Implementation of Neural Networks for studies of brain pathology in Parkinson´s disease

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ABSTRACT

- Unbiased estimation of neuron numbers within substantia nigra are a crucial part of neurobiological studies and important for experimental Parkinson's disease models
- Current techniques based on optical fractionation are extremely laborious and time-consuming
- Deep learning with context-specific convolutional neural networks (CNN) provide a powerful approach for automated and high-throughput tissue analytics
- We developed and trained a first-in-class CNN algorithm for the counting of tyrosine hydroxylase positive (TH+) neurons and Lewy bodies in substantia nigra. Results were compared to published TH+ neuron counts acquired using StereoInvestigator (Runeberg-Roos et al., 2016; Kumar et al., 2016)
- The newly developed analysis provides robust, fast (5 min per section), and reliable analysis of total number of dopamine neurons in the substantia nigra

WORKFLOW

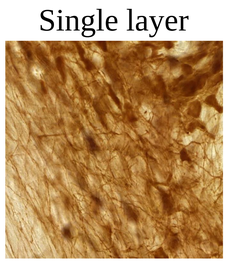
1. Immunohistochemistry

TH and pSer129 immunohistochemistry (IHC) was carried out in free-floating rat and mouse tissue sections (Penttinen et al., 2016).

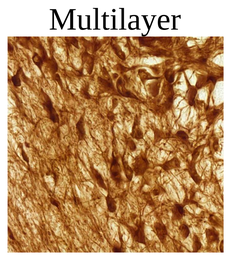
2. Whole-slide scanning

After IHC, rat brain sections were digitized using Pannoramic P250 Flash II whole-slide scanner (3DHistech, Hungary) at 0.22 µm/pixel resolution. Scan was performed using extended focus mode which renders the whole section depth in a single focal plane. A total depth of 10 µm was acquired as five focal layers with 2 µm intervals.

Single layer



Multilayer



3.

Images were uploaded to an image processing and management platform (Aiforia®, Fimmic Oy, Finland).

4. Deep Neural Network

For automatic image analysis of the samples, we used artificial intelligence on a commercially available Aiforia® Create platform platform (Fimmic Oy). The Aiforia® Create platform utilizes deep, context-intelligent convolutional neural network (CNN) algorithms for computer vision applications, i.e. analysis and classification of image features. We used a combination of deep CNNs and supervised learning to detect TH+ neurons in the whole-slide digital images of rat and mouse brain sections. The CNN algorithm was trained to detect TH+ neurons with 528 MB of image data. The trained algorithm was validated against manual counting using StereoInvestigator or direct counting from digital images in Aiforia®. Furthermore, another algorithm was trained to detect Lewy bodies.

5. Manual neuron counting

A total of 26 ROIs were selected for CNN validation in the substantia nigra of the rat brain. ROIs were selected to represent variation in terms of staining intensity and neuron body density and were excluded in the training data. TH+ neuron bodies were counted manually in the validation ROIs independently by two observers on the digital images in Aiforia®. Observers did not count the same ROIs and they were blind for the algorithm result in the validation ROIs.

CONCLUSIONS

- **The fully cloud-based Aiforia® Create platform provides automatic, fast and reproducible analysis of neuron counts in SN with minimal hands-on time**
- **The CNN algorithm shows equal performance as compared to StereoInvestigator in quantification of neurons in TH-stained SN**
- **The Aiforia® Create platform can be used for quantifying pathological markers or features from cells, such as Lewy bodies**

Kumar, A., Kopra, J., Varendi, K., Porokuokka, L.L., Panhelainen, A., Kuure, S., Marshall, P., Karalija, N., Harma, M.A., Vilenius, C., Lillevali, K., Tekko, T., Mijatovic, J., Pulkkinen, N., Jakobson, M., Jakobson, M., Ola, R., Palm, E., Lindahl, M., Stromberg, I., Voikar, V., Piepponen, T.P., Saarma, M. & Andressoo, J.O. (2015) GDNF Overexpression from the Native Locus Reveals its Role in the Nigrostriatal Dopaminergic System Function. PLoS Genet, 11, e1005710.

Penttinen, A.M., Suleymanova, I., Albert, K., Anttila, J., Voutilainen, M.H. & Airavaara, M. (2016) Characterization of a new low-dose 6-hydroxydopamine model of Parkinson's disease in rat. J Neurosci Res, 94, 318-328.

Runeberg-Roos, P., Piccinini, E., Penttinen, A.M., Matlik, K., Heikkinen, H., Kuure, S., Bespalov, M.M., Peranen, J., Garea-Rodriguez, E., Fuchs, E., Airavaara, M., Kalkkinen, N., Penn, R. & Saarma, M. (2016) Developing therapeutically more efficient Neurturin variants for treatment of Parkinson's disease. Neurobiol Dis, 96, 335-345.

