May 10, 2025

Stereotaxic injections of viral vectors and chronic optical fiber implants in mouse brains

Nature Communications

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvwe6edvmk/v1

Safa Bouabid[1,2], Mark Howe[1,2]

[1]Department of Psychological & Brain Sciences, Boston University, Boston, MA, USA;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA

Cláudia C. Mendes
University of Oxford

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwe6edvmk/v1

Protocol Citation: Safa Bouabid, Mark Howe 2025. Stereotaxic injections of viral vectors and chronic optical fiber implants in mouse brains. **protocols.io https://dx.doi.org/10.17504/protocols.io.e6nvwe6edvmk/v1**

Manuscript citation:
Bouabid *et al.*, (2025) Distinct spatially organized striatum-wide acetylcholine dynamics for the learning and extinction of Pavlovian associations.

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

Created: March 27, 2025

Last Modified: May 10, 2025

Protocol Integer ID: 125214

Keywords: chronic optical fiber implants in mouse brain, chronic optical fiber implantation in the mouse striatum, stereotaxic viral injection, surgical procedures for stereotaxic viral injection, stereotaxic injections of viral vector, stereotaxic injection, chronic optical fiber implantation, chronic optical fiber implant, acetylcholine release with tetanus toxin, suppressing acetylcholine release, mouse brain, mouse striatum, monitoring acetylcholine, mouse, dopamine, tetanus toxin, dynamics during pavlovian learning

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-020370

Abstract

This protocol outlines the surgical procedures for stereotaxic viral injections and chronic optical fiber implantation in the mouse striatum for monitoring acetylcholine, dopamine, and glutamate dynamics during Pavlovian learning and extinction using genetically encoded sensors, as well as for suppressing acetylcholine release with tetanus toxin.

Guidelines

Post-surgical care and recovery procedures ensure animal well-being before experimental data collection.

Materials

Equipment:

- Pulled glass pipette (tip diameter 30-50 µm)

Viral Vectors:

- AAV9-hSyn-ACh3.0 (WZ Biosciences, Jing et al., 2020), 2.07×10 13 GC ml -1 diluted 1:2 in PBS
- AAV9-hysn-ACh3.0-mut (WZ Biosciences, Jing et al., 2020), 2.54×10 13 GC ml -1 diluted in 1:2 PBS
- AAV5-CAG-dlight1.3b (Addgene, # 111067, Patriarchi et al., 2018), 1.7x x10 13 GC ml -1 diluted 1:3 in PBS
- pAAV2/8-hSyn-FLEX-TeLC-P2A-EYFP-WPRE (Addgene, #135391, 5.14×10 13 GC ml -1, Zhang et al., 2015) diluted 1:1 in PBS
- ssAAV-5/2-hSyn1-dlox-TeTxLC_2A_NLS_dTomato(rev)-dlox-WRPE-hGHp (Viral Vector Facility University of Zurich, 4.1×10 12 VG ml -1) diluted 1:1 in PBS
- AAV9.hSyn-FLEX.8F-iGluSnFR.A184S (Adgene, #106174, Marvin et al., 2018), 1.8 ×10 13 GC ml -1 diluted 1:1 in PBS

Troubleshooting

Anaesthetic Induction & Surgical Preparation

1 Anesthetise mice with isoflurane (3-4%) and place it in a stereotaxic frame (Kopf instruments) on an electric heating pad (Physitemp instruments).

2 Administer buprenorphine extended release for pre-operative analgesia (3.25 mg kg-1 subcutaneous, Ethiqa XR).

3 Following induction, maintain isoflurane at 1-2% (in 0.8-1 L min -1 pure oxygen) and body temperature at 37°C throughout the surgical procedure.

Injection of Viral Vectors

4 **Monitoring extracellular acetylcholine (ACh) release:**

4.1 Using a pulled glass pipette, pressure-inject the genetically-encoded fluorescent acethycholine (ACh) sensor GRAB-ACh3.0 (AAV9-hSyn-ACh3.0; **see Materials**) into the striatum of wild-type mice at 20-40 separate striatum locations.

> Note
>
> The striatum locations were chosen to maximize expression around fiber tips (200 nL at each location at a rate of 100 nL/min).

4.2 For control experiments, inject mutant version of the genetically-encoded fluorescent acethycholine (ACh) sensor (AAV9-hysn-ACh3.0-mut; **see Materials**) into the striatum of wild-type mice using the same strategy.

