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Micro-CT scanning and fiber localization

In 1 collection

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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.

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Perfusion and dissection

1 Mice were injected intraperitoneally with 400-500 mg/kg Euthasol (Covetrus Euthanasia III), and then perfused transcardially with 20mL 1% phosphate buffered saline (PBS, Fisher), followed by 20mL 4% paraformaldehyde in 1% PBS.

2 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 CT scanning

3 The brain was soaked in the 4% paraformaldehyde solution for 24h, rinsed three times with 1% PBS, and then transferred to a diluted Lugol's solution, to provide tissue contrast for computerized tomography (CT) scanning (Metscher, 2009).

3.1 The Lugol solution was prepared by diluting 10mL 100% Lugol's Solution (Carolina, 10% potassium iodide, 5% iodine) with 30mL deionized water, a dilution chosen to be approximately isotonic to biological tissues61.

3.2 Note on soak time: 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.

4 After soaking, the skulls were rinsed three times with 1% PBS, and secured in a modified centrifuge tube.

Micro-CT scanning

5 The implanted skulls were imaged 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.

CT registration and fiber localization

6 The CT was then registered 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, and then fibers were identified and mapped from the recording tip up to the grid. This process was carried out using a combination of FIJI (https://imagej.net/software/fiji/) and MATLAB (Mathworks, version 2020b) using a combination of existing MATLAB functions and custom
lab-written functions and GUIs. This pipeline is now publicly available at
https://github.com/HoweLab/MultifiberLocalization.