Effects of treadmill exercise intensity on spatial working memory and long-term memory in rats

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ARTICLE INFO

ABSTRACT

Aims: Moderate exercise promotes learning and memory. Most studies mainly focused on memory exercise effects of in the ageing and patients. There is lack of quantitative research about effect of regular exercise intensity on different memory types in normal subjects. Present study investigated the effects of different intensities of treadmill exercise on working memory and long-term memory.
Main methods: Fifty female Wistar rats were trained by T-maze delayed spatial alternation (DSA) task with 3 delays (10 s, 60 s and 300 s). Then they got a 30 min treadmill exercise for 30 days in 4 intensities (control, 0 m/min; lower, 15 m/min; middle, 20 m/min, and higher, 30 m/min). Then animals were tested in DSA, passive avoidance and Morris water maze tasks.
Key findings: 1. Exercise increased the neuronal density of hippocampal subregions (CA1, CA3 and dentate gyrus) vs. naïve/control. 2. In DSA task, all groups have similar baseline, lower intensity improved 10 s delay accuracy vs. baseline/control; middle and higher intensities improved 300 s delay accuracy vs. baseline/control. 3. In water maze learning, all groups successfully found the platform, but middle intensity improved platform field crossing times vs. control in test phase.
Significance: Present results suggested that treadmill exercise can improve long-term spatial memory and working memory; lower intensity benefits to short-term delayed working memory, and middle or higher intensity benefits to long-term delayed working memory. There was an inverted U dose-effect relationship between exercise intensity and memory performance, but exercise -working memory effect was impacted by delay duration.

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1. Introduction

Studies on patients with memory disorders [1–3], senior citizens [4,5] and animals [6] have shown that moderate exercise, especially aerobic exercise (e.g. jogging), improves learning and memory and other cognitive abilities [7]. And there were plenty of references investigated the effects and underlying mechanisms of aerobic exercise on learning and memory [7]. Memory can be classified into two basic forms: long-term memory and working memory [8]. Long-term memory, also known as reference memory, stores effective information for long period of time [8]. Whereas, as the basis of many higher brain functions, working memory is a kind of short-term memory with limited capacity responsible for transient holding and processing of newly acquired or already stored information [9,10]. These two kinds of memories have different neuroanatomical basis and molecular mechanisms [8]. Long-

term memory, especially declarative or explicit memory, is hippocampus dependent [8]. Working memory is prefrontal cortex (PFC) dependent [10,11], and relying on the involvement of hippocampus in some cases [12–15]. It has been proven that aerobic exercise benefits long-term memory [16,17] and working memory [1–4] in human participants with or without memory impairment. Animal models experiments also proved that exercise can promote expression of brain derived neurotrophic factor (BDNF) [18–21], insulin-like growth factor-1 (IGF-1) [22,23], vascular endothelial growth factor (VEGF) [18], and many other molecules or neurotransmitters [19,24], which are the crucial to enhance synaptic plasticity, neurogenesis and cerebrovascular development, then to alleviate disorders or restore the impaired brain and cognitive functions [7,20,25,26]. Therefore, exercise may also affect working memory and long-term memory by altering the structure and physiological activity of the hippocampus and the PFC in a similar way [20,21,25,27–32].

However, there is an unsolved question on the relationship between the exercise style, intensities and memory. Firstly, acute and regular exercises have different effects on memory. Compared with acute exercise,

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regular exercise can produce long-lasting influence on the physiological activities and brain plasticity [33,34]. In most reports, moderate acute exercise improves working memory [32,35,36], long-term memory [16,17], but much higher intensity exercise would temporarily suppress or permanent impair cognitive performance [37–40] and cause excessive neuronal apoptosis [41–43]. Some researchers have reported an inverted U-relationship between the acute exercise intensity and cognitive performance, and proposed several hypotheses to interpret this effect [35, 44–46]. In consideration of the long-lasting positive/negative effects of moderate/excessive regular exercise to brain and cognition of normal human or patients, we paid more attention to regular exercise and its effect in memory. Quite naturally, we could imagine a similar inverted U relationship between regular exercise and memory. Moreover, some researches have indicated this possibility [26,40,47,48]. Two papers reported that 14 days lower but not higher intensity of treadmill exercise improved spatial memory in Morris water maze (MWM) and hippocampal dendritic complexity, BDNF, cyclic AMP response element binding protein (CREB) and postsynaptic density protein 95 (PSD-95) levels in rats with traumatic brain injury or cerebral ischemia [26,47]. However, compared with detailed researched acute exercise, there is lack of systemic studies on the effect of regular exercise intensity on memory. So the first aim of present study is to investigate the inverted U hypothesis on regular exercise intensity interacting with memory performance (including working memory and long-term memory).

Delayed response task and delayed spatial alternation (DSA) task are the frequently used working memory tasks in animal research [12,49, 50]. And "delay" is an important control variable in these tasks, on behalf of relevant information is holding in working memory to direct the forthcoming behavior [10,51]. And the working memory performance often negatively correlates with the delay duration [12,49]. According to Yerkes–Dodson law [52,53], the effect of exercise intensity on working memory should be influenced by delays. Therefore, our second aim was to verify the role of delay in the exercise effect on working memory. To address these questions, we designed treadmill exercise with lower-, middle- and higher-intensities, and used spatial T-maze DSA task, MWM task and passive avoidance (PA) task to explore the effects of regular exercise intensities on (1) spatial working memory with three kinds of delays (10 s, 60 s and 300 s), (2) long-term spatial memory, (3) passive avoidance memory and (4) hippocampal neuronal density. Present study will provide first hand animal experimental data to shed some light on regular exercise intensity effect on memory.

