Generation of an immunocompetent neural assembloid model

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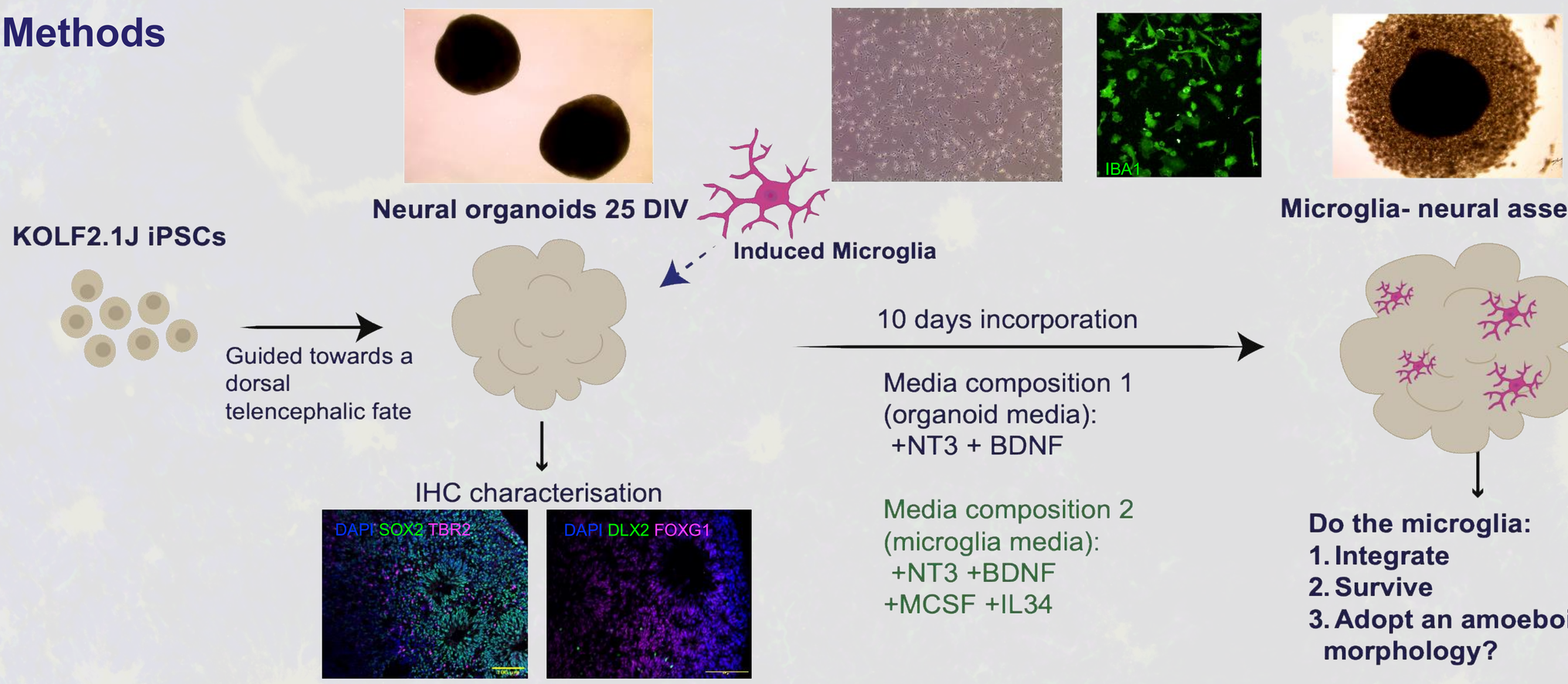
Background

- Microglia are the resident immune cells of the brain
- Microglia migrate from the yolk sac to the brain around 4.5-5.5 weeks post-conception
- Current neural organoid protocols do not contain microglia

