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Research report

Evaluation of spatial memory of C57BL/6J and CD1 mice in the Barnes maze, the Multiple T-maze and in the Morris water maze

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ABSTRACT

Evaluation of spatial learning and memory is mainly carried out using the Morris water maze as a single paradigm. We intended to test whether mice in the Barnes maze and Multiple T-maze would lead to comparable results and to test two individual mouse strains with different anxiety levels.

C57BL/6J and CD1 male mice were used in the experiments. During the acquisition phase, learning was measured using parameters latency, path length, errors in the BM and correct decisions in MTM. Mice were trained for 4 days and probe trials were performed on days 5 and 12.

Latencies reduction over the training period indicated that both strains learned all tasks. During retention phase at days 5 and 12 C57BL/6J performed the Barnes maze and Multiple T-maze task better than CD1 mice while CD1 performed better than C57BL/6J in the Morris water maze. In the BM at day 12, C57BL/6J kept the level of visits to target observed at day 5 whereas CD1 performed worse.

Strain- and task-dependent differences were observed using the three mazes. Therefore, fair evaluation of spatial memory demands application of (at least) two different test systems, a water- and a land maze. Different anxiety-related behaviour as well as stress-responses in the strains used may help to interpret the findings reported and again may propose the use of at least two mouse strains when robust evaluation of spatial memory is considered.

1. Introduction

Numerous models of behavioural tests for the assessment of spatial learning and memory have been introduced and several mouse strains were tested in various settings. [1,8,13,11,20–23,37,38]. The Morris water maze (MWM), however, is widely and mainly used as a single paradigm [14,27,28].

The Barnes maze (BM) was initially designed for testing spatial learning and memory in rats [4] and was afterwards adapted for the mouse [31]. The BM is a land maze, consisting of a large round disk with a number of holes around its perimeter permitting animals to learn the location of an escape box. The motivation to escape to a small dark escape box under the platform is represented by bright light, exposure to an open arena and/or sound.

Multiple T-maze (MTM) is also being utilised as a spatial learning task; mice have to learn a complex route to find the reward. Memory retention is evaluated as the ability of mice to locate the hidden food with decrement of latency and increment of correct decisions. [29]

The use of the MWM for the assessment of spatial learning and memory has been evaluated and used in neuropharmacology, neuropathological and physiological studies including aging [8,25]. Performance in the MWM showed variability across mouse strains [5,19] indicating the importance of the use of more than one mouse strain for MWM testing.

In comparison to the MWM, the BM is considered less aversive, anxiogenic and allowing testing under lower stressful conditions, without physical exertion [2,4]. In particular, testing in swimming tasks as in the MWM is associated with neurochemical changes including serotonergic responses [18] that may well interfere with testing cognitive functions. There is so far no published comparison between the MTM, the BM and the MWM in terms of stressful conditions. There is, however, evidence that mouse strains perform differently in the MWM and in the BM [28] and in a recent previous study the two identical strains used in this settings were tested in the MWM in our laboratory and CD1 performed better than C57BL/6J mice [30].

No systematic study on two most frequently used mouse strains in three different settings, i.e. a water and two land mazes was reported so far and this formed the rationale for the current study adding information on two land mazes to the MWM. It was considered essential for design of studies to find out whether two mouse

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strains were presenting with comparable results in the three different settings. This is not only important for design of future studies but also of importance for evaluation of previous studies that have been applying a single test for the evaluation of learning and memory only.

It was therefore the aim of the study to test learning and memory of CD1 and C57BL/6J in two land mazes and to discuss previously published data on these identical strains and the same laboratory, from the MWM. Taken together, better performance of CD1 in the MWM and better performance of C57BL/6J in the land mazes was observed.

2. Materials and methods

2.1. Animals

Two mouse strains (male, aged 12–14 weeks) were used in the study. To avoid interference between the three mazes, animals were tested only in one maze. C57BL/6J (MWM, $n = 19$; BM, $n = 16$; MTM, $n = 20$) mice and CD1 (MWM, $n = 19$; BM, $n = 19$; MTM, $n = 18$) mice were obtained from Charles River laboratory (Germany) and maintained in cages made of Makrolon and filled with wood chips in the core unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna.

