



Microglial Networks Construction & Analysis

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Background

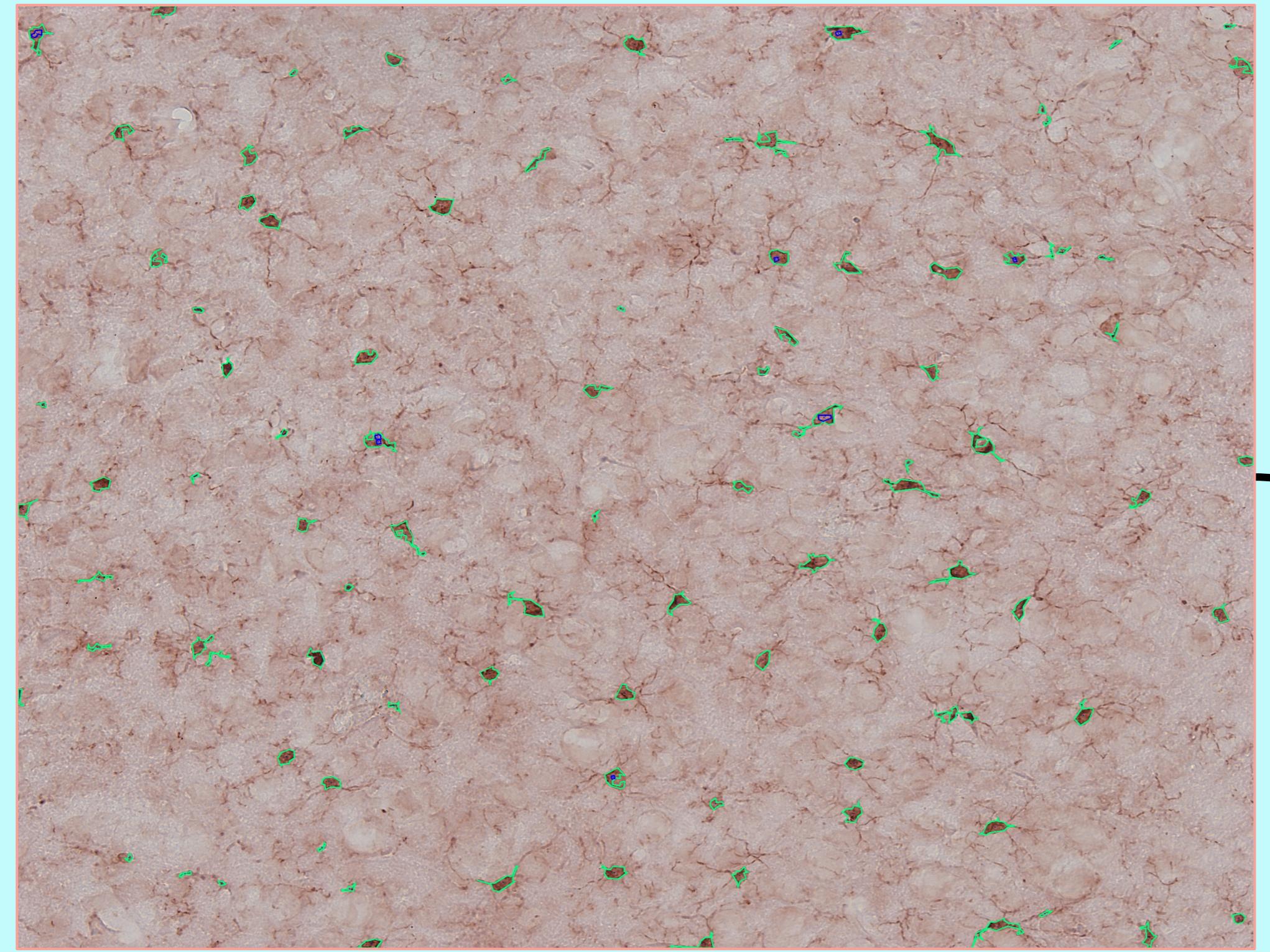
Alzheimer's disease (AD) is an irreversible neurodegenerative disorder, characterised by several neuropathological hallmarks including amyloid- β deposits, neurofibrillary tangles formation and neuroinflammation. Activated microglial cells and infiltrating leukocytes in the brain are considered key neuroinflammation processes in AD.

