INTRODUCING "FLUORESCENCE NEUROSTEREOLOGY": NOVEL METHODS FOR MAPPING THE BRAIN

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Key words. neuroscience, stereology, optical sectioning, immunofluorescence.

Aims. The aim was to combine unbiased cell quantification and immunofluorescence detection methods to quantify the number of tyrosine hydroxylase immunoreactive neurons in a midbrain nucleus.

Background. Commonly used methods of stereological estimation [1] of cell numbers limit analyses to two cell markers per tissue section [2]. We introduce here a robust and reproducible 3D stereological method using multiple fluorescence probes that makes it possible to quantify and discriminate between multiple cell types in the same specimen.

Methods. Perfusion-fixed rat brain was embedded in polyethylene glycol and serially sectioned at 35µm through the substantia nigra (SN) and ventral tegmental area (VTA) (Figure 1). A Zeiss Z1 microscope combined with an ApoTome was used for optical sectioning, and Zeiss Axiovision software for image analysis. Specimens were processed for single or multiple labelling immunofluorescence and all cell nuclei were counterstained with a fluorescent DNA marker. Sections were imaged and analysed using a systematic random-sampling strategy for unbiased stereological quantification of cell numbers [3], modified for 3D reconstructed images.

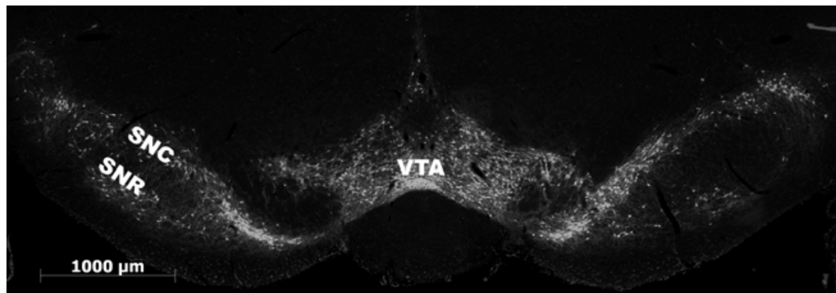

Figure 1: Midbrain nuclei displaying TH immunoreactivity.

Results:

Dopaminergic neurons in SN and VTA were identified with an antibody to the enzyme tyrosine hydroxylase and the nuclear stain DAPI. Using a semi-automated 3D sampling procedure, a total of 24,614 dopaminergic neurons was calculated in this region, a number consistent with previous methods using standard stereological methods. Double immunofluorescence labelling with the calcium binding protein, calbindin, allows analysis of neuronal subpopulations in this brain region.

Summary & Conclusions: Polyethylene glycol embedding combined with immunofluorescence and automated 3D wide-field microscopy greatly facilitates discrimination of cell types within the brain. When combined with automated sampling and 3D reconstruction this greatly enhances the power of stereological analysis to quantify multiple cell types in the same tissue sections.

Reference:

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