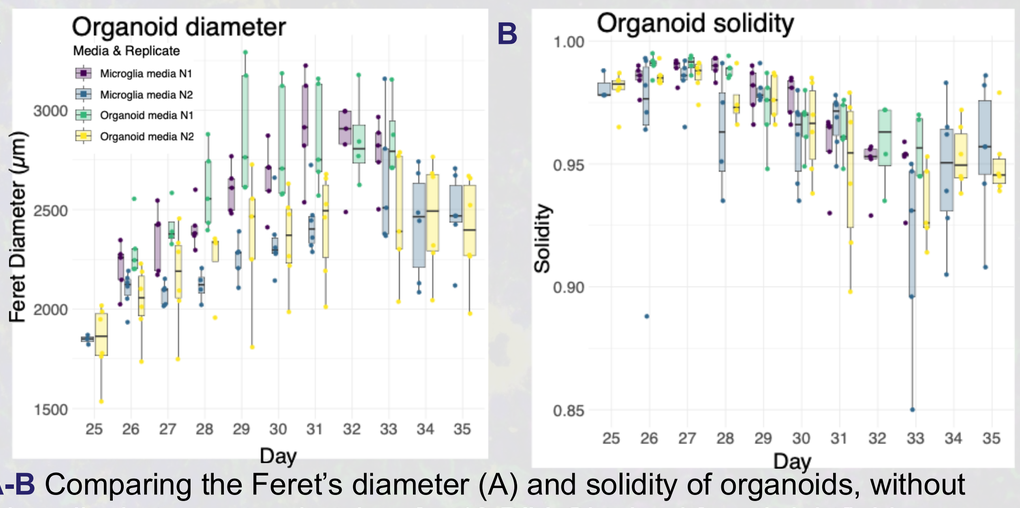
Aims

- Mimic in vivo microglia integration into the cortex
- Understand whether IL-34 and M-CSF supplementation is necessary for microglial survival