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RESULTS

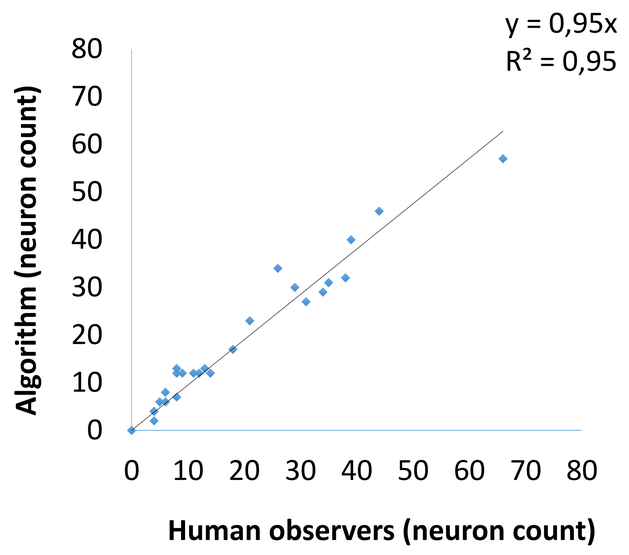
1. CNN algorithm provides reliable counting of TH-stained neurons in rat substantia nigra validated against human observers

Metrics
TP = True positive
FP = False positive
FN = False negative
TN = True negative

Metrics
Precision = TP/(TP+FP)
Recall = TP/(TP+FN)
F1-score = 2*Precision*Recall/(Precision+Recall)

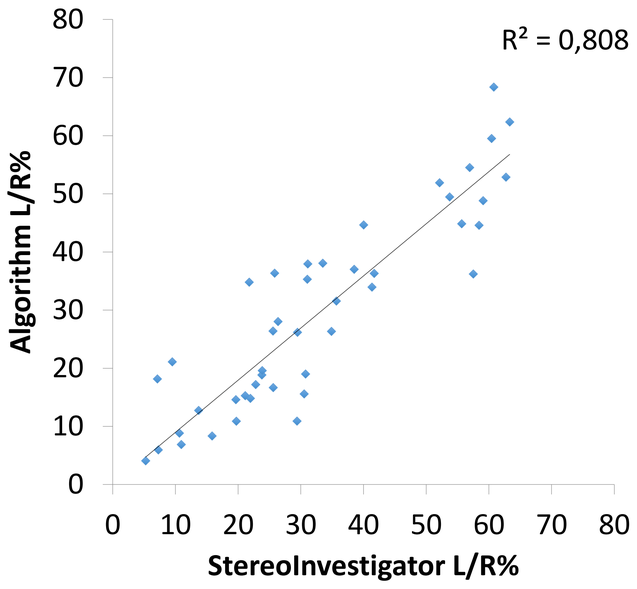
Total neuron body counts in all ROIs:	
Algorithm	489
Human observers	493
Pearson correlation	0.98

	Score (95% CI)
Precision	88.5% (85.5–91.4%)
Recall	87.8 % (84.9–90.7%)
F1-score	88.2% (85.3–91.0%)

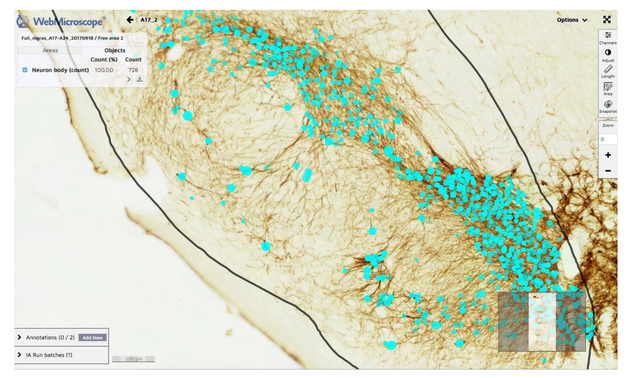


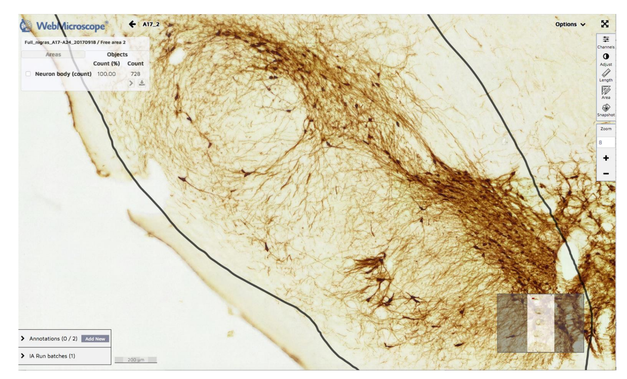
2. Aiforia® Create platform yields neuron counts comparable to StereoInvestigator in rat samples

