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Research report

Behavior on the water maze platform: Relationship to learning and open field exploration in aged and adult rats

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Abstract

In the present study we examined whether age-related deficits in the water maze could be accounted for by changes in the behavior on the platform, and whether platform behavior represents some form of exploration, akin to that seen in the open field. Twenty-seven aged and 8 adult rats (26 and 3 months old Wistar rats, respectively) were tested in an empty open field and spatial object exploration task, followed by 9 days of escape learning in a water maze. The aged as compared to the adults exhibited lower levels of open field activity and were deficient in the object displacement task. Escape deficits in the water maze and reduced activity levels on the platform were also found. In the aged, water maze performance was correlated with behavior on the platform, including quiescence, orienting-like activity and turning behavior, a form of axial rotation. In both age groups, turning behavior was also correlated with exploratory activity in the open field. In conclusion, the results support the hypothesis that age-related impairments in the water maze relate to changes in platform behavior, which, in turn, might reflect exploratory activity.

Keywords: Spatial learning and memory; Behavior on platform; Turning; Object displacement task; Exploratory activity

1. Introduction

In the water maze rats learn the position of a hidden platform on the basis of extra-maze visual and other sensory cues [14]. It was suggested that the short time periods that the rats are allowed to remain on the platform after each swimming trial are used for spatial orientation in the service of acquiring the spatial relationships between the platform and the cues in the environment [26]. Sutherland et al. observed that rotations and rearings occur especially at the beginning of hidden platform training and cease as escape becomes proficient [28]. Moreover, viewing the distal environment from the platform in advance, helped to improve escape performance during the training phase [27]. In the present study, we set out to examine the platform behavior of aged and adult rats and to analyze its relationships with learning in the water maze.

Similar to elderly humans, aged rats show variable degrees of learning impairments in spatial tasks, such as the water maze [8,12]. They do not acquire a spatial preference for the platform quadrant as readily as adults and are more persistent in swimming along the walls of the maze [22]. Exploratory behavior is related to concepts of arousal and attention and determines if and to which degree information is acquired [1,2,15]. Accordingly, the degree of information seeking would be expected to correlate with learning. Aged as compared to adult rats were found to exhibit lower levels of "exploratory" activity in an open field, i.e. lower activity levels and fewer numbers of rearings [20]. Moreover, in aged superior water maze learners, a higher number of rearings was related with spatial learning proficiency in the water maze [21]. On the other hand, in aged intermediate learners, more rearings coincided with worse learning [19]. In another study, a higher number and longer durations of open field rearings in aged and adult rats co-varied with longer swimming times near the walls of the water maze [29], which may indicate that the gathering of information about the maze environment may not only take place on the platform, but also in the periphery of the pool. This may explain why groups of animals, which were allowed to remain on the platform for either 60 or 1 s

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after each swimming trial, were shown to have similar escape latencies [10]. In the present study, the main intention was to examine whether the behavior on the hidden platform was predictive of water maze learning in aged and adult rats. Moreover, in order to assess whether platform behavior represents a form of exploration, akin to that seen in an open field, the animals were tested in an empty arena and an object exploration paradigm, and the scores were correlated with the behavior on the hidden platform.

2. Materials and methods

2.1. Subjects

The study was carried out in accordance with the German Law on the Protection of Animals and was approved by the state authority (Bezirksregierung Düsseldorf). Altogether 36 aged and 8 adult male Wistar rats (26 and 3 months old, respectively) were obtained from the animal facilities of the University of Düsseldorf. They were housed in groups of two to three and were maintained on a reversed 12-h light:12-h dark cycle (lights on at 19.00 h), with free access to food and water.

2.2. Open field

The open field was a dimly lit (ca. 0.30 lux at the center of the field) 60 cm × 60 cm dark grey Plexiglas arena with 39-cm-high walls. A video camera (SONY, DCR-TR V20E) was mounted ca. 1 m above the field; all sessions were videotaped and analyzed with EthoVision software (Noldus, Wageningen, The Netherlands). A broad-spectrum generator emitted noise at 60 dB.

The animals were placed individually into the center of the open field facing the same wall in all of the tests. They were allowed to explore the arena for 20 min on the first testing day and for 10 min the next day. On the 3rd day, they were exposed once more to the empty open field for 5 min and then removed for 1 min. During this time, two objects – blue cups of 10 cm height, 6 cm diameter on the bottom and 8.5 cm diameter on the top, with a handle on one side – were placed upside down into the open field, each of them in the middle of one of the walls, across from one another. The animals were then placed back into the field and allowed to "explore" the arena for 6 min. They were then removed once more for 1 min, during which time one of the objects was spatially moved to the middle of one of the remaining walls, diagonal to the stationary object. The animals were placed back into the maze for another 6 min. All of the available configurations of objects were equally employed and varied over the subjects. The experiments took place between 08:00 and 18:00 h. The following variables were measured to assess exploration of the empty open field: distance (cm) moved and time (s) spent in the periphery and center (30 cm × 30 cm), number of rearings. During object exploration the number of contacts with the objects (snout touching the cup) was counted.