All animals were housed in their individual home cages prior to experiments. An autoclaved standard rodent diet (Altromin 1314ff) and water were available ad libitum. Room temperature was $22 \pm 1\,^{\circ}C$ and relative humidity was $50 \pm 10\%$. The light/dark rhythm was 14:10. Ventilation with 100% fresh air resulted in an air change rate of 15 times per hour. The room was illuminated with artificial light at an intensity of about 200 lx in 2 m from 5 a.m. to 7 p.m. Behavioural tests were performed in the same room and by the same experimenter between 8 a.m. and 1 p.m. Experiments were done under license of the federal ministry of education, science and culture, which includes an ethical evaluation of the project (Project: BMWF-66.009/0152-C/GT/2007). Housing and maintenance of animals were in compliance with European and national regulations.

2.2. Barnes maze

The Barnes maze was designed for testing spatial learning and memory in rats and mice. With the Barnes maze, animals receive reinforcement to escape from the open platform surface to a small dark recessed chamber located under the platform called a "target box". The method was performed according to a recently published technique (doi:10.1038/nprot.2007.390). The escape box is prepared from thermocol (28 cm × 22 cm × 21 cm), and mice can access it through a transparent plastic tube (50 cm long, 5 cm diameter), which is found under the target hole.

The paradigm consists of a circular platform (92 cm in diameter) with 20 holes (hole diameter: 5 cm) along the perimeter. During testing, the mouse learns the spatial location of the target box. Extra-maze cues were all around the room as reference cues to learn the position of the target hole (escape hole).

On the pre-training trial, the mouse was placed in the middle of the maze in a black colored cylindrical start chamber (10.5 cm), and a buzzer (85 dB) was turned on. After 10 s have elapsed, the chamber was lifted, and the mouse was pre-trained to enter the escape box by guiding it to the escape box and remaining there for 2 min. Following the pre-training trial, the first trial started.

At the beginning of each trial, the mouse was placed in the same start chamber, and 10 s after the onset of a buzzer and light, the chamber was lifted and the mouse was free to explore the maze. The trial ended when the mouse enters the goal tunnel or after 3 min have elapsed. Immediately after the mouse enters the tunnel, the buzzer was turned off and the mouse was allowed to stay in the tunnel for 1 min. Mice were trained four trials per day/4 days. Trials were separated by 15 min. After each trial the entire maze was cleaned with 1% incidin solution (Incidin extra N, Lahmann Rauscher), and the maze was rotated to eliminate the use of intra-maze cues [6].

Trials were recorded by using computerised tracking/image analyzer system (video camcorder: 1/3 in. SSAM HR EX VIEW HAD coupled to computational tracking system: TiBeSplit).

The following parameters were recorded: errors, distance from tunnel, search strategy and time that the mouse took to escape into the tunnel. Errors were defined as nose pokes and head deflections over any hole that did not have the tunnel. The search strategies were determined by examining each mouse's daily session and defined in to three categories: (1) Direct (Spatial)—moving directly to target hole or to an adjacent hole before visiting the target. (2) Mixed—hole searches separated by crossing through the center of the maze or unorganised search. (3) Serial—the first visit to the target hole was preceded by visit at least two adjacent holes in serial manner, clockwise or counter clockwise direction.

Mice may sometimes lack motivation and explore the maze after finding the target hole without entering into it. This can be a confounding factor because although the mouse has learned the association between the spatial room cues and the escape location, the number of errors increases due to the further exploration of the maze. Some mice may even seat near the target hole without getting into it. Harrison et al. proposed a solution by calculating latency, path length and number of errors to the first encounter of the escape hole, called primary latency, primary path length and primary errors respectively [10]. Our laboratory adopted this solution [10].

Therefore it is important to measure both total and primary parameters for better understanding and interpretation of data during the acquisition phase.

