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🌐 Determining the effects of mecamylamine in the mouse striatum using a Conditioned Preference Place (CPP) paradigm

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We use this protocol and it's working

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Abstract

This protocol is to determine the effects of administering mecamylamine in the mouse brain striatum using a Conditioned Preference Place (CPP) paradigm. This model measures the increased preference for an environment associated with the administration of a drug. The goal is to create a conditioned stimulus able to evoke a specific behaviour based on the nature of the drug tested.

Troubleshooting

Before start

We performed these experiments in male C57BL/6N mice (42-50 days-old).



Preparing the mouse for surgery

- 1 Anesthetize the mouse in an induction chamber with isoflurane (5% induction; 1.5 - 2% maintenance).
- 2 Mount the mouse on the warmed stereotaxic apparatus for surgery.

Surgical Procedure

- 3 Using the stereotaxic arm, localize the cannula to the landmark of interest (bregma or dura).
- 4 Once localized, raise the cannula tip 1-2 mm above the skull surface and move to the desired coordinates. For dorsal striatum injections, we use the coordinates AP +1.0 mm; ML +/- 1.6 mm to bregma; DV -2.4 mm (from dura).
- 5 Using bilateral injection needles (O.D. 0.21 mm, I.D. 0.11 mm, RWD, China), drill through the skull at the identified coordinates to create a hole for the cannula to be inserted.
- 6 Once the procedure is complete, mice recovered for three days in a warmed home cage.

Conditioned Place Preference Testing

- 7 Place mice in a 40 cm × 40 cm transparent plexiglass arena that is divided into two equal chambers separated by doorway.

Note

The chambers were decorated with either horizontal or vertical stripes.

- 8 On day 1, allow mice to freely shuttle between two chambers to assess place preference at baseline, expressed as % time spent in right chamber.

Note

The movement of animals was recorded and analysed with Smart V3.0 tracking software.



- 9 On days 2 and 3, administer alternating bilateral striatal injection with either mecamlamine (10 $\mu\text{g}/\text{side}$) or saline vehicle (0.9%) in a volume of 0.5 μl over 1 min in AM and PM. Constrain animals respectively in the right or left chamber for 20 min.

Note

Treatments were counterbalanced for time of day.

- 10 On day 4, calculate the post-conditioning chamber preference as the % of time spent in the right mecamlamine-associated chamber compared to on pre-conditioning day 1.

- 11 For the next two days (days 5-6), administer bilateral saline injections and allow animals to explore both chambers for 20 min.

Note

Conditioned place preference should be extinguished.

- 12 Repeat the conditioning procedure with bilateral saline for both chambers, with a pre-conditioning test on day 7, two days of conditioning on days 8-9, and a post-conditioning test on day 10.

Note

To minimise place preference bias at baseline, the five animals in each test showing least place preference on the pre-conditioning day (mecamlamine 42%-58%; control 45%-55%) were selected for subsequent conditioning.

Open Field Testing

- 13 Administer bilateral striatal injection of either saline vehicle or mecamlamine (10 $\mu\text{g}/\text{side}$).

- 14 Place animals into the open field chamber and assess total running distance and average velocity within 20 minutes.



Verification of intrastriatal cannulae location in the dorsal striatum

- 15 Anesthetise mice with intraperitoneal injection of sterile Avertin (250 mg/kg body weight).
- 16 Perfuse animals transcardially with a saline solution followed by 4% paraformaldehyde (PFA) to clear blood and preserve brain for immunocytochemistry.
- 17 Dissect brains and fixed them overnight in 4% PFA.
- 18 Dehydrate samples using 30% sucrose solution for 24 hours.
- 19 Freeze brains and section it to 50 μm slices using a vibratome.
- 20 Follow **steps 2-4, 10-18** in **Protocol: Immunocytochemistry of acute brain slices used in ex vivo voltammetry recordings.**