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Research article

Verification of a multi-function closed maze for the detection of affective disorder and spatial cognitive impairment in post-weaning socially isolated rats

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ABSTRACT

Objective: To verify a behavioral device for the detection of learning, memory, and affective disorders in post-weaning socially isolated rats.
Methods: We tested the behavioral changes in post-weaning socially isolated rats using a multi-function closed maze, a self-developed behavioral device, against the classical mood disorder detection method, the IntelliCage system and Morris water maze.
Results: In the multifunctional closed maze experiment, the spatial learning and memory ability of post-weaning socially isolated rats decreased, which was consistent with the results of the water maze and IntelliCage system. Furthermore, the behavioral changes in the post-weaning socially isolated rats in the multi-function closed maze test were the same as those of the forced swimming and open field tests, indicating that the rats had depression- and anxiety-like behaviors.
Conclusion: A multi-function closed maze can detect emotional changes, spatial learning ability, and memory ability.

1. Introduction

Studies have shown that post-weaning social isolation (PWSI) rats display anxiety- and depression-like behaviors [1–3]. The spatial memory ability of rats is reduced after 12 weeks of long-term social isolation [4]. Researchers have long used experimental methods such as the forced swimming test (FST) and open field test (OFT) to evaluate anxiety- and depression-like behaviors and mood changes, and maze class instruments to detect changes in spatial memory. Several contemporary behavioral studies have aimed to develop a comprehensive instrument that could simultaneously measure emotional changes and evaluate behavioral cognition.

The Multi-function Closed Maze (MCM) is an instrument system developed by our research group to comprehensively evaluate the behavioral cognitive ability and emotional change characteristics of animals by imitating their living habitat on land. A number of invention patents have been obtained from the countries abroad. Its software, Noldus Ethovision 11.5x, can automatically detect the movement and behavior of animals using real-time video, and track and analyze animal experimental data [5]. This study compared the anxiety-and depression-like behavior changes as well as the spatial learning and memory ability of Sprague-Dawley (SD) rats with those of normal rats after 12 weeks of PWSI. We analyzed the advantages and disadvantages of these three devices in the behavioral detection of model rats. The MCM is a new behavioral device that can be used in future research on emotion-and cognition-related deficits.

2. Materials and methods

2.1. Animals and PWSI model

Three male and three female specific pathogen-free (SPF) SD adult rats weighing 250 ± 20 g were purchased from Changsheng Biological Co. Ltd (production license number: SCXK (Liao) 2015–0001). Female and male rats were caged together, and female rats were separated after pregnancy. The rats were raised routinely. The day of delivery was

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termed PND0 (postnatal day 0). After weaning, the offsprings were randomly divided into experimental and control groups, with 50% of males and females in each. There were 10 rats in each group. In the experimental group, rats were raised separately during the PND21-PND105 period. In the control group, the rats were reared in the normal groups after weaning. All animal experiments were performed in strict accordance with the PR China legislation on the use and care of laboratory animals.

2.2. Experimental method

2.2.1. MCM test

The MCM testing device comprises a cylinder with a diameter of 200 cm, a height of 80 cm, and a circular chassis with the same diameter (Fig. 1 I). The cylinder is divided into six sectors by six fan arms; under each fan arm, there are four equally spaced doors. The height of each door is 9 cm, and the width is 11 cm. The chassis is made of a metal mesh, and any animal waste can be flushed into a garbage collection system using high-pressure water. A video camera is installed directly above the maze to record data.

The experimental scheme design is illustrated in Fig. 1 II. The path design was S-shaped: A4-B1-C4-D1-E4-water bottle. For example, A4 represented door number 4 of arm A. All the doors of arm F were closed. The experiment was divided into three parts.

