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High-frequency training improves the accuracy of spatial memory but does not affect the spatial learning ability

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Abstract

Objective: To study the effect of training frequency on memory acquisition and retention.Methods: compare the time of forming a spatial memory and the accuracy of the memory information under the same total number of Morris water maze training, and define the rules of learning performance at different training frequencies.Result: Navigation experiment:Compared the escape latency to their own first escape latency,both group occurred a significant reduction at the 9–12th training session (p<0.05). At the same number of training sessions,there was no significantly different in latency between the two groups with different training frequencies (p>0.05). Spatial probe test: A significant increase in the target quadrant percentage compared to the first test results both occurred in the third and later tests. After the same number of training sessions, the target quadrant residence time percentage of the 3 / day group was significantly higher than which was in the 1 / day group in the 9–12th tests (p<0.05). Conclusion: Time of forming a memory is related to the number of training rather than training frequency.The accuracy of the memory information is related to the training frequency.

Keywords

Morris water maze test, escape latency,he target quadrant percentage,learning and memory.

1. Introduction

Learning and memory disorders is a treatment difficulty in modern medicine and a research hotspot in neuroscience [1-2], and the research on its treatment mechanism is also gradually carried out[3-4].As one of the Morris water maze experiments most commonly used to study the learning and memory drum, they are widely used in various fields of pharmacology, toxicology, psychology and so on.In the field of neuroscience, the impact of drugs or therapies on learning and memory function in animals.Morris water maze experiment works to force animals to swim, use animal survival instinct to find hidden in the water, the purpose is to successfully sail and find the platform hidden in the water, eventually escape from the water, so the behavior of the maze involves a complex learning and memory process, including spatial visual information perceptual information collection, processing, memory consolidation and then take out.Morris water maze experiment can provide more experimental indicators [5], with reliable sensitivity and easy operation, is one of the most common experimental method in the study of learning and memory mechanism.

The navigation test was mainly conducted by continuously training mice to remember the location of the platform to examine their learning ability.Different experimenters used different training methods in conducting positioning navigation experiments, training two to four times per day.However, there are many operational irregularities in the previous Morns water maze experiments and the researchers did not clearly indicate how many training times the mice will

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remember the platform, resulting in the extended experimental cycle and the inability to effectively evaluate and screen the experimental animals.Moreover, it has been shown that learning and memory effects vary in different degree of Cognitive deficits[6], but also in different cases of training methods, screening training and sub-experiments[7-9].The purpose of this experiment was to compare the effects of two different training frequencies on the learning effects of mice, by training three batches of animals for spatial learning, expecting to clarify the rules of the formation of mouse acquired learning.

2. Experimental contents

2.1. Animals and grouping

ICR mice were 160, half and half females and weighed 20±2,4–5 weeks of age.The experimental work objects were fed with standard feed, and they were asked for free feeding and drinking water.Mice were randomly divided into two groups (n=30), 1 / day and 3 / day groups, and the animals underwent 12 and 4 days, as shown in Figure 1.

