Video Article

Barnes Maze Testing Strategies with Small and Large Rodent Models

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Abstract

Spatial learning and memory of laboratory rodents is often assessed via navigational ability in mazes, most popular of which are the water and dry-land (Barnes) mazes. Improved performance over sessions or trials is thought to reflect learning and memory of the escape cage/platform location. Considered less stressful than water mazes, the Barnes maze is a relatively simple design of a circular platform top with several holes equally spaced around the perimeter edge. All but one of the holes are false-bottomed or blind-ending, while one leads to an escape cage. Mildly aversive stimuli (*e.g.* bright overhead lights) provide motivation to locate the escape cage. Latency to locate the escape cage can be measured during the session; however, additional endpoints typically require video recording. From those video recordings, use of automated tracking software can generate a variety of endpoints that are similar to those produced in water mazes (*e.g.* distance traveled, velocity/speed, time spent in the correct quadrant, time spent moving/resting, and confirmation of latency). Type of search strategy (*i.e.* random, serial, or direct) can be categorized as well. Barnes maze construction and testing methodologies can differ for small rodents, such as mice, and large rodents, such as rats. For example, while extra-maze cues are effective for rats, smaller wild rodents may require intra-maze cues with a visual barrier around the maze. Appropriate stimuli must be identified which motivate the rodent to locate the escape cage. Both Barnes and water mazes can be time consuming as 4-7 test trials are typically required to detect improved learning and memory performance (*e.g.* shorter latencies or path lengths to locate the escape platform or cage) and/or differences between experimental groups. Even so, the Barnes maze is a widely employed behavioral assessment measuring spatial navigational abilities and their potential disruption by genetic, neurobehavioral manipulations, or drug/ toxicant exposure.

Video Link

The video component of this article can be found at http://www.jove.com/video/51194/

Introduction

Spatial learning and memory in laboratory rodents was first assessed with food-deprived rats that navigated a maze of alleyways to locate a food reinforcer[1]. Several decades later, a spatial reference memory system was proposed[2]. In contrast to working memory which refers to memory within a test session or trial, reference memory refers to memory across test sessions or trials and is more closely related to long-term memory.

Several types of mazes have been developed as noninvasive assessments of this hippocampal-dependent spatial learning and memory in small and large rodents (*e.g.* water maze, multiple T-maze, radial arm maze and dry-land mazes)[3-6]. Here, we focus on the circular platform or Barnes maze, first described in 1979 by Dr. Carol Barnes[7]. This maze has been used to test spatial navigational learning and memory in a wide range of rodent models, including rats (*Rattus norvegicus*), mice (*Mus musculus*), deer mice (*Peromyscus maniculatus bairdii*), California mice (*Peromyscus californicus*), and hystricomorph rodents (*e.g.* degus [*Octodon degus*])[8-13]. Other species assessed using the Barnes maze include American cockroaches (*Periplaneta americana*)[14], corn snakes (*Elaphe guttata guttata*)[15], squamate reptiles (*e.g.* side-blotched lizards [*Uta stansburiana*])[16], and nonhuman primates (*e.g.* mouse lemurs [*Microcebus murinus*])[17]. In our labs, Barnes maze performance has been used as an index of neurotoxicity after developmental bisphenol A (BPA) or ethinyl estradiol (EE2) exposure[9-11,13]. It is also commonly used for behavior phenotyping of various mouse strains[18-21], assessment of aging effects[7,22-28], and Alzheimer's Disease-related deficits in animal models[3,29-33], as well as the effects of exercise and dietary, environmental, and metabolic alterations[34-42].

A primary advantage of Barnes maze use is that it induces less stress in the subjects relative to water mazes, such as the Morris water maze[43], although both can induce acute increases in plasma corticosterone concentrations in mice[44]. As a dry land maze, the Barnes maze may be more ethologically-relevant for terrestrial rodents[45]. Although water maze performance has been shown to be more sensitive to genetic alterations in mice[3,46,47], Barnes maze performance is more sensitive to certain other alterations[48,49]. In rodent models where water maze use is not possible, the Barnes maze may provide a fine-tuned assessment of spatial memory retention[31]. The mildly aversive stimuli typically used in the Barnes maze (*i.e.* bright lights), however, may not provide sufficient motivation for the rodent to locate the escape cage[45]. Furthermore, rodents can learn that no punishment occurs if they do not enter the escape cage. Thus, instead of actively searching for the escape cage, some rodents actively

explore the maze for long durations of each trial. As reviewed by Kennard and Woodruff-Pak[24], this increased exploration will prolong the latency to locate the escape cage, path length, and increase the number of errors. Thus, measurement of multiple parameters, including latency, error rate, time spent in the correct and incorrect quadrants, velocity, time moving, time resting, and search strategy, may collectively provide a better indicator of each subject's spatial navigational learning and memory ability[8-10]. Additionally, performance can be measured as the latency to first locate the escape cage (primary measure) or the latency to enter the escape cage (total measure). Some have argued that primary measures of performance are a more accurate reflection of spatial learning than total measures[50]. Most studies, including the examples described here, use latency to enter the escape cage to determine error rate and search strategy. Further, some tracking software systems have a three point body detection system that can measure the frequencies of sniffing the correct *vs.* incorrect holes. Finally, the maze must be thoroughly cleaned with ethanol between trials to remove olfactory cues that could provide cues or prove distracting to subsequent animals.

Barnes maze designs vary but generally each has 12 or 20 potential escape holes, only one of which leads to the home or an escape cage. The escape cage may be situated either directly below the escape hole on the maze top (for mazes without walls) or built into the surrounding wall of the maze. The cues can vary in size from approximately 16.5 cm height or width (within the maze) to a horizontal line 21.6 cm in width placed from floor to ceiling of the room wall outside the maze. **Figures 1-5** show examples of Barnes maze designs for *Peromyscus* species (**Figure 1**) and rats (**Figures 2-5**). Plugs or false bottoms must cover the nonescape holes to prevent the animal from falling out of the maze. Size of the test room can vary (~20 m^2) but it must be large enough to provide ample room for the maze, habituating the animals to the room, accommodating a computer with video set-up (if used), and a place for the experimenter to sit at a distance (at least ~122 cm) from the maze apparatus such that their presence does not interfere with the animal's performance. Assignment of escape cage location should be balanced among treatment groups and sex. While the specific procedures described here do not include rotating the maze between trials to discourage use of the intra-maze odor cues, some studies incorporate this procedure[50]. In our procedures, the maze is wiped clean with ethanol between trials to eliminate odor cues.

In locating the escape cage, three types of search strategies have been defined (originally termed "patterns" by Barnes[7]): 1) random, operationally defined as localized searches of holes separated by paths crossing the maze center, 2) serial, defined as a systematic search of consecutive holes in a clockwise or counterclockwise direction, and 3) direct or spatial, defined as navigating directly to the correct quadrant without crossing the maze center more than once and with three or fewer errors. In general, with repeated testing, rodents typically progress through the search strategies in the order listed (random, serial, and direct)[51]. A probe trial without the escape cage may also be used as a further measure of memory[50].

The protocol and representative results here were developed for two types of rodents (*Peromyscus* species- otherwise termed small rodents) and rats. While these general procedures may also hold for inbred and/or outbred mice (*Mus musculus*), other studies should be consulted on potential methodology differences for those latter species[18-21].

Protocol

1. Barnes Maze Procedure for Small Rodents

1. Turn on the overhead lights above the maze and place "Do Not Enter" signs on the outside of the lab door.
2. Bring mice in their normal home cages to the test room approximately 30 min prior to beginning the first trial to permit habituation. If the room is quiet, it may not be necessary to include white noise, otherwise this precautionary measure may be considered.
3. Set up the tracking program.
4. Gently remove the first mouse from its home cage and place in the tall covered plastic box. Place its escape (clean home) polypropylene cage (29 cm x 19 cm x 13 cm) under the designated escape hole.
    1. Ensure the paper that is blocking the tube is removed from that escape hole and all other holes are plugged.
    2. Draw curtain around the maze.
5. Place the plastic box with the mouse inside in the maze center and approximately 8 sec later, gently take the animal out of the cage and place it onto the maze.
    1. After placing the animal in the center of the maze, quietly move to the computer area (~150 cm from the maze).
    2. Initiate the appropriate tracking software program that should already be open to ensure minimal time (within a few seconds) has elapsed from the time the animal was placed on the maze until the program starts documenting its performance.
6. Observe animal's performance from computer monitor and record hole number, trial number, search strategy, and number of errors made. An error is defined as sniffing of an incorrect hole. Assessment of search strategy may be made live or later based on the tracking pattern.
    1. Search strategy is categorized as **Direct** (going directly into the escape cage with 3 or fewer errors),
    2. **Serial** (traveling along the maze perimeter until the escape cage is located), or
    3. **Random** (crossing the maze center multiple times to check various holes).
7. Stop the tracking program when the animal has all four paws inside the escape cage.
8. If the mouse fails to enter the escape cage within 5 min, gently guide it to the correct location and into the escape cage. Let the mouse remain in the escape cage for 2 min.
9. Remove mouse from the escape cage and place in home cage.
10. Spray the maze top and escape cage with 70% ethanol and wipe dry. Set the first cage/mouse aside for 30 min before running its second trial.
11. Before beginning the next mouse, plug the previously correct escape hole and remove the paper plug blocking the hole from the designated escape hole for that next subject.
12. Each mouse is tested for 2 trials/day with an inter-trial interval of approximately 30 min.