2. Materials and methods

2.1. Animal subjects

Fifty adult female Wistar rats (8 weeks, 186–225 g, purchased from Dashuo Experimental Animal Co. Ltd., Chengdu, China; license number,

SCXK (Chuan) 2014-002) were allowed to accommodate for a week before experiments. Rats were housed in an environment with 12 h/12 h dark/light cycle and at a temperature of 22 ± 1 °C. Experiments started at 09: 00. All experimental procedures were in accordance with *the Regulations of Laboratory Animals Care of Yunnan Province, China*. Animals were randomly divided into five groups with 10 rats in each group: naïve (only used for histological examination), control, lower, middle, and higher groups (Fig. 1A).

2.2. Methods

2.2.1. DSA task

2.2.1.1. Appliance. A black wooden T-maze was placed in the middle of a room on a shelf 60 cm elevated from the floor. Three sides of the room had a chest, bookshelf, etc. against the walls and one side had a window. The T-maze consisted of a major arm (90 cm × 13 cm × 20 cm) and two perpendicular side arms (65 cm × 13 cm × 20 cm). A food container (depth: 1 cm, diameter: 3 cm) was fastened at a point 2.5 cm away from the end of each side arm, respectively. An initial compartment (length: 25 cm) on the major arm was partitioned by a dodge gate. Two fluorescent lamps (40 W) were hung over the maze to illuminate and avoid casting shadows.

2.2.1.2. Experimental procedures. Discrete DSA task was applied and the procedures in reference of [12,13,49] were followed with some modification. All animals were trained by an appointed experimenter and were fed with restriction to control body weight gains. Animals were trained for 5–7 days per week and training was done before feeding. Animals were allowed to accommodate with the maze for 5 days until they could feed from the food container. During the accommodation phase, animals were allowed to explore the maze freely and the rewarding food (sunflower seed kernels) was initially scattered on all three arms, then on the two side arms, and finally placed only in the food containers. The training was started right after accommodation and each trial included one sample choice and one test choice. During the sample choice, one side arm was closed; the animal was rewarded if it enters the opened arm. After a delay with the animal in the initial compartment, the dodge gate and both of the side arms were opened, then, if the animal chose the closed arm earlier, it was rewarded for 3 s; otherwise, it was retained without reward for 3 s. The time from the opening of the dodge gate to the animal reaching the food container was recorded as the response time. The animal was placed back in the initial compartment, the dodge gate closed, and the junctions of major arm to side arms were wiped with alcohol to eliminate odors. The delay of initial training was 10 s, and 10 trials were performed per day as a session. The order of closed side arm in sample choice was arranged semi-randomly. After the average accuracy of the four groups reached 80%
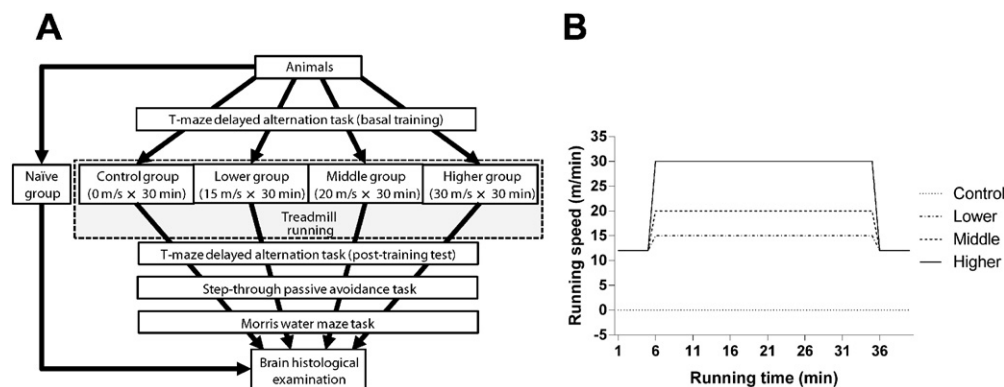

Fig. 1. Schematic diagram of animal groups and experimental timeline (A) and treadmill running speeds plan in a training phase (B) Animals in each group (n = 10) were trained by treadmill and three behavioral tasks except the naïve only for histological examination. In treadmill exercise, the running process included three processes: the first is warm-up (12 m/min for 5 min), then high speed running (30 min), and cool-down (12 m/min for 5 min) at last.

for 3 successive days, animals were respectively tested with prolonged delay of 60 s for 3 successive days, then with 300 s in the same way. Afterwards, animals were fed ad libitum and treadmill training was started. After completion of treadmill training, animals were restriction fed again and put through DSA task. After the accuracy of control group in the 10 s delay reached 80% for 3 successive days, the delays of 60 s and 300 s were tested for 3 days each. The last day's performance data of each delay in the 3 days were used for data analysis.

2.2.2. Treadmill exercise

2.2.2.1. Appliance. A six-lane flatbed treadmill (ZH-PT, Zhenghua Biological Instrument Co. Ltd., Huaibei, China) for rats was used.

