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Immunocytochemistry of acute brain slices used in *ex vivo* voltammetry recordings

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We use this protocol and it's working

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Abstract

The steps detailed in this protocol are to label choline acetyltransferase (ChAT) or tyrosine hydroxylase (TH), which are known markers for cholinergic interneurons (ChI) and dopaminergic neurons and striatal axons (DA), respectively. We use an immunofluorescent approach in 300-µm thick slices of mouse brain tissue after performing *ex vivo* voltammetry recordings.

Materials

Equipment:

- **MS 3.1 digital shaker** (IKA, SKU #0003319000)
- Gilson pipette (SKU #**FA10005M**; SKU #**FA10003M**; SKU #**FA10006M**)
- **Pastuer pipette** (VWR, SKU #612-1681)
- **Corning® Costar® TC-Treated Multiple Well Plates** (SKU #CLS3513-50EA)
- **Eppendorf™ Polypropylene Graduated Microtubes** (SKU #10509691)
- **Wet Set Quick Dry Topcoat**

Reagents:

- Picric Acid
- **Phosophate buffered saline** (Sigma-Aldrich, SKU #P4417)
- Phosphate buffer: **Sodium phosphate dibasic dihydrate** (0.2M) (Sigma-Aldrich, SKU #71643, CAS #10028-24-7) & **Sodium phosphate monobasic monohydrate** (0.2M) (Sigma-Aldrich, SKU #S9638, CAS #10049-21-5)
- **Triton-X 100** (Sigma-Aldrich, X100, CAS #9036-19-5)
- **Vectashield** (Vector Labs, SKU #H-1900-10)
- **Goat anti-ChAT primary antibody** (Millipore, SKU #AB114P, RRID #AB_2313845)
- Goat anti-ChAT primary antibody (AMCA, Jackson Immuno Research Europe)
- Rabbit anti-TH primary antibody (Sigma-Aldrich)
- Alexa Fluor 568 Donkey anti-Goat antibody (Invitrogen)
- **Donkey Anti-Goat IgG H&L (DyLight® 594)** (Abcam, SKU #ab96937, RRID #AB_10680873)
- CoraLite488 Goat anti-Rabbit antibody (Proteintech)

Troubleshooting

Before start

We first performed voltammetry recordings in *ex vivo* mouse brain slices following the steps detailed in **Protocol: Fast-scan cyclic voltammetry to assess dopamine release in ex vivo mouse brain slices**.

Fixation

1 Fix slices in 4% paraformaldehyde (PFA) dissolved in PBS containing 0.2% picric acid.

2 Leave to fixate overnight at 4°C and then store in PBS.

3 Wash x5 in PBS for 5 min.

Pre-incubation

4 Prepare preincubation solution containing PBS with 10% Normal Donkey Serum and 0.5% Triton-X during the last wash.

5 Leave to preincubate at room temperature for 1 h on an orbital shaker.

Labelling Cholinergic Interneurons (ChAT)

6 **This section details steps to label cholinergic interneurons (ChI) expressing channelrhodopsin-2 (ChR2) fused in-frame with eYFP.**

7 **Primary Antibody:**

7.1 Prepare 3% Normal Donkey Serum and 0.5% Triton-X during the preincubation period.

7.2 Add primary antibody goat anti-ChAT (Millipore, 1:100) or goat anti-ChAT (1:200, AMCA) to the above mix.

7.3 Once done, remove all the preincubation solution and add the Primary Antibody Solution.

7.4 Leave to incubate overnight (goat anti-ChAT from Millipore) or for five days (goat anti-ChAT from AMCA) at 4º C on an orbital shaker.

8 Wash x5 with PBS for 5 min.

9 **Secondary Antibody:**

9.1 Prepare PBS containing 0.5% Triton X-100 and 3% Normal Donkey Serum.

9.2 Add secondary antibody Alexa Fluor 568 donkey anti-goat (1:1000) to the above mix.

9.3 Once done, remove all the PBS from the last wash and add the Secondary Antibody Solution.

9.4 Leave to incubate for 2h at room temperature on an orbital shaker.

Labelling Dopaminergic neurons and striatal axons (TH)

10 **This section details steps to label dopaminergic neurons and striatal axons (DA) expressing (i) channelrhodopsin-2 (ChR2) fused in-frame with eYFP; (ii) genetically encoded calcium indicator GCaMP6f; (iii) voltage sensor ASAP3 without a soma-targeting signal; or (iv) optogenetic actuator Chrimson.**

11 **Primary Antibody:**

11.1 Prepare 1% Normal Goat Serum, 1% Fetal Bovine Serum and 0.5% Triton-X during the preincubation period.

11.2 Add primary antibody rabbit anti-TH (1:2000) to the above mix in the desired concentration.

11.3 Leave to incubate overnight at 4º C on an orbital shaker.

12 Wash x5 with PBS for 5 min.

13 **Secondary Antibody:**

14 Prepare PBS containing0.5% Triton X-100, 1% Normal Goat Serum and 1% Foetal Bovine Serum.

14.1 Add secondary antibody DyLight 594 Goat anti-Rabbit (1:1000) or CoraLite488 Goat anti-Rabbit (1:1000) antibody to the above mix.

14.2 Once done, remove all the PBS from the last wash and add the Secondary Antibody Solution.

14.3 Leave to incubate for 2h at room temperature on an orbital shaker.

Mounting

15 Wash x5 with PBS for 5 min.

16 Mount the individual slices on gelled slides with Vectashield mounting medium (Vector Labs).

Confocal Imaging Acquisition

17 Acquire images using a 20×/0.75 NA objective in Zeiss LSM880 confocal microscope system running ZEN black version 2.3 (Zeiss), or a confocal microscope system (FV1000 IX81; Olympus) and Fluoview software (Olympus).

18 Maximum intensity projection from a z-stack of height 30 µm was captured individually and the stack of the pictures were compressed.

Note

Red fluorescence (TH and ChAT) was captured at 638–759 nm with 633 nm excitation. **Green fluorescence** (GCaMP, ChR2, and ASAP3) was captured at 493–630 nm with 488 nm excitation.