



Barnes Maze Test ¹⁻⁸

Materials Checklist

Before starting your experiment, make sure to go over the following materials checklist:

Materials	Check
Barnes Maze	
Spray bottle with 70% ethanol	
Disposable paper towels	
Camera & video tracking system (ANY-maze)	
Clean bedding	
Remote-controlled white noise generator	
Bright overhead lights	
Plastic beaker	
Timer	
Visual cues	

Use of the video tracking software ANY-maze

Before starting experiments, please familiarize yourself with the use of the video tracking software (ANY-maze) by reading and watching the following training tutorials. You'll find printouts of the tutorials in a folder located by the computer in the testing room and a folder located on the computer desktop labeled "ANY-MAZE Tutorials":

Reading materials

- **Setting up a Barnes Maze experiment in ANY-maze** (<https://www.any-maze.com/support/guides/setting-up-a-barnes-maze-experiment-in-any-maze/>)
- **An introduction to ANY-maze** (<https://www.any-maze.com/#intro>)

Video tutorials

- **Animal Behavior Video Tracking Using ANY-maze Software -- Session 1** (<https://www.youtube.com/watch?v=dQU5yfiDg1Y>)
- **Animal Behavior Video Tracking Using ANY-maze Software - Session 2** (<https://www.youtube.com/watch?v=n8rudj4j50Y>)

Preparation

1. Open the video tracking software (Any-maze) and create a personal login account.
2. Place a "Do Not Enter – Experiment in Progress" sign on the testing room door.
3. Turn on lights above the maze. If it's too bright, dim lights to prevent interference with video camera.
4. Make sure all non-scape holes are closed.
5. Leave open only the escape hole and slide in the deep escape box/drawer.
6. Switch on white noise device.
7. To start habituation, bring mice in their home cages to the testing room 30 min before starting training.

Day 1: Habituation session



1. Place each mouse in the center of the maze and cover it with a clear 3,500-ml glass beaker for 30 s.
2. Guide mouse slowly to the target hole by sliding the glass beaker over the target hole within a span of 10–15 s.
3. Give mouse 180 s to enter the target hole by itself. In the event no entry is apparent, gently nudge it into the hole, let the mouse stay in the hole for 2 min.

Days 2-5: Acquisition training

4. Start training next day at the same time, and preferably by the same person who performed the habituation.
5. Place mouse for 15 s inside the start chamber (Box: 6.5" L x 6.5" W x 8" H) located in the center of the maze.
6. Lift the chamber, start the timer and let mouse explore the maze for 180 seconds.
7. Record latency to first nose poke at target hole and total number of errors (nose pokes at non-target holes).
8. Monitor and record any potential sign of distress (freezing, vocalization, defecation, urination, etc.).
9. Trial ends when mouse enters the target box (all four paws inside) or after 180 seconds.
10. If the mouse finds the escape box within 180 s, let it stay in the escape box for 2 min.
11. If the mouse does not enter the target hole within 180 s, gently guide it to the target hole using a glass beaker and allow it to freely enter the escape box.
12. Once mouse enters the escape box, turn off aversive stimuli (noise and light). Let mouse stay in the escape box for 2 min.
13. Pick mouse and return it to its home cage.
14. Spray platform surface with a 70 % EtOH solution, dry it clean with a paper towel and let it briefly air dry.
15. Record data: latency, errors, search strategy, path characteristics.
16. Perform 1-2 trials per training session per day.
17. When performing more than 1 trial per day, wait at least 15-20 minutes between trials (Inter-trial interval, ITI).

Notes:

- Keep escape hole location constant for each individual mouse throughout acquisition.
- Maintain consistent time of day for testing (e.g., all testing in morning or afternoon)

Day 6: Probe Trial (Short-term retention)

Objective: Assess spatial memory by removing escape box and measuring search behavior.

Step 1: Preparation

18. Wait 24 or 48 hours after the last training session to perform probe test.
19. Cover or block target hole and keep all other conditions identical to acquisition (lighting, cues, etc.). Replace the deep drawer with regular/shallow escape box/drawer)
20. Since video recording is essential during this step, make sure the digital camera is functional, properly focused and controlled by the video tracking system (ANY-maze, Stoelting Co.) is fully functional.

Step 2: Probe Trial (Short-term retention)

21. Place mouse in start chamber at center
22. Lift chamber to begin trial
23. Allow mouse to search platform for 180 seconds
24. Do NOT guide mouse or provide feedback

Day 12: 2nd Probe Trial for Long-term retention (OPTIONAL)

A 2nd probe trial can apply on day 12 to assess long-term retention. It does not include any training session between day 5 and day 12. Repeat steps 22-25.

Step 3: Data Collection

25. Record time spent in target quadrant (quadrant containing former escape hole)
26. Count number of nose pokes at target hole vs. other holes
27. Record latency to first target hole exploration



28. Take notes regarding mouse search strategy.
29. Gently remove mouse and return to home cage
30. Clean platform with a 70 % EtOH solution, dry it clean with a paper towel and let it air dry.



Timeline (6-Day Protocol: 1 habituation day + 5 training days + 1 Probe test day)

Day	Activity	Duration
Day -1	Handling and habituation	5-10 min/day per mouse
Day 1	Habituation session	5 min per mouse
Day 2-5	Acquisition training	1-2 trials/day. Total testing time: 15-20 min/mouse
Day 6	Probe trial (48 h after last acquisition)	180 s per mouse

Data collection and analysis

Latencies:

- Primary latency: Time (seconds) from trial start to entry into escape box (all four paws)
- Latency to target: Time to first nose poke or investigation of target hole
- Record for each trial during acquisition and probe

Errors:

- Total errors: Number of nose pokes at non-target holes before finding target
- Perseverance errors: Repeated visits to same incorrect hole

Probe trial:

- Target quadrant time: Percentage of time spent in quadrant containing target hole
- Target hole investigations: Number of nose pokes at target vs. other holes
- First investigation: Whether first hole investigated is target (yes/no)

Search strategies scoring:

Spatial (Efficient):

- Direct: Moves directly to target with minimal errors (<3 errors)
- Focal: Searches primarily in target area/quadrant

Serial (Intermediate):

- Serial: Systematic checking of adjacent holes in sequence until finding target

Random (Inefficient):

- Random: Unsystematic search with multiple errors and no pattern
- Thigmotaxis: Remains at platform edge with little exploration

Scoring: Review video recordings and classify predominant strategy for each trial using objective criteria.

Preferred mouse strains (Barnes maze)

Strain	Performance	Reference (PMID)
C57BL/6J	Good	12954410, 12886950, 10837506
DBA/2, CBA	Poor performance	
129S6	It learns task but shows no preference for the target hole during the probe trial	12886950
BALBc, Swiss	No apparent improvement during the acquisition phase from day 2 to day 4.	12954410



References

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