Effects of Physical Fatigue in Mice on Learning Performance in a Water Maze

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We investigated the effects of physical fatigue produced by swimming exercise on learning the Morris water maze in BALB/c mice. We measured the escape latency in the maze immediately after the swimming exercise. The control group was soaked in the water but not fatigued. For easier tasks, like one with an obvious cue flag, the escape latency was not changed by exercise fatigue. However, escape latency was increased after exercise fatigue for more difficult tasks of spatial learning. These results appear to suggest that physical fatigue impaired learning performance. The effects of swimming exercise fatigue on learning efficiency were then investigated. Mice were continuously fatigued during the spatial learning period. This increased escape latency between the first and third sessions. The results suggest that learning efficiency was impaired by exercise fatigue. This system may be useful for screening new foods used to enhance brain function during exercise.

Key words: fatigue; exercise; learning performance; Morris water maze

Diminished concentration and impaired discrimination abilities often arise with fatigue in sports. This problem has been reported frequently by many athletes. Athletes are able to maintain their performance through periods of competition and training if the exogenous administration of food, for example, is used to control reduction in these abilities. However, because possible psychological factors and pure physical exhaustion caused by exercise may be involved in these phenomena, these effects are not simple, nor are the underlying mechanisms.

Concentration and discrimination ability during exercise are brain functions, so an evaluation system must therefore be able to measure behavioral changes related to brain functioning. Some previous studies have reported on measurements of learning or cognition tasks after exercise in humans, but the results are contradictory. Some investigators have found ability to be unaffected, others that it is facilitated by physical fatigue, and yet others have found impairment. Because of the complex psychological factors present in the human experiments, evaluation of behavioral changes related to physical exhaustion in animal experiments may be a better way to gain insight into the question. Establishing such an evaluation system using rodents also has advantages in the screening of new foods for their ability to enhance brain function during exercise. The measurement of learning ability in animals is a popular and convenient behavioral method for examining at least some brain functions.

In the present study, the Morris water maze was used. It is a commonly used system for testing recognition, memory, and learning in animals. It can be used in combination with forced swimming protocols.

Materials and Methods

Animals and housing. Four-week-old male BALB/c mice (Japan Shizuoka Laboratory Center, Hamamatsu, Japan) were used. They were housed in standard cages (33 × 23 × 12 cm) under controlled conditions of temperature (22 ± 0.5 °C), humidity (50%), and lighting (lights on daily from 0600 to 1800 h). They were provided a stock diet (type MF; Oriental Yeast, Tokyo, Japan) and water ad libitum. They were housed under these conditions for several weeks prior to the start of the experiments. The care and treatment of the experimental animals conformed to the Kyoto University guidelines for ethical treatment of laboratory animals.

Water maze apparatus. The water maze consisted of a circular white plastic tank (100 cm diameter, 36 cm wall height) with a clear circular platform (7 cm diameter) located just beneath the water surface. The tank was filled with cold (15 ± 1 °C), clear water to within 5 cm of its top edge to allow the mice clear views of extra-maze visual cues. The apparatus was kept in the same position in a room (dimensions 4.5 m × 5 m) rich in extra-maze visual cues surrounding the apparatus. These cues included some fixed to the walls behind the maze, laboratory furniture, and the experimenter in a lab coat, who was situated in the same location for all trials. Mice were carefully dropped into the maze from 8 predetermined starting locations near the wall of the water tank.