2.2.2.2. Treadmill training. Control rats were placed on the uninitiated treadmill for 40 min every day at the same time. The running time and speed in exercise groups were increased progressively until the maximum speed of lower-, middle- and higher-intensity groups reached 15 m/min, 20 m/min and 30 m/min respectively and was then maintained at these levels until the 30th day of training. According to Bedford et al. [54], our design should elicit about 40–50%, 60%, or 75% of rat's maximal oxygen uptake (VO2max), respectively. The pattern of daily training included a 5 min warm up (12 m/min), 30 min speed running and a 5 min cool down (12 m/min), which is to decrease the incidence of sickness (Fig. 1B). Animals were fed ad libitum during treadmill training. The running motivation of the animals was enhanced by direct current shock (0.1–0.15 mA) at the lane terminal.

2.2.3. PA task

2.2.3.1. Appliance. The one-trial step-through method was used in PA task and the dual-purpose avoidance appliance (GEMINI, San Diego Instruments Inc., San Diego, USA) was applied in training and testing. The appliance consisted of a lit room (illuminated by overcasting incandescent lamps) and a dark room of equal size and partitioned by an automatic overhead door. The floors of the two rooms were connected with electronic stimulators.

2.2.3.2. Behavioral testing. The whole procedure lasted three days. Day 1 was the accommodating period where the animal was placed in the light room for 5 min with the door opening. Day 2 was the training period where the animal was placed in the light room against the opening door and the time (latency) from the animal being placed in the light room to entering the dark room was recorded. After the animal had its four limbs stepped into the dark room, the door was closed immediately and then the animal was shocked (electric current: 0.9 mA/3 s). The animal was returned to home cage 5 s after shock. After each trial, the floor of the appliance was wiped with alcohol to eliminate odors. The animal was exposed to the same procedure again 24 h later (Day 3) but without shock. Latency for the animal entering the dark room was recorded. If the animal did not enter the dark room within 300 s, the latency was scored as 300 s.

2.2.4. MWM task

2.2.4.1. Appliance. A circular stainless steel tank (∅ 1.8 m; depth 0.6 m) was used in the MWM task. The tank was filled with water to a depth of 30 cm and a layer of black resin particles floated on the water surface to block animals seeing the underwater platform. The water and room temperature were adjusted to 22 ± 1 °C. The tank was surrounded by a curtain ornamented with several marked visual cues. In the tank, there was a circular transparent plastic platform (∅ 12 cm). A camera above the tank was used to record the swimming track of the animal. Behavioral data were recorded and analyzed by a computer installed with a visual analyzing system (Xeye™ ABA, MacroAmbition S&T Development Co. Ltd., Beijing, China).

2.2.4.2. Long-term spatial memory training. The classic MWM training method was used [55]. The day before formal training, the animal was gently released into the water without a platform, facing the wall with its tail touching the water first. The animal was allowed to swim freely for 120 s to get used to the tank environment. The whole water surface was equally divided into four quadrants (southeast, southwest, northeast and northwest) without physical boundaries. The platform was fixed at the center of the southeast quadrant, 1.5 cm underneath the water surface. Animals were semi-randomly released into the water from east, south, west and north directions, respectively. Each animal was trained 4 times (60 s for each, interval = 5 min) per day for 6 successive days. During training, the animal was allowed to rest for 10 s when it found the platform; otherwise, if it could not find the platform within 60 s, the experimenter would lead it to the platform and let it rest for 20 s. If the animal found the platform within 10 s, we assumed that the platform location had been remembered.

2.2.4.3. Long-term spatial memory testing. When the platform was retrieved from the tank 24 h after training, the animal was allowed to explore the tank for 60 s; then the time it spent in the quadrants that had previously contained the platform, as well as the frequency that it crossed this same field, were recorded to evaluate spatial memory of platform location.

2.2.4.4. Visible platform training. The platform with a striking flag was fixed in the center of the northwest quadrant, 1 cm above the water. During training, the animal was take out from the water immediately after it found the platform; otherwise, data from the animals that failed to find the platform within 60 s were excluded from data analyses. Each animal was trained four times with 5 min intervals.

2.2.5. Histological examination

After behavioral testing, animals were euthanized by anesthesia (mebumal sodium solution, 60 mg/kg, Merck, Germany) and fixed by perfusing normal saline and formaldehyde solution (4%) through the ascending aorta. Brain tissues were fixed in formaldehyde solution (4%) for one week. After coronal slices (30 μm) were prepared using a vibratome and stained by cresyl violet (1%, Sigma, USA), the neurons in the dorsal/ventral CA1 area, CA3 area, and dentate gyrus (DG) of the hippocampus were observed under 40 × objective microscope. The number of the pyramidal neurons and granular neurons within an 180 μm long cell stripe in six hippocampal subregions (dorsal CA1 (dCA1), dorsal CA3 (dCA3), dorsal DG (dDG), ventral CA1 (vCA1), ventral CA3 (vCA3), ventral DG (vDG)) were counted.

2.2.6. Statistical analyses

All data expressed as mean ± SEM. Most data were repeated measurements and the general linear model of the multivariate test with repeated measures (GLM-RM) and paired-sample t-test were used. Comparisons among groups were conducted using independent sample t-tests and one-way analysis of variance (ANOVA) with post hoc analysis (Dunnett t-tests). P < 0.05 was considered a significant difference standard.