In this preliminary work, we studied microglia images from transgenic mice with AD-like disease (3xTg-AD), which is an animal model that develops both amyloid and tau pathologies. Particularly, we focused on microglial networks reconstruction in 3xTg-AD mice. The resulting networks were compared to microglial networks reconstructed from healthy wild-type (WT) animals.

Methodology

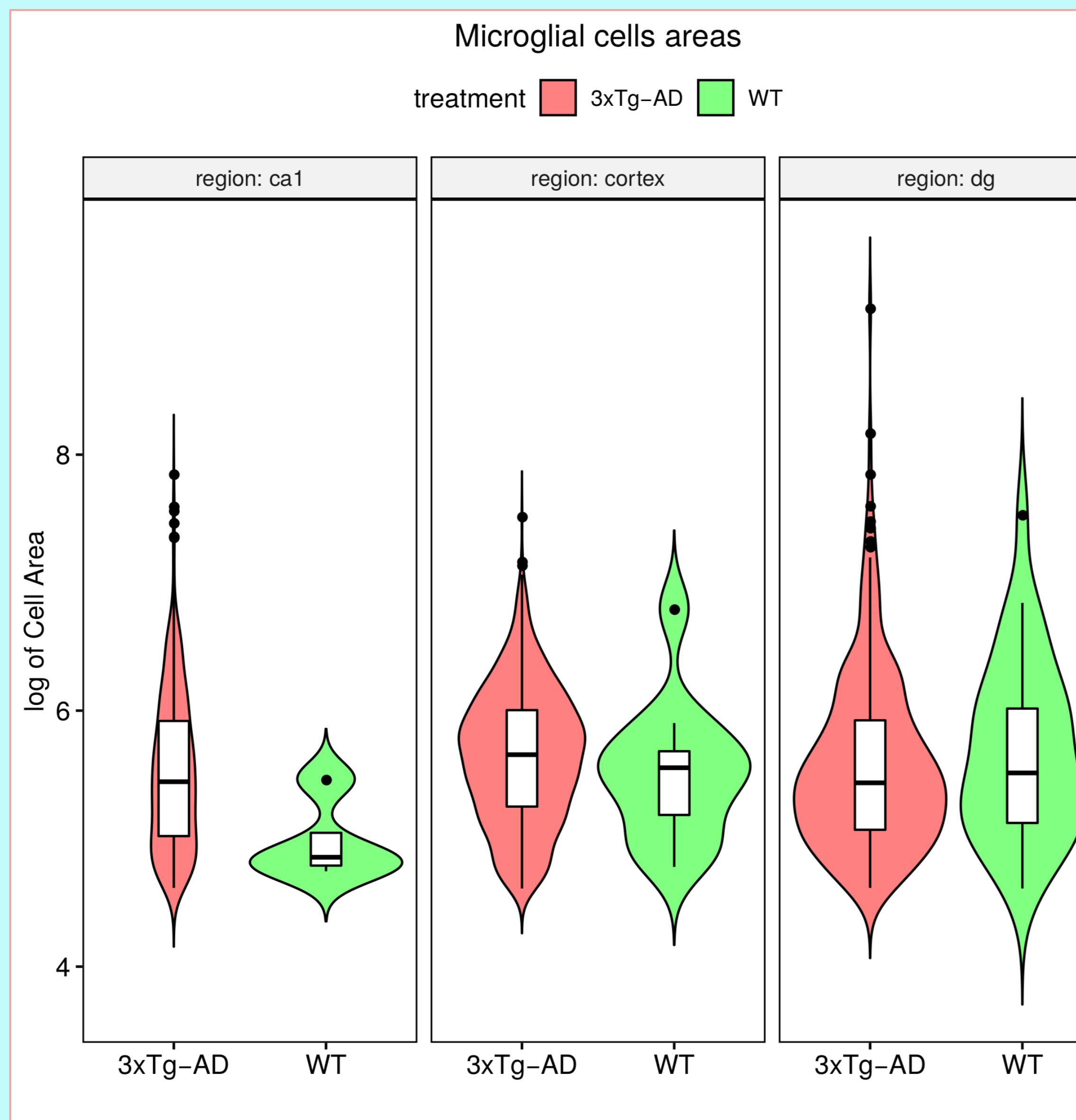
Stained microglial cells in coronal brain sections of three cerebral regions, i.e. cortex, dentate gyrus (DG) and CA1 area of the hippocampus, were analysed. The image analysis step, performed using a programming language (Python) and a very well known image analysis library (OpenCV), allowed us to detect cell bodies and to obtain spatial and morphological information describing position and area of each microglial cell in all the analysed slides.