(1) Environmental adaptation: The rats were placed in the chassis alone, and each rat explored the maze freely for 10 min without a water bottle. Based on the principles of the OFT, the central region of the starting sector (Fig. 1 II) was defined using the software package. The cumulative residence time and exploration frequency of rats in the central region and its activity frequency and cumulative time were analyzed to evaluate the anxiety- and depression-like behaviors of the rats.
(2) Directed water search: After environmental adaptation, rats were not given access to water for 12 h, but were not fasted to stimulate water exploration. The rats were trained to find the water bottle from the starting sector and drink for 10 s within 2 min. The rats that failed to complete the task had the experiment time extended until they found the water bottle and drank for 10 s. The experiment was completed over three consecutive days. At the end of the experiment, the rats were uniformly provided with water for 15 min.
(3) Spatial memory test: The water bottle was removed on the fourth day, and each rat was allowed to explore freely for 2 min. The researcher cleaned and removed any odor from the maze. The next rat was then tested, and the recorded data were analyzed.

2.2.2. IntelliCage test

IntelliCage (TSE Systems GmbH, Germany) is a quadrangular home cage environment that is used to automatically monitor the spontaneous cognition and learning behavior of rodents living in social groups. Researchers can edit different experimental programs, and run and analyze the resulting data [6,7]. The two groups of rats were regrouped according to the home-based principles of the systems. The researchers first tested four female or male rats in the control group, then four female rats in the experimental group. The experimental design was divided into the following two parts.

(1) Environmental adaptation and directional drinking: On the first day, the rats were allowed to freely explore the device for 20 min, and drink water in any of the recording rooms. Twelve hours before the test, the rats were restricted from drinking water, and a recording room was designed for each rat at the correct angle. The rats were trained for 20 min every day for three consecutive days. After the experiment, the rats were provided with water for 15 min.
(2) Spatial memory test: All water bottles were removed on the fourth day, and the rats were allowed to freely explore for 20 min after 12 h of water restriction. Data were collected and recorded.

2.2.3. OFT

The bottom of the spontaneous activity box was divided into central and peripheral areas. An incandescent lamp was centered above the box. A single rat was placed in the box at the same corner wall each time. Two