13. Repeat these steps until all mice have been tested for seven consecutive days, which may increase the likelihood of observing improved performance and/or differences between treatment groups, relative to only four days of data.

2. Barnes Maze Testing for Rats When a Tracking Program is Not Available

1. Ensure the maze is in its correct placement (directly centered below lights), false bottoms that block nonescape holes and prevent the animal from falling out are securely in the maze, and the escape cage is in the designated location for the first subject. Overhead lights above the maze should be turned on.
2. Ensure computer and camera are ready and a stopwatch is available.
3. Turn on white noise to attenuate any noises from other nearby locations. The tester's chair is approximately 122 cm from nearest edge of the maze top and remains in the same location throughout testing.
4. A timer (set to 2 min) should be available (only needed on Day 1 of testing). Timer should not "beep" or otherwise make noise. Door(s) to test room should have "Do Not Enter" sign on outside.
5. A test order sheet for the subjects will list the order of subject testing, the session number, the hole number location of the escape cage for each subject, and areas to record latency and time of day for each subject as well as an area for any necessary notes (**Figure 6**).
6. From 30-60 min before the first rat is to be tested, bring the animals in their home cages to the test room to allow for habituation.
7. The center tube that the rat is placed into at the beginning of a trial is set in the maze center. Set the cardboard sheet showing the first animal ID on top. This allows the video recording to capture the animal ID for easy identification of each subject by observing the first few seconds of the video.

Initial Testing Day 1:

1. Begin computer video recording (if used) and include approximately 5 sec of the trial with animal ID sheet for subject identification. File name (or date created) will identify day/time of testing.
2. Remove the first animal from its home cage (check identity if multiple animals are in cage) and gently place head first into the escape cage. Cover the escape cage with an extra false bottom and start the 2 min timer. This allows the animal to habituate to the escape cage.
3. After 2 min timer ends, gently remove animal from escape cage (remove false bottom cover as well and set away from maze), lift ID sheet, and immediately place the rat inside the center tube. Cover top of center tube with cardboard ID sheet.
4. Gently and slowly lift center tube with cardboard cover and set aside. Start the stopwatch as the center tube is lifted above the animal. Move to sit in the tester's chair.
5. Sit quietly in the chair, watching both the animal and the stopwatch. Each animal has a maximum of 5 min to find the escape cage.
6. If the rat finds the escape cage in less than 5 min, stop the stopwatch and record latency and time of day on test order sheet. Remove animal from escape cage and place back into home cage.
7. If the rat does not find the escape cage within 5 min, gently guide the animal to the escape cage and allow 15 sec to pass before removing and returning the animal to the home cage.
    1. This 15 sec duration can be timed using a clock with a second hand on the test room wall.
    2. Record time of day on the test order sheet and record that the rat did not find the escape cage.
8. If the rat falls/jumps off the maze, the tester should glance at stopwatch for time. The tester should then attempt to quickly retrieve the animal.
    1. If this can be done within 10 sec, replace the animal onto the center of the maze and record the time of the fall/jump on the test sheet (if the tester can distinguish between a fall or jump, this should be denoted). Continue the trial.
    2. If retrieving the animal takes longer than 10 sec, stop the stopwatch, and put animal back into the home cage. Record time of fall/jump (if the tester can distinguish between a fall or jump, note this).
    3. Data from trials in which an animal fell/jumped and could not be retrieved within 10 sec are omitted from statistical analyses.
9. Stop video recording on the computer. Record any comments about the trial.
10. Remove any urine or feces from the maze top, spray with 70% ethanol, and thoroughly wipe dry. Remove escape cage and clean with 70% ethanol.
11. Put a clean escape cage at the designated placement for the next subject. Having more than one escape cage allows each to air-dry to lessen the ethanol odor. Put a clean false bottom at the previous hole (so that all but one hole has a false bottom and that one hole contains the escape cage).
12. Set the center tube with ID sheet for the next subject in the maze center. Begin video recording on the computer.
13. Remove the next animal to be tested, place into escape cage (if Day/Session 1), and start 2 min timer (only if Day/Session 1). Continue from step 2 above. Each subject receives 1 trial/day.
14. After all animals are tested, clean the maze and escape cage, turn off overhead lights, and white noise. Remove "Do Not Enter" sign(s) from door(s).

Days 2 through 7 Testing

1. Set up test room and maze for testing as detailed above.
2. Set center tube in maze center with ID sheet on top. Begin video recording. Remove the first animal from home cage and place into the center tube.
3. This step differentiates Days 2-7 from Day 1; specifically, on Days 2-7, the subject is placed directly into the center tube after removal from the home cage and the 2 min habituation period inside the escape cage is not done.
4. Repeat procedure beginning from step 4 above.

3. Statistical Analyses for Barnes Maze Endpoints

1. Data analyses may require several statistical tests. Continuous variables, such as latency and error rate, may be analyzed as a split plot in space and time[52].
2. If some animals do not locate the escape or home cage within the maximum allotted time, the latency data can be assigned as the maximum and analyzed by using ProcLife testing in SAS version 9.2 software analysis.
    1. This statistical method is useful for behavioral data in which there is an upper limit cutoff.

3. Search strategy data may be analyzed by using a repeated measure design with PROC GLIMMIX and SAS version 9.2 software analysis.
    1. This first analysis employs a cumulative log it link and a multinomial distribution such that all three search strategies (random, serial, and direct) are included in this analysis.
    2. To determine if the animals are learning to use the more efficient search strategy (direct), a second analysis on search strategy can be performed on which the two less efficient strategies (random and serial) are combined and compared against the more efficient direct search strategy.
    3. This latter method results in a binomial distribution and also employs PROC GLIMMIX.

Representative Results

Sexually mature male deer mice are dependent upon enhanced spatial navigational ability to locate potential breeding partners, which are widely disseminated throughout the environment. Both prenatal and adult exposure to testosterone are essential in organizing and activating this later adult male behavior[53]. As such, it was presumed that early exposure to endocrine disrupting compounds might disturb this later trait in males. To test this hypothesis, male and female deer mice were developmentally exposed *via* the maternal diet to several environmentally relevant doses of BPA in a phytoestrogen free diet, a positive estrogen control (ethinyl estradiol [EE2]) in a phytoestrogen free diet, or the base control phytoestrogen-free refined diet, and were assessed for Barnes maze performance as adults. **Figure 1** shows the Barnes maze apparatus for this species. Males exposed to the two higher, but not the lowest, BPA doses demonstrated equivalent deficits in spatial learning, as manifested by prolonged latency, increased error rate, and an inability to convert to the direct search strategy over the trial period (**Figures 7-9**). However, females exposed to EE2 and the mid BPA dose, but not the other BPA doses exhibited masculinized patterns of spatial learning and memory (*i.e.* decreased latency and increased use of the direct search strategy)[9,13].

In contrast to polygamous deer mice, their related cousins, monogamous male California mice, increase their reproductive success by pair-bonding and remaining in the territory with a single female and sharing in parenting responsibilities[54,55]. Therefore, spatial navigational ability has not been subject to strong evolutionary section in California mice. Consequently, the presumption was that early BPA and EE2 exposure would not target this behavior in California mice. In support of this hypothesis, developmental exposure to BPA or EE2 did not alter spatial navigational behaviors (latency, error rate, or conversion to the direct search strategy) in males or females, which demonstrated comparable responses across all treatment groups (**Figures 10** and **11**)[10]. Compared to control deer mice, control California mice did not decrease the number of errors made over the seven consecutive test days nor did the control California male mice increase their use of the direct search strategy. This may reflect a species difference in learning ability; however, it is possible that further refinement for assessment of visuo-spatial learning and memory testing is required for California mice.