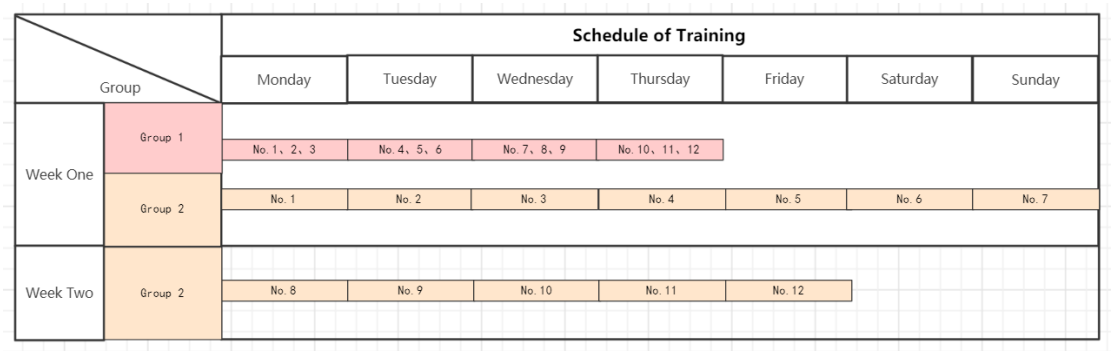

Figure 1.Training plan mode diagram

2.2. laboratory apparatus

MT-200 Morris Water Maze instrument and its video Tracking Analysis system.The Mor ri s water maze is composed of round pool and movable platform, generally suitable for the pool of 180cm diameter, 100cm height, platform of 12cm diameter and 2 cm under the water surface.The pool for mice is 150cm diameter, 50cm height, platform 8cm and 1 cm below the water surface [10].The recording and analysis system is connected to the camera and the computer. The recording system can track the movement track of the experimental animals in the water, and the analysis system can analyze the relevant data.The experimental device is usually placed in a quiet and spacious room, hanging four different shaped objects as visual clues (visual cues) in the air around the pool and above the pool.Pull the curtain around the device to block the experimenter and subject animals.The pool device and the like are divided into 4 quadrants, injected with tap water, 23cm high and water temperature at (21 ± 1) ℃; with a circular platform in the center of one quadrant.Two 40-watt bulbs are placed above the camera, and the opposite color stain (ink) is added to the water according to the animal hair color (white) used to enhance the contrast between the animal and the surrounding environment[11].The pool area is divided into four quadrants: southeast (SE), northeast (NE), northwest (NW), and southwest (SW), see Figure 2.

