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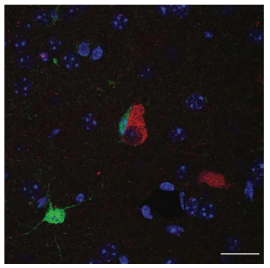
Imaging of cholinergic interneurons in post-mortem rodent tissue to identify striatal satellite astrocytes V.2



In 1 collection

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We use this protocol and it's working

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Abstract

This protocol details confocal image acquisition steps and the analysis pipeline used to calculate inter-soma distance between cholinergic interneurons/pMSNs and their nearest astrocyte. The partner **Protocol: Immunofluorescent Labelling of Post-Mortem Rodent Brain Tissue** describes how to label cholinergic interneurons/pMSNs along with striatal astrocytes in PFA perfusion-fixed, 50-µm thick, post-mortem rodent brain tissue.

Materials

Equipment:

- **ZEISS LSM 980 with Airyscan 2**

Software:

- **Zen Blue** (Zeiss, version 3.9)
- **FIJI** (version 1.54f)

Other:

- Slide with ~50 µm thick brain section with fluorescent labels.

Before start

The tissue used in the steps below were obtained after processing as described in **Protocol: Immunofluorescent Labelling of Post-Mortem Rodent Brain Tissue**.

Confocal Imaging Acquisition

1 Turn on the Leica LSM980 confocal microscope and Zen Blue imaging acquisition software.

2 Secure a glass slide containing one or more fluorescently-labelled brain section into the stage of the microscope. Take care at this step to ensure the coverslip does not break or dislodge.

3 Using a 10x objective, identify anatomical landmarks using the DAPI or ChAT/NeuN signal in the region of interest. This would include the corpus callosum for the dorsal striatum, and the anterior commissure for the ventral striatum.

Note

The DAPI signal would be visualised with a 358 nm laser. The ChAT/NeuN signal can both be visualised with a 568 nm or similar wavelength laser as appropriate for the secondary fluorescent antibody used.

4 Switch to a 63x/1.4 NA (oil immersion) objective and identify randomly selected ChAT/NeuN positive somata within the region of interest. Center the objective over this particular neuron.

Note

Only include neurons for which the full soma resides within the z-axis of the tissue (*e.g.,* no cut neurons).

Take care to identify somata while blinded to the S100β (green) channel, to prevent bias to selecting ChAT/NeuN cells that have an astrocyte (as identified by a positive S100β signal) close to them.

5 Image the identified cell across the different channels using the following lasers:

- ChAT/NeuN - 568 nm wavelength laser
- S100β - 488 nm wavelength laser
- DAPI - 358 nm wavelength laser

The laser wavelength can also be altered as appropriate for the secondary antibody that is used.

Note

The settings used were a 30 µm thick z-stack (512 x 512 pixels) at 63x magnification with 1x digital zoom, 1 µm optical thickness/optical plane, and a step size of 2 µm. The z-stack centre was set to z-coordinate where the soma is largest (*e.g.* centre of the neuron).

6 Save the images with the appropriate details of the sample and imaging settings. A .czi format is recommended but other formats could also work.

7 Move to the next cell in the region of interest. Repeat image acquisition across the desired number of cells. Sample an equal number of cells from both hemispheres.

Image Analysis

8 Open the images in FIJI (ImageJ) and convert to a maximum projection across the z axis.

9 While remaining blind to the S100β (green) channel to prevent bias on astrocyte location, draw a region of interest (ROI) around the cell in the centre of the image with the polygon selection tool. This would either be the ChAT/NeuN positive cell, depending on the sample.

10 Open the S100β (green) channel. Draw a ROI around the nearest astrocyte by identifying a signal in the S100β (green) channel (>5 µm in diameter) featuring a DAPI-positive nucleus residing closest to centre of the image.

11 From the ROIs, measure the surface area of the cells and the x and y coordinates of the centre point to calculate the intersoma distance.

12 Using the x and y coordinates obtained earlier, calculate the Euclidean distance between the centre point of the ChAT/NeuN cell and their nearest astrocyte.

13 Repeat analysis steps outlined above for all imaged cells.