Chapter 32

Animal Tests for Evaluation of Cognitive Impairment in Neonatal Mouse

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Abstract

For a long time, mice have been less popular than rats for studying cognitive impairment, mainly because much less neuroanatomical and neurochemical information was available on mice than on rats. Over the recent years, the generation of many types of transgenic mice has brought mice to the forefront of this research. Genetically modified mouse models have demonstrated useful to search memory and learning processes and the neurocircuitry and molecular mechanisms involved, as well as to extend therapies for cognitive impairment. A diversity of protocols has been developed to evaluate cognition in mice. The test models have been carefully selected according to reliability of results and disease relevance of the cognitive functions evaluated. Further criteria were ease of application and time efficiency. All tests evaluate slightly different but also interacting aspects or overlapping of learning and memory so that they can be utilized to complement each other in a comprehensive evaluation of cognitive function. In this chapter, three main protocols for evaluation cognitive/behavioral effect induced by drugs in postnatal mouse such as passive avoidance, radial arm maze (RAM), and Morris water maze (MWM) tests are described.

Key words Animal tests, Cognitive impairment, Neonatal mouse, Morris water maze, Radial arm maze, Passive avoidance

1 Introduction

Animal studies have showed that in utero drugs and chemicals exposure can produce anatomical as well as behavioral defects, which can occur at dosages lower than those required to produce somatic malformations [1, 2]. Neonatal or gestational exposure to benzodiazepines can affect brain behavior and chemistry causing learning or hyperactivity deficits [3]. A few neurobehavioral studies in animals have been conducted with antiepileptic drug. For example, in utero carbamazepine exposure did not produce hyperexcitability in primates while perinatal phenobarbital exposure in rats reduces brain weight [4]. Mice exposed prenatally to phenobarbital have reduced brain weight, neuronal deficits, and impaired development of open-field activity, schedule-controlled behavior,

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reflexes spatial learning, and catecholamine brain levels [5]. Neonatal or gestational exposure to phenytoin alters neuronal membranes in the hippocampus reduces, delays neurodevelopment, and impairs spatial learning, motor coordination, and brain weight [6]. Hyperactivity has been demonstrated in rats and primates following prenatal exposure to phenytoin. In this chapter, three main protocols for evaluation cognitive/behavioral effect induced by drugs in postnatal mouse such as passive avoidance, radial arm maze (RAM), and Morris water maze (MWM) tests are described.

2 Materials

2.1 The Morris Water Maze (MWM) Test

The Morris water maze (MWM) is an experiment of spatial learning for rodents that relies on distal cues to steer from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. Spatial learning is assessed across repeated trials and reference memory is determined by preference for the platform area when the platform is absent. Shift and reversal trials enhance the detection of spatial impairments. Trial-dependent, latent and discrimination learning can be assessed using modifications of the basic protocol. Search-to-platform area determines the degree of reliance on spatial versus nonspatial strategies. Cued trials determine whether performance factors that are unrelated to place learning are present. Escape from water is relatively immune from activity or body mass differences, making it ideal for many experimental models. The MWM has demonstrated to be a reliable and robust test that is strongly correlated with NMDA receptor function and hippocampal synaptic plasticity (Fig. 1).

2.1.1 Equipment Preparation

1. Obtain a circular pool with a diameter of 150 cm and a depth of 50 cm.
2. Arrange the room such that the animal being tested cannot see the experimenter during testing.
3. Place high contrast spatial cues about the room, and/or on the interior of the pool at a location which would be above the water surface.
4. Place a 10 cm diameter platform in the pool, a clear plexiglass.
5. Fill the pool with water until the platform is 1 cm above the water surface.
6. Let the water equilibrate to room temperature (22 °C). Hot water can be added to speed up the equilibration.