3. Results

3.1. Treadmill exercise increased neuronal density of the hippocampus

The GLM-RM results showed that, during T-maze DSA task, the body weight of subjects did not change significantly; during treadmill training, because the rats were fed ad libitum, their body weight increased ($F_{1,3} = 324.788$, P = 0.000). We assume that body weight gain was an outcome following food restriction. The treadmill exercises with different intensities had no effects on body weight ($F_{1,3} = 0.332$, P = 0.802, Fig. 2A). During treadmill exercise, most of the animals built avoidance reflex successfully and ran actively. The data from three

animals which could not run due to physical conditions were discarded. Histological checking showed that neurons in the stratum pyramidale of CA1, CA3 and stratum granulosum of DG in the six hippocampal subregions (dCA1, dCA3, dDG, vCA1, vCA3, vDG) had normal and similar shapes. However, the neuronal number in most subregions from exercise groups were larger than inactive rats (ANOVA: All $F_{4,20} \geq 3.746$, $P \leq 0.020$, vs. naive group; all $F_{4,20} \geq 4.073$ (except dCA3 and dDG), $P \leq 0.025$ vs. control group, Fig. 2B). There was no significant difference between naïve and control groups in all hippocampal subregions (All $P > 0.346$), which indicated that only behavioral training in DSA task, MWM task and PA task cannot apparently change the hippocampal neuronal density in present situation.

3.2. T-maze DSA task

3.2.1. Treadmill exercise improved DSA task performance

GLM-RM results show that the delay was one main factor affecting accuracy in DSA task ($F_{1,3} = 83.409$, $P = 0.000$), suggesting that performance was in accordance with the phenomenon whereby prolonged delay improves the task difficulty or load of working memory [49]. Strong interactions were found between 'treadmill exercise' and 'exercise intensities' ($F_{1,3} = 5.759$, $P = 0.003$), as well as among 'delay', 'treadmill exercise' and 'exercise intensities' ($F_{1,3} = 6.269$, $P = 0.002$), indicating that the accuracy of DSA task is correlated with the delay, exercise and exercise intensity. The GLM-RM results showed that although delay was not the main factor influencing relative accuracy among different groups ($F_{1,3} = 0.647$, $P = 0.427$), strong interactions were found between 'delay' and 'intensity' ($F_{1,3} = 4.696$, $P = 0.008$), indicating that exercise intensities have different effects on relative accuracy with different delays. One-way ANOVA and paired t-tests showed that the fact of taking exercise (All $T \leq 2.106$, $P \geq 0.080$) and exercise intensity (All $F_{3,30} \leq 0.774$, $P \geq 0.518$) had no effect on reaction time in the DSA task across different delays.

3.2.2. The effects of delay and exercise intensity on the accuracy of DSA task

The results of the DSA task prior to treadmill exercise showed that all animals had comparable accuracy across three different delays (ANOVA, all $F_{3,33} \leq 1.528$, $P \geq 0.226$), indicating that all the animals had comparable learning and memory ability before treadmill exercise. In tests after treadmill exercise, differences were found in sessions using delays of 10 s and 300 s, respectively (ANOVA: 10 s, $F_{3,33} = 3.600$, $P = 0.024$; 60 s, $F_{3,33} = 0.149$, $P = 0.930$; 300 s, $F_{3,33} = 5.303$, $P = 0.004$). The post hoc results showed that in sessions with 10 s delay, accuracy of the lower group were higher than that of the control ($P = 0.037$). In sessions with 300 s delay, accuracy of middle and higher groups was higher than the control (All $P = 0.017$). In sessions with 60 s delay, accuracy of all the three exercise groups was only slightly higher than the control

(all $P \geq 0.844$). Paired t-test showed that compared with basal performance, post-exercise accuracy from the lower group with 10 s delay ($T_9 = 3.051$, $P = 0.014$), the middle ($T_9 = 3.545$, $P = 0.006$) and higher ($T_9 = 2.512$, $P = 0.033$) groups with 300 s delay was increased; the control group slightly decreased across the same measures (all $T \leq 1.622$, $P \geq 0.156$; Fig. 3A–D).

To eliminate influences from basal contrasts, original data were converted into relative accuracy and similar results were obtained in comparisons among groups (10 s, $F_{3,33} = 4.384$, $P = 0.011$; 60 s, $F_{3,33} = 0.513$, $P = 0.676$; 300 s, $F_{3,33} = 3.575$, $P = 0.024$). The post hoc results showed that with a 10 s delay, the accuracy of the lower group was higher than the control group ($P = 0.007$); with a 300 s delay, accuracy of the middle group was higher than the control group ($P = 0.045$); with a 60 s delay, accuracy of all three exercise groups was only slightly higher than the control group (All $P \geq 0.524$ Fig. 3E).).

3.3. Treadmill exercise has no effect on performance during the retention period of PA task

In training phase of PA task alone with electrically shock, all animals stepped into the dark room within 10 s, no differences among groups were found ($F_{3,33} = 0.417$, $P = 0.742$), indicating that all animals were normal in motor and visual ability. Tests during the retention phase showed that most of the animals spent approximately 300 s in the lit room, significantly longer than that in training phase (paired t-test, all $T_9 \geq 21.497$, $P = 0.000$) and no differences among groups were found (ANOVA: $F_{3,33} = 0.818$, $P = 0.493$; Fig. 5).