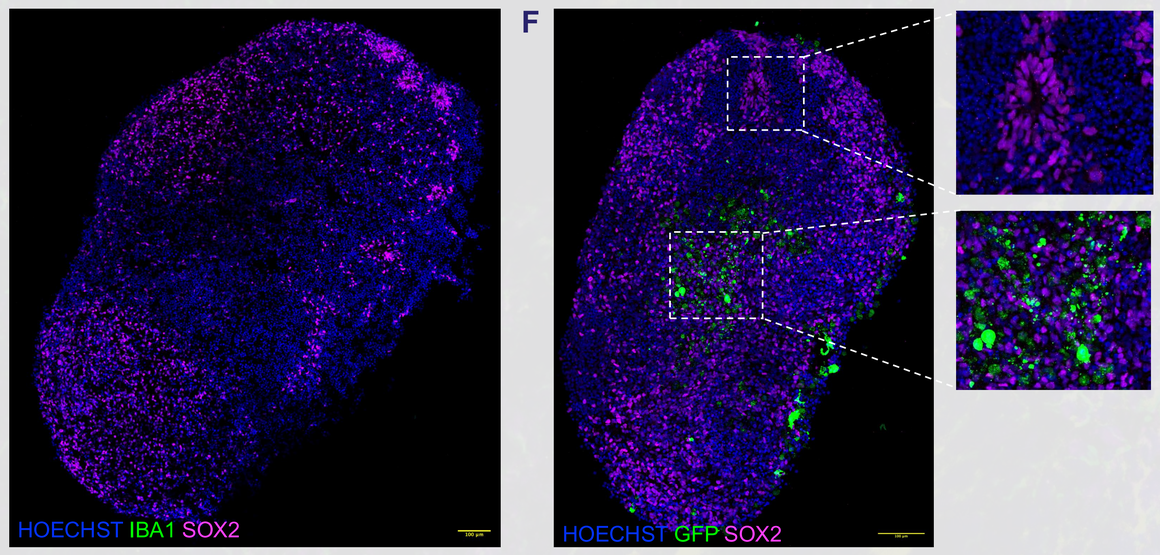
Methods



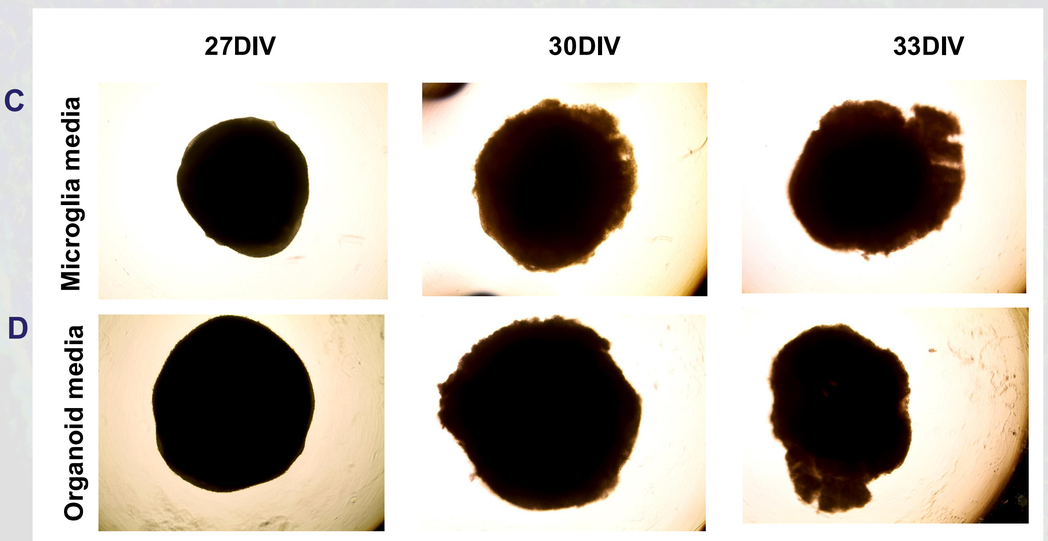
Initial results



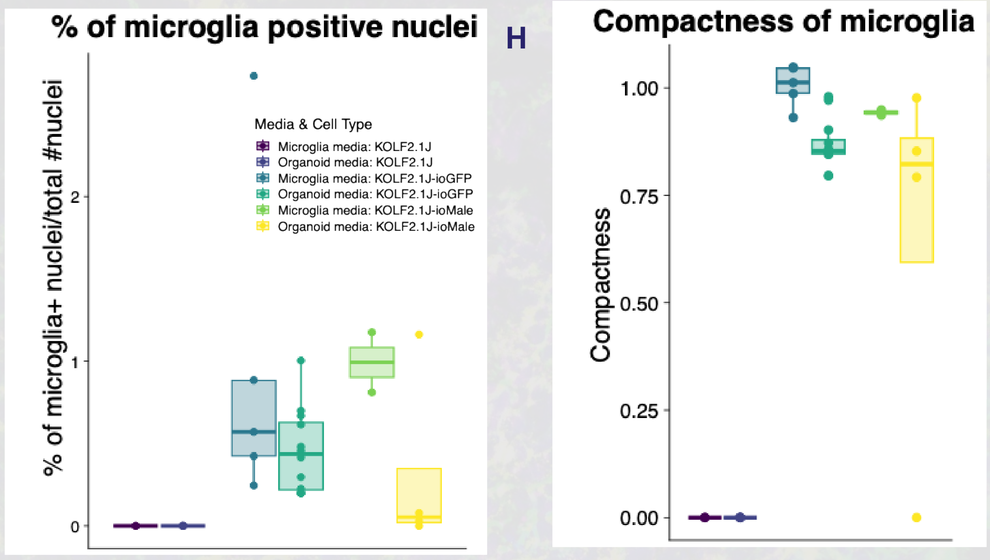
A-B Comparing the Feret's diameter (A) and solidity of organoids, without microglia, between two batches for 10 DIV. Obtained from brightfield microscopy images.



E-F Representative immunohistochemistry of an organoid without (C) and with ioMicroglia (D). Hoechst (Blue), GFP/IBA1 (Green), SOX2 (Magenta).



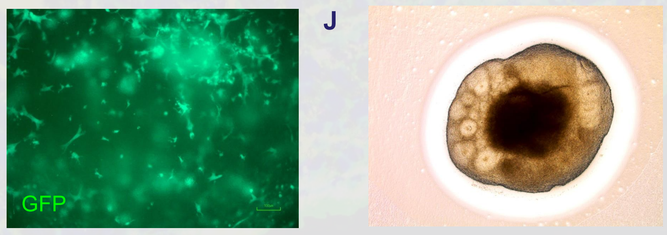
C-D Representative brightfield microscopy of organoids supplemented with IL-34 and M-CSF (termed microglia media, C), and without (termed organoid media, D). Shows the same organoid per condition across three different time points. 27, 30, and 33 days *in vitro* (from left to right).



G-H Comparing the percentage (G), morphology (H) of IBA1+/GFP+ Microglia after 10 days of incorporation with neural organoids between media compositions.

Ongoing work

- Establish optimal timing for microglia incorporation
- Determine the assembloid's immunocompetence
- Understand how microglia influence neural network activity



I Confocal microscopy of ioGFP microglia in ALI-COs
J Brightfield microscopy of ALI-COs

References

1. Buonfiglioli. A et al., 2025 A microglia-containing cerebral organoid model to study early life immune challenges. Brain, Behavior, and Immunity. 10.1016/j.bbi.2024.11.008

2. Cañizares Luna et al., 2025 Protocol for generating and using human iPSC-derived microglia-containing air-liquid-interface cortical organoid cultures. STAR Protocols. 10.1016/j.xpro.2025.103915