

Barnes Maze testing Protocol by Xuansong Mao
(Modified from Matthew W. Pitts, 2018 Bio-protocol)

Habituation phase:

1. The surround visual cues should be place. These cues should remain **unaltered** for the habituation, acquisition and probe trial phases.
2. Gently place the mouse in the escape box for **1 min** in order to familiarize the escape box and to reduce the level of anxiety. Do this before the first trial of the acquisition phase.

Clean the escape box between each animal with 70% Ethanol

Acquisition phase:

1. Allow an interval of 1-hr between habituation and the first trial of the acquisition training phase.
2. Attach the escape box to the platform at a location **different** from that used for habituation trial. The position of the escape box then remains at this fixed location for the entire duration of the training.
3. Acquisition phase consists of two training trials per day (3 min limit per trial, inter-trial interval 1 hr)
4. At the onset of each trial, the mouse is placed in a start chamber located in the center of the platform. After 15 sec, the start chamber is lifted and the mouse is allowed to freely explore the maze.
5. Each trial ends when the mouse **enters into** the escape box or 3 min elapses. (If a mouse fails to find the escape box within the 3 min period, gently guide the mouse into the escape box and allow to stay there for 15 sec prior to removal back to the home cage.

Clean the escape box between each trial with 70% Ethanol

6. For each trial, there are several parameters are recorded to assess the neurocognitive performance.
 - A. Latency to locate (primary latency) and enter (total latency) the escape box
 - B. Number of incorrect holes checked prior to locating (primary errors) and entering (total errors) the escape box
 - C. The distance traveled (path length) prior to locating the escape box and total distance for each trial.
 - D. The search strategy: spatial, serial, random

Probe trial (memory retention/short-term memory test):

1. 24 hrs after the final trial of acquisition training, mice undergo a 90 sec probe trial in which the escape box is removed from the Barnes maze apparatus.
2. The platform is divided into 4 quadrants, the time spent in the quadrant that previously had the scape box is measured.