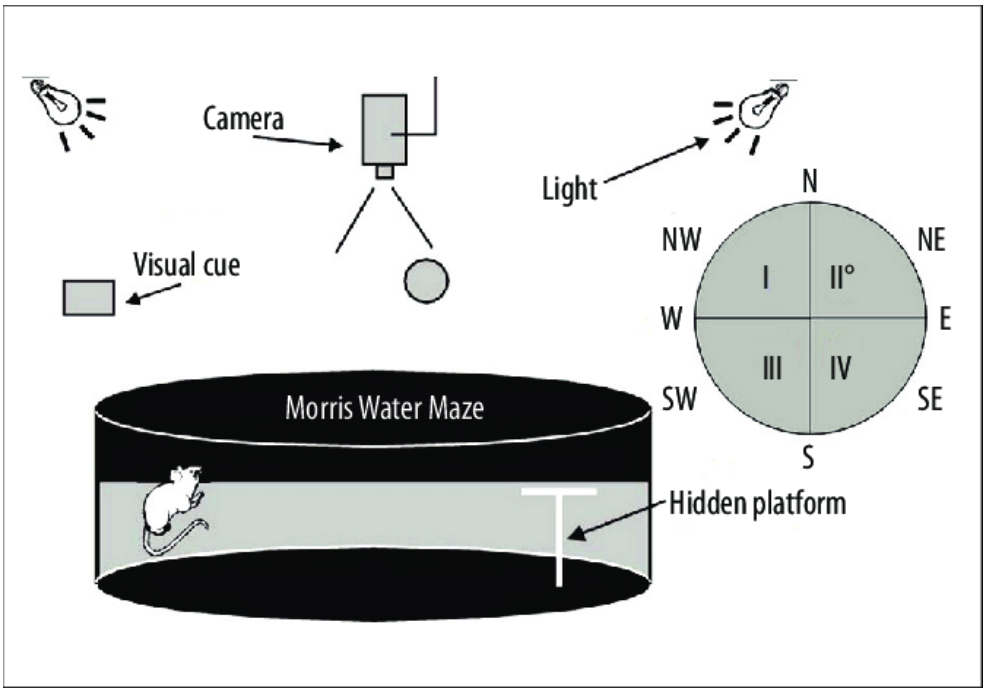

Figure 2.Morris Water Mase

2.3. Experimental procedure and observation indicators

2.3.1 Experimental procedure

Positioning navigation test is used to measure rat ability to obtain spatial information.All mice received adaptive training the day before the experiment, in the water maze with water but not the platform, and each mouse was trained and then removed and dried.For a formal test, select the center of either quadrant and place the platform at 1 to 2 cm.Animals were placed head toward the pool wall into water at random north, south, east or west, and two adjacent experiments were performed from different quadrants.The time required for the animal to enter water to find the platform was recorded as an escape latency latency (s).If the animal swam for more than 120s or still failed to climb the platform, the time was recorded as 120s and artificially placed on the platform for 30s, after which the mouse was dried and placed in the cage.Then the next mice were tested.

Spatial probe test is used to measure the retention of spatial memory. At 24h after each positioning navigation experiment to remove the platform, the animal was put from the contralateral side of the original platform quadrant into water to track its movement trajectory for 120s, recording the swimming time of the animal in the target quadrant (the quadrant of the original platform).

The mice sometimes failed to fall from the platform or then jump into the water until 30s, where the experimenter replaced the mouse back to the platform and retimed to ensure that each mouse had the same time to observe and obtain spatial information. Training or testing was performed in one or three fixed time sessions daily during the experiment. Operation was gently performed to avoid bringing additional stress stimulation to the mice.

2.3.2 Observation indicators

Escape latency: In the positioning navigation test,mice with earlier memory formation were thought to have better reference learning abilities. According to previous studies, mice with normal motor and cognitive function can form a memory after 4-9 training sessions, that is, the escape latency can be less than 70 seconds.To determine whether a group of mice form a memory to the platform, they not only reduced their escape latency significantly compared with their first escape latency, but also the 80% of mice in this group. Therefore, this study set the

standard for memory formation in a group of mice as that 80% mice can escape the latency by less than 70 seconds.

The target quadrant residence time percentage: Percentage of the time spend on target quadrant (the original quadrant of the platform)in the spatial probe test is used to measure the accuracy or learning effect of memory information. Mice with greater time spend in the target quadrant were considered to have better academic performance.

2.4. Statistical analysis

Data and data processing was performed by one-way ANOVA using statistical software. One-way ANOVA test was used within each group, with independent sample test and repeated measures ANOVA between groups. P<0.05 is significant difference and very significant difference.

2.5. Experimental result

2.5.1 Effects of training frequency on escape latency

In the positioning navigation test, the escape latency in two groups both decreased as the number of training increased. Compared the escape latency to their own first escape latency, both group occurred a significant reduction at the 9–12th training session(p<0.05). At the same number of training sessions, there was no significantly different in latency between the two groups with different training frequencies(p>0.05), as shown in Table 1, Figure 3.The escape latency below 70s reached 80% of both group on different training session — 1 / day group reached 80.25% on day 9, while the 3 / day group reached 80.29% on day 3.In the 10th – 15th experiments, mice with escape latency below 70s accounted for over 80% in this group, as shown in Table 1, Figure 4.

Table 1.The effect of the training frequency on escape latency

Escape latency(s) OR Percentage of individuals with escape latency less than 70s (X±SD,%)	Once/day（n=30)		3times/day(n=30)	
	Escape latency(s)	Percentage of individuals with escape latency less than 70s (X±SD,%)	Escape latency(s)	Percentage of individuals with escape latency less than 70s (X±SD,%)
1	107.30±27.43	3.3	103.0±30.18	0
2	99.10±41.58	3.7	110.78±38.44	4.23
3	101.29±45.34	19.75	91.12±41.51	18.31
4	82.26±56.44	28.39	85.72±41.91	36.62
5	80.57±42.41	40.74	70.31±42.98	57.75
6	78.10±41.95	56.79	71.62±42.73	64.79
7	73.11±39.57	66.67	70.58±42.90	67.61
8	74.08±41.28	71.61	68.88±46.02	76.06
9	72.26±40.44*	80.25	66.13±39.89*	81.69
10	65.10±39.69*	83.95	61.63±39.89*	85.92
11	68.70±42.53*	86.42	56.06±43.31*	88.74
12	59.73±41.99*	88.89	50.30±41.86*	90.15