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in 3 of the tasks (tasks 1, 3, and 4), and from the center of the water tank in the remaining task (task 2). The sequence of start locations was randomized for tasks 1, 3, and 4 in order to prevent the experimental mice from associating the location of the platform with a single set of cues. We measured the duration from the time the mouse was dropped into the maze to the time the mouse climbed onto the platform (the escape latency). The escape latency was recorded manually using a stopwatch. The maximum time allowed for the mouse to find the platform (the search time) was 60 s. Mice were allowed to rest on the platform for 30 s and then returned to their home cage. If they could not reach the platform within 60 s, they were led onto the platform. The water temperature of a Morris water maze is generally between 21° and 23°C. But water temperature is difficult parameter to control. We first set the water temperature to 21°C, but the mice did not attempt to find the location of the platform at this temperature. Perhaps the mice found this temperature too comfortable. Therefore we lowered the water temperature to 15°C to make the maze less comfortable. Some researchers make the water cloudy so that the mice cannot see under water, but this would have made it too difficult for us to move the platform accurately. We confirmed that BALB/c mice could not find the platform in clear water even if it was immediately in front of them. Escape latency and water clarity were not correlated in pilot experiments.

Swimming apparatus and exercise. A water pool with an adjustable current was used to exercise the mice. The details have been described previously. Briefly, we used an acrylic plastic pool (90 × 45 × 45 cm) filled with water to a depth of exactly 38 cm. The current in the pool was generated with a pump. The water temperature was strictly maintained at 34°C with an electric heater. In tasks 1 and 3, mice were forced to swim at a fixed flow rate of 7 l/min twice a day for 30 min immediately before each test session. The test sessions took place at 1000 and 1630 h. In tasks 2 and 4, mice were forced to swim at a 7 l/min flow rate for 30 min, but the flow rate was increased by 0.5 l/min for each successive session, with swimming done twice a day immediately before each test session. The sessions were at 1000 and 1630 h, as for tasks 1 and 3. If the mice could not continue swimming for the minimum stipulated time, they were allowed to rest in static water for several minutes and then returned to the current.

Water maze tasks. Task 1. Mice were trained under the condition that the location of the platform was fixed and marked by a visible cue flag consisting of a 3 cm high post (diameter 2 mm) with a black plastic flag (a board of 3.5 × 4 cm). Training was given in order to elicit practice climbing onto a platform and so that the mice learned that they could avoid the water on the platform, and learn the platform location. The experimental mice received 2 sessions of 3 trials (a total of 6 trials), each day for 9 consecutive days. The inter-trial interval was 10 min. The mice were divided into exercise and control groups, with 4 or 5 mice per group. These 2 groups achieved similar mean escape latencies within 2 sessions. The exercise group was forced to swim immediately before the 5th, 6th, 17th, and 18th sessions. The control group was soaked in still water (34°C) for 30 min at a depth that allowed the mice to stand. Mice rarely move when soaking in water. There was no difference in escape latency between the control (soaked) group and the non-treatment (unsoaked) group in the pilot experiments. The exercise and control mice were dried with paper and cooled by cold forced air, the trial starting exactly 2 min after the mice were taken from the water.

Task 2. Mice were trained under the condition that the platform was marked by a visible cue flag, as for task 1, but with 4 additional (pseudo) visible flags not attached to the platform. The pseudo-flags consisted of 3 cm high posts (diameter 1 mm) each with four 0.5 × 2 cm aluminum foil banners. The pseudo-flags were placed in various locations in the maze. The platform location was changed randomly in every session. The shape of the pseudo-flags was obviously different from the cue flag associated with the platform. The experimental mice were presented with this task in all 14 sessions (the training period). By the end of the training period, the escape latency had become short enough (about 10 s) to proceed with testing. The mice were then divided into 2 groups (exercise and control, with 7 or 8 mice in each group). The mice had similar mean escape latencies in the 2 training sessions prior to the beginning of the test period. The escape latency following exercise or soaking was measured in the 4 subsequent sessions (the test period). The exercise protocols were as described above. All mice were soaked for 2 sessions preceding the test period in order to reduce the stress associated with exposure to water. All other experimental details were similar to those in task 1.

