Published in final edited form as:

Methods Mol Biol. 2013 ; 952: 325–336. doi:10.1007/978-1-62703-155-4_24.

Behavioral Paradigms to Evaluate PPAR Modulation in Animal Models of Brain Injury

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Abstract

The use of behavioral testing has become an invaluable tool for assessing the efficacy of therapeutics for a variety of disorders of the central nervous system. This chapter will describe in detail several behavioral paradigms to evaluate the efficacy of PPAR agonists to modulate cognitive impairments in rodent models. When used together as a battery these procedures allow for a global assessment of cognition. These tests are explained in detail below, and include: (1) Novel Object Recognition (NOR), (2) Morris Water Maze (MWM), (3) Delay Match to Place (DMP), and (4) Cue Strategy.

Keywords

PPAR agonists; Cognition; Novel object recognition; Morris water maze; Delay match to place; Cue strategy

1. Introduction

PPAR agonists appear to have therapeutic potential in the treatment of neurologic diseases including: Alzheimer's disease (1), multiple sclerosis (2, 3), Parkinson's disease (4–7), excitotoxic injury (8, 9), AIDS dementia (10, 11), brain tumors including glioma (12, 13), and radiation-induced brain injury (14, 15). The therapeutic implications for PPAR agonists necessitate the use of animal models and procedures to evaluate potential efficacy.

Several behavioral paradigms have been successfully used to evaluate the efficacy of PPAR agonists to modulate cognitive impairments. These involve procedures that allow for the interrogation of several different brain regions in order to globally assess cognition. These tests are explained in detail below, and include: (1) Novel Object Recognition (NOR), (2) Morris Water Maze (MWM), (3) Delay Match to Place (DMP), and (4) Cue Strategy.

2. Novel Object Recognition (NOR) Task

Rodents are attracted by novel objects and engage readily in exploration using their vibrissae, nose, and forepaws. The premise of the NOR is that rodents are able to discriminate a novel from a familiar object; they can also detect a mismatch between the past and present location of a familiar object (object in place recognition), and can discriminate different objects in terms of which object has been encountered earlier (temporal order memory) (16). The version of NOR task described below is mainly a perirhinal cortex (PRh)-dependent version (17).

2.1. Testing Apparatus

The arena should be sufficiently large enough to allow for ample exploration. For our rat studies we use an opaque white polypropylene container that is 24 × 18 × 20 . A video camera is mounted directly above the arena. The NOR can be scored using an automated system; however, care must be taken to ensure that only true exploration is counted. The

standard practice is to record “object exploration” when the animal’s nose is within 1–2 cm of the object and the vibrissae are moving. Object exploration is not recorded when the animal uses the object to prop itself up while looking around and sniffing, or when the rat is touching the object with some part of the body but headed in another direction. There are systems, such as the Noldus EthoVision® (Leesburg, Virginia, USA) that allow for precise tracking of the nose in order to collect object exploration data, as well as multiple parameters to evaluate other non-object directed behavior (e.g., rearing, grooming, etc.). Several other vendors market similar systems that would be suitable for acquisition and analysis of the NOR task (Stoelting Co., Wood Dale, IL, USA; TSE Systems Inc, Chesterfield, MO, USA). Alternatively, a trained observer, who is blind to group membership, can easily conduct the scoring from the previously recorded video. When setting up your testing paradigm, special consideration should be given to the color of the rats to be tested, as the tracking systems typically need contrasting backgrounds for successful video acquisition (e.g., we used brown rats in a white arena). The objects should be secured to the arena floor so the animal cannot displace them during exploration. We use Velcro patches for this purpose, which also ensures that the objects are always placed in the same location within the arena.

2.2. Methodology

The NOR task is comprised of three phases: familiarization, delay, and test phases. Before testing, each animal is habituated to the testing arena by allowing it to explore the empty arena for 5 min a day for 4 days. After habituation, the following sessions are divided into a sample phase and a test phase. During the sample phase (test 1), the rodent is allowed to explore and become familiar with two identical 3-D objects located within the testing arena for 3 min (Fig. 1). Following the sample phase, a delay is introduced (we use a 5 min delay) during which the animal is removed from the arena and returned to its home cage. A test phase (test 2) follows in which one of the two objects from the sample phase is paired with a novel object (Fig. 1). The subject is left in the arena for a total of 3 min during the test phase and activity is recorded. The objects are secured to the arena floor so the subject cannot displace them during exploration. Velcro patches can be used for this purpose; these patches also serve as fiduciary marks that ensure the objects are always placed in the same location within the arena. Recognition memory is inferred from the preferential exploration (measured as time) of the novel object compared with that of the familiar object.