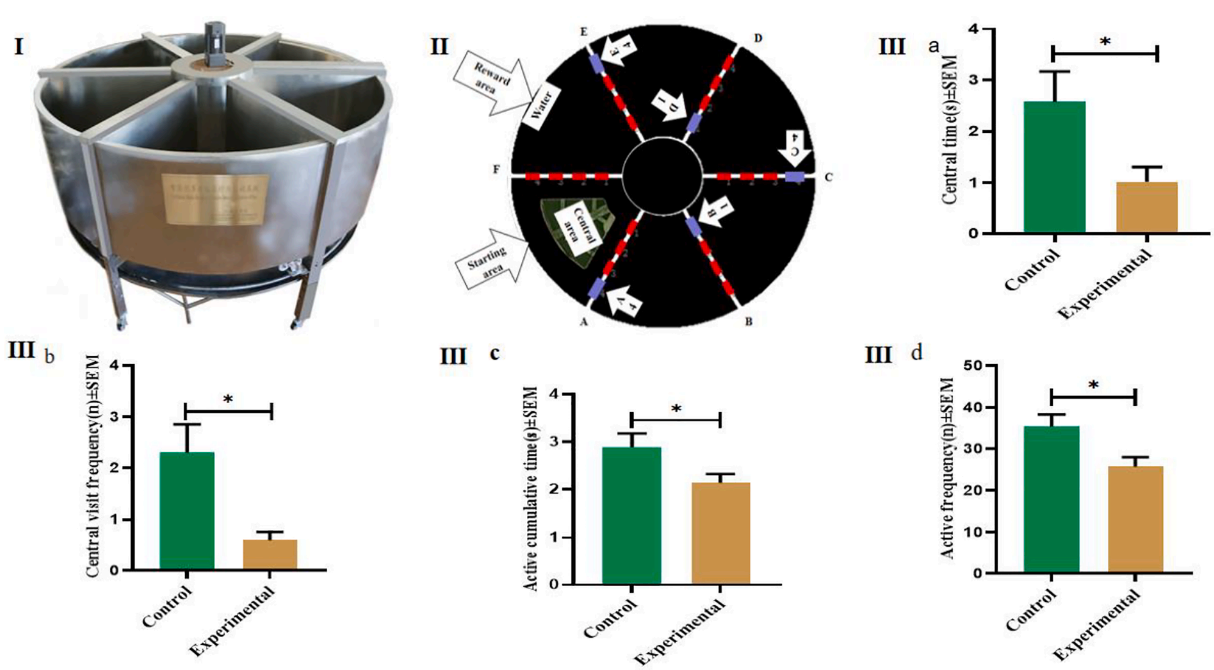

Fig. 1. MCM detects anxiety and depression-like behavior changes in PWSI model mice. Fig. 1 MCM detects anxiety and depression-like behavior changes in PWSI model mice. I: MCM appearance diagram, II: The central area of the starting sector of the MCM path. III:a: Time in the central area, b: Exploration frequency in the central area, c: Cumulative time of activity, d: Frequency of activity. Note: Compared with the control group, *P < 0.05, n = 10 per group.

observers counted the time the rats spent in the central area of the active field and the number of times they stood in the central area within 5 min. After the experiment was completed, the odor was cleaned and removed [8].

2.2.4. FST

The rats were placed separately in a transparent cylinder device with 40 cm deep water at 23–25 °C. On the first day, the rats were allowed to swim for 5 min to adapt to the environment. On the second day, the swimming time was 15 min. Immobility swimming time (when a rat floats without struggling with its hind limbs or performing minimal movements to keep its head above the water) and climbing behavior (when a rat uses its front paws to climb up along the inner wall of the pool) were recorded and statistically analyzed by two observers [9].

2.2.5. MWM test

The MWM device is a black circular pool with a diameter of 160 cm and a height of 60 cm, and is equipped with a movable safety platform, with a diameter of 10 cm, fixed in the center of the fourth quadrant, 2 cm below the water surface. The experiment was divided into the following three parts.

(1) Environmental adaptation and directional navigation: On the first day, each rat explored the device freely for 2 min without a secure platform to adapt to the environment. The rats were trained to find the safety platform from the starting point within 2 min and stand on the platform for 5 s. The rats that did not complete the task were guided by the experimenter until they found the safety platform.
(2) Space exploration: The safety platform was removed on the fourth day of the experiment, and each rat explored freely for 2 min. The experimental results were analyzed using the Shanghai Jiliang Behavioral Software System [10].

2.3. Statistical analysis

The data were completely randomly designed for both samples, and the variables were numerical in type. The data are expressed as mean ± SEM (standard error of the mean). The Shapiro-Wilk test was used to determine data normality. The Levene test or F test (*t*-test) was used to determine the homogeneity of the variance data. When the data conformed to both normal distribution and homogeneity of variance, a *t*-test of the two independent samples in the parameter test was used for comparison. If the homogenization of variance was not met, the data were compared using the approximate *t*-test of the Satterthwaite method. The test level was set at $\alpha = 0.05$, bilateral test. $P < 0.05$ was considered statistically significant. GraphPad Prism version 8.0 was used to generate statistical graphs.

3. Results

3.1. Effect of PWSI on bodyweight

Compared with the control group, the body weight of the experimental group did not change significantly before the offspring rats were isolated ($t = -0.355$, $P = 0.727$). However, after 12 weeks of PWSI, the experimental group exhibited a significant increase in weight ($t = 2.56$, $P < 0.05$).

3.2. MCM test of the effect of PWSI on anxiety- and depression-like behaviors

Compared with the control group, the experimental group showed a significant decrease in the accumulation time in the central area of the starting sector ($t = 2.332$, $P < 0.05$; Fig. 1 IIIa) and frequency of exploration in the central area ($t = 2.92$, $P < 0.05$; Fig. 1 IIIb). The experimental group showed reduced activity ($t = 2.199$, $P < 0.05$; Fig. 1 IIIc) and activity frequency ($t = 2.665$, $P < 0.05$; Fig. 1 IIId), and the difference was significant.

3.3. MCM test of the effect of PWSI on spatial learning and memory

Compared with the control group, the latency of water-finding of the experimental group increased significantly (Training Day1: $t = -0.874$, $P > 0.05$; Training Day2: $t = -8.97$, $P < 0.001$; Training Day3: $t = -5.657$, $P < 0.001$; Fig. 2 I), and the accumulated time in the target sector decreased significantly (Training Day1: $t = -0.433$, $P > 0.05$; Training Day2: $t = 4.325$, $P < 0.01$; Training Day3: $t = 5.794$, $P < 0.001$; Fig. 2 II). In the test phase, the frequency of exploring the target sector of the experimental group decreased significantly ($t = 3.969$, $P < 0.01$; Fig. 2 III).

