



# Robustness of microglial networks in Alzheimer's disease

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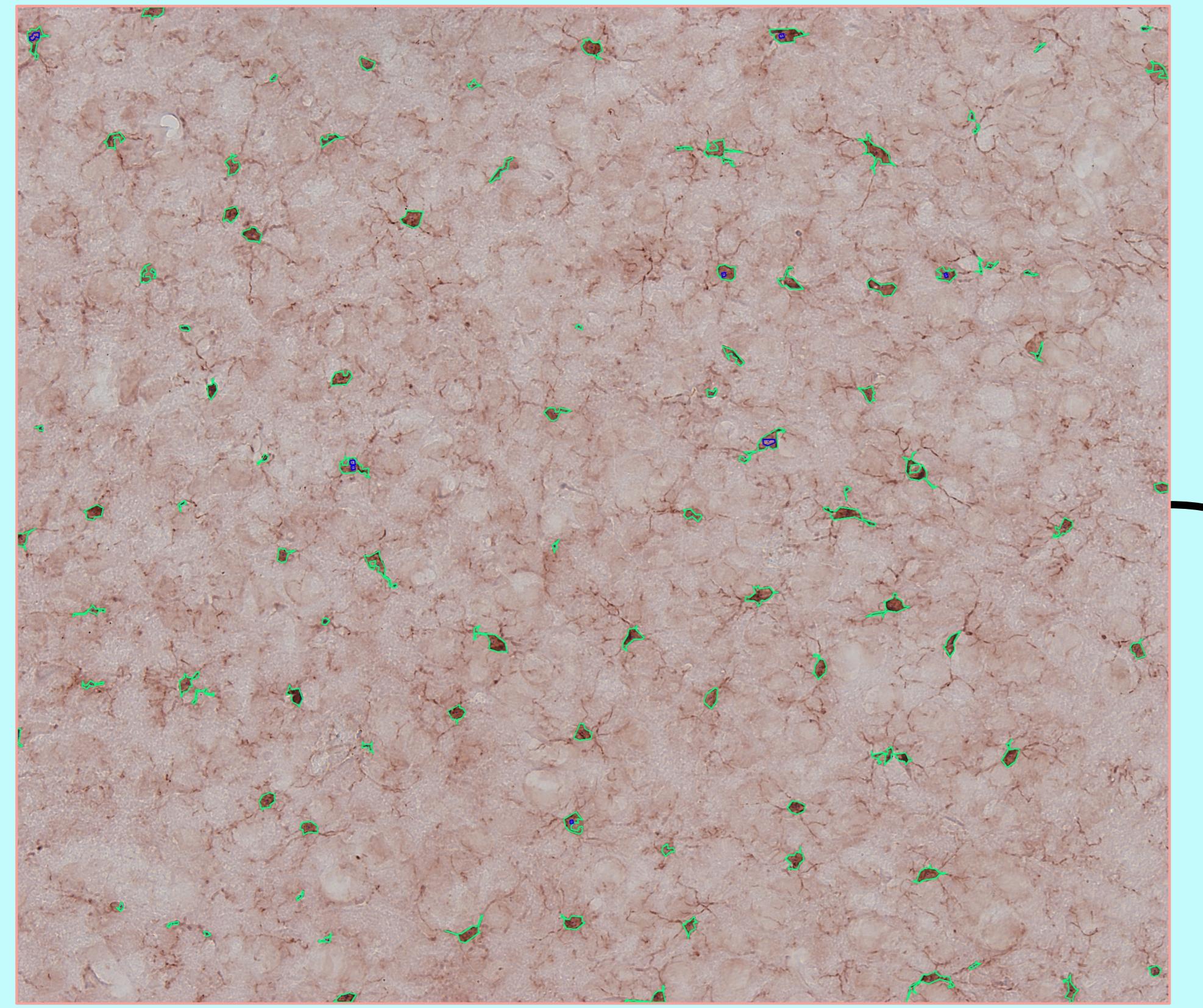
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## Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by neuronal loss, neurofibrillary tangles formation, gliosis, and amyloid- $\beta$  accumulation. Chronic neuroinflammation is thought to play a key role in AD pathology and several studies indicated that microglia activation promotes neurodegeneration processes in AD. A recent work by Spangenberg et Al. [1], suggested that microglia depletion directly affects amyloid- $\beta$  deposition leading to a reduction of neuropathological changes. Hence, we studied the properties of microglial cell-cell interaction networks in wild type (WT) healthy mice and 3xTg-AD transgenic mice with AD-like disease.



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.

