Research report

BALB/c mice are not so bad in the Morris water maze

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Abstract

We compared the learning performances of BALB/c mice subjected to the Morris water spatial task under two different lighting conditions. In the first one, the experimental room was lit by neon tubes (direct and bright illumination) and in the second one by a halogen lamp directed to the roof (diffuse illumination). The scores of BALB/c mice in the diffuse illumination condition clearly demonstrated that these mice could learn to escape to a hidden platform while they could not under direct illumination condition. Moreover, they were able to acquire the task by means of spatial cues. These results are interpreted in terms of a decrease of anxiety levels. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Morris water maze; BALB/c; Lighting condition; Spatial learning

1. Introduction

Compared with many other strains, BALB/c mice are classically described as poor learners in tests requiring spatial abilities, like a water escape task [23], the radial maze [21] and the Morris water maze [11,26]. These poor performances were sometimes attributed to hippocampal particularities described in this strain [21,24]. However, BALB/c mice are also described as very anxious [1], and we know that anxiety may influence the performances in the Morris water maze task [14,20] and that it may vary according to lighting conditions [8,25]. Furthermore, these mice are albinos and therefore probably highly susceptible to bright illumination. As a consequence, high levels of illumination might conduct to high levels of anxiety, thus leading to impairment during learning task. It is then unfortunate that in the literature concerning spatial performances, whatever the apparatus is used, there is usually no precise description of the lighting conditions of the experimental room. As far as we know, only one study investigated the effects of intra-maze contrast and brightness on the performances of BALB/c mice in the Morris water maze [15]. Some of the conclusions suggested that these mice could have good performances when the distance swum was considered. They also presented data of the probe trial, which supported this notion.

The aim of the present investigation was, therefore, to compare the performances of BALB/c mice subjected to the place learning version of the Morris water task [18,19] under two different lighting conditions. The first one was the condition used for testing other strains (see [6] for example) i.e. the device was illuminated by means of neon tubes fixed on the ceiling of the experimental room. In the second one, while appeared to us more favourable for the albino mice, the water maze was diffusely illuminated by means of a halogen lamp.

2. Materials and methods

2.1. Animals

A total of 32 Balb/cByJeo mice (eight males and eight females in each experimental condition) were
used. The strain was purchased from IFFA Credo (France). All the animals were born and reared in the laboratory. During the experiment, they were placed in groups of eight of the same sex, in standard rearing cages (28 × 12 × 15 cm) maintained in an air-conditioned room (22 ± 1°C). The diurnal cycle was reversed (12 h light, 20:00–08:00 h; 12 h dark, 08:00–20:00 h). Water and food were delivered ad lib.

2.2. Apparatus

A water maze derived of the apparatus described by Morris [18] and adapted for mice was constructed. The maze consisted of a green plastic pool, 55-cm in diameter and 40 cm in height, filled with water (24 ± 2°C) to a depth of 20 cm. A platform (7 cm in diameter) was placed at a fixed location during the experiment, 1 cm below the surface of the water, in the middle of a quadrant. Small plastic pieces were placed on the surface of the water in order to make this platform invisible. The pool was always kept in the same position in the room. Many extramaze cues surrounding the pool were available including cues fixed to the walls behind the maze, laboratory furniture, and the experimenter, situated in the same area for all the trials.

In a first condition, the room was lit by a halogen lamp directed to the roof, placed at a corner of the room, which delivered diffuse, dim, white lighting (80 lux at the water surface). In a second condition, lit was delivered by neon tubes placed directly 120 cm behind the centre of the pool. In the latest condition, the light intensity of the light was kept at 120 lux.

According to previous experiments [4,6], the test was divided into three phases, the pretraining session, the learning phase, and the probe test.

2.2.1. Pretraining session

The pretraining session began with the mouse being placed on the platform for 20 s. It was then placed in the water and allowed to swim for 60 s and then returned to the platform for an additional 20 s. Each subject was then given practice for swimming and climbing onto the platform, which was done by placing the mouse in the water with its forepaws against the platform edge. All mice readily climbed onto the platform when so placed. Each mouse was allowed to do this three times.