Task 3. The mice were first trained with the platform marked by a visible cue flag as in task 1. The platform location was changed randomly in every session. The experimental mice were presented with this task throughout the 15-session training period. After training with the visible cue, the escape latency became sufficiently short (about 10 s) to begin testing. The mice were then divided into 2 groups (exercise and control, each with 7 or 8 mice). Mice in each group had similar mean escape latencies in the 4 sessions prior to the beginning of testing. The first test session, the cue flag was removed and the platform was placed at a fixed location. The mice were tested on the hidden-platform task for spatial learning, and the escape latencies immediately after exercise were measured throughout the next 8 sessions (the test period). The exercise protocols were as described above. Before and after the
hidden-platform test period, a probe trial was administered to investigate whether the mice showed a preference for the platform location previously learned, in the absence of the platform. The probe test trial was analyzed by measuring the time spent in the quadrant of the pool where the platform had been located. The time was recorded manually using a stopwatch and the quadrant was marked by drawing a line on the bottom of the tank. All other details were similar to those for task 1.

Task 4. We believe the results show that the mice that completed task 3 learned the location of the platform spatially, and we continued to use these mice for task 4. The mice were presented with hidden-platform training for spatial learning in a further 5 sessions to solidify their learning. After a training period to establish a short escape latency (about 10 s), the exercise and control groups in task 3 were exchanged to avoid exercise-training effects. The escape latency following exercise was measured in the next 6 sessions (the test period). The exercise protocols were as described above. All other details were similar to those for task 1.

Physical fatigue by exercise. The maximum swimming speed for all trials in tasks 1, 3, and 4 was 24.4 cm/s, and the speed was equivalent to a flow rate of about 9 l/min in the adjustable-current water pool. If the mice could swim at a 9 l/min flow rate for 60 s after swimming for 30 min at a 7 l/min flow rate, the implication is that the impairment in learning performance caused by exercise was not due only to peripheral fatigue. Mice were soaked (the controls), or forced to swim at a 7 l/min flow rate for 30 min, then paper dried and cooled by cold forced air. Two minutes later the mice were forced to swim at flow rates of 8, 9, 10, or 12 l/min (4 mice at each flow rate). We measured the swimming time to fatigue. The maximum swimming time allowed was 3 min because the maximum search time allowed in the water maze was 60 s.

Statistical analysis. Individual trial data were averaged for each session (3 trials per session) and the session data were analyzed. Data are expressed as means ± SE. For comparisons between 2 groups at given flow rates, the unpaired Student’s t-test was used in tasks 1, 2, and 4. The comparison between the eighth session and the other sessions in the same group was performed by one-way repeated measures analysis of variance (ANOVA) followed by Fisher’s test in task 3. The paired Student’s t-test was used for probe tests. Statistics were calculated using the Stat View software package (Macintosh Version J 5.0; Abacus Concepts, Berkeley, CA). A level of p < 0.05 was used as the criterion for statistical significance.

Results

Effects of exercise on well-experienced mice

Task 1

During the 5th, 6th, 17th, and 18th sessions, the exercise group was forced to swim at a 7 l/min flow rate (Fig. 1). During the 5th and 6th sessions, there was a weak tendency for the escape latency in the exercise group to increase, as compared with the control group (P = 0.13, 0.17). But the escape latency was the same as that for the control group in the 17th and 18th sessions.

Task 2

The escape latencies for the both groups shortened progressively throughout the 14 sessions (data not shown). The mice appeared to learn to distinguish the cue flag from the pseudo flags. Figure 2 shows the effects of exercise on the escape latencies in well-learned mice. The non-exercise bars refer to the 14th session. Swimming at 7–8 l/min flow rates had no significant effects in this task.
Fig. 2. Effects of Swimming Exercise on Mean Escape Latencies Using the Visibly Cued Platform and the 4 Visible Pseudo-flags, but without the Platform (Task 2).

Each bar represents the mean score per session (mean of 3 trials). The platform location was changed randomly at every session. The exercise group was forced to swim at 7, 7.5, 7.5, and 8 l/min flow rates for 30 min before the maze task. The statistical significance values (p) are indicated. Values are means ± SE for 7–8 mice.