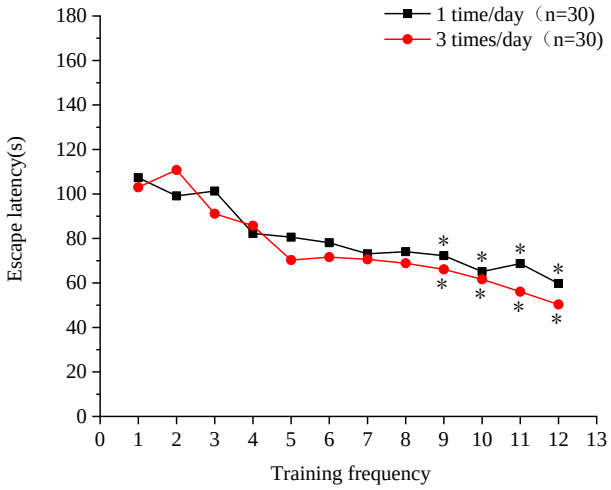

Figure 3.The escape latency in two groups

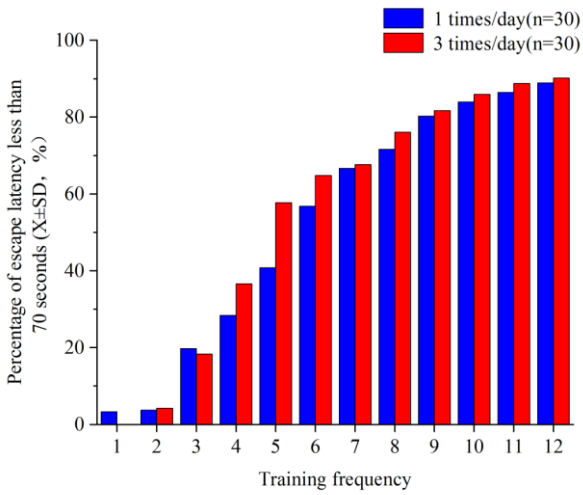

Figure 4.The percentage of individuals with escape latency less than 70s

2.5.2 Effects of training frequency on the target quadrant percentage

The metrics of 1 / day and 3 / day group were combined into two large samples for data analysis, and the data are shown in Table 2.In the first three times, target quadrant residence time percentage increased with the number of training times, then tend to stabilize with serrated irregular fluctuations.The trajectory plots of the two groups of mice in the spatial exploration test can visually show the differences between the two groups,as we can see in the Figure5(A).A significant increase in the target quadrant percentage compared to the first test results all occurred in the third and later tests($p<0.05$).Comparing both mice with the same number of training times, the target quadrant residence time percentage of the 3 / day group was significantly higher than which was in the 1 / day group at 9–12th tests($p<0.05$),as we can see in the Figure5(B).

Table 2.The effect of the training frequency on the target quadrant percentage

Time spend on target quadrant(%)	Once/day(n=30)	3times/day(n=30)
1	23.05±11.39	24.85±11.37
2	24.13±11.85	27.01±12.98
3	29.61±15.28*	30.88±16.91*

4	29.13±12.17*	31.38±17.25*
5	29.89±12.65*	35.93±12.69*
6	33.64±18.76*	32.85±16.29*
7	32.00±16.70*	33.32±15.71*
8	31.89±14.16*	34.74±21.62*
9	31.93±14.39*	36.41±17.39*
10	31.14±16.49*	37.68±17.15*
11	33.57±18.78*	38.73±15.64*
12	34.71±19.11 *	39.77±24.89*

