

- SOP No:** AHT 14
- SUBJECT:** Elevated Plus Maze Test – Mice (September 2015)
- REASON FOR USE:** This is a behavioural test designed to determine brain function
- POLICY:** This technique may only be performed by operators skilled in the technique.
- PRECAUTIONS:**
- EQUIPMENT:** Maze in a “plus” shape comprising four arms and a central platform made of Perspex and elevated above the floor. Two opposite arms are enclosed with walls; two other arms are open.
- PROCEDURE:**
1. Adjust light in the experimental room according to the experimental protocol. Generally, the brighter the light, the less time animals tend to spend on the open arms. Ensure even lighting of the same type of arms.
 2. Prepare for digital recording. If Noldus Ethovision or other automatic tracking program is to be used, set up all necessary parameters and check successful tracking settings.
 3. Bring animals into the room and allow them to acclimate for 30 min.
 4. If pharmaceutical intervention is required, administer required substances according to the AEC approved experimental protocol.
 5. Prepare the trial. Depending on the setup, either start the trial/recording or the trial will start automatically when the animal is placed in the maze. Place animal in the centre of the maze facing one of the open arms. Retreat behind the curtain and observe on the screen. Be prepared to intervene if the animal is going to jump off the maze.
 6. Trial usually lasts 10min. In this time the animal is allowed to freely explore all four arms and the centre of the arms.
 7. At the end of the trial, stop recording, remove animal from the maze and return to the home cage. Thoroughly clean the maze with 70% alcohol solution and allow to dry. Repeat steps with the next animal.

Assessment:

Main measures in the Elevated Plus Maze include time spent in open arms or % of time spent, time spent in closed arms, number of arm entries plus behavioural measures. Behavioural measures include head dips, rearings, groomings and stretch attend postures. In addition, time spent inactive can be calculated. It is useful to record number of defaecations and urinations occurring during the trial. The behaviour measures might be further sub-divided into occurring in the open or closed arms. Generally, the ratio of time spent in open arms compared to time spent in other parts of the maze is interpreted as a measure of anxiety; the number of defaecations is another measure of anxiety. The distance travelled in the closed arms and number of entries into the closed arms is considered to be measures indicating locomotor activity. Behavioural/ethological measures provide additional information.

RECOMMENDATIONS:

1. Carefully select lighting conditions suitable for the particular research purpose. Avoid conditions resulting in "ceiling" or "floor" effects.
2. After the lighting conditions are selected, record them and keep them consistent between inter-connected experiments as well as time of the day when experiments are conducted.
3. If automated animal tracking is employed, take particular care in setting up subject detection and tracking.
4. Do not interfere with the trial. Retreat behind the curtain and observe on the screen; avoid noise.
5. Run all manual scoring in a blind manner. Behavioural scoring requires practice; scoring by two independent observers might improve detection.

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CHAIR OF AEC

REFERENCES

1. Carobrez, AP and LJ Bertoglio (2005). Ethology and temporal analysis of anxiety-like behaviour: The elevated plus-maze model 20 years on. *Neuroscience and amp; Biobehavioural Reviews* 29(8): 1193-1205.
2. Harms LR, Eyles DW et al. (2008). Developmental vitamin deficiency alters adult behaviour in 129/SvJ and C57BL6J mice. *Behavioural Brain Research* 187(2):343-350