May 09, 2025

Micro-CT scanning for post-implant localization of multi-fiber arrays in mouse striatum

Forked from Micro-CT scanning and fiber localization

Nature Communications

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.36wgq6955lk5/v1

Mai-Anh Vu[1,2], Safa Bouabid[1,2], Mark Howe[1,2]

[1]Department of Psychological and Brain Sciences, Boston University, Boston, MA, United States;
[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.36wgq6955lk5/v1

Protocol Citation: Mai-Anh Vu, Safa Bouabid, Mark Howe 2025. Micro-CT scanning for post-implant localization of multi-fiber arrays in mouse striatum. **protocols.io https://dx.doi.org/10.17504/protocols.io.36wgq6955lk5/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 25, 2025

Last Modified: May 09, 2025

Protocol Integer ID: 125042

Keywords: Micro-CT, fiber localization step, moving mice, mouse striatum

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

Abstract

We have developed a new micro-fiber array approach capable of chronically measuring and optogenetically manipulating local dynamics across over 100 targeted locations simultaneously in head-fixed and freely moving mice, enabling investigation of cell-type and neurotransmitter-specific signals over arbitrary 3-D volumes . This protocol includes the micro-CT scanning and fiber localization steps.

Please contact us (mwhowe@bu.edu) if you are interested in using this technique.

Materials

SOLUTIONS:

Preparing Lugol solution

- dilute 10mL 100% Lugol's Solution (Carolina, 10% potassium iodide, 5% iodine) with 30mL deionized water

Note: This dilution was chosen to be approximately isotonic to biological tissues.

Troubleshooting

Before start

Multi-fiber arrays were fabricated and calibrated following the **Protocol: Fabrication and calibration of multi-fiber arrays to monitor dopamine release in the mouse striatum**.

PFA Fixation by Intraperitoneal Perfusion & Brain Dissection

1 Inject mice intraperitoneally with 400-500 mg/kg Euthasol (Covetrus Euthanasia III).

2 Perfused transcardially with 20mL 1% phosphate buffered saline (PBS, Fisher), followed by 20mL 4% paraformaldehyde in 1% PBS.

3 After perfusion and decapitation, the lower jaw and front of the skull were removed in order to allow diffusion of solution into the brain while still keeping the implant intact.

Preparation for Micro-CT Scanning

4 Soak brain in 4% paraformaldehyde solution for 24h, rinse three times with 1% PBS, and then transfer to a diluted Lugol's solution (**see Materials**) to provide tissue contrast for computerized tomography (CT) scanning (**Metscher, B., 2009**).

Note

Initially, samples were soaked in this diluted Lugol's solution in a foil-wrapped 50mL conical centrifuge tube on an orbital shaker plate for 10-14 days. We have more recently found that using 4 oz specimen cups instead of the 50mL conical centrifuge tubes enables better diffusion of the Lugol's solution, and adequate contrast can be achieved in three to four days.

5 After soaking, rinse skulls three times with 1% PBS, and secure them in a modified centrifuge tube.

Micro-CT Scanning

6 Image the implanted skulls in a micro-CT scanner (Zeiss Xradia Versa 520, a core instrument of the Boston University Micro-CT and X-ray Microscopy Imaging Facility) with the following parameters:

- 140kV
- 10W
- HE1 filter
- 0.4X objective
- 2s exposure time
- 1001 projections
- 12-micron voxel size

Micro-CT Registration and Multi-Fiber Localization

7 Register the micro-CT to the Allen Mouse Brain Common Coordinate Framework 3D 10-micron reference atlas (**Wang et al., 2020**) to bring individual mice into a common coordinate system.

8 Identify and map fibers from the recording tip up to the grid.

This process was carried out using FIJI (**https://imagej.net/software/fiji/**) and MATLAB (Mathworks, version 2020b) using a combination of existing MATLAB functions and custom-written functions and GUIs publicly available on **GitHub**.

Protocol references

Metscher, B.D. (2009). **MicroCT for developmental biology: A versatile tool for high-contrast 3D imaging at histological resolutions.** Dev. Dyn. *238*, 632–640. 10.1002/dvdy.21857.

Wang, Q., Ding, S.-L., Li, Y., Royall, J., Feng, D., Lesnar, P., Graddis, N., Naeemi, M., Facer, B., Ho, A., *et al.* (2020). **The Allen Mouse Brain Common Coordinate Framework: A 3D Reference Atlas.** Cell *181*, 936-953.e20. 10.1016/j.cell.2020.04.007.