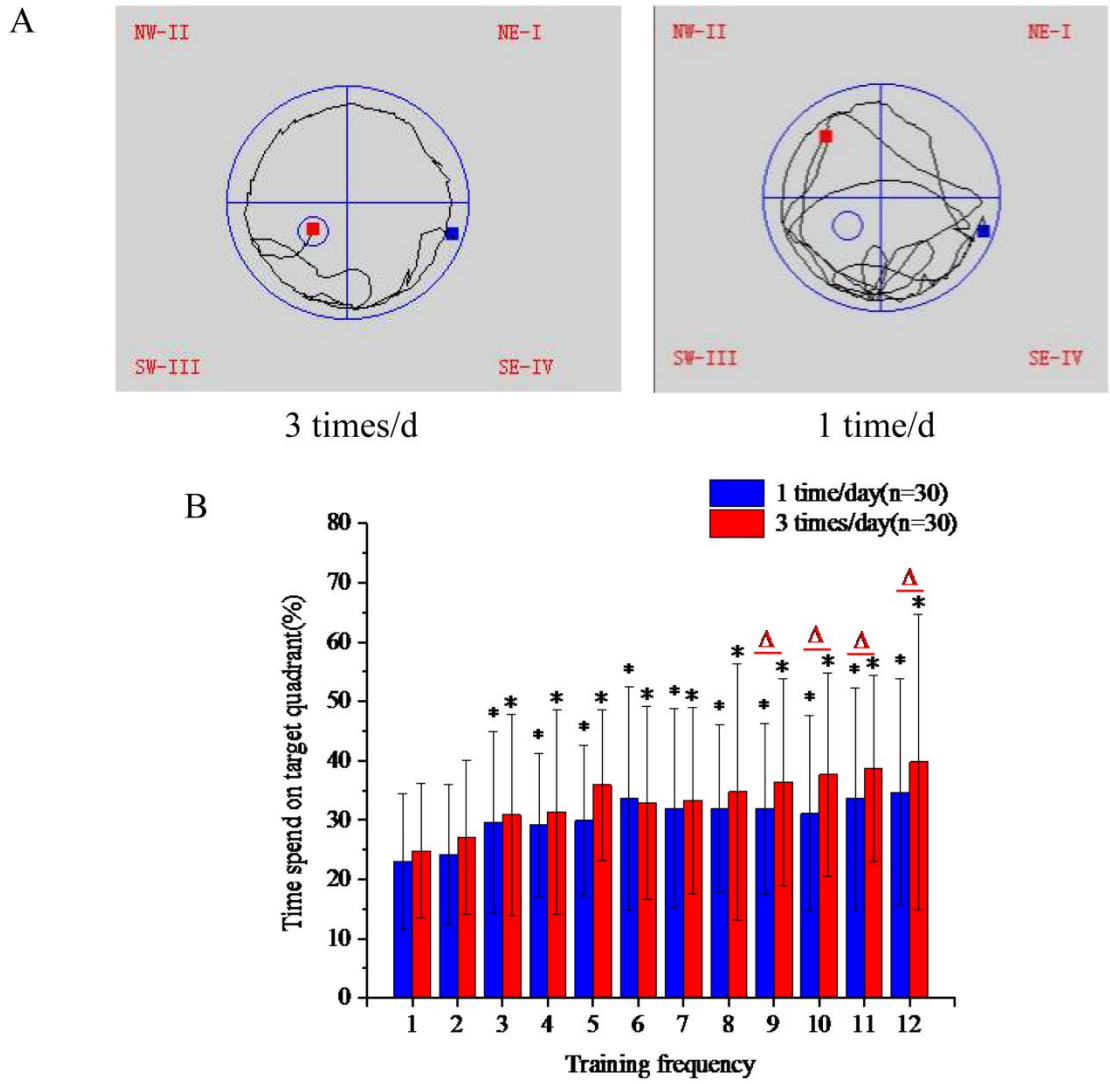

Figure 5.The time spend on target in two groups

3. Conclusion

Time of forming a memory is related to the number of training rather than training frequency (training interval)-over 80% of mice can remember the location after nine positioning voyage training, and mice with high training frequency can remember the location of the platform earlier. The accuracy of the memory information is related to the training frequency- the mice

trained three times per day performing better on the spatial probe test than the mice trained once per day.

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