The Barnes maze apparatus and associated hardware for rats are shown in **Figures 2-5**. This apparatus was used to assess spatial learning and memory of male and female Sprague-Dawley rats on postnatal days 47-51 (5 consecutive days, 1 trial/day). On the last day (*i.e.* day 5), the escape cage was moved 180° from its original location on days 1-4. These subjects had previously been assessed for the righting reflex and slant board behavior (preweaning), and play behavior, open field activity levels, and motor coordination. Their dams had consumed 3 small pieces of vanilla wafer onto which was dispensed 1 ml/kg body weight of water on gestational days 6-21. The subjects themselves were orally treated with 1 ml/kg body weight of water twice daily on postnatal days 1-21. At weaning, they were pair-housed with a same-sex sibling. However, only 1/sex/litter was assessed for Barnes maze performance. **Figure 12** shows average latency to locate the escape cage for each sex on each of the 5 test days. Significant main effects of sex ($p < 0.04$) and session ($p < 0.01$) indicated shorter latencies in females and shorter latencies on days 2-5 relative to day 1. Others have also reported shorter latencies in female rats[56]; however, similar sex effects have not always been noted in our lab[11]. Thus, a consistent sex effect in rats is yet to be determined. Endpoints other than latency are not yet available; however, tracking software is in use in a similar study to examine error rate and search strategy in rats.

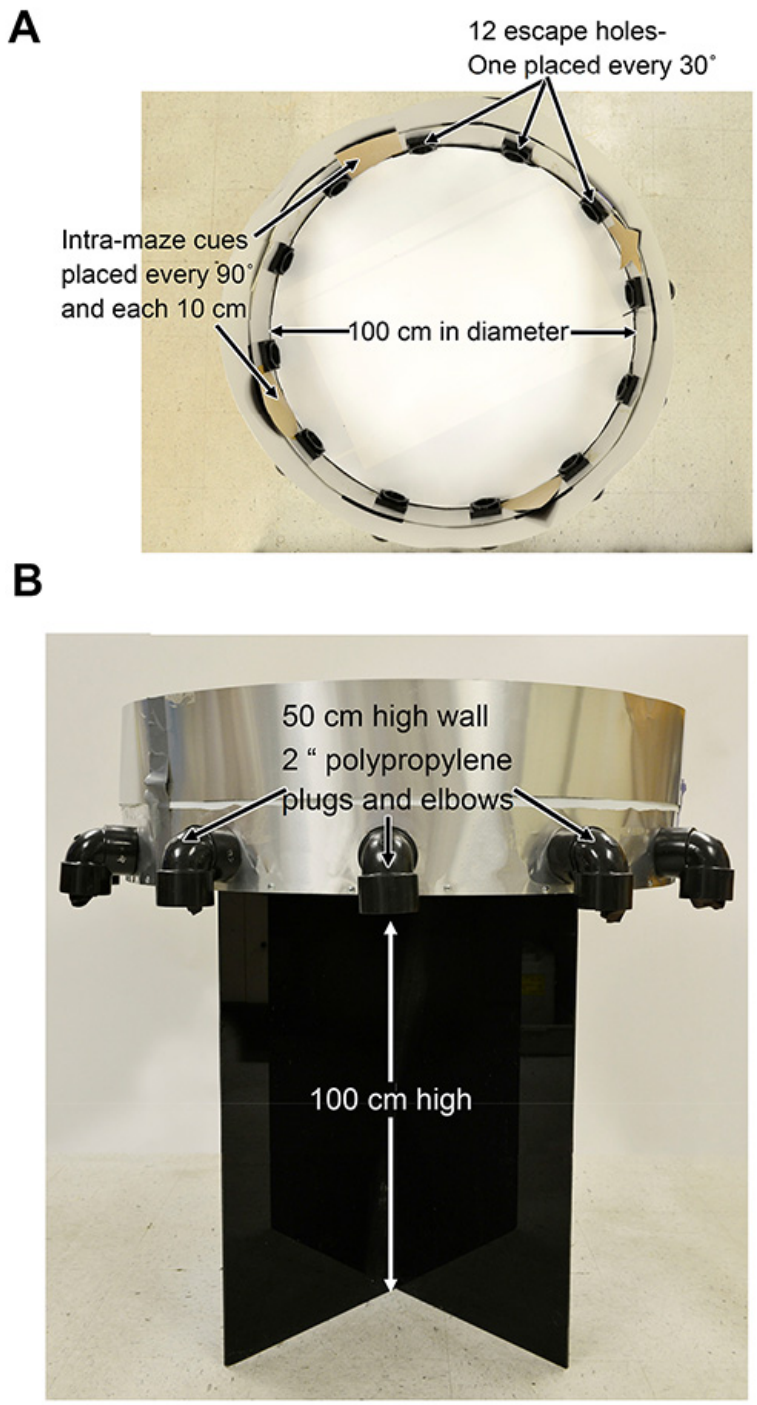


Figure 1. The Barnes maze apparatus for *Peromyscus* species. A) The intra-maze geometric cues (*e.g.* circle, square, triangle and star) are placed inside the maze wall every 90°, there are 12 escape holes placed every 30°, and the maze is surrounded by a black curtain (not shown). **B)** The maze top is placed on a polypropylene stand and elevated 100 cm above the floor. Click here to view larger image.

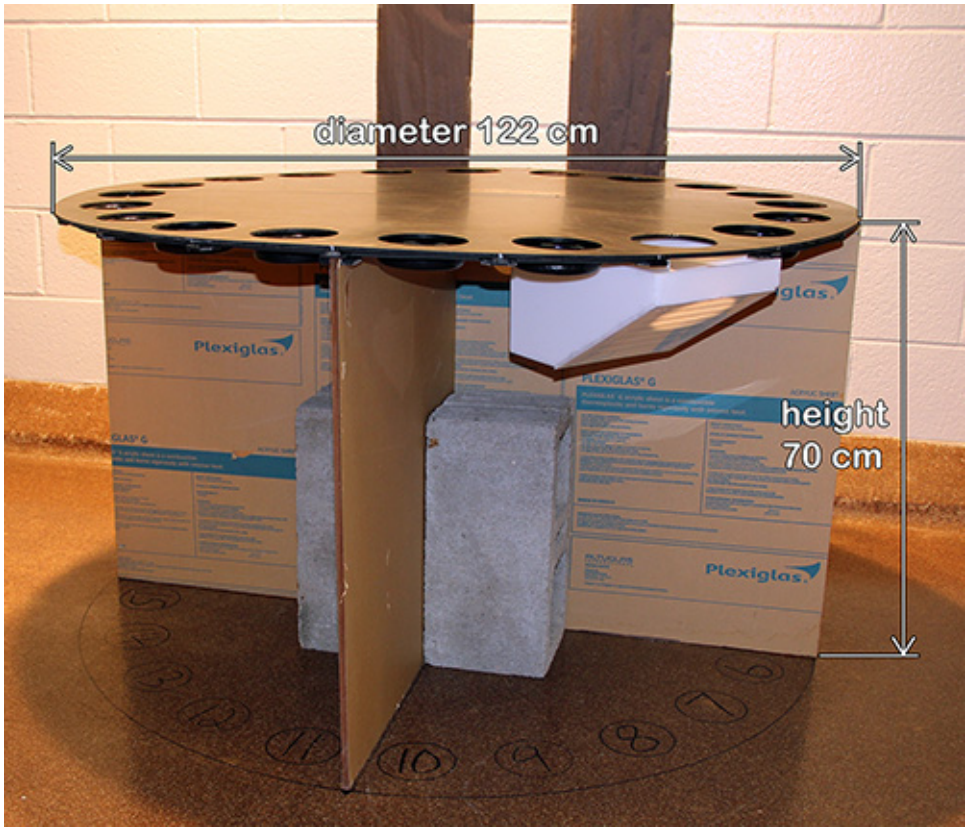


Figure 2. The Barnes maze apparatus for rats. The maze top and supporting stands can be seen with maze top diameter and height from floor shown. Numbers on the floor indicate hole numbers and allow the tester to place the escape cage in the designated location (the floor numbers cannot be seen by the subject). One of the extra-maze visual cues can be seen on the far wall (*i.e.* black vertical stripes). Click here to view larger image.

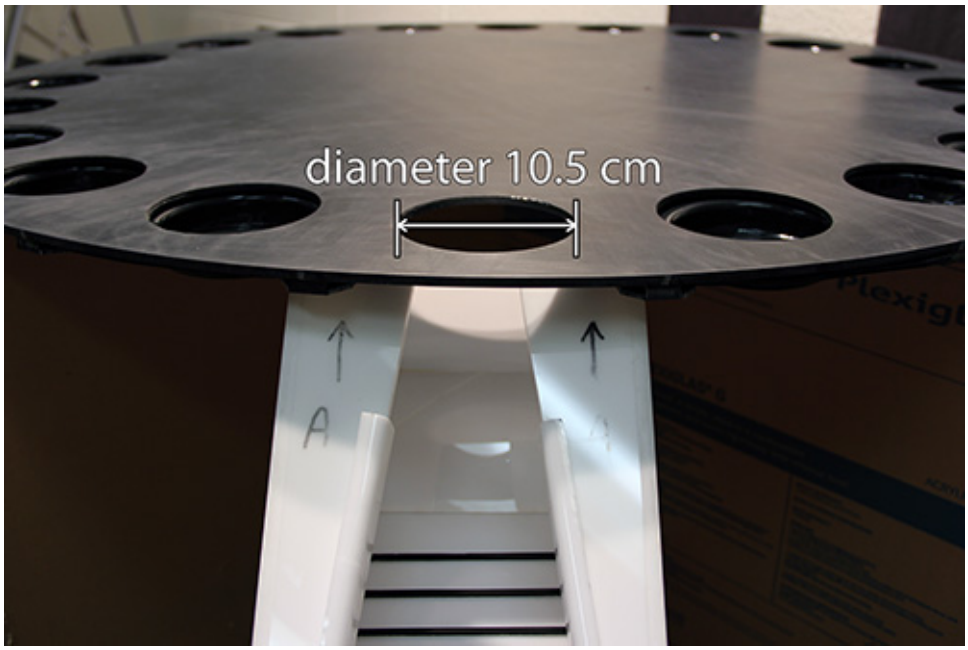


Figure 3. A closer view of the Barnes maze apparatus for rats. The white escape cage slides into grooves on the underside of the maze top. Similar grooves are located on the underside of the maze top for each perimeter hole. Click here to view larger image.

