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Optogenetic Silencing of Projection Terminals

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

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Abstract

This protocol describes the combination of protocols and techniques that are employed to optogenetically silence projections from various areas in the brain.

Safety warnings

Wear appropriate PPE as required by your institution.

Ethics statement

Prior ethics approval (e.g. IACUC) should be obtained before performing these experiments. Approval was obtained by the Stanford University IACUC before any procedures were performed.

Before start

Read all protocols and linked protocols beforehand to ensure understanding when combining protocols together.

Intracranial Virus Injection & Optic Fiber Implantation

1 Follow steps 1-11 in protocol for viral injection of desired virus before implanting optic fiber: **dx.doi.org/10.17504/protocols.io.8epv5r514g1b/v1**

2 Follow protocol starting on step 7 for optic fiber implant: **dx.doi.org/10.17504/protocols.io.n2bvj9yrwlk5/v1**
NOTE-viral injection and optic fiber implantation occur in the same surgery.
A head plate is be placed if a mouse is planned to be used in a head-fixed behavior (lever-pushing task).

2.1 Wait 2 weeks for recovery before any behavior and recording experiments.

3 These experiments are to test behaviors when SNr or DCN projections are silenced in different behavior paradigms. A mouse will only perform one behavior (e.g. either lever-pushing task or rotarod task).

STEP CASE

Optogenetic Silencing & Lever-Pushing Task 6 steps

4 Follow procedure in protocol **dx.doi.org/10.17504/protocols.io.eq2ly63pwgx9/v1** for task habituation and training process.

5 Mice have two optogenetic silencing sessions.
At the start of each new session/day: a baseline is collected for 2-3 minutes without inhibition.

6 Please refer to protocol dx.doi.org/10.17504/protocols.io.eq2ly63pwgx9/v1 for details on trial structure.

7 To initiate the silencing using the inhibitory opsin eOPN3, a laser is turned on for 3 minutes with 500 ms pulses at 0.1 Hz and a power of 10mW from the fiber tip.

8 The silencing period is quantified starting at 1 minute after optogenetic laser onset as 'inhibition period'.
The 2 minute window starting 1 minute after optogenetic laser was turned off is the 'post-inhibition washout period'.

9 Mice are trained for another 9-10 minutes following inhibition.