Spatial information, i.e. X and Y coordinates, were used to reconstruct a mono-dimensional model using a network-based approach, for each image we analysed. To this aim, nodes and edges represent detected cells and their distances, respectively. Each node was integrated with morphological information which, currently, only describe the area of the corresponding detected cell. Eventually, we used Cohen's D estimate to evaluate the effect size and *t*-test to evaluate the significance of our findings. The network reconstruction and the data analysis were performed using the R statistical language.



Representative immunohistochemistry image analysed using an in-house algorithm written in Python that takes advantage of OpenCV image analysis library. The contours of each detected microglial cell are coloured in green, whereas in blue are coloured the areas under the threshold that fall into a cell body.

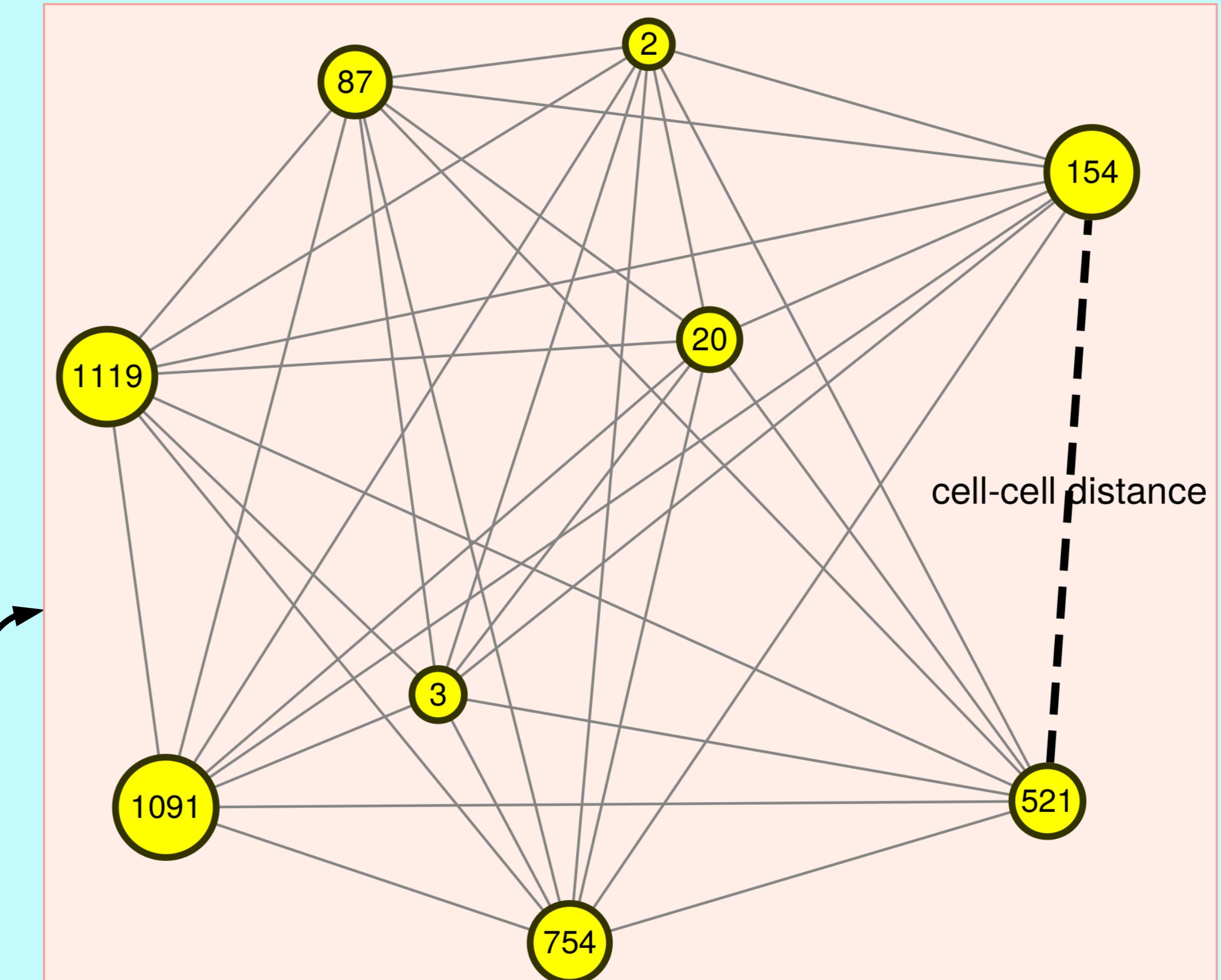
Cell detection was performed through the use of thresholds. Our algorithm transformed the images from RGB to black and white according to the threshold and, eventually, found the objects which satisfied the imposed constraints, such as the minimum and maximum area.



