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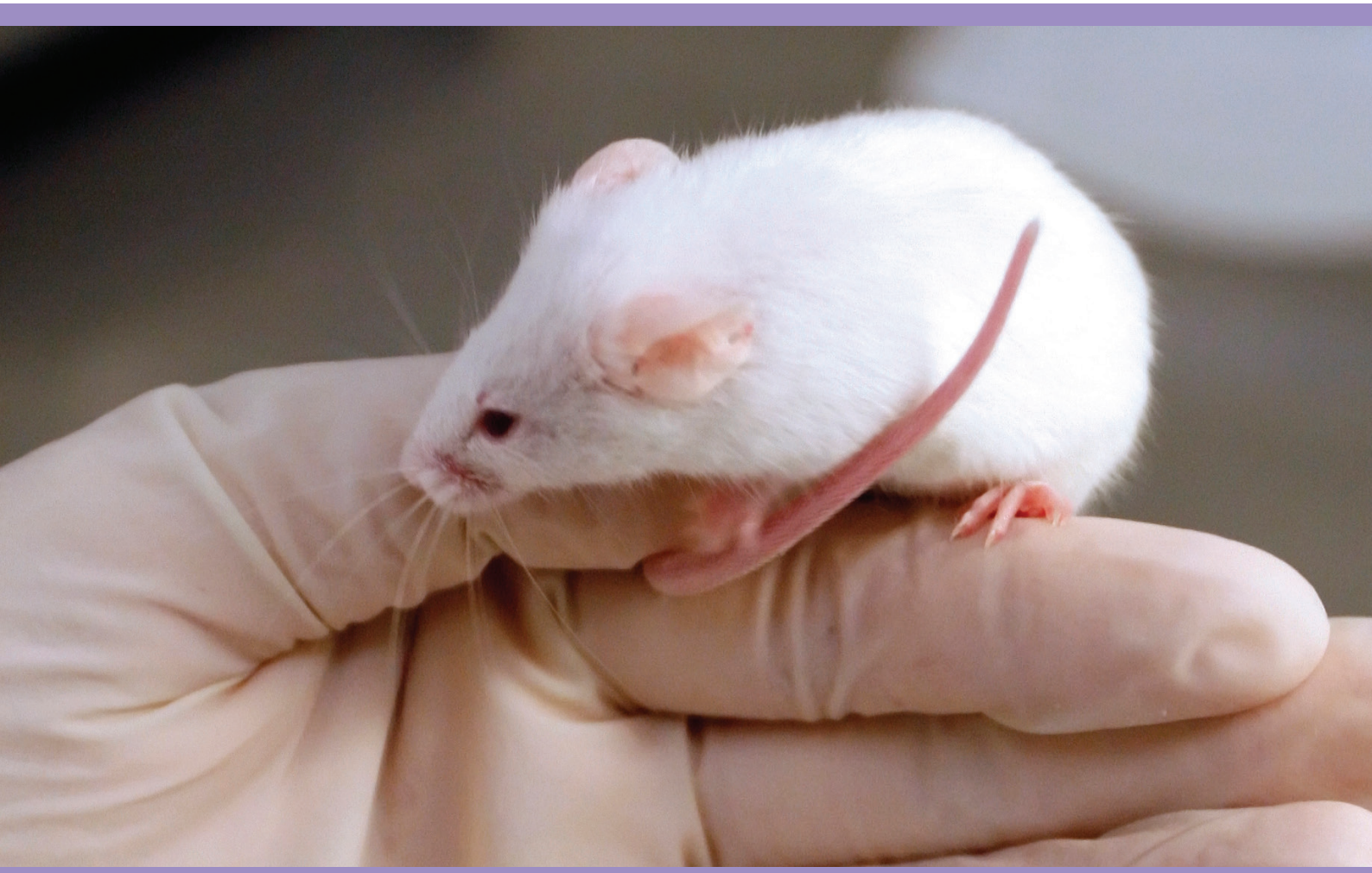
How to perform a Barnes maze test



This is a chapter from the e-book:

Basic behavioral neuroscience in rodents.

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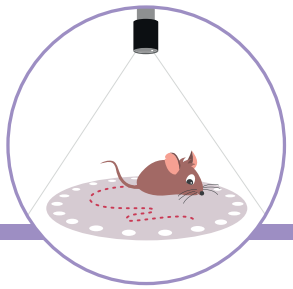
THE BARNES MAZE



The Barnes maze is a dry-land environment for testing learning, memory and cognitive flexibility. Carol Barnes designed this behavioral paradigm in 1979 to test memory deficits in aging rats, while only being adapted to mice as late as 1995. This test is regarded as an alternative to the Morris water maze, which relies on swimming behavior potentially confounding learning and memory readouts. Although being coined as an alternative, there are some clear advantages and disadvantages towards using either a Barnes maze or Morris water maze to test learning and memory which will be listed here.