Figure 4. The center tube with sample subject identifying sheet on the top of the Barnes maze apparatus for rats. The cardboard cover lifts away to place the rat inside the tube and is then replaced. The handle on the center tube allows easy lifting to begin the trial. Click here to view larger image.

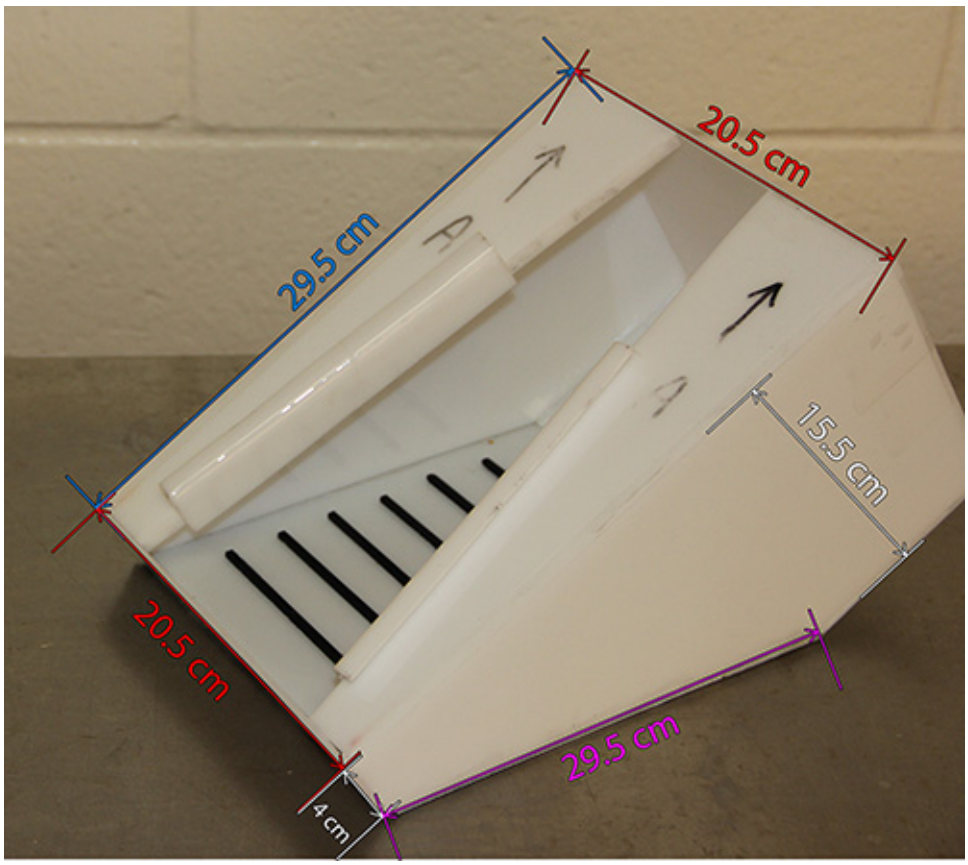
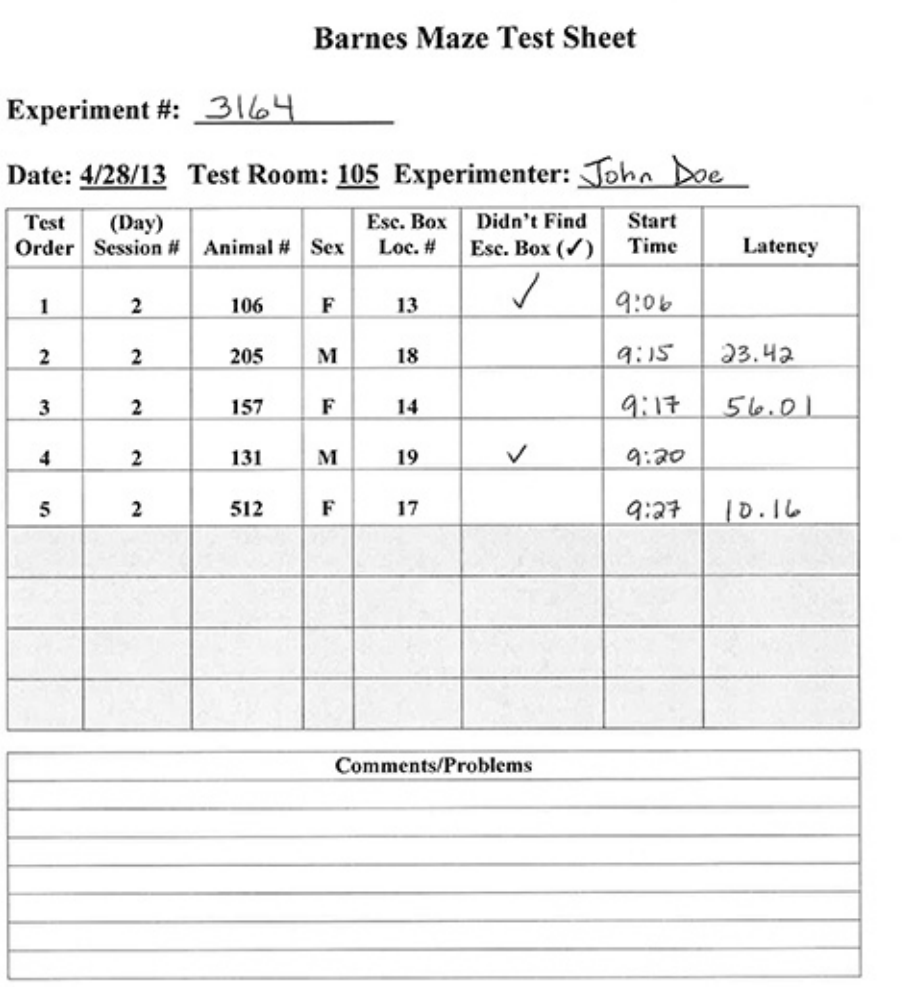


Figure 5. The escape cage for rats with dimensions. Small treads on the downward ramp provide traction for the rat when entering. Click here to view larger image.



Barnes Maze Test Sheet

Experiment #: 3164

Date: 4/28/13 **Test Room:** 105 **Experimenter:** John Doe

Test Order	(Day) Session #	Animal #	Sex	Esc. Box Loc. #	Didn't Find Esc. Box (✓)	Start Time	Latency
1	2	106	F	13	✓	9:06	
2	2	205	M	18		9:15	23.42
3	2	157	F	14		9:17	56.01
4	2	131	M	19	✓	9:20	
5	2	512	F	17		9:27	10.16

Comments/Problems

Figure 6. Sample test sheet for Barnes maze. The handwritten information is recorded at the time of the test session while other information is generated beforehand. The columns are: 1) test order so that the animal cages may be arranged in order and the experimenter knows the order of testing, 2) session number (typically, 5-7 consecutive days), 3) animal ID, 4) sex (may not be necessary on the test sheet), 5) location of the escape cage for each subject, 6) as indicated by check marks in this column, animal numbers 106 and 131 did not find the escape cage within the allotted time, 7) the time of day for the beginning of each animal's trial, and 8) latency to locate the escape cage (in sec). At the bottom of the test sheet is ample space for the experimenter to record comments and notes, such as any noises or interruptions that might have occurred during the session. Click here to view larger image.