Comparison of 44 rat substantia nigra (Left/Right ratios)	
Pearson correlation	0.90

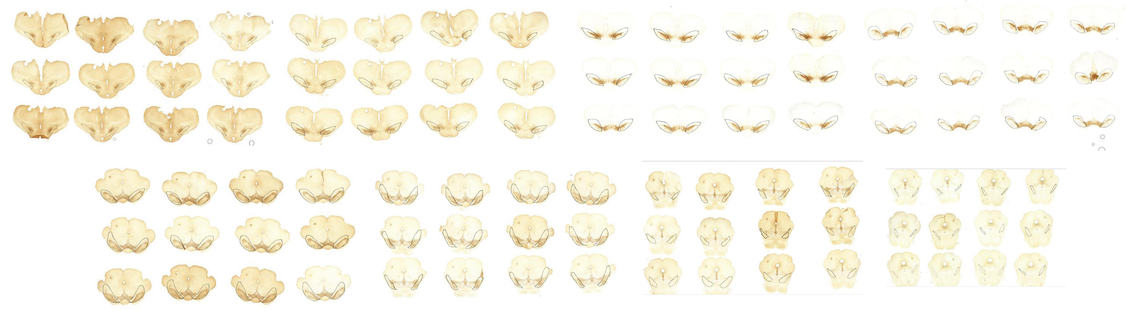


Aiforia® Create platform allows robust analysis with an easy visual validation of results. In this example, the CNN algorithm detected 728 neurons whereas only 17 neurons were detected in StereoInvestigator analysis (incorrect by visual validation).

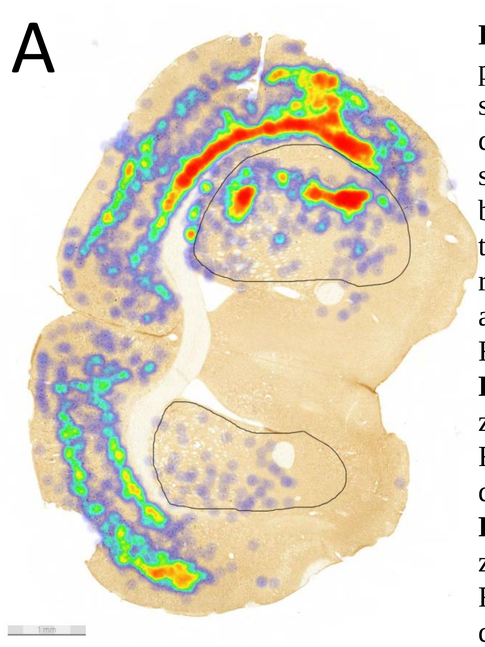


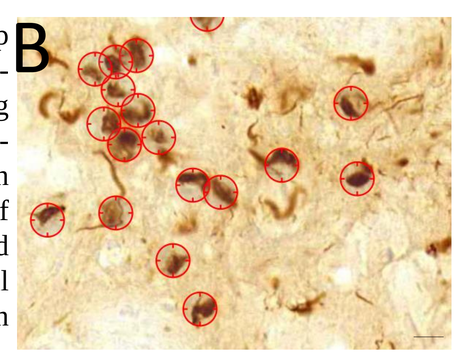


3. Analysis of a whole TH-stained substantia nigra of a rat (96 sections) shows a total of 29 689 TH+ cells. The full analysis was completed in 3 hours.



4. CNN algorithm is able to recognize Lewy bodies. Mouse brain sections stained with pSer129 (marker for phosphorylated α-synuclein, the major component of Lewy bodies).





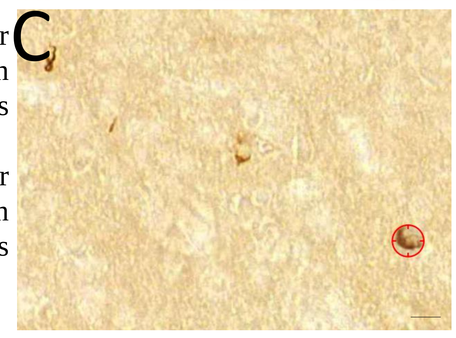


Figure A. A heatmap presentation of the whole-section analysis showing differential expression of α-synuclein positive neuron bodies in the two sides of the brain. The marked regions indicate striatal areas that are analyzed in Fig B and Fig C.

Figure B. High power zoom of the upper side in Fig A. 702 neuron bodies detected in the upper area.

Figure C. High power zoom of the lower side in Fig A. 55 neuron bodies detected in the lower area.

Figs B,C Scale bar: 10 µm