MOUSE BARNES MAZE

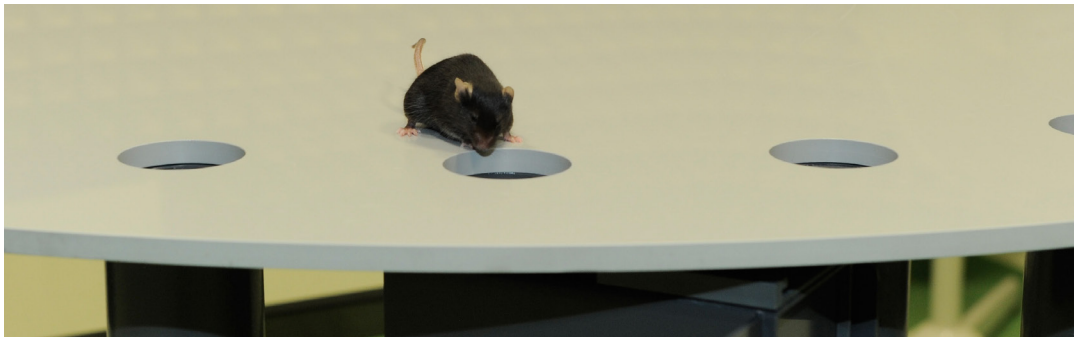
Diameter is 100 cm
20 holes, 5 cm diameter

RAT BARNES MAZE

Diameter is 130 cm
20 holes, 10 cm diameter

The performance in the Barnes maze, and the Morris water maze, are also highly sensitive to anxiety in rodents, which can be induced (or of greater levels) in animals subjected to for example pharmacological and/or genetic manipulation. This can be mitigated for example by decreased light intensity.

Generally the Barnes maze is a well-established behavioral paradigm, and is used, as stated, to test learning and memory in rodent models for, for example, Autism Spectrum Disorder, Alzheimer's disease and ageing, to name a few. Performance in this test is primarily linked to allocentric spatial learning, since the animals learn the position of an 'escape box' relative to other positions on the maze. This is made clear by the construction of the Barnes maze, which is a circular table with circular holes around the circumference. As mentioned, the goal of this test is that the subject (rat or mouse) reaches an escape box that is positioned beneath one of these holes. This task relies on visual cues, which thus prompts spatial learning and memory. Learning the position of this box beforehand is essential to this test, which we thus call the acquisition phase. This can be done in a number of ways, food reward is a popular choice. However it has been seen that motivating animals to indeed look for the escape box can be challenging. Non-responders are not uncommon in this test. These are animals that simply ignore the tendency to reach the escape box. Oversaturation can also take place when there are a large number of acquisition trials.



A C57BL/6J mouse looking down into the escape hole of a Barnes maze.

Barnes maze or Morris water maze, which one should I choose?

Advantages of the Barnes maze

1. *The Barnes maze does not involve swimming, which can be perceived as stressful and increases corticosterone levels.*
2. *Swimming in the Morris water maze also causes reductions in core body temperature which can affect performance, this can be circumvented somewhat by regulating the water temperature properly.*
3. *While in a swimming task, rodents can take to floating, which is thought to represent a state of behavioral despair, a common readout of depressive-like behavior in the forced swim test.*

Disadvantages of the Barnes maze

1. *A lack of stressful stimuli in the Barnes maze can result in slow learning. To increase the motivation to escape a mild stressor such as white noise or a buzzer sound can be played.*
2. *The Barnes maze requires a bit more training/ trials than the Morris water maze. Where a Barnes maze generally takes 15-20 acquisition trials, even having protocols of up to 40 trials. The Morris water maze is generally performed over ± 12 trials.*
3. *It is said that the Barnes maze is somewhat limited to the use of young (3-5 months of age) adult C57BL/6J mice.*

Concluding

Both the Barnes maze and the Morris water maze are well validated behavioral paradigms for studying learning and memory. Both have clear advantages and disadvantages. It should thus be pragmatically considered which one is the best for you. Do you have a validated setup of one or the other in your lab? Have you previously published with one of these tests? Or do you simply want to avoid swimming, for example in mice which are less-than-natural swimmers compared to rats? Think practically about these things when writing/planning your study design.

