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Research report

Spatial learning by mice in three dimensions

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HIGHLIGHTS

- Mouse spatial memory was tested on a novel 3D radial arm maze.
- Mice exhibited learning on working and reference memory tasks on the 3D maze.
- Working memory was not impaired on the 3D maze when compared with a 2D analogue.
- Reference memory was impaired on the 3D maze when compared with the 2D maze.
- This may be explained by a differential encoding of vertical and horizontal space.

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ABSTRACT

We tested whether mice can represent locations distributed throughout three-dimensional space, by developing a novel three-dimensional radial arm maze. The three-dimensional radial maze, or "radiolarian" maze, consists of a central spherical core from which arms project in all directions. Mice learn to retrieve food from the ends of the arms without omitting any arms or re-visiting depleted ones. We show here that mice can learn both a standard working memory task, in which all arms are initially baited, and also a reference memory version in which only a subset are ever baited. Comparison with a two-dimensional analogue of the radiolarian maze, the hexagon maze, revealed equally good working-memory performance in both mazes if all the arms were initially baited, but reduced working and reference memory in the partially baited radiolarian maze. This suggests intact three-dimensional spatial representation in mice over short timescales but impairment of the formation and/or use of long-term spatial memory of the maze. We discuss potential mechanisms for how mice solve the three-dimensional task, and reasons for the impairment relative to its two-dimensional counterpart, concluding with some speculations about how mammals may represent three-dimensional space.

1. Introduction

The ability to accurately navigate through the world is vital for the survival of mobile animals, and requires the perception and encoding of spatial cues associated with important locations such as food nesting location. Studies over many years have concluded that mammals create an internal representation of space (sometimes known as the cognitive map; [1]). This work has primarily focused on spatial navigation in horizontal planar environments: however, recently, interest has been growing in the means by which larger and more dimensionally complex spaces might be represented and used in navigation (see Jeffery et al. [2] for review). Few laboratory studies of three-dimensional spatial encoding have been conducted to date, and so the aim of the present experiment was to determine whether mice could perform a 3D version of a widely used laboratory-based navigation task, the radial maze task [3].

The advantage of the Olton radial maze paradigm is that it can test both long-term (reference) and short-term (working) memory concurrently. In the 3D version of the maze, which because of its spherical symmetry we have named the radiolarian maze,[1] food rewards are located at the end of arms that project from

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[1] Radiolaria are zooplankton having radial symmetry: see https://en.wikipedia.org/wiki/Radiolaria.

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a sphere, rather than the usual disc, so that for each horizontal arm coordinate there are two vertical coordinates. Thus, animals have to remember the distribution of rewards by encoding both horizontal and vertical components simultaneously, and also dynamically update this representation as trials progressed and the arms become depleted. We used mice because they are lightweight, are skilled climbers and have a naturally 3D ecology.

The animals were trained on two versions of the radial maze task, the standard version in which all arms begin by being baited and are depleted without replacement as the trial progresses, and a reference memory task in which only some of the arms are ever baited. We reasoned that if mice cannot encode the vertical component of the space then they should have difficulty learning both versions of the task due to confusion between the upper and lower arms at a given azimuth (horizontal direction). Comparisons with a version of the original radial arm maze and with a two-dimensional analogue of the radiolarian maze revealed that mice are equally able to learn the working memory version of the task in the radiolarian maze as in two-dimensional mazes. In the reference memory task the rate of reduction in reference memory errors were comparable for the radiolarian maze and its two-dimensional analogue; however, overall learning was reduced in the radiolarian maze. We suggest that mice are able to simultaneously represent both vertical and horizontal components of a spatial task, but that having to encode both components creates difficulties for them. We conclude with a discussion of how the 3D structure of the maze task may be represented.

2. Methods

2.1. Subjects

Subjects were 40 male C57BL/6J mice obtained at 8–10 weeks of age from Charles River Laboratories, individually housed and mildly food restricted to maintain body weight at 90% of free feeding weight. A 12 h reversed light/dark cycle was used with 30 min simulated dawn at 23:30 and simulated dusk at 11:30; all mice were trained during their dark cycle between 12:30 and 15:00. Experiment 1 used three cohorts of mice (each $n = 8$) while Experiment 2 used two (each $n = 8$). All mice were naïve to the experimental apparatus prior to habituation. All procedures carried out during these experiments were licensed by the UK Home Office, subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act of 1986.