Fig. 3. Effects of Swimming Exercise on the Hidden-platform Task in Mice That Had Previously Learned the Location of the Platform (Task 4).

Each point represents the mean score per session. The exercise group was forced to swim at 7, 7.5, 8, 8.5, 9, and 9.5 l/min flow rates for 30 min before the session with the platform location fixed. The escape latency tended to be higher in the exercise group swimming at the 7.5 l/min flow rate compared with the control group (p = 0.07). Similar results were obtained at the 8–9 l/min flow rate. The statistical significance values (p) are indicated. Values are means ± SE for 7–8 mice.

**Task 4**

The escape latency of the hidden-platform test in the exercise group swimming for 30 min at the 7.5 l/min flow rate tended to increase (P = 0.07), compared with the control group (Fig. 3). Similar results were obtained at the 8–9 l/min flow rate. The non-exercise bars refer to the immediately previous session at 7 l/min, during which neither group was exercised.

**Effects of exercise on inexperienced mice throughout learning**

**Task 3**

The mice were trained to find the platform using the visible cues throughout the 15 sessions. The task was then changed to one employing the hidden platform for 8 sessions. The escape latency for the hidden platform in the exercise group was significantly extended (p < 0.05) between the first and the third sessions compared to the eighth session (Fig. 4). In the eighth session, the escape latency decreased significantly and the escape latency between exercise and control groups was very similar. On the other hand, the escape latency in the control group did not show significant differences in all sessions of the hidden-platform task. In the probe test, the time spent in the quadrant was measured before and after the hidden-platform test period for both the control and the exercise group. In control group, the time spent in the quadrant after the hidden-platform test period increased significantly over the time spent in the quadrant before the hidden-platform test. On the other hand, the time spent in the quadrant after the hidden-platform test period did not increase significantly over the time spent in the quadrant before the hidden-platform test period for the exercise group (Table 1).

**Physical fatigue by exercise**

Both the exercise and control groups were able to
swim for over 60 s at the 8 to 10 l/min flow rate (Fig. 5). Therefore, they could swim at a speed of 24.4 cm/s, the maximum swimming speed in all trials, even if they were tested after swimming.

Discussion

We investigated how learning performance in mice was affected by forced swimming protocols. We assumed that “learning performance” measured in terms of escape latency for the water maze was affected by some aspects of brain functioning (e.g., learning, recall, concentration, discrimination, etc.). The features of each task were different. Tasks 1, 2, and 4 assessed the effects of exercise (physical fatigue) on escape latency in well-experienced mice. Task 3 assessed the successive effects of exercise on mice learning the maze spatially. We examined whether exercise has negative effects on learning efficiency in task 3.

Task 1 was too easy for the mice and the escape latency was not affected by fatigue (Fig. 1). The mice
learned to find the platform using the cue flag as well as the objects around the maze. Task 2 required the mice to discriminate between a correct flag shape (associated with the platform) and pseudo-flags. We did not observe impairment in learning performance in this task, either (Fig. 2). Perhaps the cue made the task too easy. Gerlai et al. found that the visible-platform task was often easy for rodents to solve.\textsuperscript{40} Task 4 required spatial learning, the mice having to find the platform using the objects located around the maze. This task appears to have been more difficult, and we observed impairment in learning performance with fatigue (Fig. 3). This task is used repeatedly, because we can use the same well-experienced mice. We believe that this task is suitable for screening new foods for their ability to enhance some aspects of brain functioning during exercise.