3.4. IntelliCage test of the effect of PWSI on spatial learning and memory

Compared with the control group, the water-finding latency of the experimental group was significantly longer (Training Day 1: $t = -0.293$, $P > 0.05$; Training Day 2: $t = -2.813$, $P < 0.05$; Training Day 3: $t = -2.869$, $P < 0.05$; Fig. 3 Ia), and the time to find the correct angle was significantly less (Training Day 1: $t = -0.031$, $P > 0.05$; Training Day 2: $t = 3.244$, $P < 0.01$; Training Day 3: $t = 5.074$, $P < 0.001$; Fig. 3 Ib). In the test phase, the number of nose contacts at the correct angle of the experimental group was significantly lower ($t = 2.765$, $P < 0.01$; Fig. 3 Ic).

3.5. Effect of PWSI on the OFT and FST

Compared with the control group, the experimental group showed a significant decrease in the cumulative exploration time ($t = 2.460$, $P < 0.05$; Fig. 3 IIa) and standing times in the central area of the OFT($t = 2.555$, $P < 0.05$; Fig. 3 IIb). The FST immobility time of the experimental group increased ($t = -2.893$, $P < 0.01$; Fig. 3 IIc), and the climbing time decreased ($t = 2.474$, $P < 0.05$; Fig. 3 IId).

3.6. MWM test of the effect of PWSI on spatial learning and memory

Compared with the control group, the swimming latency of the experimental group increased (Training Day 1: $t = -2.350$, $P < 0.05$; Training Day 2: $t = -4.141$, $P < 0.001$; Training Day 3: $t = -3.799$, $P < 0.01$; Fig. 4 I), while the proportion of time in the target quadrant decreased (Training Day 1: $t = 2.558$, $P < 0.05$; Training Day 2: $t = 4.523$, $P < 0.001$; Training Day 3: $t = 3.553$, $P < 0.01$; Fig. 4 II). During the space exploration phase, the number of times the experimental group crossed the safety platform was significantly reduced ($t = 2.449$, $P < 0.05$; Fig. 4 III). The track in the target quadrant was significantly reduced (Fig. 4 IV).

4. Discussion

In this study, three instruments were used to test the behavioral changes in PWSI rats. The results showed that SD rats experienced anxiety- and depression-like behavior changes after 12 weeks of social isolation, and their spatial learning and memory abilities were impaired.

The MCM provides sufficient space for land exploration, and each sector of the MCM can be regarded as an open field box. By analyzing the data of the environmental adaptation stage using the open field principle in the MCM, we found that the results were consistent with those of the OFT. In the MCM, the depression-like behavior changes of rats in different areas of the maze can be tracked more objectively by analyzing the differences in the activity of the animals. Classical tests of depressive behavior include the FST and tail suspension tests [11]. However, both tests induce an acute stress response [12].