The basic measurement is the time the rat spent exploring an object, defined as placing his/her nose within 2 cm of the object and actively exploring it. The following parameters are generally obtained, E1: the total time spent exploring the identical objects A1 and A2 in the sample phase (3 min); E2: the total time spent exploring object A3 (identical to A1 and A2) and the novel object B1 in test phase (3 min); and D1: the index of discrimination defined as the difference in time spent exploring objects A3 and B1 in the test phase (i.e., $B1 - A3$). Rats with an $E1 < 10$ s are eliminated from further analysis due to insufficient exploratory behavior. The discrimination ratio ($D1/E2$), a measure of the rat’s recognition memory, is then calculated, and the average discrimination ratio for each treatment group compared.

2.3. Special Considerations

Care must be taken to ensure that scent-marks and trails are removed between each phase and animal, which can be done with thorough cleaning of the arena as well as by using identical copies of the objects during the test phase. These measures will ensure that the animal is recalling visual characteristics of the object from the familiarization phase as opposed to olfactory cues. Pairing stimuli that are constructed of similar material (e.g., glass with glass) further reduces the possible influence of olfaction during the recognition test. Because rodents exhibit innate preferences for certain objects, counterbalancing the familiar

and novel objects is required. The position (left or right) of the novel object in the test phase is balanced between sessions to avoid any spatial preference.

3. Morris Water Maze (MWM) Task

The MWM has been the most extensively used and accepted task to assess and compare spatial learning and memory in rodents (18). The MWM task is a challenging procedure for rodents that employs a variety of sophisticated mnemonic processes (19). In order to successfully navigate the maze and find the hidden platform, the animal must acquire and spatially localize relevant visual cues and then process, consolidate, retain, and then retrieve these cues (18, 19). The general processes used for “visuospatial navigation” in rats also appear to contribute considerably to human day-to-day cognitive processes and thus the MWM task has been used to investigate models relevant to the study of a variety of neurological illnesses where cognition is impaired (e.g., Alzheimer’s disease, Parkinson’s disease, schizophrenia, and radiation-induced brain injury) (6, 20–28).

3.1. Invisible Platform Tests

In the classic design, rats are repetitively released in a circular pool filled with opaque water, in which the only escape opportunity is provided by a small submerged platform. The platform is invisible and occupies a fixed position throughout training. To escape, rats must learn and remember the position of the platform relative to visible cues placed around the arena.

3.1.1. Testing Apparatus—MWM testing should be conducted in a large circular pool (rats, diameter: 68 , height: 24 ; mice, diameter: 40 , height: 12) made of plastic. The pool should be filled to a depth of ~30 in. of water (mice, ~10) to cover a white 4.25 diameter platform (mice, ~2) and maintained at $25^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$. The platform is submerged approximately 1.0 cm below the surface of the water and placed in the center of the fourth quadrant. The water is rendered opaque by the addition of a white food coloring agent. The pool should be located in an isolated room, with the pool surrounded by a curtain. Attached to the curtain should be a number of extramaze visual cues, including highly visible contrasting geometric images (squares, triangles, circles, etc.). The curtain also allows for the experimenter to be hidden from the rat undergoing testing, reducing distraction of the rat during testing (Fig. 2). Swimming activity of each rat is monitored via a video camera mounted overhead, which relays information including latency to the platform, total distance traveled, time and distance spent in each quadrant, etc., to a video tracking system. When setting up a testing paradigm, special consideration should be given to the color of the rats to be tested, as the tracking systems typically need contrasting backgrounds for successful video acquisition (e.g., we used brown rats in a white pool, with white opaque water). Our laboratory has used the Noldus EthoVision® system (Leesburg, VA, USA) with good results; however, there are several other vendors which market similar systems (Columbus Instruments, Columbus, OH, USA; TSE Systems Inc., Stoelting Co.).