One day after the acquisition phase, subjects received a probe trial for 90 s to check the short-retention memory. During probe trial the tunnel leading to the target box was closed. Mice were allowed to explore the maze and visits in to the target hole and in the adjacent holes, strategy used, and latency to reach the target hole for the first time were recorded.

On the 12th day, subjects once again received the probe trial for 90s to check long-term retention memory. Mice were not trained during the time period between 5th and 12th day.

2.3. Multiple T-maze

The MTM is one of the spatial learning tasks in which animals learn to find the goal box based on their memory of previously visited arms. The MTM was constructed of wood and consisted of a wooden platform with seven choice points and the dimensions 150 cm × 130 cm × 15 cm and a path width of 8 cm [24,29]. Prior to testing mice were deprived of food for 16 h to motivate food searching. Mice were placed in a start box in a black cylindrical start chamber (diameter: 10.5 cm; [32]). After 10 s elapsed, the chamber was lifted and the first trial was started. Mice were searching for the reward and the trial was completed when mice had reached the goal box or, if failed, after 5 min. Arriving in the goal box, mice were allowed to consume a small piece of a food pellet as provided reward and transferred to their home cage. Immediately after each trial the entire maze was cleaned with 1% incidin solution (Incidin extra N, Lahmann Rauscher). After testing, animals were given food as per body weight (120 g/kg) into the home cage, representing the amount to preserve their body weight but keeping them hungry for the following day for MTM tests. Mice were trained with three trials per day for 4 days. Trials were carried out using 20 min intervals.

Trials were recorded by using computerised tracking/image analyzer system (video camcorder: 1/3 in. SSAM HR EX VIEW HAD coupled to computational tracking system: TiBeSplit). The system provided the following parameters, correct or wrong decision (wrong means a path ending), path length, speed and latency to reach the goal box.

On the fifth experimental day (short-term retention memory), subjects were undergoing a probe trial for 5 min. Mice were allowed to explore the maze and path length, time to reach the goal and correct and wrong decisions were recorded.

On the twelfth day, subjects again performed a probe trial for 5 min to check long-term retention memory. Mice were not trained during the time period between 5th and 12th day.

2.4. Morris water maze

MWM consisted of a circular pool in which mice were trained to escape from water by swimming to a hidden platform as described previously [30]. MWM consisted of a circular pool (122 cm diameter, walls 76 cm depth) in which mice were trained to escape from water by swimming to a hidden platform (1.5 cm beneath water surface) whose location could be only identified using distal extra-maze cues attached to the room walls. Visual cues had different colors and dimensions and were kept constant during the whole experiment. Water temperature was maintained at $21 \pm 1\,^{\circ}C$.

The pool was divided into four quadrants (compass locations: NE, NW, SW and SE) by a computerised tracking/image analyzer system (video camcorder: 1/3 in. SSAM HR EX VIEW HAD coupled to computational tracking system: TiBeSplit). The platform was placed in the middle of the SW quadrant and remained at the same position during the whole experiment.

The spatial acquisition phase consisted of 16 training trials: 4 training trials per day and 4 training days with an inter-trial interval of 20 min. Mice were released randomly with their heads facing the pool wall from the four compass locations, and allowed to swim and search for the platform for 120 s. If mice did not locate the platform after 120 s, animals were manually placed on the platform and allowed to remain on it for 30 s.

On the first training day, mice were given an acclimatization training session in the water maze; mice were placed on the hidden platform, were allowed to swim for 30 s, and were guided subsequently back to the platform. The latency and path length to reach the hidden platform and speed was recorded.

One day after the acquisition phase, subjects received a probe trial, in which the platform was removed. Mice were released from the NE start point and were allowed to swim freely for 60 s. The path the mouse swam was tracked and analyzed for the proportion of swim time and/or path length spent in each quadrant of the pool and swim speed was recorded [17].

On the 12th day, subjects once again received the probe trial for 60 s to check retention memory. Mice were released from the NE start point and were allowed to swim freely for 60 s. The path the mice swam was tracked and analyzed for the