Research report

Barnes maze performance of *Octodon degus* is gender dependent

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ABSTRACT

Gender differences in spatial navigation have been widely reported in nocturnal rodent species. Here, for the first time we report gender differences in spatial learning and memory of *Octodon degus*, a long-lived diurnal hystricomorph rodent. In the present study, 16 months old male and female *O. degus* were tested in the 18-holes Barnes circular maze. The acquisition session consisted of four daily 4 min trials, during 10 days. Seven days later, the retention test was performed. To avoid the effect of hormonal fluctuation on spatial navigation, both the acquisition and the retention tests, were performed in 21-day regular cycling females in a period that corresponds to the diestrus phase of the estrus cycle. At the beginning of the acquisition, female *degus* were significantly slower than males to find the escape hole, but the situation reversed afterwards. Moreover, during the course of acquisition, females made significantly less reference memory errors, working memory errors as well as omission errors, than males. In both sexes, motivation and learning ceiling effects were reached at days 5–6 of the training. During the acquisition, females used more frequently a spatial strategy, while males preferably applied either serial, random or opposite strategies. The observed cognitive differences between male and female *O. degus* existed only during the acquisition period but not during the retention, indicating that acquisition and consolidation are differently influenced by gender.

1. Introduction

Spatial learning, defined as coordinated and goal-directed movement through the environment [10], is one of the most essential cognitive abilities. Although the existence of gender dimorphism in spatial navigation has been questioned, a male advantage in spatial navigation ability is commonly observed in several mammalian species, including humans [e.g., for review see [2,23]]. Experimental studies in animals were usually performed in nocturnal rodents (rats and mice), having a short lasting estrus cycle (4–5 days), during the rest period of their daily activities. The results obtained from those studies are inconsistent. In several studies, male rats outperformed females in the Morris water maze [7,14,22,36], the radial maze [22] and the Barnes maze [1]. On the other hand, there are studies that indicate no significant differences between genders in performance of the Morris water maze [9,17] and the radial maze [40]. Interestingly, in one study it has been demonstrated that females outperform males in the Barnes maze [46]. Mouse studies have shown that male mice are superior to females in the water-escape radial maze [16] and the spatial novelty task [13], superior or equal to females in the Morris water maze [[4,44], respectively] and superior [30] or equal [18,33,29] to females in the Barnes test.

It has been postulated that hormone fluctuations across the estrus cycle may account for apparent inconsistent male advantages. Several reports have shown that spatial learning and retention ability in the Morris water maze of female rats [14,17,45] and mice [12] depends on the phase of the estrus cycle, suggesting that the diestrus is a period in which females do not differ significantly from males [14,17]. In some other studies, no differences in navigation performance by female rats across the estrus cycle were found in the Morris water maze [5,9] and the radial maze [38]. Furthermore, gonadally intact female rats outperformed ovariectomized females during acquisition of the radial maze, but not during acquisition and retention in the water maze [11].

We hypothesize that a study in a diurnal rodent that has a longer lasting ovarian cycle and testing the animals during their active period would be more comparable to studies performed in humans. Therefore, the aim of the present study was to evaluate gender-related differences in spatial behaviour in *Octodon degus*, a

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long-living diurnal hystricomorph rodent (life span up to 10 years) where females display an ovarian cycle of 21 days, with a luteal phase of approximately 14–15 days [25]. *Degus* has been found suitable to investigate diurnal rhythm [15,26,41–43], social behaviour [3] and age-related neuropathology and behavioural alterations [20,35,39]. Moreover, in this specie exists sex differences in the circadian rhythms [19,25,27,28] as well as in brain morphology [21,34,48,49], but not behavioural changes [8] as response to early-life adverse emotional experience. Recently, it has been reported that *degus* can be successfully trained to use tools with the fore-limbs to retrieve a distant food reward. When given a choice, tools can be of different size, colour, or shape, and animals are reluctant to use tools with a non-functional blade [32]. Data about the spatial navigation abilities of these animals are still lacking. Since our previous data indicated that *degus* expressed thigmotaxis in the open field [35], we presumed that the Barnes circular maze could be a suitable model to test its spatial navigation. Here we present data indicating that in this particular low-stressful and non-food motivated test, female *degus,* more than males, use spatial navigation strategies.

2. Material and methods

2.1. Animals

O. degus, of both sexes (eight males and 10 females), at the age of 16 (±1) months, were obtained from the Animal Service of the University of Murcia. The animals were individually housed in Plexiglas cages in an isolated room (Chrono-lab), with controlled humidity (60%) and temperature (23 ± 1 °C), and under a 12:12 light/dark cycle (light on from 08:00 to 20:00 h). Light was provided by fluorescent lamps controlled by an electronic timer (DataMicro, Orbis), with a light intensity of 350–400 lx at the cage level. The *degus* were fed *ad libitum* throughout the experiment, using a commercial rat chow (A04 rat–mouse maintenance Panlab).

The experiments were performed during the light period (09:00–15:00 h) and were recorded using a webcam to enable subsequent evaluation. The recordings were analyzed by two researchers (one of them was kept blind concerning the animal groups). To avoid the effect of hormonal fluctuation on spatial navigation, both the acquisition and the retention tests in 21-day regular cycling females were performed in a period that corresponds to the diestrus phase of estrus cycle.