3.1.2. Methodology—Rats are tested individually by being placed into various quadrants of the pool, and the time elapsed and/or the distance traversed to reach the hidden platform recorded. With each subsequent entry into the maze the rats progressively become more efficient at locating the platform, thus escaping the water by learning the location of the platform relative to the distal visual cues. The learning curves are then compared between groups. An illustration of a typical MWM set-up (as used in our laboratory) is shown in Fig. 2. The MWM consists of two testing phases; a standard phase followed by a reversal phase. In the standard phase spatial learning and reference memory are measured. The escape platform is placed in quadrant 4 throughout all training trials. At the beginning of each

training trial, the rats are introduced into the quadrants in a systematically random pattern, and each rat's performance is recorded using an automated tracking system during the 90 seconds (s) trial. Two training trials are given each day for 2 days followed by the third day which includes one training trial followed by one probe trial (1 block; Table 1). During each training trial, the rat is allowed to search for 90 s to locate the platform. Once the rat has found the platform it is allowed a 30 s "rest" period, in order for the rat to obtain spatial visual cues after each trial before being removed. If the rat does not locate the hidden platform, then a 90 s time is recorded for that rat and it is led and placed on the platform for 30 s, in order to ensure all rats have the opportunity to obtain spatial cues equally. After the completion of each trial, the rat is dried with a towel to remove excess water and returned to their home cage. The measurements acquired during this phase include: (1) the latency to the platform, (2) total distance traveled, and (3) the time and distance spent in each quadrant. The latency and distance traveled should decrease over the sample blocks, indicating acquisition of the task (Fig. 3). During the probe trial, the platform is lowered beyond reach; the rat is then allowed to search for 30 s. The time elapsed and distance swam in the previous platform quadrant (quadrant 4) is recorded. In addition to measuring the quadrant, an annulus ring can be placed around the previous platform location (via computer program) to localize the area more precisely. The number of crossings through this region is typically recorded and compared between groups. This schedule is repeated for four blocks.

In the reversal phase of the MWM, the platform location is moved to the opposite quadrant (quadrant 2) with the visual cues remaining in the same positions. This task measures spatial bias for the previous platform location and requires the rats to inhibit their previously learned response and form a new strategy to find the escape platform in a novel location. This is accomplished by measuring the percentage of time spent (and distance swam) in the previous target quadrant as well as the number of crossings over the previous platform location. These assessments provide a second estimate of the strength and accuracy of the memory of the previous platform location.

3.2. Visible Platform Tests

A visible platform test is performed to determine if alterations in visual acuity confound the data analysis, since the successful completion of the task depends on the use of visual cues. However, care must be exercised to ensure that certain behaviors that might be interpreted as impaired visual acuity are taken into account (19). For example, the absence of search behaviors or thymgotaxis (swimming constantly along the perimeter of the pool) might be misinterpreted as visual deficits since the animal does not locate the platform in a reasonable period of time (19). Thus, animals must make attempts to cross the pool and then be impaired at locating the platform in order for an interpretation of visual deficits to be made (19).

Immediately following the final probe trial, the platform is placed in the pool in the quadrant located diametrically opposite the original position. A visible cue (we use an inflated glove) is attached to the platform which is raised slightly above the surface of the water. Each rat is allowed one trial in order to acclimate to the new set of conditions and locate the platform visually. This is accomplished by lowering the rat into the water into quadrant 4 and allowing the rat to locate the platform; no time limit is placed on this first trial. Once the platform is located, allow the rat 30 s on the platform. The rat should then immediately be given a second trial in the same manner and the latency to the platform measured as a comparison of visual acuity.

4. Delay Match to Place (DMP)

The DMP version of the MWM is sometimes referred to as a working memory protocol. In this DMP version, the platform is moved to a new location each day resulting in the animal not knowing where the platform is hidden on trial 1 of each day (Fig. 4). Once the animal finds the platform, it can generally encode this new location in one trial. This is shown by the animal finding the platform much faster on trial 2 and subsequent trials of that day (29).

In our studies, we use a DMP procedure that starts on the last day of the standard MWM (Table 1). The reversal day of the standard MWM is the first day of the DMP and lasts for a total of 4 days. Each DMP testing day consists of four trials, a sample trial (trial 1) and three match trials (trial 2–4; Table 2, Fig. 4). The hidden escape platform in the sample phase each day is located 2 cm under the water surface, in a unique location (Fig. 4), and remains in the same location for the remaining match trials for that day. The delays between trials are constant within days and are as follows across the 4 days of testing: 20 min, 15 s, and 15 s (Table 2).

5. Cue Strategy Task

A spatial/place strategy, which involves the flexible use of spatial cues, is dependent upon intact hippocampal circuitry. Conversely, a response/cue strategy, or the formation of associations between discrete cues and behavioral responses, is dependent upon intact striatal circuitry.

Strategy preference can be assessed 24 h after the last day of DMP training. A visible platform is placed in the quadrant opposite of the quadrant where the hidden platform was previously located. The rats are given two 60 s strategy probe trials.