3.4. Treadmill exercise improved performance in a MWM task

After the 6-day training all animals were able to find the hidden platform within 10 s. GLM-RM results showed that the times of training has a significant effect on the time spent for searching for the platform ($F_{1,3} = 42.491$, $P = 0.000$) and swimming distance ($F_{1,3} = 54.526$, $P = 0.000$), but no differences were found between groups (Time, $F_{1,3} = 0.134$, $P = 0.939$; Distance, $F_{1,3} = 0.376$, $P = 0.771$, Fig. 5A). During testing, all groups spend most of the time in the target quadrant (the quadrant with the platform), indicating that all of them acquired memory of the location of the target quadrant (paired t-test, all $T \geq 3.516$, $P \leq 0.005$, compared with the quadrant at the opposite angle); no differences were found between groups (ANOVA: $F_{3,32} = 1.303$, $P = 0.291$, Fig. 5). However, differences among groups were found for the frequency that animals crossed the platform field (ANOVA: $F_{3,32} = 6.438$, $P = 0.002$). Post hoc results showed that the middle group got across the platform field more often than control ($P = 0.002$), indicating that these animals acquired more accurate spatial memory of the platform location (Fig. 5C–D).
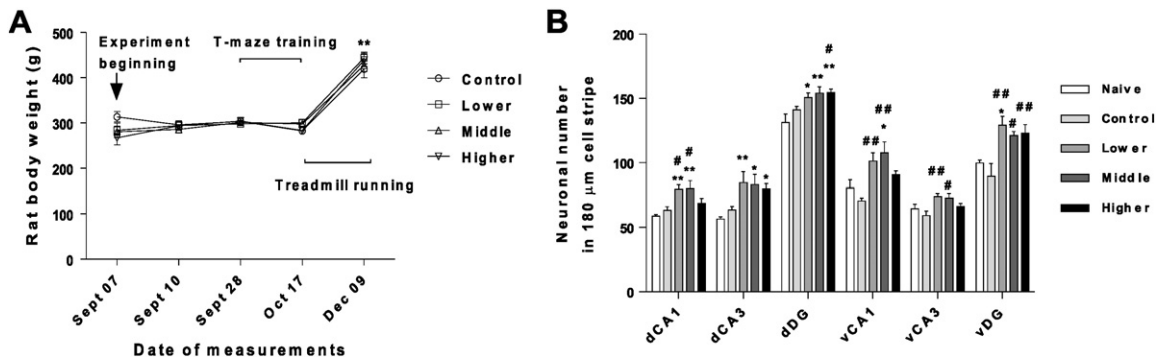

Fig. 2. Animal weight changing (A) and the effects of treadmill exercise on relative accuracy of T - maze DSA task (B). A. All rats habituate laboratory and were food restricted to T-maze delayed spatial alternation (DSA) task, so the body weight did not increase before treadmill exercise (feed freely). B. Histological examination showed the hippocampal neuronal density significantly increased in the dorsal/ventral CA1/CA3 and dentate gyrus (DG) subregions after exercise in all exercise group, esp. under the middle and lower intensity. **, P < 0.01 vs before treadmill exercise training (A); *, P < 0.05, **, P < 0.01 vs. Naïve (B); #, P < 0.05, ##, P < 0.01 vs. Control; all data were shown as mean ± SEM.
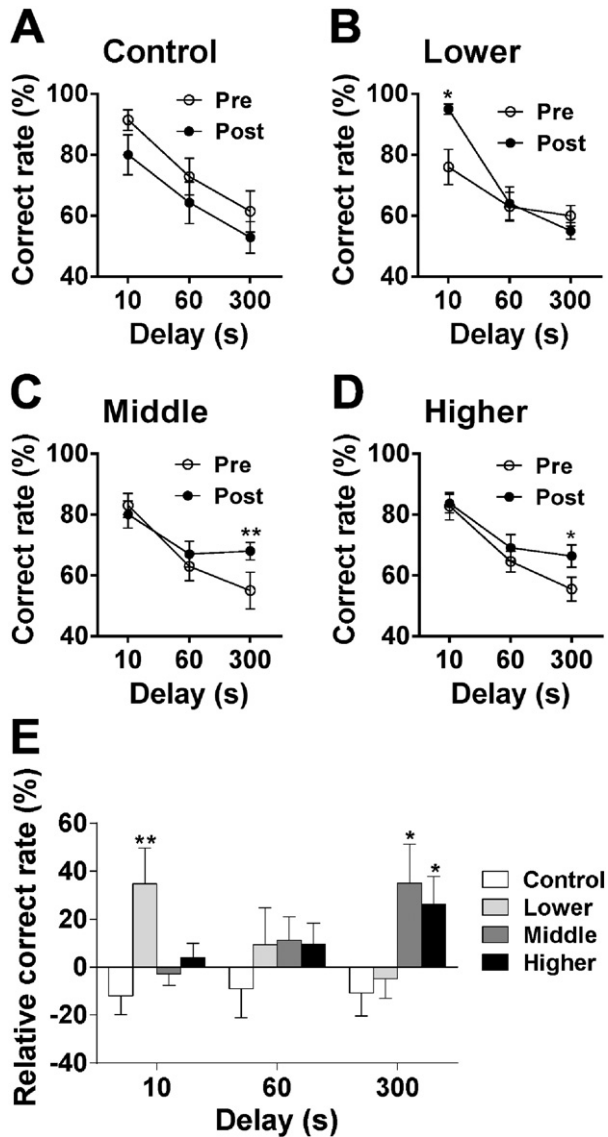
Fig. 3. The absolute accuracy (correct rate (%)) pre- and post-treadmill exercise (A–D) and the relative accuracy of T - maze delayed spatial alternation task with different delays (E). Lower intensity at 10 s delay (B), and middle (C) and higher (D) intensities at 300 s delay improved performance than pre-exercise. And the relative performance was also improved at 10 s delay (lower) and 300 s delay (middle and higher) than control (E). Pre, pre-exercise; post, post-exercise; *, P < 0.05, **, P < 0.01, vs. pre-exercise (A–D); *, P < 0.05, **, P < 0.01, vs. control (E); all data were shown as mean ± SEM.

