Spiked up microglia-containing neural organoid model

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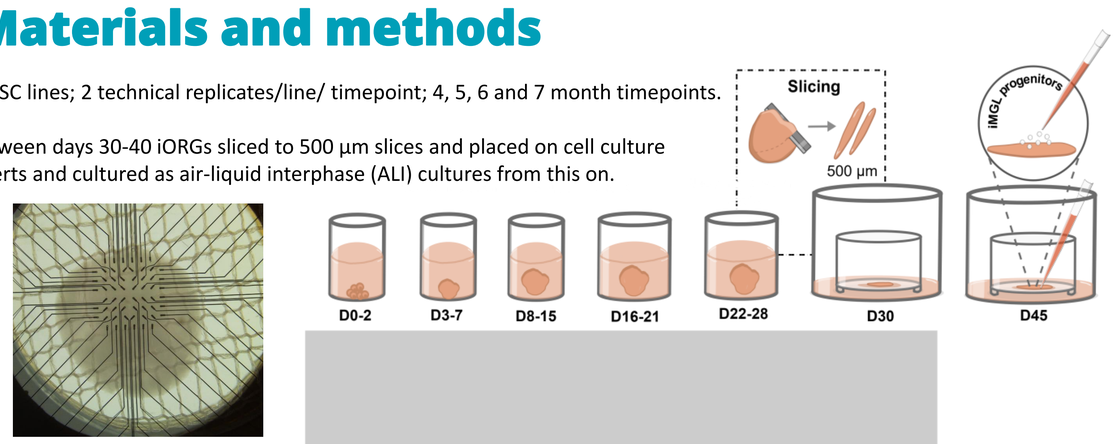
Introduction

- Human induced pluripotent stem cells (iPSC) offer an unlimited access to human brain cells
- Neurons and astrocytes develop from ectoderm but microglia are mesodermal. Thus, they need to be incorporated separately
- By incorporating microglia progenitors into developing cerebral-like organoids, we will be able to mimick the co-maturation of microglia and neurons in developing brain-like structure.

- **AIM: To develop an optimized microglia-containing organoid model (iORG) that show enhanced neuronal network activity and is suitable to studies of neurodevelopment.**

Materials and methods

- 5 iPSC lines; 2 technical replicates/line/ timepoint; 4, 5, 6 and 7 month timepoints.
- Between days 30-40 iORGs sliced to 500 µm slices and placed on cell culture inserts and cultured as air-liquid interphase (ALI) cultures from this on.



- iMG progenitors differentiated from same iPSC lines and incorporated after slicing (day 45) on top of the iORGs,
- IHC samples fixed with 4% PFA, cryoprotected with 30% sucrose and frozen as OCT blocks on dry ice.
- MEA carried out with MEA2100-Mini-60-System (MultiChannel Systems, MCS) using 3D electrodes (60-3DMEA250/12/100iR-Ti). NMDA applied using a fast perfusion system.
- Whole-cell patch clamp technique applied in both voltage and current clamp modes.

Results

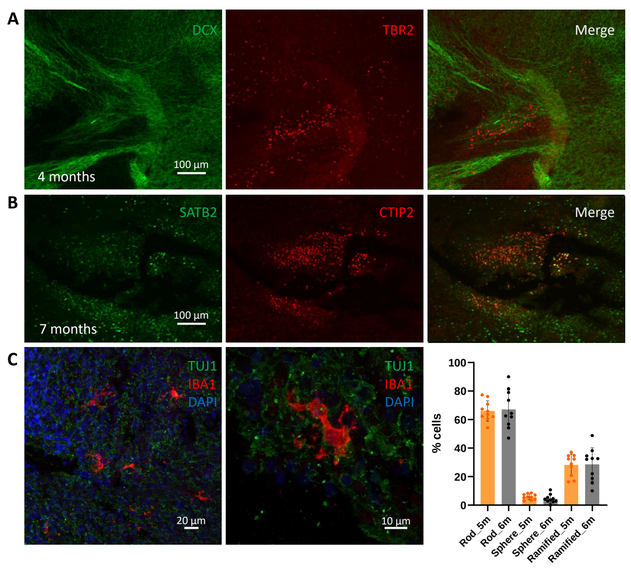


Figure 1. iORGs express early neuronal markers doublecortin (DCX) and TBR2 at 4 months of age. Expression of these markers decrease over time **(A).** iORGs express cortical pyramidal neuron markers SATB2 (upper layer) and CTIP2 (deep layer). Expression of these markers increases over time **(B).** iORGs have iMG during all developmental phases and they are mostly rod-like or ramified **(C).** n=10/group.

Figure 3.

(A). Quantification of frequency of spontaneous excitatory post-synaptic currents (sEPSC) over time.