5 **Monitoring extracellular dopamine (DA) release:**

5.1 Inject genetically encoded dopamine sensor dLight1.3b (AAV5-CAG-dlight1.3b; **see Materials**) into the striatum of wild-type mice at 10-40 total locations (200-800nl at each location) using the same procedure as above.

6 **Suppressing acetylcholine (ACh) release from cholinergic interneurons with tetanus toxin light chain (TelC):**

6.1 Drill circular craniotomies bilaterally above the injection sites (from bregma, in mm; AP: 1, ML: ± 1.4).

6.2 Inject bilaterally Cre-dependent Tetanus toxin light chain (TelC) viral vectors (pAAV2/8-hSyn-FLEX-TeLC-P2A-EYFP-WPRE or ssAAV-5/2-hSyn1-dloxTeTxLC_2A_NLS_dTomato(rev)-dlox-WRPE-hGHp; **see Materials**) in the anterior dorsal medial striatum (aDS) of ChAT-Cre mice at 4-12 sites per hemisphere (300nl/site at a rate of 100nl/min) at the following coordinates in mm; AP: 0.8, ML: ± 1.25, DV: -2.5 and -3; AP:1, ML: ±1.4, DV:-2.75 and -3.

6.3 Control ChAT-Cre mice were injected with saline using the same strategy as above.

6.4 Seal craniotomies with Kwik-Sil (WPI), and seal the skull with Metabond (Parkell) and a metal head plate.

7 **Monitoring extracellular glutamate release from cholinergic interneurons:**

7.1 Drill craniotomies above the injection sites in the right hemisphere (from bregma, in mm; AP: 1, ML: 1.4).

7.2 Inject genetically encoded glutamate sensor (iGluSnFR) in aDS of ChAT-Cre mice at 6 sites (300nl/site at a rate of 100nl/min) at the following coordinates in mm: AP: 0.8, ML: 1.5, DV: -2.75, -3.25 and -3.75; AP: 1.1, ML: 1.5, DV: -2.75, -3.25 and -3.75.

7.3 Attach a 100 µm core diameter optical fiber (MFC_100/125- 0.37NA) to a zirconia ferrule (Doric).

7.4 Slowly lowered the diameter optical fiber into the medial region of the aDS (AP:1, ML:1.4) to a final depth of 3 mm from bregma.

7.5 Seal craniotomies with Kwik-Sil (WPI), and secure the optical fiber and a head plate to the skull with Metabond (Parkell).

Implantation of Multi-Fiber Arrays

8 Mount the multi-fiber array onto the stereotaxic manipulator.

9 Remove dura gently, and slowly lowered the multi-fiber array into position.

10 Seal craniotomy with a thin layer of Kwik-Sil (WPI), and secure the multi-fiber array to the skull surface using Metabond (Parkell).

Head-Fixation

11 Secure a metal head plate and ring (Atlas Tool and Die Works) to the skull with Metabond, and cover the implant surface with a mixture of Metabond and carbon powder (Sigma Aldrich) to reduce optical artefacts.

12 Protect the fiber bundle by a cylindrical plastic tube, extending ~ 1-2 mm above the fiber bundle, and secure around the bundle using a mixture of Metabond and carbon powder.

Post-Operative Recovery

13 Place each mouse in an individual cage with a heating pad and perform post-operative injections of meloxicam (5 mg kg -1 subcutaneous, Covertus) and 1 mL of saline per day subcutaneously for 4 days after surgery.

14 Allow them to recover in their cages for at least 2 weeks after surgery.

Protocol references

Jing, M., Li, Y., Zeng, J., Huang, P., Skirzewski, M., Kljakic, O., Peng, W., Qian, T., Tan, K., Zou, J., et al. (2020). **An optimized acetylcholine sensor for monitoring in vivo cholinergic activity.** Nat Methods 17 , 1139–1146.

Patriarchi, T., Cho, J.R., Merten, K., Howe, M.W., Marley, A., Xiong, W.-H., Folk, R.W., Broussard, G.J., Liang, R., Jang, M.J., et al. (2018). **Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors.**Science 360 , eaat4422.

Zhang, Y., Zhao, S., Rodriguez, E., Takatoh, J., Han, B.-X., Zhou, X., and Wang, F. (2015). **Identifying local and descending inputs for primary sensory neurons.** J Clin Invest 125 , 3782–3794.

Marvin, J.S., Scholl, B., Wilson, D.E., Podgorski, K., Kazemipour, A., Müller, J.A., Schoch, S., Quiroz, F.J.U., Rebola, N., Bao, H., et al. (2018). **Stability, affinity, and chromatic variants of the glutamate sensor iGluSnFR.** Nat Methods 15 , 936–939.