This study used the MCM to design evaluation indexes, including the

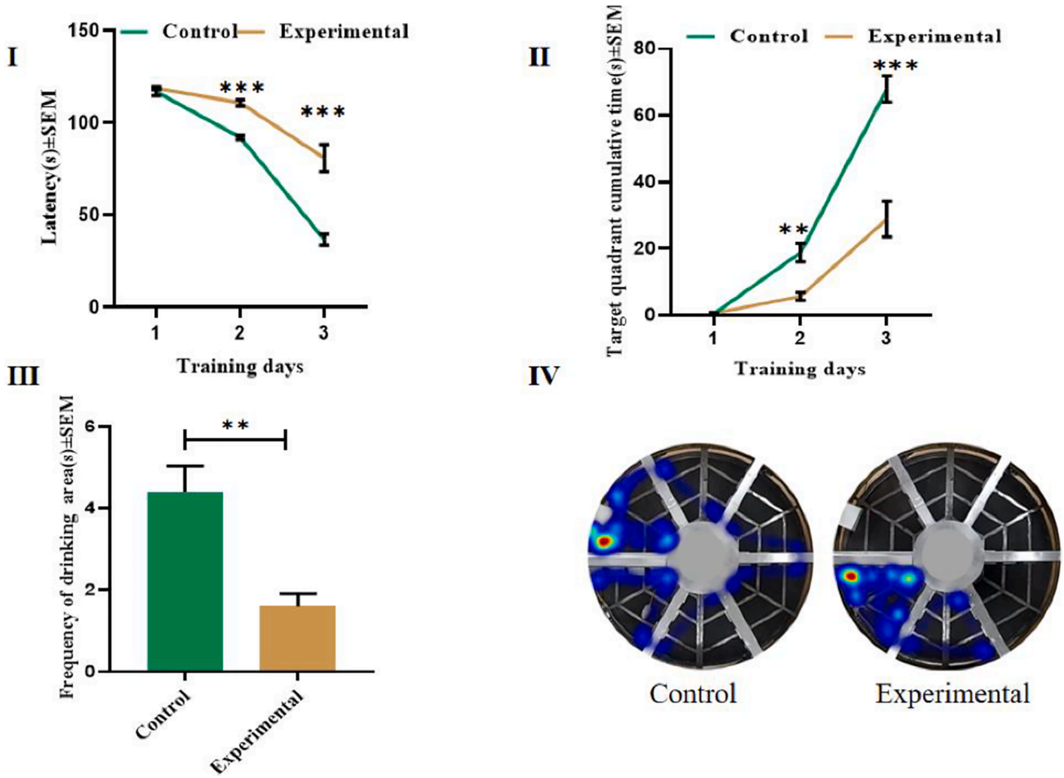

Fig. 2. MCM detects the spatial learning and memory ability of PWSI model mice. Fig. 2 MCM detects the spatial learning and memory ability of PWSI model mice. I: Water-finding latency. II: Target quadrant cumulative time. III: Frequency of drinking water area. Ⅳ: Trace diagram of the hot zone during the test phase. Note: Compared with control group, **P $<$ 0.01, ***P $<$ 0.001, n = 10 per group.