2.2.2. Learning phase

Training began 1 h after the pretraining session. Each mouse was subjected to three trials per session for four consecutive sessions, with each trial beginning from a different point at the perimeter of the pool (N, S, E, and W). At the beginning of each session, the mouse was placed on the platform for 10 s. A trial began with the experimenter removing the mouse and placing it in the water facing the maze wall. It ended when the mouse climbed onto the platform. The latency before reaching the platform was recorded manually with a stopwatch. The three latencies of a session were added (LAT) to analyse the performances of the mice. If the mouse failed to climb onto the platform within 60 s, the trial was stopped and the mouse was removed from the water and placed upon the platform. The intertrial interval was 10 s, the animal remaining on the platform during this period before beginning the new trial. At the end of the three trials, the mouse was removed and placed under a lamp for warmth. The inter-session interval was 2 h.

2.2.3. Probe test

One hour after the last training trial, the animals were subjected to a probe test, during which the mouse swam, for 60 s in the absence of the training platform. This trial was recorded from a video camera. The probe test trial was analysed by measuring the time spent in each quadrant of the pool (TIME).

3. Results

A 2 × 2 × 4 analysis of variance (ANOVA) with repeated measures on the last factor was conducted for the two lighting conditions (halogen or neon), the two sexes (male or female) and the four learning sessions. The lighting condition main factor, *F*(1, 28) = 9.99; *P* < 0.005, and the session main factor, *F*(3, 84) = 11.28, *P* < 0.001, were significant, but not the sex main factor, *F*(1, 28) = 0.4, *P* > 0.1. None of the interactions was significant: lighting condition × sex, *F*(1, 28) = 1.34, *P* > 0.1; lighting condition × session *F*(3, 84) = 0.66, *P* > 0.1; sex × session, *F*(3, 84) = 0.62, *P* > 0.1; lighting condition × sex × session double interaction, *F*(3, 84) = 1.65, *P* > 0.1. As shown in Fig. 1, irrespective of sex and sessions, mice performing in the halogen

![Fig. 1. Mean escape latencies (in seconds) on successive sessions. Each point represents the cumulative score per session (the sum of three trials). *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.](image-url)
light condition had lower escape latencies in the Morris water maze than mice performing in the neon light condition.

During the probe test, a three-way ANOVA was conducted with the two lighting conditions, the two sexes, and the four quadrants. The lighting condition × quadrant interaction was significant, $F(3, 84) = 5.51$, $P < 0.002$. As shown in Fig. 2, mice in the halogen light condition spent more time in the training quadrant than mice in the neon light condition. In the diffuse condition, mice spent more time in the training quadrant than in it was expected by chance. In contrary to the more direct condition, mice performed at chance level (about 15 s).

4. Discussion

The poor performance of BALB/c mice in the neon light condition was consistent with results from previous studies where the lighting condition was not specified [11,26]. The good performance of the same strain in the halogen light condition is consistent with the results of Klapdor and van der Staay [15]. Moreover, the final performances of BALB/c mice and found no intergroup performance difference in the radial maze. In the same way, Fancis et al. [11] found no impairment in albino CD-1 mice in water maze learning. This result suggests that poor visual acuity is not the only factor responsible for the bad spatial learning abilities of BALB/c mice in comparison to many other strains such as pigmented C57BL/6 mice. Nevertheless, the results of our study indicated that the lighting condition was an important factor in the spatial performance of BALB/c mice. It was likely that the diffuse homogeneous lighting provided by the halogen lamp allowed the animals to see better the extramaze visual cues than the neon light condition did. In the latter condition, the light reflects more brightly in certain areas of the pool than in others and may impair the ability of an albino strain to see the room cues.

Moreover, a high level of anxiety may cause the poor performance of BALB/c mice in that condition, as fear or stress has been shown to affect Morris maze performance in rats [14,20]. It has previously been shown that BALB/c mice are more anxious than other strains in various tests such as the elevated plus-maze [1,2,13]. Additionally, lighting conditions affect the behaviour of BALB/c mice in different anxiety tests [5,8,25]. For example, the fact that BALB/c mice are more active in an open field when exposed to red lights than when exposed to bright white lights is an indication that this latter condition is more fearful for BALB/c mice. According to data provided by Zaharia et al. [27], we can then assume that the anxiety level could be one of the main explicative factors for the bad performances of BALB/c mice in water maze task. So, it is possible to think that we can increase learning performances by decreasing anxiety level, and it has been demonstrated that rearing in enriched environment, which decreased emotional reactivity [5,9] increased learning performances (see [22] for a review).

Data from pharmacological studies tend to be inconsistent with this assertion. Indeed, pharmacological compounds such as benzodiazepine receptor agonists, which reduced anxiety level [10], decreased spatial performances [3,17,27]. However, it is well known that benzodiazepine has both anxiolytic and amnestic effects. The link between anxiety and memory remains to be clarified.

References


