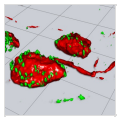

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Confocal microscopy and characterization of synaptic boutons associated with ganglion neurons

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.bakdics6

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ABSTRACT

This protocol describes confocal microscopy and image analysis procedures for characterizing neuronal cell bodies and their associated synaptic boutons in thick (50 µm) cryosections. The protocol has been applied to rat pelvic ganglia, where neuronal cell bodies have been identified using immunohistochemical markers of specific neuron populations and/or fluorescent retrograde tracer.

Confocal microscopy

1 To optimize visualization of synaptic boutons, image using a 60x 1.4 NA oil immersion objective with 3x digital zoom. Multi-channel images were collected by sequential imaging of channels, with four scans averaged per image.

- If a particular channel is susceptible to photobleaching (e.g., retrograde tracers such as FluoroGold), image this channel first.
- For these studies, a Zeiss LSM880 was used in Fast Airyscan mode.

LSM880 with Airyscan
Confocal microscope
Zeiss LSM880

2 Using Zen Black (Zeiss software), batch process Airyscan output to 16-bit 1260 x 1260 pixel images.

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Image analysis

3 Airyscan processed images were converted to Imaris (.ims) files for analysis using the Surface Rendering module.

Imaris
source by Bitplane

4 For the channel showing the neuronal cell body of interest, create a mask by first creating the neuronal surface and processing in a defined volume. Use parameters tailored to your specific immunolabeling signal requirements. Examples of parameters for manual adjustment include background subtraction, split touching objects and threshold.

5 Delete all other objects from this channel, select a surface of the neuron and merge all surfaces to create an appropriate mask of the neuron.

For studies of pelvic ganglion cell bodies, surface detail is set to 0.2 µm.

6 For the channel showing synaptic boutons, create mask and surface of the boutons as above.

For studies of synaptic boutons associated with pelvic ganglion, surface detail is set to 0.05 µm.

7 To analyse the distance between the neuronal cell body surface and the synaptic boutons, apply the Distance Transformation extension. This calculates the closest distance between two surfaces, and the surface area and volume of all objects with rendered surfaces.

8 Export data separately from each channel (neuronal cell body, boutons) to Excel.

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