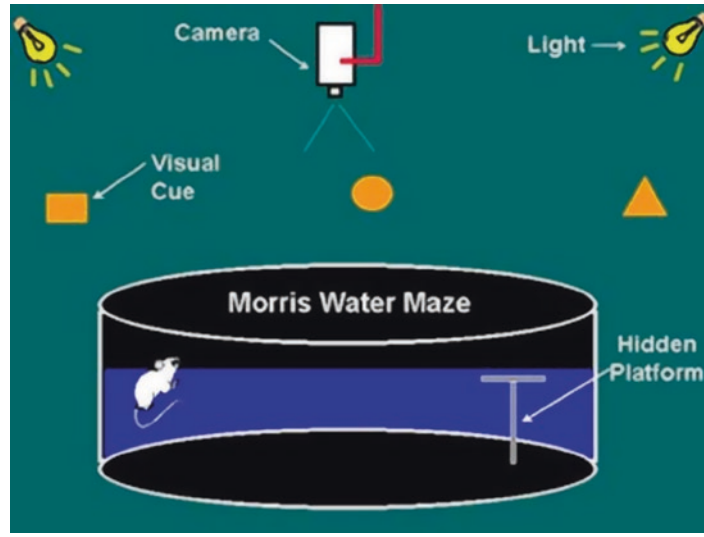

Fig. 1 Morris water maze (MWM) test

2.1.2 Software Preparation

1. Calibrate the pool in the computer software so the camera can create physical distance information from pixel-based information (*see* **Note 1**).
2. Divide the pool into four quadrants. Specify the platform zone as a variable zone which can change with each trial.
3. Create five platform subzones—one in each quadrant, and one in the center of the pool. Save the calibration and use it for the remaining test days.
4. Set the maximum trial time as 90 s.
5. Specify the program to begin tracking automatically, when the experimenter exits the testing area. Utilize any "reflection minimization" options your software package provides (*see* **Notes 2** and **3**).
6. Track path length, escape latency, and time spent in each quadrant (*see* **Note 4**).

2.2 The Radial Arm Maze (RAM) Test

The Radial Arm Maze (RAM) was designed by Samuelson and Olton (1976) to measure spatial memory and learning in rodents. It is a device consisting of eight horizontal equidistantly spaced arms radiating from a small circular central platform (30 cm in diameter) elevated (70 cm) off the floor. At the entrance of each arm there is an opaque door and at the end of each arm a small food cup is placed, which is not visible from the central platform. Experimental animals are placed on the central platform from which they have to collect the hidden baits placed at the end of the arms. Animals are habituated to the environment by exploring the maze for 15 min per day for 3 days. After that animals are trained

one session per day for 8 consecutive days. Each session lasts 10 min or until all eight arms have been entered or 2 min has passed since the animal's last arm entrance. In order to analyze the animal's performance, the following are considered: the number of sessions to reach the criterion of one error or no errors, averaged over 4 consecutive days of training; total time to complete the session divided by the total number of arm entries; the number of correct choices in the first eight arm entries of each session; the number of adjacent arm entries in each session; and the number of errors in each session and the total number of errors across eight sessions. Recently, variations of the RAM have developed, and all of them have confirmed that it is a consolidated paradigm for the evaluation memory of and learning.

2.2.1 RAM Test

1. Mice.
2. Food reward (10-mg pellet of chow or sweetened breakfast cereal) (*see* **Notes 5** and **6**).
3. Radial arm maze (Fig. 2), handmade or fully automated.

2.3 Passive Avoidance Test

This procedure measures the basic ability to learn and remember the presence of a shock stimulus that requires minimal training and produces rapid learning with exquisite control over the unconditioned aversive stimulus. In accordance with the guidelines of the American Psychological Association, the shock intensity used in this task should be the minimal amount needed to motivate the animal. The shock is very brief and only one training trial is used for each animal. Mice are tested in the passive avoidance apparatus only after they have undergone other less stressful testing. This ensures that all mice tested in this test are capable of perceiving and responding to the shock in a normal manner. Mice exhibiting any abnormalities indicative of locomotor disabilities or pain perception (i.e., hyperalgesia) must be excluded from testing (Fig. 3).

2.3.1 Passive Avoidance Test

1. Dual compartment testing apparatus.
2. Electroshock generating device.
3. Mouse.

3 Methods

3.1 MWM Test

3.3.1 The First Day

1. Transfer the mice from their housing facility to the behavior room.
2. Keep the mice in an area where they cannot see the pool or spatial cues.
3. Let them adjust to the new environment for at least 30 min before testing.