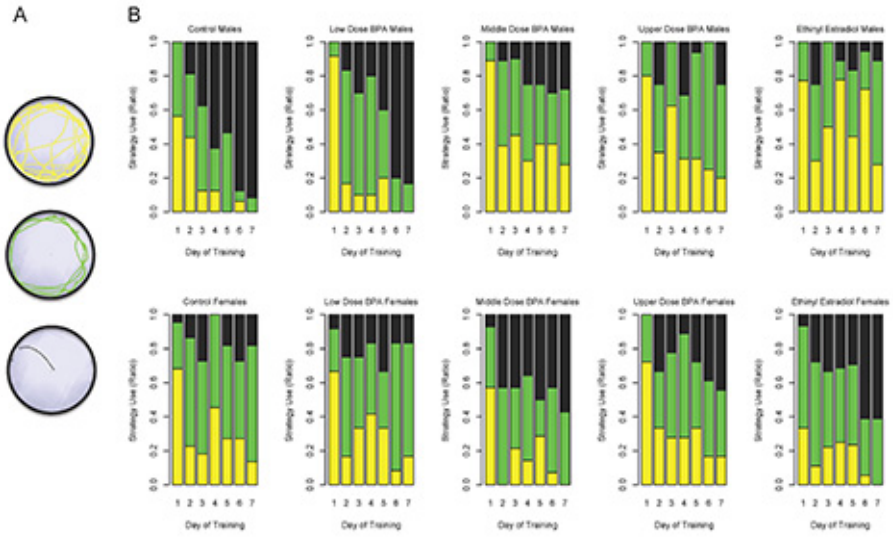


Figure 7. Effects of developmental exposure of male and female deer mice to BPA or EE2 on search strategy in Barnes maze. **A)** Example diagrams illustrating the three defined search strategies: random (top), serial (middle), and direct (bottom). **B)** Percentage of BPA, EE2 and control mice employing random (yellow), serial (green), or direct (black) search strategies across acquisition testing. CTL males utilized the direct search strategy more commonly over the 7 consecutive day test period than all other groups except low dose BPA males and EE2 females (all P values < 0.05). CTL = control; EE2 = ethinyl estradiol, BPA = bisphenol A**.** Adapted with permission from [13]. Click here to view larger image.

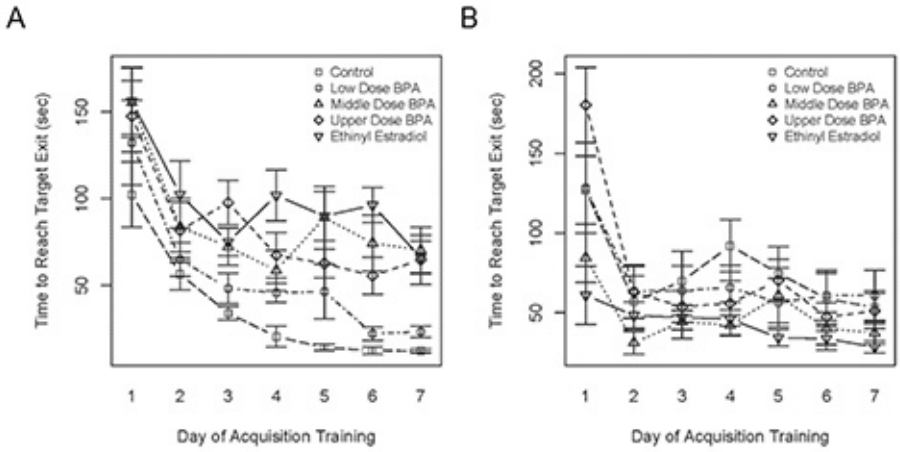


Figure 8. Effects of developmental exposure of male and female deer mice to BPA or EE2 (same groups as in Figure 3) on latency to locate the escape cage in the Barnes maze. A) Males. **B)** Females. CTL males more rapidly located the correct escape cage, as exemplified by shorter latencies, than CTL females (P = 0.0103), EE2-exposed males (P < 0.0008), and upper and middle-dose BPA males (P = 0.03, P = 0.02, respectively). CTL males, however, showed similar responses as low dose BPA males and EE2 females (P's > 0.05). In contrast, EE2 females had decreased latency periods across the trial period than EE2 exposed males (P = 0.0013). Data are presented as the mean ± SEM. Adapted with permission from Jasarevic *et al.*[13] Click here to view larger image.

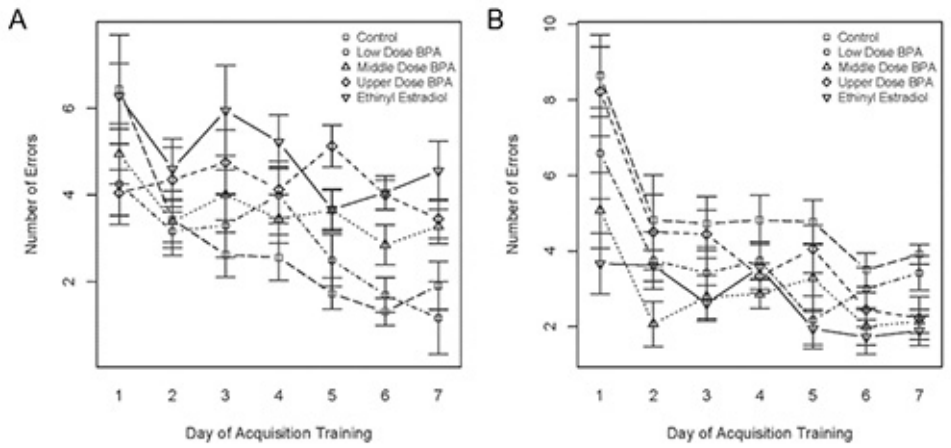


Figure 9. Developmental exposure of male and female deer mice to BPA and EE2 (same groups as in Figure 3) on escape errors. **A)** Males. **B)** Females. CTL males demonstrated approximately half of the number of errors or entries into incorrect holes compared to CTL females (P = 0.0002) and EE2 males (P = 0.02). Moreover, the CTL males committed fewer errors than upper dose BPA males (P = 0.02) but did not differ in error rate (P > 0.05) from either middle or low dose BPA males. On the other hand, EE2 females demonstrated a masculinized response such that this group possessed the same error rate as CTL males and reduced errors (P = 0.002) than EE2 males. Middle dose BPA-exposed females also showed less errors than low dose BPA and CTL females (P = 0.0005 and 0.01, respectively). Data are presented as the mean ± SEM. Adapted with permission from Jasarevic *et al.*[13] Click here to view larger image.

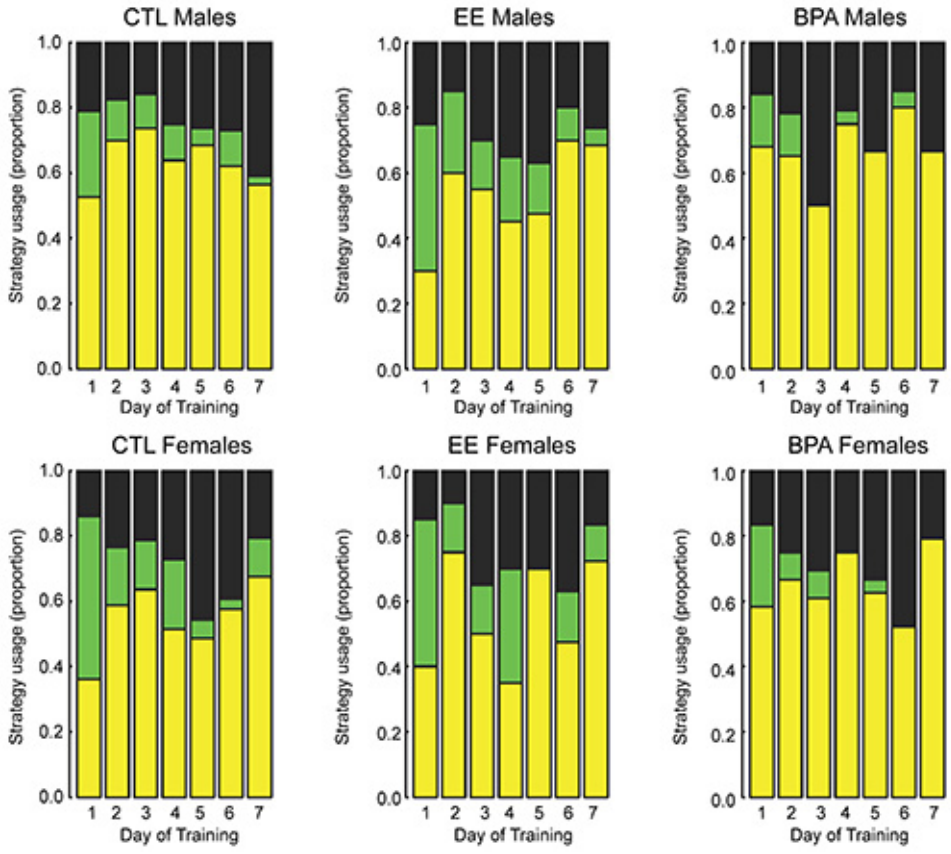


Figure 10. Search strategies of male and female California mice in Barnes maze testing. Search strategies are color-coded: random (yellow), serial (green), and direct (black). During the 7 day testing period, there were no significant effects of toxicant or sex on search strategy use for these animals. Adapted with permission from Williams *et al.*[10] Click here to view larger image.

