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Intracellular neuromelanin quantification

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Miquel Vila¹

¹Vall d'Hebron Research Institute

Vilalab Public



Miquel Vila

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for quantifying intracellular neuromelanin in coronal sections of the rodent brain, in HE stained sections (NM-occupied area) and unstained sections (NM OD).

H&E-stained sections (NM-occupied neuronal area)

- 1 Scan H&E sections using the Panoramic Midi II FL, HQ SCIENTIFIC 60x and section images were acquired with CaseViewer software at an objective magnification of 63x.
- 2 Acquire SNpc images at 63x with CaseViewer.
- 3 Upload individual images at Image J software.
- 4 Click on Adjust canvas size and adjust it at 1596×1198.
- 5 Click on Invert image.
- 6 With the free hand selections tool, draw a neuromelanin-pigmented neuron cytoplasm (excluding the nucleus) and measure the area.
- 7 With the free hand selections tool, draw the neuromelanin pigment of the neuron and measure the area.
- 8 Calculate the percentage of the neuronal area occupied by the pigment using the formula: neuromelanin pigment area/neuronal cytoplasm area.

Unstained sections (NM optical density)

- 9 For unstained sections, take pictures of different fields in the pigmented area using the Zeiss Imager.D1microscope coupled to an AxioCamMRc camera.
- 10 Upload individual images at Image J software.
- 11 Click on Invert image.
- 12 With the free hand selections tool, draw the intraneuronal neuromelanin pigment of a neuron and measure the optical density. Measure approximately 30-50 neurons per



animal, depending on the brain region.

- 13 Calculate the optical density of the neuromelanin pigment for each animal using the mean values of the pigmented neurons in the unstained sections.