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Immunohistology

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Mai-Anh Vu[1], mwhowe[1]

[1]Department of Psychological and Brain Sciences, Boston University, Boston, MA, United States

Team Cragg

Mai-Anh Vu

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 immunohistology steps we used to confirm viral expression, and/or stain for neurons after our micro-CT scanning and contrast agent soaking procedure.

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Removing Lugol's contrast agent from tissue

1 In preparation for immunohistology, Lugol's solution was removed from brain tissue after CT imaging by soaking the implanted brains in a solution of 5% sodium thiosulfate (STS, Carolina) in 1% PBS for 4-6 days (Hopkins et al., 2015).

2 The implant was then removed, the brain returned to the STS solution for one hour.

Slicing

3 The brains were then moved to a solution of 30-40% sucrose in PBS.

4 Once the brains sank, coronal sections (50 µm) were sliced with a cryostat (Leica CM3050 S) and transferred to PBS for storage.

Immunostaining

5 To stain for dLight1.3b or ChR2 expression:

5.1 Sections were then blocked and treated in a 5% normal goat serum (NGS) and 0.2% PBS triton (Sigma Aldrich) solution (PBST).

5.2 Treated sections were then incubated for 24-48 hours at 4°C in 5% NGS, 0.2% triton, and a GFP primary antibody (chicken polyclonal antibody; 1:1000, ThermoFisher Scientific, No. A10262).

5.3 Slices were then washed, and incubated for 2 hours at room temperature in 5% NGS, 0.2% PBST, and an Alexa 488 secondary antibody (goat anti-chicken; 1:200, ThermoFisher Scientific, No.A-11039).

6 For neuronal staining:

6.1 Sections were then blocked and treated in a 5% bovine serum albumin (BSA) and 0.2% PBS triton (Sigma Aldrich) solution (PBST).

6.2 Treated sections were then incubated for 24-48 hours at 4°C in 5% BSA, 0.2% triton, and a NeuN primary antibody (guinea pig antibody; 1:500, Synaptic Systems, 266 004).

6.3 Slices were then washed, and incubated for 2 hours at room temperature in 5% BSA, 0.2% PBST, and an Alexa 647 secondary antibody (goat anti-guinea pig; 1:200, ThermoFisher Scientific, No. A-21450).

Mounting and imaging

7 Finally, stained sections were mounted onto glass slides using Vectashield Mounting Medium with DAPI (Vector Laboratories, H-2000).

8 Confocal images were acquired with a Zeiss LSM 800.