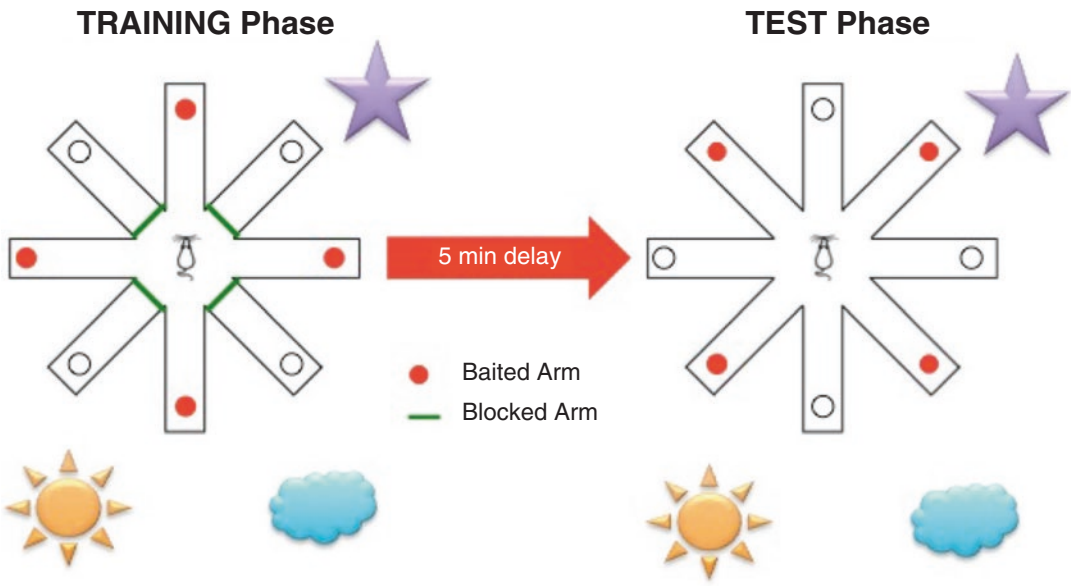

Fig. 2 The radial arm maze (RAM) test

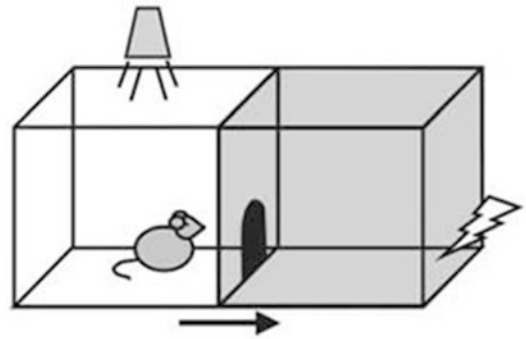

Fig. 3 Passive avoidance test

4. Place a visible flag on the platform to increase its visibility.
5. To begin testing, lift the mouse by the base of the tail and gently place the mouse into the water, facing the edge of the pool.
6. Quickly leave the testing area.
7. If the mouse finds the platform before the 90-s cutoff, allow the mouse to stay on the platform for 5 s then return it to its home cage.
8. If the mouse does not find the platform, place the mouse on the platform and allow it to stay there for 20 s before returning it to its home cage.
9. When testing is complete, return the mice to their housing facility. Mice are dried off.

10. In preparation for the following day, remove the flag from the platform and add additional water to the pool to submerge the platform to 1 cm below the surface.

3.1.1 The Second to Fifth Days: Hidden Platform

1. Load the pool calibration into the tracking software.
2. Create five trials, with an inter-trial interval appropriate for your experiment.
3. Program the platform location to remain in the same position throughout all trials and days, but have the starting direction differ with each trial, each day.
4. For black mice, add white, nontoxic powdered tempera paint to the pool and mix thoroughly.
5. Use enough paint such that the submerged platform is not visible from the surface of the water.
6. For white mice, a black pool with clear water and a clear plexiglass platform should be used.
7. To begin testing, lift the mouse by the base of the tail, gently place the mouse into the water, facing the edge of the pool.
8. Quickly leave the testing area.
9. If the mouse finds the platform before the 90-s cut-off, allow the mouse to stay on the platform for 5 s then return it to its home cage.
10. If the mouse does not find the platform, place the mouse on the platform and allow it to stay there for 20 s before returning it to its home cage.
11. When testing is complete, return the mice to their housing facility. Mice are dried off.
12. In preparation for the following day, remove the flag from the platform and add additional water to the pool to submerge the platform to 1 cm below the surface.

3.1.2 The Sixth Day: Probe Trial

1. Load the pool calibration into the tracking software.
2. Create one trial with no platform zone, and one starting direction. The starting direction farthest from the platform quadrant used on days 2–5 is preferred. Set the trail length to 60 s.
3. Remove the platform from the pool.
4. To begin testing, lift the mouse by the base of the tail, gently place the mouse into the water, facing the edge of the pool.
5. Quickly leave the testing area.
6. If the mouse finds the platform before the 90-s cut-off, allow the mouse to stay on the platform for 5 s then return it to its home cage.

7. If the mouse does not find the platform, place the mouse on the platform and allow it to stay there for 20 s before returning it to its home cage.
8. When testing is complete, return the mice to their housing facility. Mice are dried off.
9. In preparation for the following day, remove the flag from the platform and add additional water to the pool to submerge the platform to 1 cm below the surface.

3.1.3 Data Analysis

1. For each day and each mouse, average the five trials to give a single path length and escape latency for each test subject. Calculate the combined error appropriately.
2. For day 6, simply collect the path length, escape latency, and time spent in the platform quadrant for each mouse.
3. If any differences exist between groups on day 1, it is likely a problem with vision rather than learning and memory. Only proceed with analysis if no differences are seen on day 1.
4. Compare the learning curves for days 2–5 using statistics appropriate for your data set.
5. A steeper curve represents faster task acquisition; a shallower curve represents a deficit in task acquisition.
6. The data from day 2 to day 5 are analyzed using ANOVA.
7. For day 6, compare the percent of time spent in the previously learned platform quadrant, using statistics appropriate for your data set.
8. A higher percentage of time spent in the platform quadrant is interpreted as a higher level of memory retention.