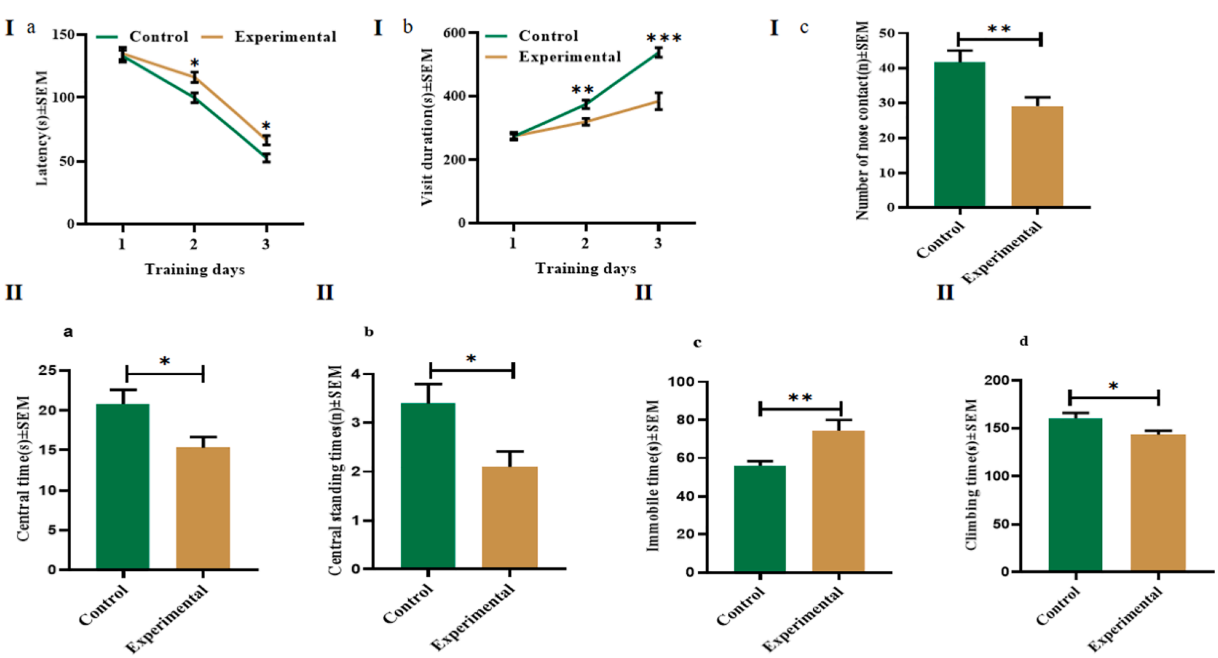

Fig. 3. The effect of IntelliCage test, OFT and FST on PWSI model rats. Fig. 3 I: IntelliCage test of PWSI: a: Water-finding latency, b: Correct angle visit duration time, c: In the test phase, the number of nose contacts at the correct angle. II: Effect of the OFT and FST on PWSI: a: Cumulative time in the central open field, b: Number of times standing in the central open field. c: Forced swimming immobility time, d: Forced swimming climbing time. Note: Compared with the control group, *P $<$ 0.05, **P $<$ 0.01, ***P $<$ 0.001, n = 10 per group.

latency to the reward area, accumulated time and frequency to the reward area, visiting times, and accumulated time in the reward area. These indexes can correspond to the classic indexes of the MWM, as the commonly used detection indicators include the escape latency, proportion of time in the target quadrant, and time to cross the platform [13,14]. This experiment confirmed that the results were consistent with those of the MWM. Common learning and memory evaluation devices include the T-maze [15], Y-maze [16], O-maze [17], eight-arm maze

