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

In 1 collection

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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 administrating 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.

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 mecamylamine (10 µg/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 mecamylamine-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 (mecamylamine 42%-58%; control 45%-55%) were selected for subsequent conditioning.

Open Field Testing

13 Administer bilateral striatal injection of either saline vehicle or mecamylamine (10 µg/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.**