Workflow & Results

cell	X	Y	area
20	21	1695	579
744	897	330	331.5
154	275	1451	394.5
521	1236	1041	595
1091	466	358	351
1119	118	333	401.5

Cell spatial and morphological information were retrieved by Python algorithm used for contour detection. Coordinates were then used to build the microglial networks. This network construction step was performed by using the R statistical language.



In our model, nodes represent cells while edges do not represent cell-cell physical interaction but describe the distance in the bidimensional, i.e. (x, y), space, between two microglia cells.

Discussion

Our preliminary results showed that the average cell-cell distance in microglial networks from WT control animals was significantly higher for both CA1 and cortex, when compared to the average distance of 3xTg-AD transgenic mice. In both cases the results were substantial since the effect size had a very high coefficient and the *t*-test scored a very low p-value (as shown in the tables). Furthermore, the average area of WT networks was substantially smaller with respect to the average area of 3xTg-AD microglial networks, for CA1. On the contrary, the results we obtained in the other analysed regions, i.e. DG and cortex, according to both *t*-test and Cohen's D, were not statistically significant (lightblue rows in the tables). Since the methodology rely on images, their quality is a crucial aspect. In fact, to detect cell contours, it is necessary to set a threshold that should work for all the images. Hence, the lack of homogeneity in colours, presence of artifacts or technical inaccuracies may strongly affect the model reconstruction and the subsequent data analysis.

Conclusion

The methodology we propose is still under development. However, the cell-cell distance parameter was highly informative reflecting the inflammation status in the 3xTg-AD brains. To add a further layer of information, we are planning to compute several other parameters that will better characterise each network and each type of animal model. Since our model is network-based, we are working to extract common metrics, already established in network analysis. Finally, we are also working to increase the number of brain sections and animals for this type of analysis and extend the methodology to other imaging studies we are currently performing in our lab.

Acknowledgements

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