Task 3 involved continuous spatial learning with exercise. In the cue-training period, the location of a platform with a cue flag was randomized, with the result that the mice did not learn the platform location. This contrasts with the hidden-platform task, in which the mice had to learn the platform location spatially. We demonstrated the effects of fatigue over several sessions in this task (Fig. 3). The results of the probe test also indicate that learning efficiency was attenuated by fatigue (Table 1). The target quadrant dwell time for the exercise group was not increased significantly probably due to the delay of spatial learning. The escape latencies for the control group did not change even in the first hidden task, but those of the exercise group increased significantly in that task. It may appear that the escape latencies of the control group did not increase significantly, but the well-trained mice were able to reach the platform accidentally within 20 s even in the hidden task. The exercised mice appeared not to seek the platform eagerly and did not learn the location of the platform well even though they reached it. Fatigue may have negative effects on some aspects of brain functioning such as discrimination or learning ability. This task could not be imposed repeatedly. We were, however, able to evaluate the effects of fatigue produced by exercise on learning efficiency during the learning period.

It is possible that physical fatigue itself affected swimming capacity and escape latency. To check this, we examined the swimming capacity of the mice after swimming. We found that the mice could swim for over 90 s at a flow rate of 8 to 10 l/min after swimming for 30 min at a flow rate of 7.1 l/min (Fig. 5). This result, and the difference in the results between tasks 2 and 4, indicate that the increase in escape latency was not due only to physical fatigue.

Thirty years ago investigators reported that exercise fatigue impairs brain functions such as learning and cognition in humans.\textsuperscript{32–37} But other investigators reported the contrary conclusions: that fatigue either does not affect learning ability,\textsuperscript{1–12} or indeed facilitates it.\textsuperscript{13–31} This question appears to be unresolved at present. Perhaps it was too difficult to evaluate such effects on human subjects because the experimental conditions were not the same. We can, however, strictly control the experimental conditions in animal experiments (e.g., exercise intensity, time, and learning tasks). Our results using animals show that fatigue produced by exercise significantly impairs learning performance. We observed similar results in a preliminary experiment using human subjects. In these experiments, regularly trained healthy males and females (19–26 years old) were exercised for 1 h (mainly in a running task), then tested with the Kuraepelin test, which evaluates calculation speed and accuracy.\textsuperscript{31} We found that the number of corrections increased significantly compared with the sedentary control group (unpublished data). This result supports the results for mice reported here.

We note that the Morris water maze is popular in the evaluation of animal learning ability and memory.\textsuperscript{38} It seems particularly appropriate for testing rodents due to their escape behavior in water.

The exercise intensity, the exercise time, and the interval before the maze test were critical factors in this experiment. It is necessary carefully to control them. Five-week-old BALB/c mice were able to swim for 30 to 60 min at a flow rate of 7.1 l/min in preliminary experiments. For this reason, we configured our experiments with 2 swimming periods a day at a flow rate of 7.1 l/min (60 min in the morning, with 30 min in the afternoon for tasks 1 and 3). But the mice gradually adapted to this protocol, making it inappropriate for the long-term experiments. Therefore in tasks 2 and 4 the current speed was increased 0.5 l/min in every session; this always fatigued the mice. Increasing the current might have been expected to increase fatigue, but this was not the case. Almost all the mice were fatigued within about 15 min at 9 or 9.5 l/min; therefore resting time had to be increased and the extent of fatigue was lower than for flow rates of 7.5 or 8 l/min. It appears that a flow rate of 7 to 8.5 l/min is optimal because at this rate mice require about 30 min to become fatigued. Does the extent of fatigue correlate with the effects on learning performance? In tasks 2 and 4, the mice were forced to swim at a flow rate of 7 to 9.5 l/min for 30 min for each successive session and the escape latency was most increased at a flow rate of 7.5 l/min, at which the mice were presumably most fatigued. Considering this, the results of tasks 2 and 4 (Fig. 2, 3) show a correlation between extent of fatigue and effects on learning performance.

In conclusion, learning performance in mice did not change using an easy task like that using an obvious cue (the visible flag), but it was impaired significantly in the relatively difficult spatial learning task. This test system may be useful for the screening of new foods intended to enhance some aspects of brain functioning during exercise. These results also resolve the classical problem of whether learning performance is impaired by physical fatigue in mice.
References