Although remarkable training effects (GLM-RM, time, $F_{3,9} = 22.274$, P = 0.000; distance, $F_{3,9} = 23.718$, P = 0.000) were observed during the visible platform test, no differences were found among groups (time, $F_{1,3} = 2.154$, P = 0.113; distance, $F_{1,3} = 2.062$, P = 0.125) (Fig. 5E), indicating that all animals were normal in vision and were able to locate the platform according to visual cues.

Analysis of swimming speed during MWM task showed that swimming speed was relatively stable during training (33.91 cm/s), faster during memory testing (40.51 cm/s) and slow during visible platform testing (25.77 cm/s). In training phase, differences in swimming speed were found for training effects (GLM-RM, $F_{1,3} = 9.119$, P = 0.005) and among groups ($F_{1,3} = 5.325$, P = 0.004). Post hoc results showed that swimming speeds in the lower and middle groups were higher than the control group (all P ≤ 0.046). In memory testing phase, differences among groups were found, i.e., the speed of the middle group was slower than control group (GLM-RM: training effect, $F_{1,3} =$

16.866, P = 0.247; among groups, $F_{1,3} = 2.958$, P = 0.047), and in accordance with post hoc results (P = 0.034). Training effects were also found in the visible platform tests (GLM-RM: training effect, $F_{1,3} = 28.331$, P = 0.000), but no differences among groups ($F_{1,3} = 2.534$, P = 0.074). In summary, swimming speeds in the exercise groups were higher than the control group during the training period, but were lower than that of the control group during memory testing and visible platform testing (Fig. 5F).

4. Discussion

We showed that among groups with different exercise intensities no differences could be found in body weight (Fig. 2A), latency to PA training (Fig. 4), searching time for the visible platform (Fig. 5E). Present results mainly indicate that one month regular treadmill exercise improved spatial long-term memory, working memory and hippocampal neuronal density, which were consistent with many previous reports [18,20,21,25,31,48,56–58]; and the alternating body weight, motor ability should not important factors in memory enhancement in present study.

4.1. Effects of treadmill exercise on hippocampal neuronal density

The hippocampal neuronal density enhancement should be an important basis for improved learning and memory [25], which should be derived by adding effects of two aspects: neurogenesis including increase of newborn neuron survival and decrease of existing neuron apoptosis [28,29,59]. In present study, the neuronal density in the six subregions of hippocampus from the three exercise group was higher than that in control or naïve rats, suggested regular treadmill exercise can promote neurogenesis (such as in the DG) or protect the existing neurons from apoptosis (such as in the CA1 and CA3 areas). This result was consistent with previous work from adolescent [18] or adult rodents [25,29,59].

4.2. Effects of treadmill exercise on different types of long-term memories

In the MWM task, the animal needs to remember the location of the hidden platform, which is a typical spatial memory task [55]. In present study, no differences were found in the performance of animals during MWM and PA training, suggesting that learning ability (MWM; consistent with [60,61]) and motor and visual functions were not affected by regular exercise. In MWM task, although we did not observed the difference of spatial learning and time spent in the target quadrant, as reported by two papers [60,61], but the more crossing times through platform field in testing phase indicated middle intensity rats formed more accurate spatial memory than others. These results are consistent with lots of previous studies, in which moderate exercise can improve of spatial
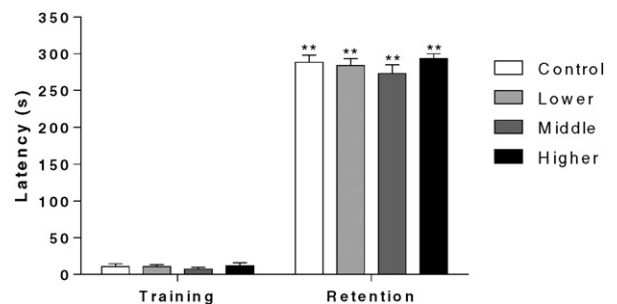