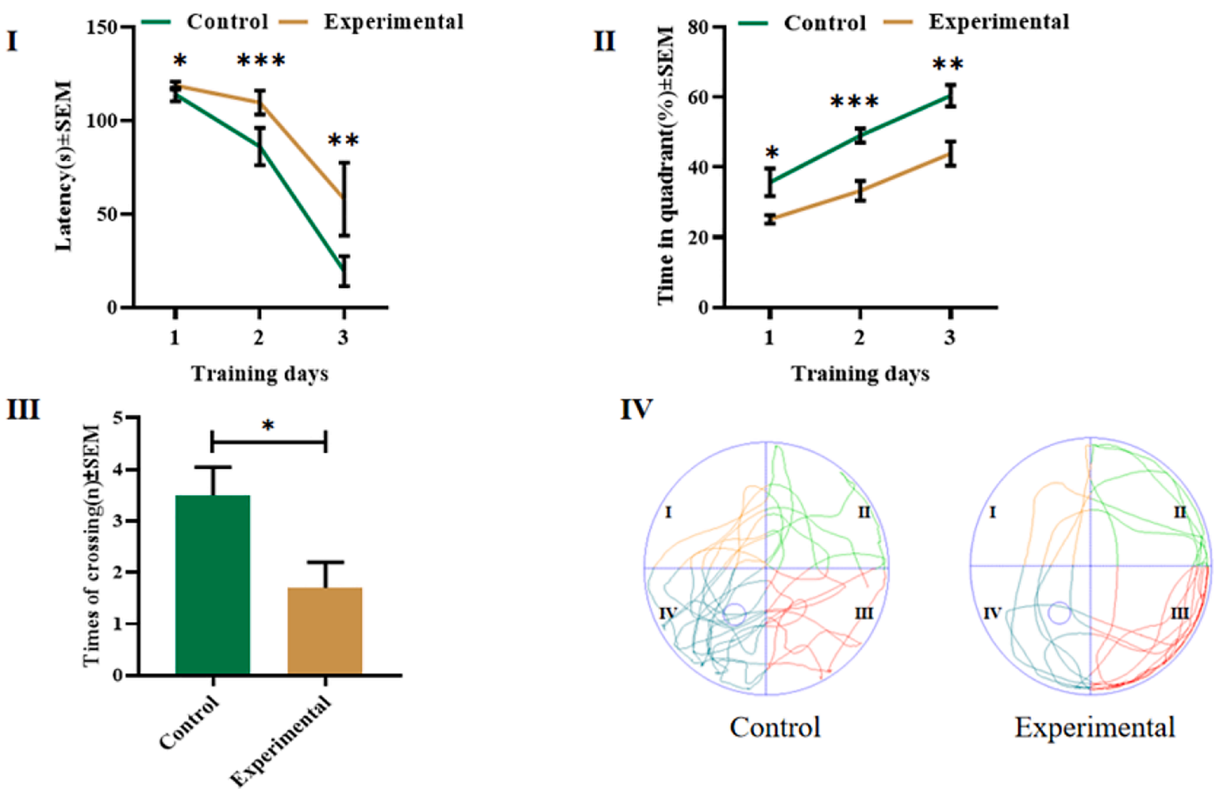

Fig. 4. MWM detects the spatial learning and memory ability of PWSI model mice. Fig. 4 MWM detects the spatial learning and memory ability of PWSI model mice. a: The escape latency to reach the safety platform, b: The percentage of time in the target quadrant (%), c: The safety platform crossings during the test phase, d: In the test phase, the track in the target quadrant. Note: Compared with the control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 10$ per group.

[18], Barnes maze [19] and MWM. In the three days of directed water search of the MCM test, the development of spatial memory was observed as some rats remembered the water bottle's location in the target quadrant or went straight to the target. In the test phase, by analyzing the results of the two maze tracks, we found that the track results for the MWM and MCM tests both showed impaired spatial cognition in PWSI rats [20]. In the MWM test, rats are forced to swim continuously or expand their swimming area to find a safe platform due to their strong need for survival. Studies have shown that the feeling of anxiety and fear caused by MWM affects spatial memory ability in animals [21]. Furthermore, it has been reported that socially isolated rats are particularly sensitive to fear [2,22]. More importantly, in the MWM test, animal performance is also reduced when the presence of water is considered more stressful than thirst because of the rats' motivation to escape [23]. However, in the MCM test, if the rats find the reward area but no reward (water), the exploration time in the reward area is significantly increased (shown as a deep color in the heat area map). This shows that the MCM does not incite animals' fear of water, and that the animals can undergo testing with less pressure and without exerting physical energy.

The MCM provides a wide range of exploration and selection conditions, enabling researchers to monitor the living habits of rats. However, all IntelliCage indicators were produced only when the animal entered the recording chamber [24]. In addition, aggression has been reported in male animals trained for a long time with the IntelliCage system [25]. These studies suggest that the social environment affects the results of the test, which is particularly important in the study of PWSI rats. Therefore, the InttelliCage experiment cannot measure the spatial memory of PWSI rats according to the parallelism principle. It is well known that animals often hoard food [26], and such a phenomenon cannot be automatically observed in real-time with the test.

In summary, we compared the differences between the three instruments by studying spatial memory and emotion-like behavioral changes in PWSI rats. We found that the MCM was the most suitable instrument. As a type of land maze, the MCM avoids the survival pressure of the MWM and improves the shortcomings of IntelliCage system, such as a lack of video tracking. We simultaneously detected changes in mood and memory levels in PWSI rats using the MCM. Our findings can be used as a basis for future research on the influence of emotion on memory. Our results provide new ideas for the future development of MCM behavior science. However, as a new behavioral instrument, the MCM has some limitations in terms of the hardware and software equipment used. The MCM is not accurate enough to simultaneously identify multiple animals at the same time. In the future, we will use chip recognition technology to improve the equipment and facilitate the simultaneous detection of changes in the behavior of multiple animals. So far, the MCM does not have automated door opening and closing; in the future, we will explore automatic door opening and closing. The improved MCM will be used in experiments such as circadian rhythm, location preference, new object recognition, and high-throughput screening of pathological animal models.

CRediT authorship contribution statement

Guangwen Cao: Writing – original draft, Data curation. **Xi Wei:** Software. **Wenbo Li:** Formal analysis. **Haoran Yin:** Formal analysis. **Wenxuan Lang:** Investigation, Validation. **Pengsheng Wei:** Investigation, Validation. **Qiwen zhu:** Project administration. **Ge Jin:** General experiment design, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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