

JAN 13, 2023

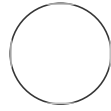
Elevated plus maze protocol

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ABSTRACT

This protocol describes steps for assessing anxiety-like behavior in rodents using an elevated plus maze test.

The elevated plus maze (EPM) is one of the widely used behavioral assays to measure anxiety-like behaviors in rodents. In the EPM test, rats or mice are placed at the intersection of a plus-shaped maze with two open arms and two closed arms. The rodents show natural preference to stay in closed areas and avoid open and elevated areas. This expression of unconditioned anxiety is well captured in this assay. The amount of time spent in the open arm vs the closed arm is typically measured to assess anxiety-like behavior. The anxiolytic or anxiogenic effects of pharmacological agents and genetic manipulations can be investigated using this method.

IMAGE ATTRIBUTION

Representative ANYmaze track plot of an animal in an elevated plus maze during a 5-min session.

GUIDELINES

- Male and female mice should be tested separately on different days to avoid the effect of pheromones on behavioral output.
- It is suggested that the researchers performing the behavioral assays be blinded for the mouse genotype to remove experimenter bias.

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.dm6gpj8kdgzp/v1

Protocol Citation: Archana Venkataraman, Holly Ingraham 2023. Elevated plus maze protocol. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.dm6gpj8kdgzp/v1>

MANUSCRIPT CITATION:

<https://www.biorxiv.org/content/10.1101/2022.04.04.486775v1.full>

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Protocol status: Working
We use this protocol and it's working

Created: Sep 16, 2022

Last Modified: Jan 13, 2023

PROTOCOL integer ID:
70161

Keywords: elevated plus maze, mice, anxiety-like behaviors

MATERIALS

- The elevated plus maze (EPM) apparatus, consisting of two open arms and two closed arms, is set up 50 cm above the floor. The dimensions of each arm are 35 cm × 5 cm, with the closed arms having a 15 cm high wall, and a center area of 5 cm × 5 cm.
- An over-head camera is used to obtain a full view of the entire maze and is connected to a computer installed with video tracking software ([ANY-maze, Stoelting, Wood Dale, IL, USA](#)) to enable automated tracking of the animal's movements.
- Detailed procedure on using the software for conduction elevated plus maze test is available at <https://www.protocols.io/view/any-maze-protocol-elevated-plus-maze-v6-2-5qpvo5o1xl4o/v1>

BEFORE START INSTRUCTIONS

Pre-handling the mice by the experimenter at least for 3-5 days prior to testing is strongly suggested.

- 1 Make sure the equipment is clean and dry before use.
- 2 Set up the overhead camera and ensure that the open arms and closed arms are appropriately selected in the 'zone' tab of the ANYmaze program.
- 3 Enter all animal information such as animal ID, status, and treatment, in the ANYMaze software.
- 4 Allow experimental animals to habituate to the behavior testing room at least 45 min prior to beginning of the test.
- 5 *Drug administration:* In experiments involving chemogenetic manipulation, deschloroclozapine (DCZ) injections (75 µg/kg) were administered intra-peritoneally 10 mins before testing. For alosetron pre-treatment, mice were subcutaneously injected with 0.1 mg/kg of the 5HT₃ antagonist 15 mins prior to the DCZ injections.



6 Once ready to start the experiment, place a mouse in the center area of the maze.

7 Place the animal such that the head is oriented towards the closed arm and away from the experimenter. Always handle each animal consistently throughout all experiments.



8 Immediately after the animal is placed in the maze, start the experiment on the ANYmaze software.

9 Allow the animal to explore undisturbed for 5 min.

10 Each animal is given one trial and should not be subjected to repeated testing.



11 After each trial, all arms and the center area were cleaned with 70% ethanol, to remove olfactory cues.

12 The time spent and distance traveled in the open and closed arms were determined using ANY-maze software and these measures serve as an index of anxiety-like behavior.