Fig. 4. Effects of treadmill exercise on the latencies in training and retention phases of passive avoidance task. All animals can normally learn the passive avoidance task. And there was no difference among groups. **, P < 0.01, vs. training phase; all data were shown as mean ± SEM.
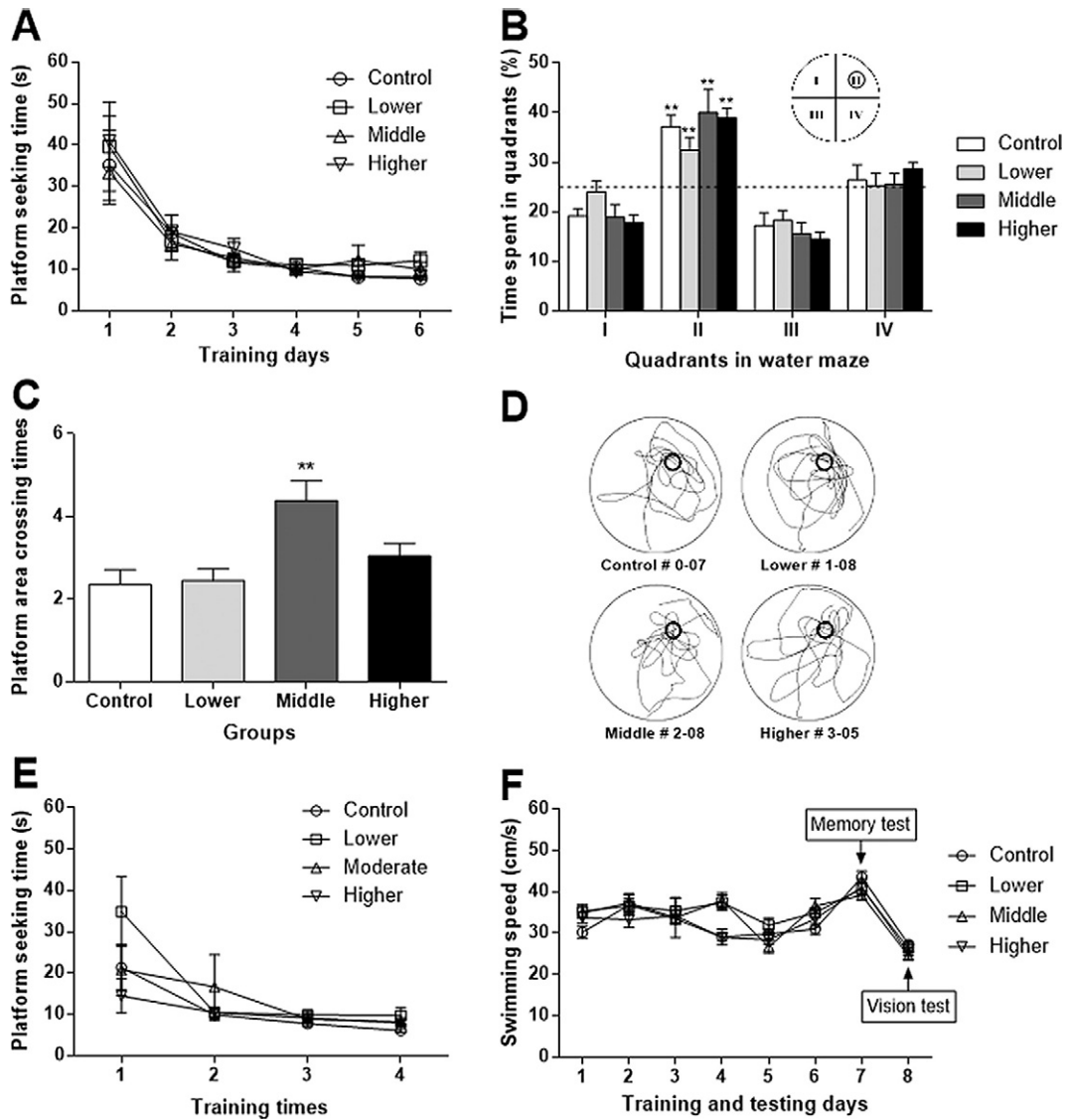
Fig. 5. Effects of treadmill exercise on the Morris water maze task. A. Time of seeking the submerged platform in training phase; B. Time (%) spent in quadrants in memory testing phase, all groups spent more time in the target quadrant (quadrant II) than in diagonal quadrant (**, P < 0.01); C. Crossing times through the platform field in memory testing phase, middle group showed more crossing times than control (**, P < 0.01); D. Examples of the track diagram in memory testing phase; E. Time of seeking the visible platform; F. Swimming speeds in the total MWM task. All data were shown as mean ± SEM.

memory in normal animal or animal models with memory disorders [47,48,56,57,60,61].

In PA task, the animal needs to learn to relate the pain and fear caused by electric shock with the environment of the dark room for building PA memory. Therefore, PA memory is a fear-related long-term memory. The retention latency to enter the dark room in all animals in PA task was increased over training phase, but no difference was found among groups. These data show that the training intensities used here did not affect PA memory; or perhaps the PA task is insensitive to present exercise intensity (which may be the outcome of a "ceiling effect" because the PA task is so easy for rats to learn). Present neuronal density data and PA performance in normal animal are consistent to the works of Kim et al. [29, 59], in the latter, treadmill exercise did not affect the high retention latency of normal rats in passive avoidance task, but increased neurogenesis and suppressed neuronal apoptosis in hippocampus.

The data of present study suggest that middle intensity treadmill exercise is beneficial to long-term spatial memory, but the effect on PA long-term memory requires further work. According to our present MWM data and previous work [47,48], an inverted U relationship

should be exist between the regular exercise intensity and spatial long-term memory performance which was identical to our hypothesis.

