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Validating Responsiveness of AAV-DIO-hM3Dq-DREADD

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We use this protocol and it's working

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Abstract

To confirm responsiveness of AAV-DIO-hM3Dq-DREADD in DAT^{IRESCre} mice. Activation of DREADD receptors in dopamine neurons with CNO should induce a robust increase in locomotion.

Materials

- DAT^{IRESCre} mice bilaterally injected in the midbrain with AAV-DIO-hM3Dq-DREADD
- Med Associates wireless activity wheels (ENV-047)
- Med Associates Hub (DIG-807)
- Med Associates software (SOF-860)
- Clozapine-n-oxide (CNO; Tocris 4936-MTO)
- Sterile saline

Troubleshooting

Preparation

1 Mice should be at least two-weeks post-surgery.

2 Habituate mice to wireless running wheels in individually-housed cages.

2.1 See Victoria Vance, Katerina Rademacher, Ken Nakamura 2023. Behavior Tracking with Running Wheels. protocols.io https://protocols.io/view/behavior-tracking-with-running-wheels-cymrxu56

3 Prepare CNO for IP injection.

3.1 0.5mg/kg CNO dissolved in sterile saline, 500µL volume per mouse.

3.2 Prepared fresh the day of injection.

Procedure

4 Note: All injections should be performed at the same time of day, at least 2–3 hours before the dark cycle.

5 Day 1: IP inject mice with 500µL of sterile saline.

6 Day 2: IP inject mice with 500µL CNO.

7 Day 3 or 4: IP inject mice again with 500µL CNO.

8 Responders increase their running wheel activity in the 10-60min following IP injection of CNO. Exclude mice that do not respond to IP CNO from further analysis.