1394
doi:10.1017/S1431927622005682
Microsc. Microanal. 28 (Suppl 1), 2022

Using Computational Methods and 3D Volume EM Reconstructions to Examine Interactions Between Microglia and Oligodendrocyte Precursor Cells in Mouse Cortex

JoAnn Buchanan[1]*, Jenna Schardt[1], Forrest Collman[1], Stephen J Smith[1], Dwight E. Bergles[2], Jenna Glatzer[2], H. Sebastian Seung [3], R. Clay Reid[1] and Nuno da Costa[1]

[1.] Allen Institute for Brain Science, Neural Coding, Seattle, WA, USA.
[2.] Solomon Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA.
[3.] Princeton Neuroscience Institute, Princeton University, Princeton, NJ, USA.
* Corresponding author: joannb@alleninstititue.org

Glia play complex and wide-ranging roles in the developing and adult brain, including maintaining homeostasis, synaptic pruning, regulating neuronal function, myelinating axons, and clearing cellular debris while reacting to injury and disease. Glial cell types found in the brain include astrocytes, microglia, oligodendrocytes, and oligodendrocyte precursor cells (OPCs). Microglia, astrocytes and OPCs react to nervous system injury by the formation of the glial scar and their disfunction can lead to diseases including MS, mood disorders, tumors and Alzheimer's disease[1].

Microglia actively remove dead or dying OPCs and oligodendrocytes while eliminating myelin debris via phagocytosis[2,3]. Little is known about the interactions between OPCs and microglia in the developing brain and how their mechanisms of phagocytosis and synapse removal might overlap. Here, we used computationally reconstructed datasets created from large volume serial section electron microscopy(SSEM) samples of mouse visual cortex to examine the widespread evidence of the physical contacts between these glia[4]. We focused specifically on contacts between neurons, microglia and OPCs, with attention to their ramified branches and morphological features at large-scale and fine structural levels.

The two glial types could be distinguished by their ultrastructure- the cytoplasm of OPCs was electron lucent and contained numerous organelles and vesicles while the cytoplasm of microglia was electron dense and granular with long stretches of endoplasmic reticulum and fewer organelles in the branches. The endings of the two cells were often intertwined and made extensive contact with each other (Fig.1a-e). Signs of engulfment namely phagosomes and phagolysosomes were observed and both cell types (Fig 1 e, asterisks) but were more numerous in OPCs. The phagolysosomes of each cell type were morphologically different from each other which may reflect different phagocytic mechanisms[5].To better identify the types of ingestions in OPCs, we used array tomography to stain synaptic elements and Lamp2 immunostaining to identify lysosomes and phagolysosomes.

Microglia and OPCs were predominantly found in satellite positions, with their nuclei closely opposed to the soma of their host neurons. Both OPCs and microglia made contacts with somas of neurons, with their branches even wrapping the entire circumference. An example of the two cell types contacting the soma of a pyramidal neuron is shown in Fig.1d. Microglia are smaller than OPCs, and several individual microglia and OPCs could touch an individual neuron. In Fig.1f, one neuron was contacted by 6 microglia and 5 OPCs. There were many fine scale interactions between microglia and OPCs at the level of synapses, nodes of Ranvier and neuronal somata that were observed throughout the datasets. These

morphological observations raise questions about the type and nature of communications that may be present and whether OPCs and microglia work in concert to orchestrate circuit refinement or neuronal activity and what types of mechanisms they use to do so. Visit https://www.microns-explorer.org/ to see the data and find out more[6, 7].

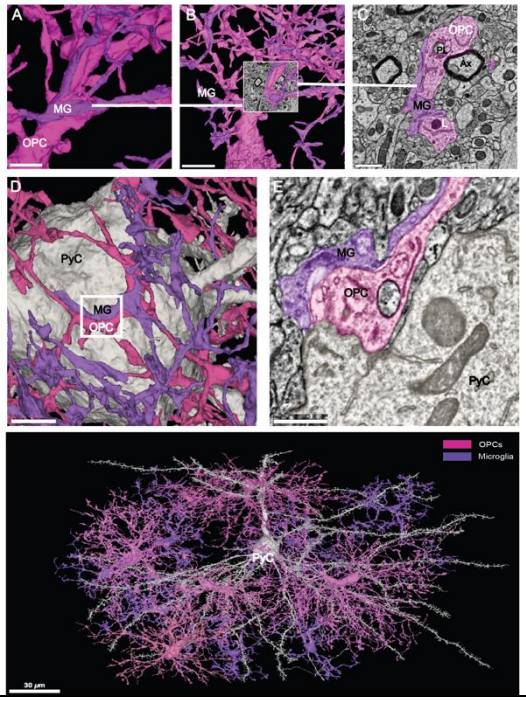

Figure 1. Interactions between OPCs and microglia.
a) Branches of OPC(pink) and microglia(MG)(purple) are intertwined. Scale bar 1.5µm. **b)** Thin section slice area in 3D reconstruction of the two cells shows their close proximity. Scale bar 3 µm. **c)** Higher magnification of thin section slice of the OPC (pink) and microglia (MG in purple) branches indicates their overlap. Phagolysosome (arrow) and lysosome (L) are evident in the OPC branch. Two myelinated axons (Ax) are close by. Scale bar 750 nm. **d)** 3D rendering of the branches of OPC (pink) and microglia (MG)(purple) overlap on the surface of a pyramidal neuron soma (PyC). Scale bar 3 µm. **e)** Higher magnification of thin section slice of the same area demonstrates the overlap of the processes and phagosomes (asterisks) within each branch. Scale bar 750 nm. **f)** A pyramidal neuron (PyC) is contacted by 5 OPCs and 6 microglia with their branches often intertwining. Scale bar 30 µm.

References:
[1] S Hong, L Dissing-Olesen and B Stevens, Curr Opin Neurobiol, **36** (2015), p. 128. doi:10.1016/j.conb.2015.12.004
[2] EN Santos and RD Fields, Sci Adv **7 (**2021). doi:10.1126/sciadv.abk1131
[3] HS Domingues et al., Front Cell Dev Biol, **4** (2016), p. 711. doi: 10.3389/fcell.2016.00071 eCollection 2016.
[4] CM Schneider-Mizell et al., Elife, **10** (2021) doi: 10.7554/eLife.73783
[5] J Buchanan et al., bioRxiv (2021). doi:10.1101/2021.05.29.446047
[6] https://www.microns-explorer.org/
[7] The authors acknowledge support by the Intelligence Advanced Research Projects Activity (IARPA) via Department of Interior/ Interior Business Center (DoI/IBC) contract numbers D16PC00003, D16PC00004, and D16PC0005.We thank the MICrONS Consortium and the Allen Institute founder, Paul G. Allen, for his vision, encouragement and support.