All procedures related to animal maintenance and experimentation were in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the guidelines issued by the Spanish Ministry of Agriculture, Fishing and Feeding (Royal Decree 1201/2005 of October 21, 2005) and were approved by the Institutional Animal Ethics Committee. Efforts were made to minimize the number of animals used, as well as their suffering.

2.2. Barnes maze test

The Barnes maze consisted of a circular platform 160 cm in diameter raised 75 cm from the ground and surrounded with 45 cm high wall. The platform was made of white plexiglas. Eighteen circular holes (8 cm in diameter), were bored through the platform equidistant from each other (16 cm), and 5.5 cm from the outer edge. All holes except the target one were blocked with mesh. A plastic transparent escape box (31 cm long × 16 cm wide × 13 cm high), was positioned under the escape hole. The start box was a white, open-ended cylinder (20 cm in diameter and 15 cm high, easily lifted from the platform to the roof, approximately 3 m above. The maze was positioned in a room with many extra-maze cues (e.g., geometric figures, pictures, cage racks, furniture, etc.) to permit orientation of *degus*. The testing room was illuminated by fluorescent lights located on the ceiling (normal room lighting) such that the platform was exposed to uniform illumination of 210 lx. A web camera was secured on the roof, directly above and focused on the maze surface, and plugged directly into the data acquisition computer. This allowed the experimenter while seated outside of the field of view of *degus*, to raise the start box, and immediately begin recording of its behaviour.

2.3. Habituation session

During habituation period, the escape box was filled with the bedding from the home cage. The habituation session was divided into three parts. It began with placing the animal into the transparent escape box for 2 min. In the second part, the animal was placed near the escape hole surrounded by the start box and left for 1 min to escape. If the animal did not enter into escape box, it was gently picked
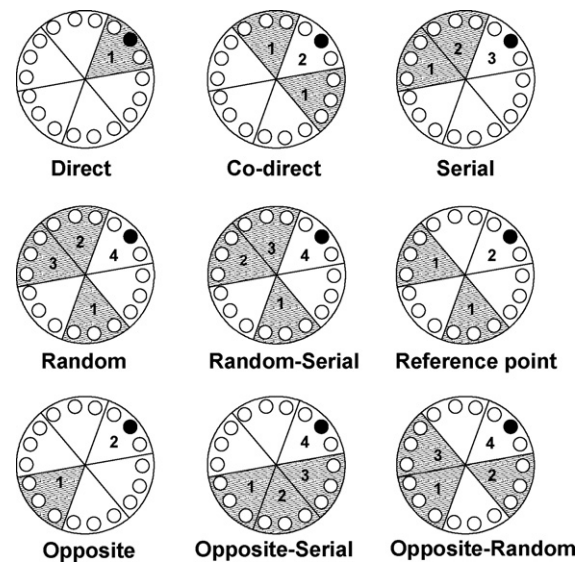
Fig. 1. Strategy type. Schematic representation of the 18-hole Barnes maze. The maze is divided into six segments of three holes each. The numbers in the segments indicate the order in which areas were visited for each of the nine differentiated strategy types. The escape hole is indicated in black.

up and put through the target hole into the escape box. The animal was left in the escape box for 2 min. Finally, in the third part, the animal was put in the centre of the maze, left during the following 4 min to enter into escape box. In case the animal did not enter in the escape box, it was put into the escape box as mentioned above and left there for 2 min. Each part of habituation session was separated by a 5 min resting phase, which *degus* spent in its home cage. During this period, the maze and start box were cleaned thoroughly with 70% alcohol solution to remove odours.

2.4. Learning and memory

Two days after the habituation session, the animals were trained for 10 days and then exposed to a retrieval session, 7 days later. Each training or retrieval session consisted of four consecutive 4 min trials, separated by a 5 min resting phase in the animal home cage. At the beginning of each trial, *degus* was confined for 30 s in the start box in the centre of the maze until a trial was initiated by lifting the cylinder. Each *degus* was permitted to explore the maze freely for the 4-min session. If the *degus* did not entered the escape box within the allotted time it was gently picked up and placed into the escape box. The animal was left in the escape box for 2 min before being returned to its home cage for 5 min. The escape hole remained at a constant position throughout all trials and sessions. Between trials, the surfaces of the maze platform and start box were thoroughly cleaned with alcohol.

The following parameters were recorded:

- Number of entrance into escape hole.
- Latency to the first visit of any hole (visit a hole was defined as searching a hole, including nose pokes and head deflections).
- Latency to the first visit of escape hole.
- Decision time of entrance into the escape box: the period that passed from initiation of exploration of the escape hole and entrance into the escape hole.
- Latency to escape.
- Number of visit of escape hole as the first visited hole.
- Number of omission errors (visit of the escape hole without escape was scored as an 'omission error').
- Number of correct responses (the response was defined as 'correct' when the degus visits the escape hole and enters into the escape box at the first instance).
- Number of reference memory errors to the first visit of escape hole (on each trial, every first visit of a non-escape hole was scored as a 'reference memory error').
- Number of reference memory errors per omission error.
- Number of working memory errors to the first visit of escape hole (repeated visits to the same non-escape hole on the same trial were scored as 'working memory errors').
- Number of working memory errors per omission error.
- Distribution of start point by hole: the incidence of the first visit of each hole.
- Distribution of errors by hole: the number of total errors per each hole.
- Search strategy to find the escape hole.

The strategy was determined by observing the overall search pattern. For this we divided the maze into six areas (each corresponding to three holes). Nine strategy types were defined (Fig. 1). The first visit of the target area (target hole or one hole