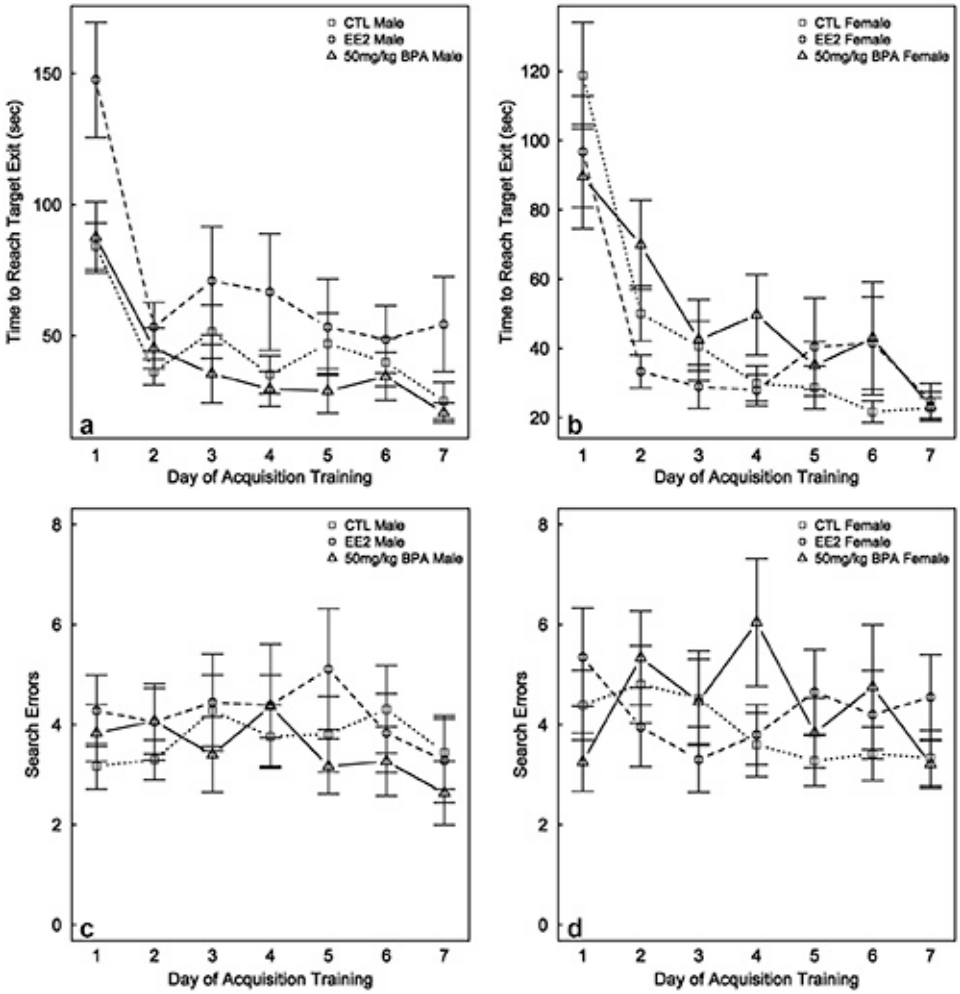


Figure 11. Latency to locate the escape cage and escape errors in Barnes maze testing for male (A & C) and female (B & D) California mice (same groups as in Figure 6). **A** and **B)** Latency. **C** and **D)** Escape errors. During the 7 day testing period, there were no significant effects of toxicant or sex on search strategy use for these animals. Data are presented as the mean ± SEM. Adapted with permission from Williams *et al.*[10] Click here to view larger image.

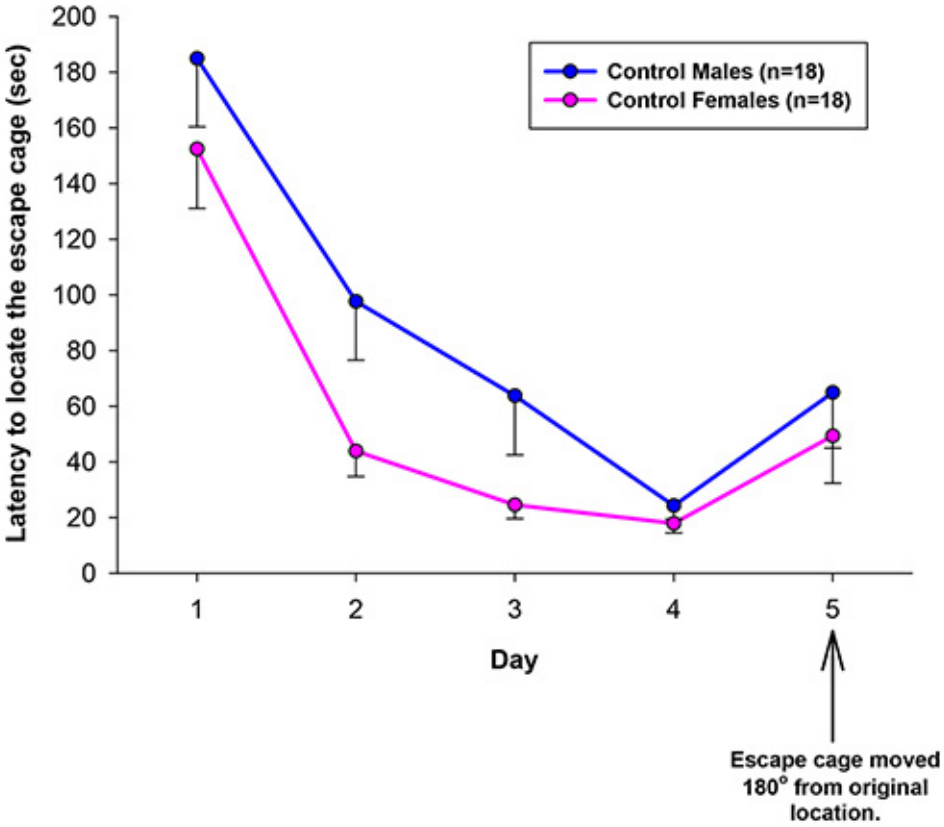


Figure 12. Latency to locate the escape cage for male and female Sprague-Dawley rats assessed on postnatal days 47-51 (1 trial/day). On the last day (day 5), the escape cage was moved 180° from its original location. Females exhibited significantly shorter latencies than males and latencies on days 2-5 were significantly shorter than latency on day 1. Data are presented as the mean ± SEM. Click here to view larger image.

Discussion

Critical steps in Barnes maze testing procedures include: 1) providing the proper mildly aversive stimulus to motivate the animal to locate the escape cage, 2) ensuring uniform conditions are maintained across the animal trials (*e.g.* test time, testing personnel, external noise control, and other stimuli that might affect performance), 3) if trials are video recorded, optimizing and ensuring proper video recording and file back up, and 4) cleaning of the maze with 70% ethanol to remove olfactory cues between trials.

Identifying the best stimuli to motivate the subject to locate the escape cage can require some modifications and/or troubleshooting. The typical stimulus is bright illumination overhead. However, this may not be sufficient for some species. Although only noted anecdotally by us, rats that have been extensively behaviorally assessed (and therefore, extensively handled) seem less motivated under the standard Barnes maze conditions, likely because they become more docile and habituated to different apparatus and/or environments. Auditory stimuli (*e.g.* predator sounds) may be considered but this limits the ability to habituate simultaneously other animals to the testing room. Other stimuli that have been used successfully include overhead fans to direct air at the maze top[57,58] or modifying the Barnes maze to be appetitive, instead of aversive[56].

Extra-maze visual cues are the norm for Barnes maze testing with rats. In typical laboratory mouse species, it has been suggested that extra-maze cues may produce better results than intra-maze cues[59,60]. However, deer mice can successfully use intra-maze cues to locate the escape cage and do successfully convert over the testing period to use of the direct search strategy[8,9]. Moreover, an external wall prevents the animals from falling or jumping from the maze. As California mice are easier to handle and about 2-3x larger than deer mice, others have successfully tested this species in the Barnes maze without the use of a wall[40,61]. However, the maze in that case was smaller (65 cm in diameter) with 16 holes that were placed more inward (1.3 cm).