Start locations are on either side of the tank, equidistant from the visible cue platform and the prior hidden platform location. A “place strategy” is recorded if the rat crossed the annulus of the prior hidden platform location before escaping to the visible platform. The annulus is defined as a 5 cm perimeter around the prior hidden escape platform location. A “cue strategy” is recorded if the rat did not cross the prior hidden platform annulus before swimming to the visible platform. Data are scored by a blinded observer using recorded swim paths (Ethovision, Noldus).

Acknowledgments

Supported by National Institutes of Health grants CA112593 and CA113267.

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Fig. 1.

The novel object test phases. Test 1 is the sample phase: the rodent is allowed to explore and become familiar with two identical 3-D objects located within the testing arena for 3 min. A delay is introduced between test phases and the animal is returned to its home cage. A test phase (test 2) follows in which one of the two objects from test 1 is paired with a novel object; the subject is left in the arena for a total of 3 min during which activity is recorded.

Fig. 2.

The standard set-up for the Morris water maze (MWM). The subjects are repetitively released in a circular pool filled with opaque water, in which the only escape opportunity is provided by a small submerged platform. The platform is invisible and occupies a fixed position throughout training. To escape, subject must learn and remember the position of the platform relative to visible contrasting cues placed on a curtain that surrounds the arena. The curtain also allows for the experimenter to be hidden from the subject undergoing testing, reducing distraction during testing. A camera is mounted directly above the arena to allow for automated tracking and data collection.

Fig. 3.

A typical measurement of task acquisition of the MWM. The representative plot shows the expected trend for escape latency (time to locate the hidden platform) over the duration of the trials; escape latency decreases over time indicating task acquisition. The circular diagrams represent the MWM arena and the relative path lengths from the beginning, middle, and end of the training trials. Overall path lengths decrease and the path becomes more direct as the subject progresses through the trials.

Fig. 4.

The delay match to place (DMP) task set-up for MWM. Following the standard MWM, the DMP version can be performed. On Day 1 of the trial, the submerged platform is moved to the opposite quadrant (dark circle) from where it was in the previous standard MWM trial (dotted circle). The subject is introduced to the arena at various quadrants throughout the training and trials on Day 1 (*arrows*) and for all other days (Days 2–4). The platform location is changed each day (dark circle is new location; dotted circle is location from previous day).

Table 1

The experimental protocol and timing for the standard Morris water maze. The experimental paradigm is 13 days which are divided between four blocks, each containing of 3 days. Each day consists of two trials a day, with the third and final day of each block consisting of one trial (trial 5) and a probe trial. The very last trial (Day 13) is the reversal, where the platform is moved to the opposite quadrant; this is also Day 1 of the delayed-match-to-place task

Standard Morris Water Maze		
Block 1	Day 1	Trial 1
		Trial 2
	Day 2	Trial 3
		Trial 4
	Day 3	Trial 5
		Probe
Block 2	Day 4	Trial 1
		Trial 2
	Day 5	Trial 3
		Trial 4
	Day 6	Trial 5
		Probe
Block 3	Day 7	Trial 1
		Trial 2
	Day 8	Trial 3
		Trial 4
	Day 9	Trial 5
		Probe
Block 4	Day 10	Trial 1
		Trial 2
	Day 11	Trial 3
		Trial 4
	Day 12	Trial 5
		Probe
	Day 13	Reversal

Table 2

The experimental protocol and timing of the delayed match to place task. The table shows the timing of the DMP for four rats (Rats: A,B,C, and D). The DMP procedure starts on the last day of the standard MWM, the reversal day. DMP is performed over a total of 4 days with each testing day consisting of four trials: a sample trial (trial 1) and three match trials (trial 2–4). The hidden escape platform in the sample phase each day is located 2 cm under the water surface, in a unique location and remains in the same location for the remaining match trials for that day. The delays between trials are constant within the 4 days of testing and are as follows: 20 min, 15 s, and 15 s

Delayed match to place version of the Morris Water Maze			Starts on reversal (Day 13) of MWM
40 min Testing period			Trial
20 min Interval	0 --> 5	Rat A	1
	5 --> 10	Rat B	1
	10 --> 15	Rat C	1
	15 --> 20	Rat D	1
2 min			
15 s Interval	20 --> 25	Rat A	2
			3
			4
	25 --> 30	Rat B	2
			3
			4
	30 --> 35	Rat C	2
			3
			4
	35 --> 40	Rat D	2
			3
			4