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MANUSCRIPT CITATION: Optical deep brain stimulation can reactivate NM-laden neurons and rescue behavioral phenotypes.

Optogenetic modulation of dopaminergic neurons

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ABSTRACT

This protocol describes an optogenetic activation of neuromelanine-laden dopaminergic neurons in the SNc-VTA. We induce neuromelanin (NM) in the SNc-VTA through viral expression of a humane tyrosinase virus. Furhtermore we express a red-absorbing opsin in one hemispherend and blue absorbing in the other hemisphere. We detail how we optognetic stimulateanimals in over four weeks.

THis optogenetic deep brain stimulation induces a reduction in neuromelanine levels in dopaminergic neurons, and rescues behavioral phenotypes as evaluate in the grip strength test.

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

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Viral Injection

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

Viral Constructs:
1.- pAAV-hSyn1-dlox-ChrimsonR_tdTomato(rev)-dlox-WPRE

2.- pAAV-hSyn1-dlox-hCHR2(H134R)_mcherry(rev)-dlox-WPRE

3.- pAAV_Ef1a_DIO_hTyrHA_minBack

Injections:
- Bilateral injections were performed.
- Right hemisphere: viruses 1 and 3.
- Left hemisphere: viruses 2 and 3.
- Viruses were combined 50:50 and injected a total Volume of 600ul per hemisphere

Steroetactic coordinates:
- Subtantia nigra

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

Injection set-up:
- Hamiliton syringe couple t oa WPI injector (UMP3T-1) (link)

- Injection speed 100nl/min

Experimental Timeline

2 - Animals were left to accumulate neuromelanin for 6 or 10 weeks.
- Animals were stimulated for 30 minutes for 4 weeks. Stimulation during 5 days and two days rest.
- The stimulation was 635nm, 5ms pulse, 10 Hz at at 10mW. 3 seconds ON 10 seconds OFF.

Optical stimulation

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- fibers are attached bilaterally to the animals
- the animal is placed in open field arena
- stimulation is controlled via PulsePal (OpenEphys link) on a MRL_III-635L laser