Methodologically, there are minor details which could affect the Barnes maze procedure and intrepretation of the results. The maze top for rodents is relatively large and the test room must be large enough to allow the tester to move freely around the maze. Placing the maze in a corner is not recommended as the tester must be able to move around the perimeter to retrieve the rat and place the escape cage in the appropriate location. Anxiety levels of the rodent, as evidenced by increased plasma corticosterone concentration[44], are elevated during testing and extraneous stimuli could be exacerbating. Rodents typically freeze at sudden auditory stimuli and thus, it is important that the testing environment is not located in noisy area. Because this can be a lengthy assessment on a given day and across days, it can be challenging for the tester to remain attentive to the trial; however, direct attention to the subject's behavior is essential. For this reason and to avoid circadian effects on performance, it is optimal to test a limited number of animals for a select window of time (*e.g.* in the morning or afternoon) on a given

day. Finally, the odor of ethanol may be aversive to the subject, although this has not been explicitly tested. Several escape cages and additional false bottoms are suggested so that the cages have time to air dry after being sprayed with ethanol.

The primary advantages of the Barnes maze are its ease of use relative to other maze types and the additional endpoints that can be obtained that may provide a more comprehensive assessment of experimentally-induced impairments. Additionally, this dry land maze may better recapitulate the natural environment for land-dwelling rodents. The multiday testing period could provide more robust evidence of altered performance, as evidenced by latency, error rate, and conversion over the course of testing from an inefficient search strategy (random or serial) to a direct search strategy.

Results from the Barnes maze can be verified with other tests of spatial navigation. In addition, it is important to establish that potential Barnes maze performance deficits are not a result of alterations in anxiety, activity, or motor abilities. Thus, results of anxiety and/or locomotor assessments, such as elevated plus maze or open field behavior, may determine if Barnes maze impairments reflect true alterations in spatial navigation. However, common murine tests of anxiety may not always be predictive of Barnes maze performance[44]. If true spatial navigation alterations are present, molecular, histopathological, electrophysiological, or synaptogenic changes might be apparent in the hippocampus, entorhinal cortex, or other cortical areas, as those brain regions appear to govern this learning and memory response[62-64].

Disclosures

This document has been reviewed in accordance with United States Food and Drug Administration (FDA) policy and approved for publication. Approval does not signify that the contents necessarily reflect the position or opinions of the FDA nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the FDA. The authors have no competing interests and nothing to disclose.

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References

1. Tolman, E., Gleitman, H. Studies in spatial learning: place and response learning under different degrees of motivation. *J. Exp. Psychol.* **39**, 653-659 (1949).
2. Olton, D. S. & Papas, B. C. Spatial memory and hippocampal function. *Neuropsychologia.* **17**, 669-682 (1979).
3. Stewart, S., Cacucci, F. & Lever, C. Which memory task for my mouse? A systematic review of spatial memory performance in the Tg2576 Alzheimer's mouse model. *J. Alzheimers Dis.* **26**, 105-126, doi:10.3233/jad-2011-101827 (2011).
4. Sharma, S., Rakoczy, S. & Brown-Borg, H. Assessment of spatial memory in mice. *Life Sci.* **87**, 521-536, doi:10.1016/j.lfs.2010.09.004 (2010).
5. Brown, W. The effects of intra-maze tetanizing shock upon the learning and behavior of the rat in a multiple-T maze. *J. Genet. Psychol.* **76**, 313-322 (1950).
6. Morris, R. Development of a water-aze procedure for studying sptial learning in the rat. *J. Neurosci. Methods.* **11**, 47-60 (1984).
7. Barnes, C. A. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psychol.* **93**, 74-104 (1979).
8. Jasarevic, E., Williams SA, Roberts RM, Geary DC, Rosenfeld CS. Spatial navigation strategies in *Peromyscus*: a comparative study. *Anim. Behav.* **84**, 1141-1149 (2012).
9. Jasarevic, E. *et al.* Disruption of adult expression of sexually selected traits by developmental exposure to bisphenol A. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11715-11720, doi:10.1073/pnas.1107958108 (2011).
10. Williams, S. A. *et al.* Effects of developmental bisphenol A exposure on reproductive-related behaviors in California mice (*Peromyscus californicus*): A monogamous animal model. *PLoS ONE.* **8**, e55698, doi:10.1371/journal.pone.0055698 (2013).
11. Ferguson, S. A., Law, C. D. & Abshire, J. S. Developmental treatment with bisphenol A causes few alterations on measures of postweaning activity and learning. *Neurotoxicol. Teratol.* **34**, 598-606, doi:10.1016/j.ntt.2012.09.006 (2012).
12. Popovic, N., Madrid, J. A., Rol, M. A., Caballero-Bleda, M. & Popovic, M. Barnes maze performance of Octodon degus is gender dependent. *Behav. Brain Res.* **212**, 159-167, doi:10.1016/j.bbr.2010.04.005 (2010).
13. Jasarevic, E. *et al.* Sex and dose-dependent effects of developmental exposure to bisphenol A on anxiety and spatial learning in deer mice (*Peromyscus maniculatus bairdii*) offspring. *Horm. Behav.* **63**, 180-189, doi:10.1016/j.yhbeh.2012.09.009 (2013).
14. Brown, S. & Strausfeld, N. The effect of age on a visual learning task in the American cockroach. *Learn. Mem.* **16**, 210-223, doi:10.1101/lm.1241909 (2009).
15. Holtzman, D. A., Harris, T. W., Aranguren, G. & Bostock, E. Spatial learning of an escape task by young corn snakes, *Elaphe guttata guttata. Anim. Behav.* **57**, 51-60, doi:10.1006/anbe.1998.0971 (1999).
16. Ladage, L. D., Roth, T. C., Cerjanic, A. M., Sinervo, B. & Pravosudov, V. V. Spatial memory: are lizards really deficient? *Biol. Lett.* **8**, 939-941, doi:10.1098/rsbl.2012.0527 (2012).
17. Languille, S., Aujard, F. & Pifferi, F. Effect of dietary fish oil supplementation on the exploratory activity, emotional status and spatial memory of the aged mouse lemur, a non-human primate. *Behav. Brain Res.* **235**, 280-286, doi:10.1016/j.bbr.2012.08.014 (2012).
18. Patil, S. S., Sunyer, B., Hoger, H. & Lubec, G. Evaluation of spatial memory of C57BL/6J and CD1 mice in the Barnes maze, the Multiple T-maze and in the Morris water maze. *Behav. Brain Res.* **198**, 58-68, doi:10.1016/j.bbr.2008.10.029 (2009).