## Methodology

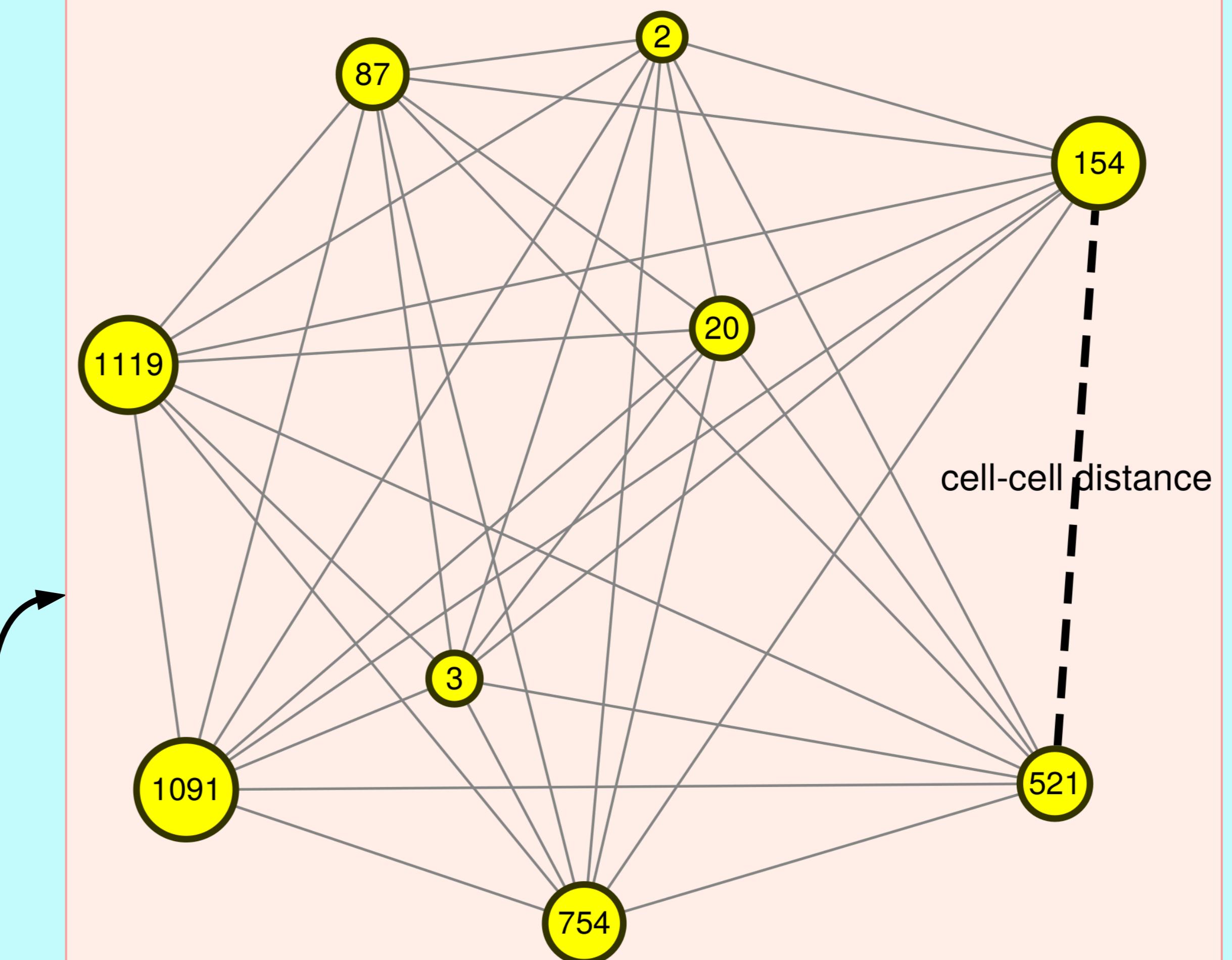
Images of stained microglial cells in coronal brain sections of cortex and dentate gyrus (DG) and CA1 area of hippocampus were obtained from immunohistochemistry experiments using an advanced microscopy platform (Zeiss) and Zen software. The images were analysed by using an automated algorithm to detect stained microglial cells. Next, spatial information, i.e. X and Y coordinates of every cell, were used to reconstruct a fully connected network for each image. Network nodes were represented by microglial cells whereas edges were the physical distance between them. Then, for each network, we iteratively removed the longest edge, or equally long edges, until the network structure became disrupted, i.e. more than one isolated component formed.

The network reconstruction and the data analysis were performed using the R statistical language. 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

