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Immunofluorescence on acute hippocampal slices following electrophysiology

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We use this protocol and it's working

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Abstract

This protocol describes protein detection using immunofluorescence on hippocampal slices obtained from adult mice following electrophysiology recordings. Here, we detail the fixation, cryopreservation, slicing and staining methods to produce reliable detection of fluorescently labeled proteins. It is intended to enable spatial quantification of proteins (e.g. glutamate receptors) following electrophysiology recordings.

Materials

Reagents:
Phosphate-buffered saline (PBS)
Paraformaldehyde 4% (in PBS)
Triton-X
Horse serum (or other blocking agent)
Sucrose
Optimal cutting temperature compound (OCT)
Dry ice
Mountant (e.g. ProLong™ Glass Antifade, ThermoFischer Scientific P36980)

Equipment:
Cryostat, chucks and blades
Microscope slides
Culture dishes
Pasteur pipettes
Cryomolds
Parafilm
Tissue spatula
Tweezers
PAP pen
Coverslips
Clear nail polish

Troubleshooting

Safety warnings

If you choose to use this protocol, you do so at your own risk and must ensure that any local guidance is adhered to.

Ethics statement

Experiments involving animals must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s). Animal care and experimental procedures were reviewed and approved by the Animal Ethics and Compliance Program at the University of Toronto and conducted in accordance with the Canadian Council on Animal Care (CACC) guidelines.

Fixation

1w 0d 2h 15m

1 Immediately after electrophysiology recording is complete, transfer hippocampal slice from recording solution to 2-3 mL fixative solution (4% PFA in PBS) in a small culture dish using a pasteur pipette. Leave on rocker at Room temperature for 02:00:00

2h

2 Wash 3 x 00:05:00 with 1 X PBS

15m

3 Replace PBS with 30% sucrose solution (w/v in PBS) and seal culture dish with parafilm

4 Store at 4 °C for 1-2 weeks

1w

Cryo-embedding

5m

5 Transfer slice to a cryomold, placing it flat at the bottom using a spatula. Include orientation markers on outside of mold

6 Using a rolled kimwipe, soak up any excess sucrose solution without directly touching the slice

7 Pour Optimal Cutting Temperature compound (OCT) overtop of slice and fill the mold, being careful not to displace the slice. Remove any bubbles in the OCT carefully using a pair of tweezers or spatula

8 Embed cryomold in crushed dry ice to flash freeze

5m

9 When frozen, wrap cryomold in parafilm and/or a small plastic bag, label and store at -80 °C until processing

Cryo-sectioning

2h

10 Place sample and tools in cryostat for 00:30:00 to acclimatize to -22 °C 30m

11 Pour OCT on specimen chuck and allow it to freeze. When nearly frozen, place a weight on top to flatten surface of OCT

12 Remove OCT-embedded sample from cryomold. Place a small amount of OCT on flat surface of chuck and immediately place sample flat on top, with the tissue facing upwards.

13 Tighten the chuck in the chuck holder, orienting the tissue with the longest side facing the blade

14 Adjust the chuck holder knobs such that the OCT is flush with the blade before slicing

15 Slice 20 µm sections, ensuring that the blade remains flush with the sample (not on an angle). Adjust as needed before proceeding.

16 Collect each slice from under the anti roll plate and orient it appropriately. Pick slice up swiftly with a Room temperature microscope slide

17 Collect 16-20 slices across 4-5 microscope slides and allow slides to dry for 01:00:00 at Room temperature 1h

18 Store slides at -20 °C until staining *

Immunostaining - Primary Ab Incubation 2h 30m

19 Allow slides to acclimatize to Room temperature for 01:00:00 1h

20 Use a hydrophobic barrier PAP pen to outline sections on microscope slide and allow to dry 5m

21 Gently wash slides 5 x 00:05:00 with 0.1% Triton-X in PBS (PBS-T) using a pasteur pipette (~ 250 µL /slide) 25m

22 Incubate in blocking solution (10% horse serum in PBS-T) for 01:00:00 at Room temperature 1h

23 Incubate in primary antibody cocktail (typically 1:1000 dilution) in blocking solution overnight at 4 °C

Immunostaining - Secondary Ab Incubation 4h 20m

24 Wash slides 5 x 00:05:00 with PBS-T 25m

25 Incubate in secondary antibody cocktail (typically 1:500 dilution) in blocking solution for 02:00:00 at Room temperature protected from light 2h

26 Wash slides 5 x 00:05:00 with PBS-T, keeping slides protected from light in between washes 25m

27 Incubate in DAPI (1:500 in PBS) for 00:10:00 at Room temperature protected from light 10m

28 Wash slides 2 x 00:05:00 in PBS, keeping slides protected from light in between washes 10m

29 Let slides dry 00:10:00 on an angle protected from light 10m

30 Mount slides with an appropriate mounting medium and coverslip

31 Wait 00:30:00 for mountant to solidify 30m

32 Seal edges of coverslip with nail polish to prevent from drying out and allow to dry 30m

33 Store in fridge until imaging

Imaging

34 Clean slides with ethanol

35 Image slices using a confocal microscope. When possible, image and quantify slices that have visible electrode marks to identify the recording plane within the slice to assess differences between electrophysiological conditions