PROTOCOL SUGGESTION (MULTIPLE DAYS)

Preparation

- Place the maze in your testing room at the appropriate lighting intensity for your tests. You might have to decrease this intensity a bit, since this will improve performance on this test in animals with higher levels of anxiety.
- Place three or four distal visual cues (length/width ~30 cm) surrounding the arena, visible to the animals while subjected to the test

Habituation (day 1)

- Transport the animals, preferably in their home cages, into the testing room and allow the animals to acclimate to this room for a minimum of 30 minutes prior to starting the test.
- Attach the *escape box* to the platform.
- Remove a single animal from the home cage with your preferred handling technique: tail handling, full hand handling, tube handling and place the animal in the escape box for 1 minute.
- After this, place the animal in the center of the maze, allow it to explore for 5 minutes or until it reaches the escape box.

Training/acquisition phase (days 1 - 10)

- Allow an interval of at least 1 hour between the habituation trial and the first training session.
- Attach the escape box to the maze, at a different location than the habituation trial! This position will remain the same for all training/acquisition trials.
- Daily two training session can be performed with an average length of 3-5 minutes per session (your preferred choice).
- Remove a single animal from the home cage with your preferred handling technique: tail handling, full hand handling, tube handling and place the animal in the center of the maze
- The trial is over when the maximum time for the test has elapsed, or if the animals has found the escape box. If the animal does not find the escape box within the given timeframe, place the animal in the escape box for 15 seconds after the trial and note the maximum time.
- After each trial remove all fecal pellets and wipe up all spots of urination. Spray the maze with 30-70% ethanol and wipe down with a clean paper towel.

A standard Barnes maze is always elevated from the floor. Credits: He, S. and Corscadden, L. (2022). Maze Engineers.

Probe trial

- Three days after the final session of acquisition training, perform a probe trial for 1 minute. In this trial the escape box should be removed from the apparatus.

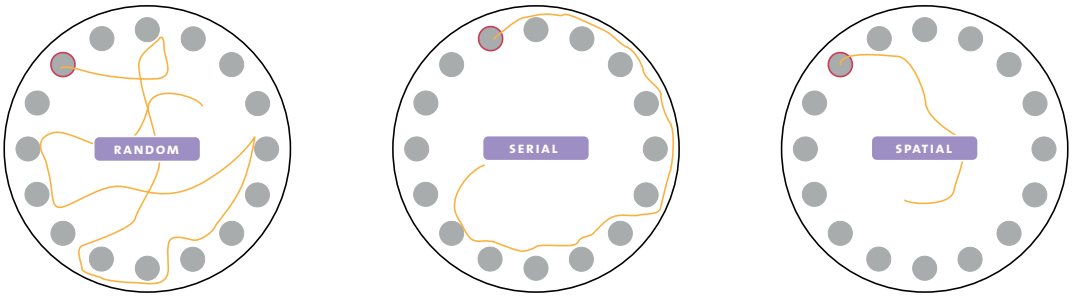
SETUP IN ETHOVISION XT

In EthoVision XT there is a predefined template for the Barnes maze, making tracking and analyzing as easy as dragging and dropping the circles (for the escape holes) to the correct place in the video image. Specify which hole has the goal box underneath, and you are ready to acquire your data. Record the following parameters: latency to locate the escape box, latency to enter the escape box, number of (incorrect) holes checked before locating and escaping to the box and distance traveled prior to locating the escape box (and total distance traveled).

INTERPRETATION OF THE RESULTS

Investigating the animal's searching strategy over consecutive trials is an interesting readout of the Barnes maze. For example, some animals randomly search for the correct hole while in the acquisition phase, while other animals stick to a certain pattern when systematically checking each hole. These search strategies are classified as random, serial, or direct (or spatial).

In general, with repeated testing, rodents typically progress through the search strategies in the order listed (random, serial, and direct). This progression over time can be used to visualize the increase in searching performance during the acquisition phase of this test. However some animals, even with direct visual markers at the escape box, keep preferring the serial search strategy over the direct (spatial strategy). However these strategies aren't necessarily slower and/or faster compared to each other. Rodents, particularly mice, can walk rapidly over the edge of the maze, employing the serial search strategy, and showing no particular difference in time to reach the escape box, and keep using this method in all trials. This does however result in significantly more errors, since they generally check more incorrect holes. Visualizing and classifying this strategy is thus of upmost importance for the quantification of this test, since total number of errors will give a skewed representation of the animals' performance.



Visualizing searching strategies on the Barnes Maze with EthoVision XT is critical for the interpretation of this test.

Want to read more about search strategies in the Barnes maze? Check out this interesting publication by Harrison *et al*.