19. Koopmans, G., Blokland, A., van Nieuwenhuijzen, P. & Prickaerts, J. Assessment of spatial learning abilities of mice in a new circular maze. *Physiol. Behav.* **79**, 683-693, doi:S0031938403001719 [pii] (2003).
20. Holmes, A., Wrenn, C. C., Harris, A. P., Thayer, K. E. & Crawley, J. N. Behavioral profiles of inbred strains on novel olfactory, spatial and emotional tests for reference memory in mice. *Genes Brain Behav.* **1**, 55-69 (2002).
21. Youn, J. *et al.* Finding the right motivation: genotype-dependent differences in effective reinforcements for spatial learning. *Behav. Brain Res.*. **226**, 397-403, doi:10.1016/j.bbr.2011.09.034 (2012).
22. Barrett, G. L., Bennie, A., Trieu, J., Ping, S. & Tsafoulis, C. The chronology of age-related spatial learning impairment in two rat strains, as tested by the Barnes maze. *Behav. Neurosci.* **123**, 533-538, doi:10.1037/a0015063 (2009).
23. Prut, L. *et al.* Aged APP23 mice show a delay in switching to the use of a strategy in the Barnes maze. *Behav. Brain Res.* **179**, 107-110, doi:10.1016/j.bbr.2007.01.017 (2007).
24. Kennard, J. A. & Woodruff-Pak, D. S. Age sensitivity of behavioral tests and brain substrates of normal aging in mice. *Front. Aging Neurosci.* **3**, 9, doi:10.3389/fnagi.2011.00009 (2011).
25. Stouffer, E. M. & Yoder, J. E. Middle-aged (12 month old) male rats show selective latent learning deficit. *Neurobiol. Aging.* **32**, 2320 e2311-2324, doi:10.1016/j.neurobiolaging.2010.04.021 (2011).
26. Barreto, G., Huang, T. T. & Giffard, R. G. Age-related defects in sensorimotor activity, spatial learning, and memory in C57BL/6 mice. *J. Neurosurg. Anesthesiol.* **22**, 214-219, doi:10.1097/ANA.0b013e3181d56c98 (2010).
27. Barnes, C. A. & McNaughton, B. L. An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. *Behav. Neurosci.* **99**, 1040-1048 (1985).
28. Bach, M. E. *et al.* Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation *in vitro* and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5280-5285 (1999).
29. O'Leary, T. P. & Brown, R. E. Visuo-spatial learning and memory deficits on the Barnes maze in the 16-month-old APPswe/PS1dE9 mouse model of Alzheimer's disease. *Behav. Brain Res.* **201**, 120-127, doi:10.1016/j.bbr.2009.01.039 (2009).
30. Reiserer, R. S., Harrison, F. E., Syverud, D. C. & McDonald, M. P. Impaired spatial learning in the APPSwe + PSEN1DeltaE9 bigenic mouse model of Alzheimer's disease. *Genes Brain Behav.* **6**, 54-65, doi:10.1111/j.1601-183X.2006.00221.x (2007).
31. Yassine, N. *et al.* Detecting spatial memory deficits beyond blindness in tg2576 Alzheimer mice. *Neurobiol. Aging.* **34**, 716-730, doi:10.1016/j.neurobiolaging.2012.06.016 (2013).
32. Walker, J. M. *et al.* Spatial learning and memory impairment and increased locomotion in a transgenic amyloid precursor protein mouse model of Alzheimer's disease. *Behav. Brain Res.* **222**, 169-175, doi:10.1016/j.bbr.2011.03.049 (2011).
33. Banaceur, S., Banasr, S., Sakly, M. & Abdelmelek, H. Whole body exposure to 2.4 GHz WIFI signals: effects on cognitive impairment in adult triple transgenic mouse models of Alzheimer's disease (3xTg-AD). *Behav. Brain Res.* **240**, 197-201, doi:10.1016/j.bbr.2012.11.021 (2013).
34. Fedorova, I., Hussein, N., Baumann, M. H., Di Martino, C. & Salem, N., Jr. An n-3 fatty acid deficiency impairs rat spatial learning in the Barnes maze. *Behav. Neurosci.* **123**, 196-205, doi:10.1037/a0013801 (2009).
35. King, M. R., Anderson, N. J., Guernsey, L. S. & Jolivalt, C. G. Glycogen synthase kinase-3 inhibition prevents learning deficits in diabetic mice. *J. Neurosci. Res.* **91**, 506-514, doi:10.1002/jnr.23192 (2013).
36. Enhamre, E. *et al.* The expression of growth hormone receptor gene transcript in the prefrontal cortex is affected in male mice with diabetes-induced learning impairments. *Neurosci. Lett.* **523**, 82-86, doi:10.1016/j.neulet.2012.06.050 (2012).
37. Agrawal, R. & Gomez-Pinilla, F. 'Metabolic syndrome' in the brain: deficiency in omega-3 fatty acid exacerbates dysfunctions in insulin receptor signalling and cognition. *J. Physiol.* **590**, 2485-2499, doi:10.1113/jphysiol.2012.230078 (2012).
38. Li, J., Deng, J., Sheng, W. & Zuo, Z. Metformin attenuates Alzheimer's disease-like neuropathology in obese, leptin-resistant mice. *Pharmacol. Biochem. Behav.* **101**, 564-574, doi:10.1016/j.pbb.2012.03.002 (2012).
39. Teixeira, A. M. *et al.* Exercise affects memory acquisition, anxiety-like symptoms and activity of membrane-bound enzyme in brain of rats fed with different dietary fats: impairments of trans fat. *Neuroscience.* **195**, 80-88, doi:10.1016/j.neuroscience.2011.08.055 (2011).
40. Steinman, M. Q., Crean, K. K. & Trainor, B. C. Photoperiod interacts with food restriction in performance in the Barnes maze in female California mice. *Eur. J. Neurosci.* **33**, 361-370, doi:10.1111/j.1460-9568.2010.07528.x (2011).
41. Walton, J. C. *et al.* Photoperiod-mediated impairment of long-term potention and learning and memory in male white-footed mice. *Neuroscience.* **175**, 127-132, doi:10.1016/j.neuroscience.2010.12.004 (2011).
42. Wong-Goodrich, S. J. *et al.* Voluntary running prevents progressive memory decline and increases adult hippocampal neurogenesis and growth factor expression after whole-brain irradiation. *Cancer Res.* **70**, 9329-9338, doi:10.1158/0008-5472.can-10-1854 (2010).
43. Holscher, C. Stress impairs performance in spatial water maze learning tasks. *Behav. Brain Res.* **100**, 225-235 (1999).
44. Harrison, F. E., Hosseini, A. H. & McDonald, M. P. Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. *Behav. Brain Res.* **198**, 247-251, doi:10.1016/j.bbr.2008.10.015 (2009).
45. Sunyer, B., Patil, S, Hoger, H, Lubec, G. Barnes maze, a useful task to assess spatial reference memory in mice. *Nat. Protoc.* **198** (2007).
46. Takeuchi, H. *et al.* P301S mutant human tau transgenic mice manifest early symptoms of human tauopathies with dementia and altered sensorimotor gating. *PLoS ONE.* **6**, e21050, doi:10.1371/journal.pone.0021050 (2011).
47. Mathis, C., Bott, J. B., Candusso, M. P., Simonin, F. & Cassel, J. C. Impaired striatum-dependent behavior in GASP-1-knock-out mice. *Genes Brain Behav.* **10**, 299-308, doi:10.1111/j.1601-183X.2010.00666.x (2011).
48. Lewejohann, L. *et al.* Role of a neuronal small non-messenger RNA: behavioural alterations in BC1 RNA-deleted mice. *Behav. Brain Res.* **154**, 273-289, doi:10.1016/j.bbr.2004.02.015 (2004).
49. Raber, J. *et al.* Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. *Radiat. Res.* **162**, 39-47 (2004).
50. Harrison, F. E., Reiserer, R. S., Tomarken, A. J. & McDonald, M. P. Spatial and nonspatial escape strategies in the Barnes maze. *Learn. Mem.* **13**, 809-819, doi:10.1101/lm.334306 (2006).
51. Vorhees, C. V. Methods for detecting long-term CNS dysfunction after prenatal exposure to neurotoxins. *Drug Chem. Toxicol.* **20**, 387-399, doi:10.3109/01480549709003895 (1997).
52. Steel, R. G. *Principles and Procedures of Statistics: A Biometrical Approach*. 3rd edn, 400-428 McGraw-Hill Higher Education (1996).
53. Galea, L. A., Kavaliers, M. & Ossenkopp, K. P. Sexually dimorphic spatial learning in meadow voles Microtus pennsylvanicus and deer mice *Peromyscus maniculatus*. *J. Exp. Biol.* **199**, 195-200 (1996).
54. Gubernick, D. J. & Teferi, T. Adaptive significance of male parental care in a monogamous mammal. *Proc. Biol. Sci.* **267**, 147-150, doi:10.1098/rspb.2000.0979 (2000).

55. Gubernick, D. J. & Alberts, J. R. The biparental care system of the California mouse, *Peromyscus californicus. J. Comp. Psychol.* **101**, 169-177 (1987).
56. Williams, M. T. *et al.* Long-term effects of neonatal methamphetamine exposure in rats on spatial learning in the Barnes maze and on cliff avoidance, corticosterone release, and neurotoxicity in adulthood. *Brain Res. Dev. Brain Res.* **147**, 163-175, doi:S0165380603003353 [pii] (2003).
57. Inman-Wood, S. L., Williams, M. T., Morford, L. L. & Vorhees, C. V. Effects of prenatal cocaine on Morris and Barnes maze tests of spatial learning and memory in the offspring of C57BL/6J mice. *Neurotoxicol. Teratol.* **22**, 547-557, doi:S0892-0362(00)00084-2 [pii] (2000).
58. Pompl, P. N., Mullan, M. J., Bjugstad, K. & Arendash, G. W. Adaptation of the circular platform spatial memory task for mice: use in detecting cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease. *J. Neurosci. Methods.* **87**, 87-95, doi:S0165027098001691 [pii] (1999).
59. O'Leary, T. P. & Brown, R. E. The effects of apparatus design and test procedure on learning and memory performance of C57BL/6J mice on the Barnes maze. *J. Neurosci. Methods.* **203**, 315-324, doi:10.1016/j.jneumeth.2011.09.027 (2012).
60. O'Leary, T. P. & Brown, R. E. Optimization of apparatus design and behavioral measures for the assessment of visuo-spatial learning and memory of mice on the Barnes maze. *Learn. Mem.* **20**, 85-96, doi:10.1101/lm.028076.112 (2013).
61. Bredy, T. W., Lee, A. W., Meaney, M. J. & Brown, R. E. Effect of neonatal handling and paternal care on offspring cognitive development in the monogamous California mouse (*Peromyscus californicus*). *Horm. Behav.* **46**, 30-38 (2004).
62. Foster, D. J. & Knierim, J. J. Sequence learning and the role of the hippocampus in rodent navigation. *Curr. Opin. Neurobiol.* **22**, 294-300, doi:10.1016/j.conb.2011.12.005 (2012).
63. Lipton, P. A. & Eichenbaum, H. Complementary roles of hippocampus and medial entorhinal cortex in episodic memory. *Neural Plast.* **2008**, 258467, doi:10.1155/2008/258467 (2008).
64. Wolbers, T. & Hegarty, M. What determines our navigational abilities? *Trends Cogn. Sci.* **14**, 138-146, doi:10.1016/j.tics.2010.01.001 (2010).

