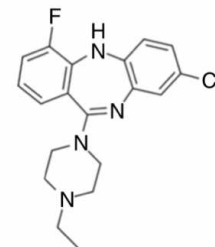


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Chemogenetic modulation of catecholaminergic neurons

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Cristian González-Cabrera¹, Csilla Novák¹, Maria P. Contreras¹, Ernesto Durán¹, Matthias Prigge¹

¹Neuromodulatory Network Group, Leibniz Institute for Neurobiology, Magdeburg

TeamPrigge



Team Prigge

Leibniz Institute for Neurobiology

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Protocol status: Working

We use this protocol and it's working

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Keywords: Chemogenetics, hM4Di, hM3D, DREADDs, long-term modulation, ASAPCRN, chemogenetic modulation of catecholaminergic neuron, catecholaminergic neuron, dopaminergic neuronal activity in the lc, neuromelanin, preparation of the j60 agonist, stereotactic injection techniques for these chemogenetic tool, chemogenetic modulation, dopaminergic neuronal activity, j60 agonist, dependent accumulation through daily agonist administration, neuronal activity, chemogenetic tool, stereotactic injection technique

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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



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)

Stereotactic coordinates:

- Substantia 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:

- Hamilton syringe coupled to a WPI injector (UMP3T-1) ([link](#))
- Injection speed 100nl/min

Expression

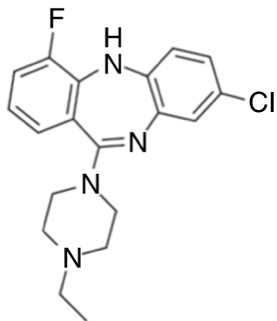
- 2 Assess accepted pain management protocol for injected animals, Start experiment as early as 3 weeks post-injections

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](#)))

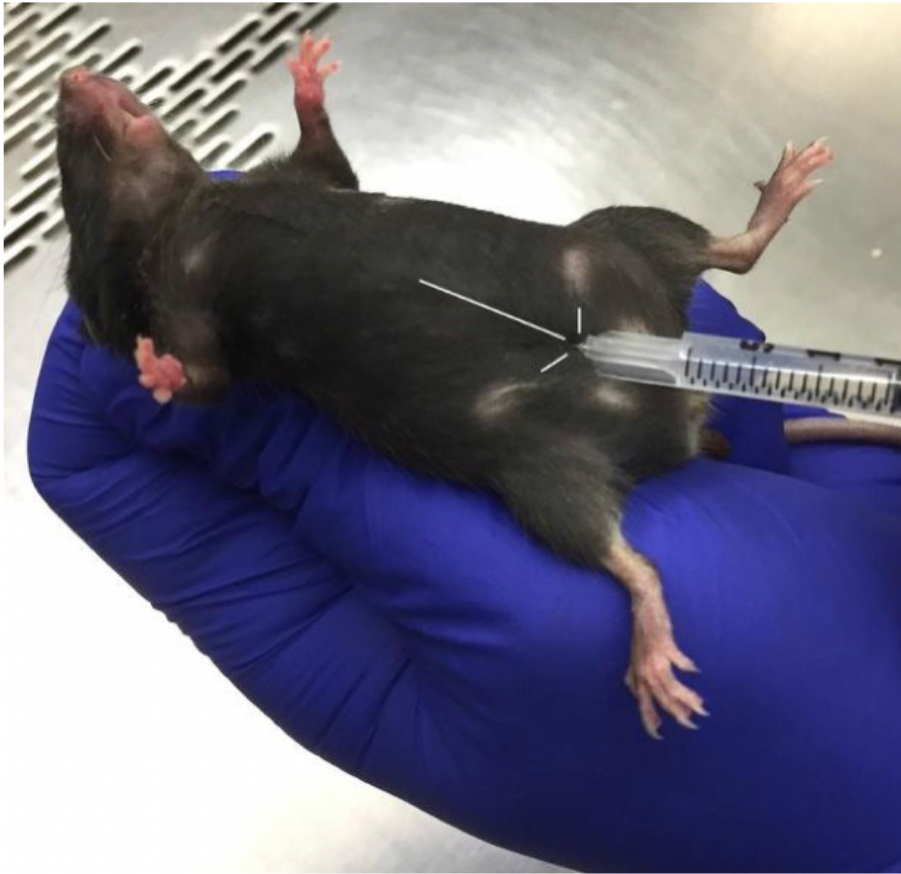
The solubility is very good, so by vortexing for a couple of seconds it should be already dissolved.

- 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.

Protocol references

Bonaventura J, Eldridge MAG, Hu F, Gomez JL, Sanchez-Soto M, Abramyan AM, Lam S, Boehm MA, Ruiz C, Farrell MR, Moreno A, Galal Faress IM, Andersen N, Lin JY, Moaddel R, Morris PJ, Shi L, Sibley DR, Mahler SV, Nabavi S, Pomper MG, Bonci A, Horti AG, Richmond BJ, Michaelides M. High-potency ligands for DREADD imaging and activation in rodents and monkeys. *Nat Commun.* 2019 Oct 11;10(1):4627. doi: 10.1038/s41467-019-12236-z. PMID: 31604917; PMCID: PMC6788984.