SOP#:	Date Issued: 8/03	Date Revised: 7/08
TITLE:	**Animal behavior**	
SCOPE:	Research Personnel	
RESPONSIBILITY:	BORC staff	
PURPOSE:	To outline the proper procedures for use and maintenance of the three animal behavior instruments.	

1 PURPOSE

This SOP explains how to apply Animal Behavior in biomedical research.

2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

3 BEFORE CONDUCTING YOUR EXPERIMENT

4 PROCEDURES

4.1 Morris Water Maze

Place or spatial learning is the most basic MWM procedure. The concept behind it is that the animal must learn to use distal cues to navigate a direct path to the hidden platform when started from different, random locations around the perimeter of the tank.

4.1.1 Train mouse

Mice (the number will be decided by investigators) will be trained using a visible platform/hidden platform version of the water maze task.

1) Prior to each training session, mice will be removed from their group cages and individually housed in holding cages for the duration of that day's training (approximately 6 h).

2) An 8 X 8 cm clear Plexiglas platform with an attached 7.5 X 7.5 X 7.5 cm black Plexiglas cube (visible platform) will be centered in a randomly selected quadrant of a circular, 92 cm inner diameter polyethylene tank filled with warm water made opaque using white tempura-based paint (DryTemp powder tempura). Multiple objects will be placed in the testing room to act as distal cues; these cues remained constant through all experiments.

3) In one training block, a mouse will be gently transported from its holding cage and released into the water facing the maze wall.

4) The trial will end when the mouse mounted the platform with all four paws or if the mouse do not mount the platform after 60 s of swimming. Animals not finding the platform within this time limit are either placed on the platform or guided to it.

All mice will spend 20 s on the platform before returning to the holding cage. Following a 1-min rest interval, the mouse will be returned to the maze at a different starting quadrant and the above process will be repeated until all four quadrants had been tested over four trials.

5) On subsequent days, repeat the trials. The number of days over which to repeat testing depends on the learning curve. With four trials per day, 5–6 days (20–24 trials) is typically sufficient to reach asymptotic performance;

4.1.2 Reference memory: probe trial

The object of the probe trial is to determine whether or not the animal remembers where the platform was located.

1) On trial day, remove the platform.

2) Place the animal in a novel start position in the maze, facing the tank wall — for example, 180^{o} from the original platform position. Swimming paths will be videotaped and analyzed using a commercially available software package (EthoVision, Noldus Inc.).

Use a novel start position during the probe trial to ensure that its spatial preference is a reflection of the memory of the goal location rather than for a specific swim path.

3) Remove the animal after a fixed interval (usually 30 or 60 s). The memory is determined by several parameters, including number of platform-site crossovers, time and distance spent in the target quadrant compared with the other quadrants, time in a pre-defined annulus surrounding the target that is larger than the target itself, average distance to the target site, angle (bearing) to the target site, latency to first target-site crossover, and mean search difference and mean zone difference scores (see ref. 62 for a description of the latter measures). Percent time or percent distance in the target quadrant is used most frequently.

4.1.3 Finish

When finished with the water maze, drain the tank. Disinfect the inside of the tank with Clorox wipes

4.2. Barnes Maze Testing

A. The maze platform is white, circular, 0.95 m in diameter, and had 12 evenly spaced 10-cm holes at its edges. The platform is suspended approximately 1.4 m off the ground, which we found to be high enough that rodents did not spontaneously jump from the platform to the ground.

B. A darkened escape chamber similar in texture to the animal's home-cage is maintained under one of the holes at the edge of the platform. This escape chamber remains in a constant position relative to the room throughout testing, although the platform itself is rotated to confuse any possible scent trails.

Animals are habituated to the maze for 1 day prior to the beginning of testing. This habituation consisted of placing the animal in the escape chamber for 2 min, then placing it directly in the hole that led to the escape chamber and allowing it to remain in the chamber for another 2 min, and finally placing the animal in a small, four-walled chamber containing the escape hole that allowed the animal to escape from this one-choice test. The amount of time that an animal took to enter the hole in the one-choice test is measured as a control for the motivation to enter the hole.

C. Automate your behavioral testing using the most versatile, easy to use video tracking system available. SDI's ANY-Maze is designed to test in a wide range of mazes and enclosures, allowing you to choose whether to track the head, body, tail, or whole animal. Flexible in every way, the maze will set up quickly with a Firewire or USB connection to the PC and tests automatically with virtually any camera.

4.3. Eight-Arm Radial Maze

A. The radial maze is made of beige lusterless Perspex (each arm, 50 cm long × 10 cm wide × 40 cm tall), placed in a dimly lit room (50 lx), with room temperature maintained at 23 ± 1 C°.

B. The maze itself has no landmarks; the only landmarks are outside the radial maze on the surrounding walls.

C. During four consecutive days, food treat (2 g) is scattered in home cages to avoid neophobia. Mice are familiarized with the maze for 3 d before training: on the first day, mice are placed in the maze for 20 min with scattered food reward (60 mg food treat).

D. On the second and third days, mice are placed in the maze for 15 min and are able to consume the reward only from the food cups at the ends of the arms (15 mg in each arm).

E. During the trial period, food is deprived for 16 h before each experimental session. Trial begins as the mouse is placed in the central arena within an opaque cylinder that is removed at the beginning of each trial. In the learning phase, mice are given daily training trial over a period of 7 to 8 d and expected to learn to visit baited arms (not adjacent arms).

F. Trial ends when one of the following conditions is reached: all four baited arms have been visited or the trial lasted for more than 10 min.

G: After each mouse clean the maze with Clorox disinfecting wipes, wipe down with a damp cloth, and dry.

5 MAINTENANCE

6 REFERENCES

Refer to the manufacturer's manual for additional information.

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