2.3. Morris water maze

Water maze training began 3 days after the last open field test. A circular 185 cm diameter swimming pool made of black polyethylene was filled 30 cm deep with 20 ± 1 °C water. A 18 cm diameter black PVC platform was submerged 1.5 cm under the water surface level. It was randomly located in the center of one of four equally large virtual quadrants (platform quadrant) for all rats, but was maintained in a constant position throughout training for each animal. A sequence of four equidistant starting points was randomly varied on each test day, but was the same for all animals during a given test day. Cues located around the maze, such as posters, a blackboard and cupboard, were available for spatial orientation and highlighted by four 75 W bulb lights. Diffuse ceiling lighting provided additional illumination (ca. 6.5 lux at the surface of the pool). Computer-based systems for behavioral analysis were placed behind a barrier remote from the maze. A camera was mounted 2 m above the maze.

The rats were brought to a waiting room at least 30 min before the experiments and were kept in holding cages shortly before testing. At the beginning of each trial, they were individually placed into the pool facing the wall. One adaptation trial of 2 min was run without a platform present and was followed by 9 days of training with the platform hidden. A training trial was terminated when the animal escaped onto the platform or after a maximum of 2 min, after which the rat was guided to the platform by an experimenter. Once on the platform, it was left there for 30 s. Between trials the animals were kept in holding cages for 60 s. After two trials, the rats were dried with paper towels and heated by two 75 W light bulbs. Two training trials were run in the morning and two in the afternoon, every 3rd day. All swimming trials were recorded on video and analyzed via Ethovision (Noldus, Wageningen, The Netherlands). Testing took place during the animals' active night cycle between 9.00 and 17.00 h.

Time [22] and distance (cm) swum to the hidden platform were measured for each acquisition trial; four trials were averaged for each training day. The decrease in centimeter required to locate the platform over the course of 9 training days was taken as an index of learning. Higher as compared to lower mean escape distances averaged over all days of training (levels of learning) were derived and used for comparison of the age groups. Distance moved in the platform quadrant and near the walls of the pool was also quantified. For statistical evaluations, distance moved in the platform quadrant was expressed as percent of the distance moved in all four quadrants before the animal reached the platform (escape distance). Distance swum near the walls of the pool, designated as "thigmotactic swimming", was also expressed in terms of percentage of the distance moved in the maze. For measuring the latter, the maze was divided into three equidistant circles (as measured by diameter to the midpoint of the pool), including an outer, a middle, and an inner circle (each of them 30.83 cm wide). Distance moved in the outer circle was taken as an index of "thigmotactic swimming".

Platform behavior was also analyzed for each trial and averaged per day as well as for all 9 training days. Quiescence (s) on the platform was defined as the lack of motion of the whole body. Activity was taken to be all behavior excepting quiescence, and thus, represents the inverse of quiescence. Different kinds of active behavior could be discerned: orienting-like activity (s) included head and body movements but not headshakes (s) and grooming (s), which were measured separately. The duration (s) of rearings comprised a very small percentage of all activity (aged overall mean ± S.E.M.: 0.09 ± 0.02; adult: 0.24 ± 0.06), so that it was included with the measure "orienting-like activity". Turning behavior (s) was a defining feature of the activity on the platform, and was measured when the animal rotated at least 360° around its own axis. A turn could include not only orienting-like behavior, but also short bouts of quiescence, headshakes and grooming.

2.4. Statistical analysis

All data used for statistical evaluations were checked for normality and equal variance of the distributions, using the Shapiro–Wilks test and Levene-Statistic, respectively. Results did not allow for parametric testing to be used in most cases. Accordingly, group comparisons were performed using the Mann–Whitney *U*-test. For within-group comparisons, Friedman or Wilcoxon tests were applied. Spearman rank-order correlation procedures were also used to examine the relationships between variables. Due to the large number of tests performed, *p*-values ≤ 0.05 are presented solely as measures of effect, taking into account the probability of occurrence of Type I error.

3. Results

3.1. Subjects

Of the 44 animals obtained, 27 of the aged and all 8 adult animals were used for statistical evaluation. The other 9 animals showed obvious signs of physical weakness or illness during experimentation; 4 animals could not complete the water maze task and the other 5 were found to have tumors when their brains were removed several weeks after the behavioral tests.