the beginning of the experiment, mice were acclimated to a control diet for 7 days and then were mated. During mating, one male and one female were housed together. After a vaginal seminal plug was observed, male was removed and the pregnant female left alone. Litter size was adjusted to 8 pups with 4 males and 4 females. All offspring were weaned at 3 weeks of age. For all comparisons shown, sibling males were divided into two groups: the control group (C) and the exercise group (E). Mice randomized to the E group underwent several acclimation exercise sessions on a motorized treadmill (electrical stimulus) at 10 m/min (0% grade) for 20 min during the first week. Thereafter, the mice underwent 6 weeks of treadmill training at 12 m/min (75% VO2max) for 60 min/day, 5 days/week on a 0% grade [40]. Meanwhile, the females were kept sedentary. After 6 weeks, mice were mated as shown above. After weaning, male offspring from the control (CM, $n = 18$) or the exercise group (EM, $n = 16$) were housed individually and underwent behavioral assessment. Body weight was obtained and recorded once per week. Each mouse was placed in the experimenter's hand for 5 min sessions, 2–3 times per day. We found that considerable handling was required for mice to habituate to the experimenter and the general testing conditions. Beginning at postnatal day 22, each cohort of mice was tested in behavioral tests. The inter-task interval was 2 days. Following the behavioral experiments, four mice from each group were randomly chosen for anatomical assays. Animals were deeply anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine) prior to undergoing transcardial perfusion with 4.0% paraformaldehyde in 0.1 M phosphate buffered saline (PBS). After removal, brains were post-fixed in buffered 10% formalin at room temperature, embedded in paraffin, and then cut into sections 6 μm thick. For molecular and protein analysis, hippocampus were rapidly dissected out, immediately placed on dry ice, and frozened with liquid nitrogen, then stored at −80 °C until use (CM, $n = 14$; EM, $n = 12$). All experimental procedures were approved by Tianjin Institute of Physical Education Animal Care and Use Committee under the guidelines of the Chinese Academy of Sciences.

2.2. Open field test

Locomotor activity was measured using an open field test system. The open field test was performed according to a previously described protocol [41]. The open field consists of a round arena (Diameter = 100 cm) covered by a white plastic floor, surrounded by a 50 cm high sidewall made of white polypropylene. The floor of the arena was divided into 25 equal squares. Male offspring at 22 days old (CM, $n = 18$; EM, $n = 16$) were tested. The test started by placing a mouse at the center of the arena and the testing sessions lasted for 5 min. The number of crossings (defined as at least three paws in a quadrant) and the number of rearings (standing upright on the hind legs, while forepaws were free) were scored manually. During the interval of the test the apparatus was cleaned with a 10% ethanol solution. The test was carried out in a temperature, noise, and light-controlled room.

2.3. Morris water maze

To evaluate the effect of paternal exercise on memory functions, all male pups at 25 days old were tested for spatial memory acquisition and retention using the MWM. As previously described [42,43], the water maze model was performed in a circular tank (diameter 0.9 m) filled with opaque water. A platform (6 cm × 35 cm) was submerged below the water's surface in the center of the target quadrant. The swimming path of the mice was recorded by a video camera and analyzed by Videomot software (Huaibei Zhenghua Company, China). For each training session, the mice were placed into the maze consecutively from four random points of the tank.