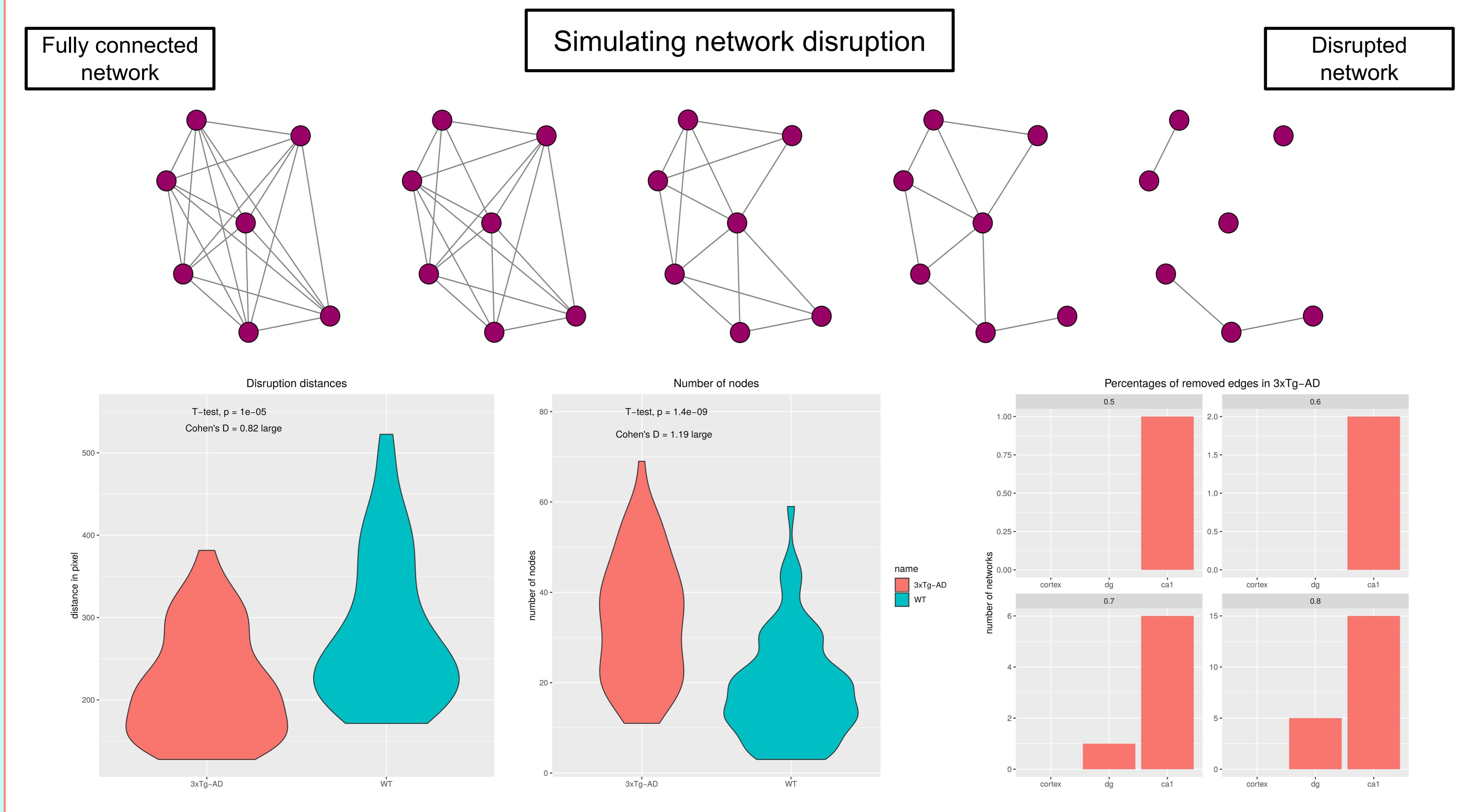
Cell spatial and morphological information were retrieved by Python algorithm used for contour detection. Coordinates were then used to build the microglial networks. This network construction step was performed by using the R statistical language.



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 results showed that the number of nodes, i.e. microglial cells, was significantly higher in networks from AD mice comparing to WT controls. Also, the maximum distances allowed for an interaction, before the network disruption, were smaller in 3xTg-AD mice. On the other hand, the smaller number of cells found in WT mice led to longer edges between fewer nodes. Interestingly, following progressive edge removal, we observed that hippocampus, a brain structure involved in memory formation, showed a weaker microglial network structure, comparing to the cortex in both WT and 3xTg-AD mice. Indeed, hippocampus microglial networks were disrupted with a smaller amount of removed edges, suggesting that increased vulnerability of microglial networks may contribute to hippocampus dysfunction leading to memory deficits in AD patients.



Increased vulnerability of microglial networks in the hippocampus of mice with AD-like disease. The networks were disrupted by progressively removing the longest edges, until two or more islands appeared. Network example (top) show that, starting from a fully connected network, node removal leads to islands generation, two represented by an isolated node. The violin boxplots (left) show disruption distances and number of nodes, i.e. microglial cells, in microglial networks in WT and 3xTg-AD mice. The bar plots (right) show the number of networks disrupted at different percentages of removed edges in the cortex and dentate gyrus (dg) and CA1 (ca1) regions of the hippocampus in 3xTg-AD animals.

## References

- [1] Elizabeth Spangenberg et al. "Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model". In: Nature communications 10.1 (2019), pp. 1-21.

## Conclusion

The methodology we propose is still under development. However, the data also indicate that our network model may be useful to investigate brain cell-cell interactions and better understand neuropathological processes in AD. Finally, the model can be improved by including other key AD hallmarks such as amyloid- $\beta$  deposition and tau pathology, to increase its predictive power. 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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