

SOP No: AHT 23

SUBJECT Object recognition test – rodents (April 2015)

POLICY: This technique may only be performed by operators skilled in the technique

PRECAUTIONS:

EQUIPMENT: The object recognition test will take place in the apparatus of the Open Field; a circular arena with a diameter of 100cm and white plastic walls 30 cm high for rats. (Arena smaller for mice).
A virtual central circle of 66cm diameter is created in the middle of the arena.
The light levels at the middle of the arena (~90 lux) and the time of experiment must be noted in lab book. To facilitate tracking, use uplights, not the fluorescent downlights which cause reflections.
At all times during the experimental testing, the experimenter can watch the trial on a television while being in the same room, but removed from the immediate experimental area (behind a curtain screen or a door).
Objects such as coloured blocks, toys or shapes are required, of which **AT LEAST TWO** are identical (see Figure 1)

PROCEDURE: Carry animals into the room (with lighting levels which will be used in the experiment with doors shut) at least 30 mins before beginning experiment.
Program the software (eg Ethovision)
Drugs/compounds should be given at appropriate absorption times prior to introducing rats/mice to the apparatus
At the end of each trial, remove the animal and thoroughly clean the arena with 70% alcohol solution, and dry with paper towelling.

Habituation

Start recording, and identify subject with white board placed in front of camera for ~3 secs.
Gently place animal in the outer field facing the centre field
Trials last for 10 minutes.

Training

Performed 24h after habituation

Protocol essentially the same as habituation, except two alike objects (i.e. coloured blocks) are placed at strategic positions in the centre circle prior to placing the animal in the outer arena (note objects MUST be the same)

Trials last for 10 minutes.

Trial

Performed either 15min, 90min or 24h after training (will isolate differences in short, intermediate and long-term memories) as previously described [1, 2]

Protocol essentially the same as training.

One of the objects (from training) in the centre field is replaced by a DIFFERENT object (i.e. MUST be of different colour and/or shape)

Objects MUST be in the same positions as training

Trials last for 10 minutes.

Analysis

Manually, or using ethological software (eg. Ethovision (Noldus, The Netherlands))

Assessments:

An inspection is operationally defined as approaching, sniffing and looking at object from a distance of $\leq 2\text{cm}$ (can be programmed as a 'zone' in Ethovision)

Habituation

Time taken to initially enter the inner zone

Time spent in centre zone

Training

Time taken to initially enter and inspect the objects

Time taken inspecting each object

Time spent in centre zone

Testing

Time taken to initially enter and inspect the objects

Time taken inspecting each object ($\geq 50\%$ of time inspecting the familiar suggests memory deficit)

Distance travelled

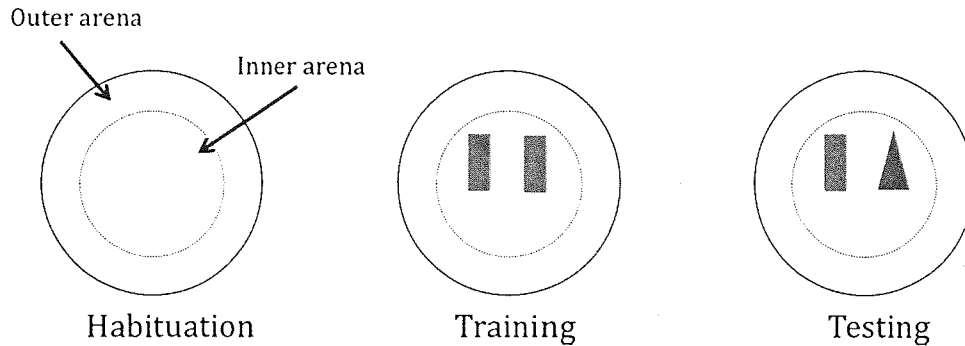
Entries into centre

Note; sitting on object not considered an inspection

Percent time inspecting; $T_F / (T_N + T_F) \times 100$, [T_F =time spent exploring familiar object; T_N =time spent exploring the novel object]

Note; Protocol for mice is essentially the same, except the dimensions of the arena and objects may vary (i.e. smaller).

Basic arena set-up



RECOMMENDATION

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01.04.2015



CHAIR OF AEC

REFERENCES

1. Costa, M.S., et al., (2008) *Caffeine improves adult mice performance in the object recognition task and increases BDNF and TrkB independent on phospho-CREB immuncontent in the hippocampus.* *Neurochem Int.*, 53(3-4): p. 89-94.
2. Costa, M.S., et al., (2008) *Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tirosine kinase receptor (TrkB) content in mice.* *Neuroscience.*, 153(4): p. 1071-8.

