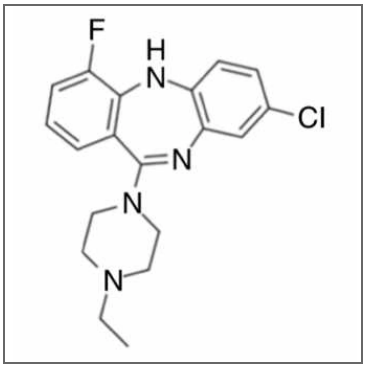

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Protocol status: Working
We use this protocol and it's working

Chemogenetic modulation of catecholaminergic neurons

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ABSTRACT

This protocol outlines a method for modulating noradrenergic and dopaminergic neuronal activity in the LC and SNc-VTA, respectively, using Cre-dependent hM3D and hM4D expression. It details stereotactic injection techniques for these chemogenetic tools and the preparation of the J60 agonist for intraperitoneal delivery. Developed for longitudinal studies spanning up to four weeks, the protocol aims to assess neuromelanin's activity-dependent accumulation through daily agonist administration.

MATERIALS

JHU37160 aka J60

Created: Jan 30, 2024

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PROTOCOL integer ID: 94402

Keywords: Chemogenetics, hM4Di, hM3D, DREADDs, long-term modulation

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Stereotactic injection

1 Stereotactic injection of viruses into DbH-Cre (JAX# 033953) or DAT-Cre (JAX# 006660) animals

Viral Construct:
pAAV-hSyn-DIO-hM4D(Gi)-mCherry (Addgene link)
Volume: 400ul
pAAV-hSyn-DIO-hM3D(Gq)-mCherry (Addgene link)
Volume: 400ul

(in our experiment we mix our DIO-hTyr Virus 1:1 with DREADDs)

Steroetactic coordinates:

- Subtantia nigra

ML: 1.4mm AP: 3.25mm DV: 4.0mm (blunt needle / NF34BL-2)

- Locus coeruleus

ML: 1.2mm AP: 5.2mm DV: 3.5mm (blunt needle / WPI NF34BL-2)
ML: 1.5mm AP: 5.2mm DV: 4.2mm (beveled needle / WPI NF34BV-2)

Injection set-up:

- Hamiliton syringe couple t oa WPI injector (UMP3T-1) (link)
- Injection speed 100nl/min

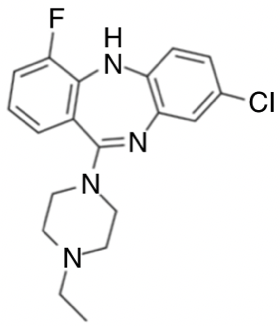
Expression

2 Assess accepted pain management protocol for injected animals, Start experiment as earlyas 3 weeks post-injections

Preparation agonist solution

3 **Preparation of agonist solution**

Several agonists are available for chemogenetic modulation with advantages and disadvantages, i.e., clozapine N-oxid (CNO), clozapine, or JP60. We are using J60 (JHU37160)
Please look at the reference for more detail (Bonaventura et al., 2019)



JHU37160

chemical structure JHU37160 aka J60 (Bonaventura et al., 2019)

- Solve entire vial (1mg) in 10ml saline (0.9% NaCl)
  (Compound can be ordered from Torcris (link))

  The solubility is very good, so by vortexing for a couple of seconds it should be already dissolved.

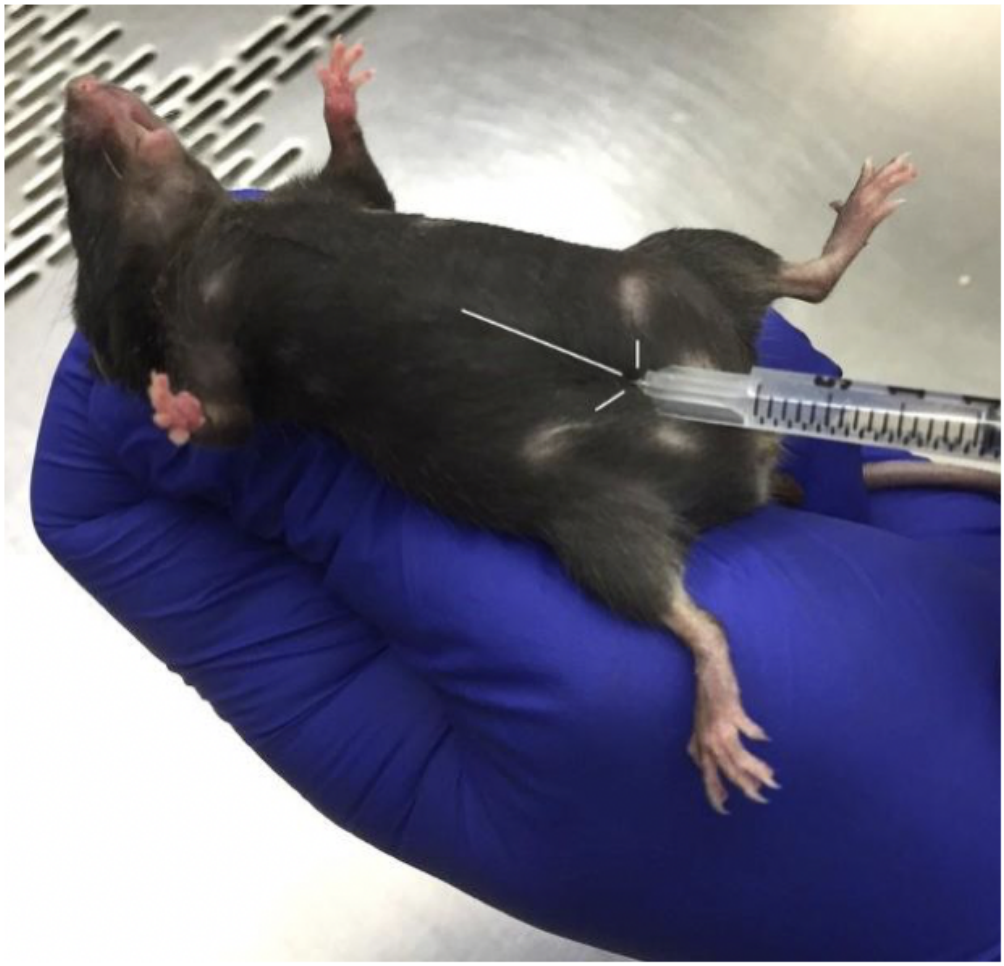
- Aliquot in a volume of 0.3ml and freeze at -20degree

DO NOT USE IT FOR MORE THAN 1 MONTH!

Modulation of Neuronal Activity

4

- Animals are injected at a dosage of 1mg/kg body weight. Therefore, each aliquot is the maximum for one animal. The injections are intraperitoneal, as shown in the picture. When administering repeated injections, use different locations in the belly to avoid the same injection site.



Intraperitoneal injection of mice. Please consult a local vet for more details.