3.2 RAM Test

1. Weigh each mouse daily throughout training and testing to monitor health and degree of food deprivation.

3.2.1 Training Trial

2. Restrict food available to mice so that its body weight attains 85% of that prior to training (*see* **Note 7**).
3. Allow mice to become comfortable with the experimenter.
4. Give food reward in home cage for a few days prior to training in order to acclimate the mice to the reward in a familiar environment.
5. Set up radial arm maze.
6. Place a well-handled pair of mice on the maze at the same time.
7. Spread food rewards around the entire maze to encourage exploration.
8. On subsequent days, place food only on the arms, then only at the ends of the arms.

9. Finally, place mouse alone on maze and food only in the food cup at end of arms.
10. Testing can begin when mouse is comfortable being picked up by the experimenter and, when placed alone on the maze, explores without hesitation and without excessive defecation or urination.

3.2.2 Testing Trial

1. Place food reward at end of each arm before each test session.
2. Place mouse on central platform with all guillotine doors closed.
3. Raise all doors simultaneously. Allow mouse to enter an arm. Close doors to all other arms.
4. Allow mouse time to eat food and to return to central platform.
5. Close door to that arm and confine mouse to the central platform area for a set time (from 0 s to many minutes).
6. Repeat **steps 3–5** until all food pellets have been retrieved or until a predetermined length of time has elapsed.
7. Record which arm the mouse entered each time and whether it received a food reward. Time elapsed between the beginning of the test session and the mouse's obtaining all eight food rewards. Number of correct arm choices: i.e., those that are chosen the first time. Number of incorrect arm choices: i.e., visits to the same arm more than once during a single test session (*see* **Note 8**).

3.2.3 Data Analysis

1. Performance for all groups is typically expressed.
2. The percentage of correct choices made in each test session in relation to the total number of arms entered.
3. The absolute number of correct choices made in the first 8–12 choices of each test session.
4. The percentage of correct choices made in relation to the number of incorrect choices.
5. The data are best presented as a line drawing comparing a performance measure for each group versus daily test sessions.
6. Data from 2 or 4 days of testing can also be averaged into blocks.

3.3 Passive Avoidance Test

3.3.1 Training Trial

1. The testing apparatus is a trough-shaped alley divided into two distinct compartments that are separated by a sliding door.
2. The white, brightly lit compartment is free of aversive stimulation whereas the black, dark compartment is equipped with shock capability.
3. The apparatus is cleaned with 70% ethanol before use.

4. The training trial begins by placing the animal in the white compartment facing the door.
5. The door is opened to allow access to the dark compartment.
6. The latency to enter the dark compartment is recorded.
7. When the animal steps into the dark compartment with all four paws, the door is closed and a 1–2 s footshock is delivered (0.2–0.5 mA shock, minimum required to elicit flinching and/or vocalization).
8. The animal remains in the dark compartment for an additional 10 s after the termination of the aversive stimulus, before being removed and placed back into its home cage.
9. The apparatus is cleaned with 70% ethanol in between animals.

3.3.2 Testing Trial

1. At the time of the testing trial (usually 1–7 days after training), the animal is again placed inside the white compartment and the door is raised to allow access to the dark compartment.
2. The latency to reenter the dark compartment is recorded; however, there is no aversive stimulus applied to animal upon reentry into the dark compartment during testing.

3.3.3 Data Analysis

1. The latency to reenter the dark compartment is recorded and compared.

4 Notes

1. A tracking camera, positioned ~200 cm above the center of the pool, can be used to quantify the distance swam on each trial and thereby determine swimming speed when combined with latency measurements.
2. The tracking system can also display swim path and distance and provide additional information on search efficiency and exploration patterns during acquisition and probe trials.
3. This equipment and associated computer software can be obtained from several commercial manufacturers.
4. Additional analyses utilizing sophisticated computer tracking programs can classify the spatial location of the animal with regard to the platform in order to provide information on the spatial pattern of the mouse's search during both the training and testing phases.
5. The food reward is typically a small piece (10 mg) of normal chow or a flavored (chocolate is a favorite) or sweetened breakfast cereal.

6. Liquid rewards, such as chocolate milk or water, can also be used. Liquid rewards are preferred if the rat will be given a drug, e.g., scopolamine, that might make swallowing dry food uncomfortable.
7. A typical rat will be ready for testing—i.e., food-restricted and acclimated to the maze-within ~7 days. Animals should be run in the maze once a day every day (including weekends, ideally) during training and testing.
8. Longer waits make the task more difficult to solve, increase the length of time for which the rat must remember which arms it has entered.

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