4.3. Treadmill exercise and working memory

To successfully complete the mission in the DSA task the animal is required to transiently hold and process new spatial location information [9,12]. In the DSA task here, we tested working memory at three different delays (10 s, 60 s and 300 s). In general, the longer the delay the more difficult it is to remember, and the poorer the DSA performance [49]; this was observed in present results (Fig. 3A–D). We found that different intensities had different effects on DSA task performance with different delays: the DSA scores at 10 s delay and 300 s delay were increased by lower-intensity exercise and middle-/higher-intensity exercise, respectively, and the improvement effects from higher-intensity exercise were weaker than that of middle-intensity exercise (Fig. 3A–E).

Previous studies on human have found that acute or regular aerobic exercise improves working memory in teenagers and adults with poor

working memory performance [1,36,58,62–64]. Meta-analysis of McMorris et al. [35] reported that acute moderate exercise can enhance working memory, and there was an inverted U relationship on exercise intensity-working memory effect. Dief et al. [21] reported 30 days swimming exercise rescued the impaired T-maze DSA task performance by shorting response time and increasing accuracy in stressed rats. Present data agree with Dief et al. [21], suggested that regular exercise could improve working memory in rats.

Present study, for the first time, found that regular lower-intensity treadmill exercise and middle-/higher-intensity treadmill exercise are beneficial to working memory with a short delay (10 s) and long delay (300 s) in normal adult rats, respectively. We assumed that in a certain range, training intensity and working memory delay/load are positively correlated, i.e., lower-intensity exercise improves working memory with a short delay, whereas, higher-intensity exercise improves working memory with a long delay. As shown in Fig. 6, we assume that the effects of exercise intensity on working memory follow an inverted U dose-effect curve. Working memory with specific delay is supposed to have an optimal functional range, which means that under or over training will be either useless or lead to impairments. Here, the optimal functional ranges of lower-intensity and middle-/higher intensity on working memories were at delays of 10 s and 300 s, respectively.

According to the law of Yerkes–Dodson raised by Yerkes & Dodson 100 years ago [52,53], working memory tested by DSA task and spatial memory tested by MWM task should be relative complex tasks, so the exercise intensity related arousal-performance effect accords with the inverted U relationship. In the light of their theory, more difficult task would need a milder motive arousal to get to be proficient [52,53]. But our present data on the DSA task seemingly disagree with that. Generally, the longer delay means more working memory load/difficulty (see Fig. 3A–D, the delay dependent decline of accuracy here and from others [12,49] supports this account), subjects need to maintain information for longer time and suppress the irrelevant noises at the same time. Although the PFC is the center of working memory [10,11], we think the 300 s delay has exceeded the duration limitation of the PFC can hold. Under such situation, subjects need the involvement of the hippocampus, as what reported by Kesner and his colleagues [14,15]. In present paper, 300 s delay perhaps need a higher intensity exercise (such as the middle and higher intensity in present study) to arouse the spatial information from the hippocampal to the PFC in support of working memory [12–14]. The effects of exercise intensity on working memory with 300 s delay are similar to long-term spatial memory in MWM task indicated there would be a common underlying basis. Perhaps
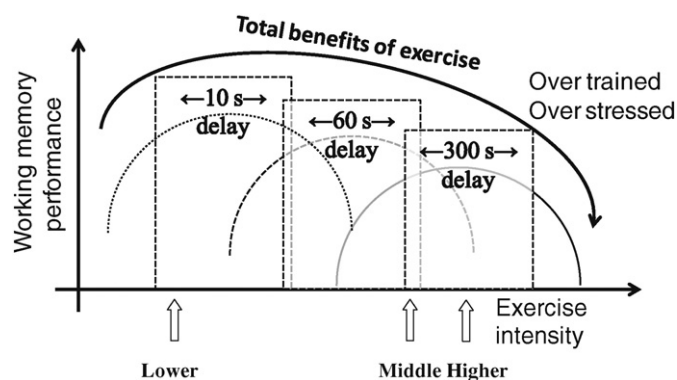
the increased neuronal density in hippocampus should make such a contribution, supported important status of the hippocampus in spatial memory.

However, the working memory with 10 s delay should be mainly completed via the PFC [12,13,50,65]. Ten seconds delay is so brief that the PFC must frequently update and hold the relevant information, inhibit the irrelevant information interfering and urgently prepare to make a choice, in which it would be demanding more executive resources [10]. Thereby, the lower exercise intensity probably meet the optimal range of working memory with 10 s delay. But to 60 s delay, maybe the three intensities are not on the optimal range, so that the DSA task performance improvement is not significant. The underlying mechanism of exercise intensity effect on working memory would be the further research aim.

4.4. Conclusion

In general, present study offered new animal experimental data to suggest that regular exercise can improve working memory, long-term spatial memory in rats in an inverted U exercise intensity - performance relationship. And the increased hippocampal neuronal density in exercise groups would play a crucial role in long-term spatial memory and long-term working memory. And the most interesting finding in present paper was that the exercise–working memory performance relationship was impacted by the delay duration. Understanding the neural effects and mechanisms of exercise would be helpful to design suitable exercise style and intensity for improvement of human neural and mental health.

Acknowledgments

Fig. 6. Schematic diagram of hypothesis on the relationship of working memory performance and exercise intensity. Each delay of the working memory task has the optimal intensity range of exercise (such as the 10 s, 60 s and 300 s delays). If the exercise intensity is too low (at the left side of the curve line), the memory efficiency is too small and not obvious. But on the other side (right side), if the exercise intensity is too strong (over trained or over stressed), there will be damage effects. On the whole, with the increase of exercise intensity, the benefits of exercise effect will firstly increase and then decrease gradually.

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