Quantification of frequency sIPSCs **(D).** n=5 cells per group. 2-way ANOVA. * p<0.05.

Figure 4

(E, F). n=10/group, 5 different cell lines. Mean +/- SD. Student's t test. **** p<0.0001.

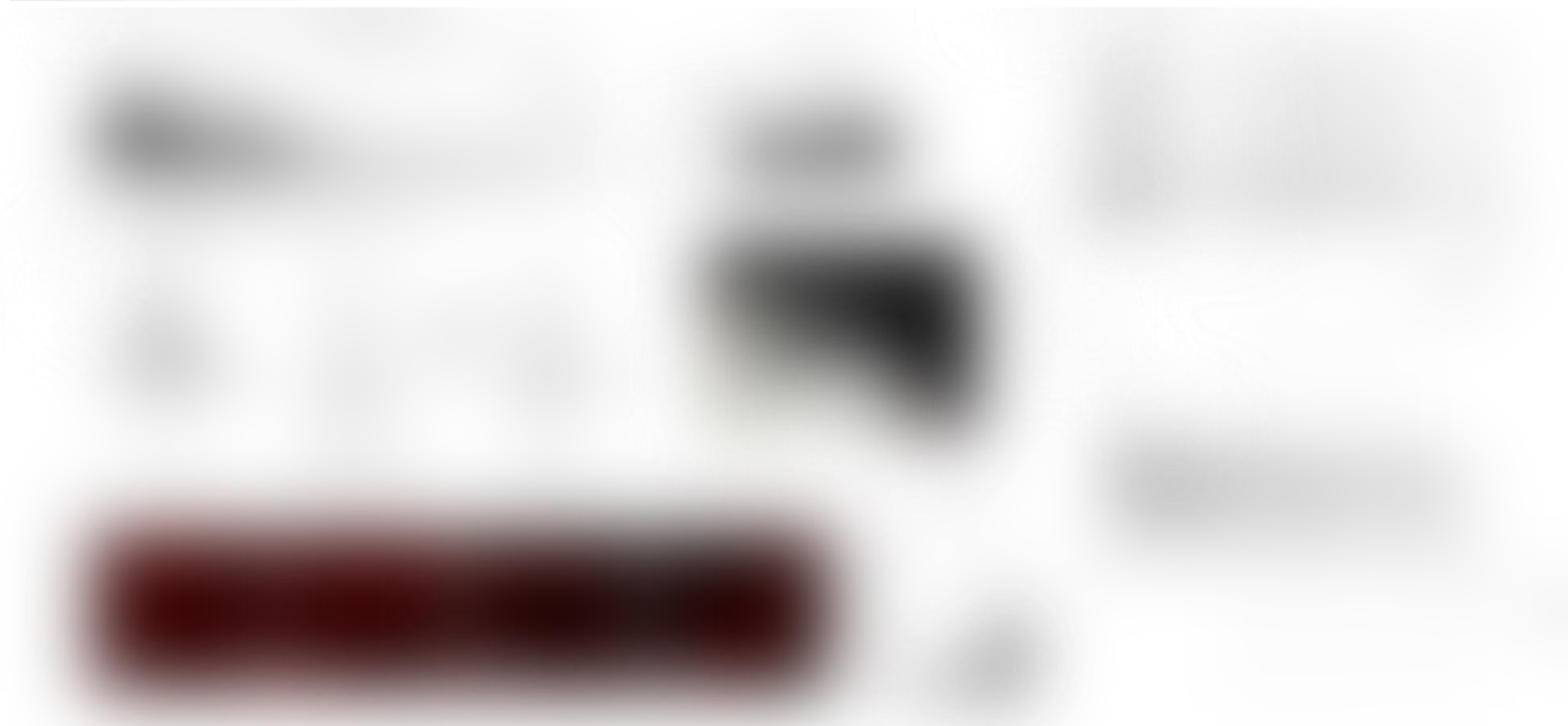


Figure 5. Developmental GABA switch from excitatory to inhibitory interneurons has already occured at 7 months

(E). Representative pictures and quantification of parvalbumin-positive inhibitory interneurons in iORGs over time. n=10 iORGs/group. Mean +/- SD. One-way ANOVA. *p<0.05, **p<0.01 **(F).**

Conclusions

- Microglia accelerate neuronal maturation at early developmental stages
- Microglia promote the development of neuronal network

- Neuronal responses begin to resemble mature human adult brain after 6 months in culture
- Synaptic GABA release is inhibitory at 7 months of age
- Our novel microglia-containing organoid model provide a promising platform to study cell-cell interactions during neurodevelopment, associated pathologies, genetic factors and environmental exposures in clinically relevant 3D environment

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