Automated quantification of dopaminergic immunostained neurons in substantia nigra using freely available software

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Resumen

Computerized techniques for image analysis are critical for progress in cell biology. The complexity of the data in current methods eliminates the need for manual image analysis and usually requires the application of multiple algorithms sequentially to the images. Our aim was to develop a software for immunohistochemical analysis of brain dopaminergic neurons combining several computational approaches to automatically analyze and quantify their number in the substantia nigra after a neurotoxic injury. For this purpose, we used a Parkinson's disease animal model to test our application. The dopaminergic neurotoxin, 6-hydroxydopamine, was administered in adult male rats to damage dopaminergic neurons in substantia nigra and to induce hemiparkinsonism. The lesion was corroborated by behavioral evaluation in response to apomorphine and amphetamine. The animals were euthanized and their brains processed for tyrosine hydroxylase immunohistochemistry for dopamine neuron

identification. Neurons positive for tyrosine hydroxylase were evaluated in substantia nigra by light microscopy. The images were used to show quantification applicability. To test our software counting accuracy and validity, automatic dopamine neuron number was correlated with the data obtained by three independent observers. Several parameters were used to depict neuronal function in dataset images from control and lesioned brains. In conclusion, we could perform an automated quantification of dopaminergic neurons and corroborate the validity and accuracy of a freely available software.

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