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Anatomical Verification (Mendonça et al 2024)

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Marcelo D Mendonça^{1,2,3}

¹Champalimaud Research, Champalimaud Foundation, Lisbon 1400-038, Portugal;

²Champalimaud Clinical Centre, Champalimaud Foundation, Lisbon 1400-038, Portugal;

³NOVA Medical School | Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisbon 1169-056, Portugal



Christine Weber-Schmidt

Allen Institute

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for anatomical verification (sample prep, immunostaining and imaging) in Mendonça et al 2024.

Materials

Standard perfusion setup

Vibrotome

Before start

Animals were euthanized after completion of the behavioural tests.

Sample Preparation

- 1 Anaesthetize mice with isoflurane, followed by intraperitoneal injection of ketamine-xylazine (5 mg/kg xylazine; 100 mg/kg ketamine).
- 2 Perfuse transcardially in 1% phosphate buffered saline (PBS) and then 4% paraformaldehyde (PFA) in PBS.
- 3 Dissect brains after perfusion is completed.
- 4 Keep brains in 4% PFA solution overnight and then transferred to 1x PBS solution.
- 5 Section brains coronally in 30 to 50-um slices (using a Leica vibratome VT1000S) and keep in PBS before mounting or immunostaining.

Immunostaining for TH (Day 1)

- 6 Rinse 3 times with TBS (10 min each)
- 7 Peroxides treatment (inhibition peroxidase and permeabilization) **5 min**
 - 1.5 mL methanol
 - 450 μ L H_2O_2
 - 13.05 mL TBS(this step is for DAB staining but we kept it because it favors the penetrance of the Ab)
- 8 Rinse 3 times with TBS (10 min each)
- 9 Membrane permeabilization treatment **15min** (or 20min for Ab that don't penetrate well)
 - 300 μ L Triton 10%
 - 14.7 mL TBS
- 10 Rinse 3 times with TBS (10 min each)
- 11 Blockade with BSA 3% or other blocking solutions **1h** (if needed)



- 12 Primary Antibody (diluted in TBS): **overnight at 4°C** (or longer period if needed)
TH (1:1000) Pel-Freez, Rogers, AR

Immunostaining for TH & Mounting (Day 2)

- 13 Rinse 3 times with TBS (10 min each)
- 14 Secondary Antibody (diluted in TBS without) **40-45 min Room Temperature** (1:400)
- 15 Rinse 2 times with TBS (10 min each) AND Rinse 2 times with TB (10 min each)
- 16 Mounting with **Vectashield or PVA DABCO**

Imaging

- 17 Images were taken using a wide-field fluorescence microscope (Zeiss Axiolmager).