2.2. Apparatus

The apparatus comprised three versions of the classic radial arm maze: a 3D version named the radiolarian maze (Fig. 1A), a 13-arm version referred to throughout as the classic maze (Fig. 1B), and a two-dimensional analogue of the radiolarian maze, named the hexagon maze (Fig. 1C). Arms were baited with condensed milk applied to dressmakers' pins inserted at the end of each arm. All three mazes were used for Experiment 1, and just the radiolarian and hexagon mazes for Experiment 2. All experiments were carried out in the same well lit room, with consistent visual extramaze cues available to mice throughout data collection.

The radiolarian maze (Fig. 1A) was constructed from lightweight materials and coated with papier-mâché followed by crèpe bandage, to provide grip. The central section comprised a 30 cm diameter sphere from which radiated 14 equidistantly placed cylindrical arms, each 14 cm in length and 3.5 cm in diameter. The maze was suspended by nylon line in the centre of an empty 19-inch rack, with the lowermost arm 30 cm above the floor of the rack.

The classic maze (Fig. 1B) was a 13-arm version of the standard Olton radial arm maze, constructed using MDF and covered with crèpe bandage so as to maintain consistency with the radiolarian maze. The central section comprised a 30 cm diameter circle from which radiated 13 evenly spaced arms, each 14 cm in length and 3.5 cm in diameter. The maze was raised 30 cm above a table, and was placed in the centre of the experimental room.

The hexagon maze (Fig. 1C), a two-dimensional analogue of the radiolarian maze, had 12 arms. The maze constituted a hexagonal ring, with 30 cm sides each 3.5 cm in width, with six 14 cm arms, with a width of 3.5 cm, extended outwards and six 14 cm arms extended inwards from the corners of the ring. Thus, on returning from an arm excursion mice would have four choices—turn left, turn right, go straight ahead or turn back. This maze was therefore more geometrically similar to the radiolarian maze than was the classic maze. The maze was covered in crèpe bandage, so as to maintain consistency with the radiolarian and classic mazes, and was again raised 30 cm above a table in the centre of the experimental room.

2.3. Habituation

For both experiments, subjects were habituated to each maze for 5 days before training commenced. In the first 2 days no arms were baited and mice were allowed to freely traverse the maze for 15 min. In the final 3 days each mouse was introduced to the maze from each of the arms in turn. Once it made its way from an arm to the centre of the maze it was removed and placed on another randomly selected arm. This was repeated until all animals willingly navigated from each of the arms to the centre within 1 min.

2.4. Experiment 1—working memory task

2.4.1. Subjects and training

Each cohort of mice ($n = 8$ per cohort) was trained on one of the three mazes only.

Once habituation was completed, the working memory phase of training began. For the working memory task, all of the arms of each of the mazes were baited with condensed milk. Mice were required to retrieve food from all arms of the maze. An arm visit was only recorded when an animal's head reached the end of an arm. The number of re-entry errors (repeated visits to an already-depleted arm) and omission errors (number of unvisited arms) and order of visits were scored manually by two experimenters, who sat in opposing corners of the experimental room. Mice were removed from the maze after either 15 min or once they had collected the food reward from all arms of the maze, whichever was soonest. Mice were trained on this task for one trial per day for at least 7 days or until the number of omission and re-entry errors had reached a 3-day plateau, defined as a non-significant difference between the final 3 days of trials (using repeated measures ANOVA).

2.4.2. Analysis

Paired *t*-tests comparing the first three trials to the last three trials were used to assess learning. Values for task latency, the total number of visits, the number of omission errors and the rate of re-entry (working memory) errors as a percentage of total visits were compared between mazes using repeated measures ANOVA.

For the movement pattern analysis on the radiolarian maze, the lower vertically projecting arm was removed from analysis as this arm was only ever visited by two mice, and was consistently the last to be visited. For comparison of movements within and between layers, seven arms comprised the upper layer—the upwards projecting arm at the top of the maze and the six outwards projecting arms on the top section of the maze. The six arms projecting outwards in the lower half of the maze comprised the lower layer. The