Modelling of glia-neuron crosstalk in vitro to facilitate drug discovery for Alzheimer's disease.

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Abstract

Objectives: Neuroinflammation has been implicated in the pathogenesis of Alzheimer's disease and potential new therapeutic targets have been identified in microglia and astrocytes. New in vitro models modelling neuroglia interactions are therefore required to facilitate drug discovery by enabling target identification studies as well as drug screening.

Methods: We first developed assays amenable to pharmacological modulations in rodent coculture of neurons and astrocytes. We then developed an approach to generate a quantitative and reproducible primary rodent triculture system that is suitable for pharmacological studies. The protocol was optimised to generate tricultures containing neurons, astrocytes and microglia by culturing in a serum-free medium designed to support all three cell types and adding exogenous microglia to cocultures. Immunocytochemistry and multi-electrode array (MEA) recordings using the Maestro Pro MEA system (Axion BioSystems) have been used to characterise the model and to test tool compounds.

Results: We showed that, while astrocytes increase neuronal activity, microglia in the triculture model suppress neuronal activity in a dose-dependent manner. Furthermore, increased neuronal activity in cocultures and suppressed neuronal activity in tricultures correlated with different density of dendritic spines and of the postsynaptic protein Homer1 along dendrites, indicative of a direct or indirect effect of astrocytes and microglia on synapse function. These models were then used to investigate the effect of tool compounds on neuronal activity.

Conclusions: In summary, our models provide robust and reliable tools for studying the role of glia-neuron crosstalk in the regulation of neuronal activity. They allow for pharmacological manipulation of the system in a high-throughput manner and have the potential to be used for disease modelling, drug screening and target validation.

Development of a rodent triculture system.

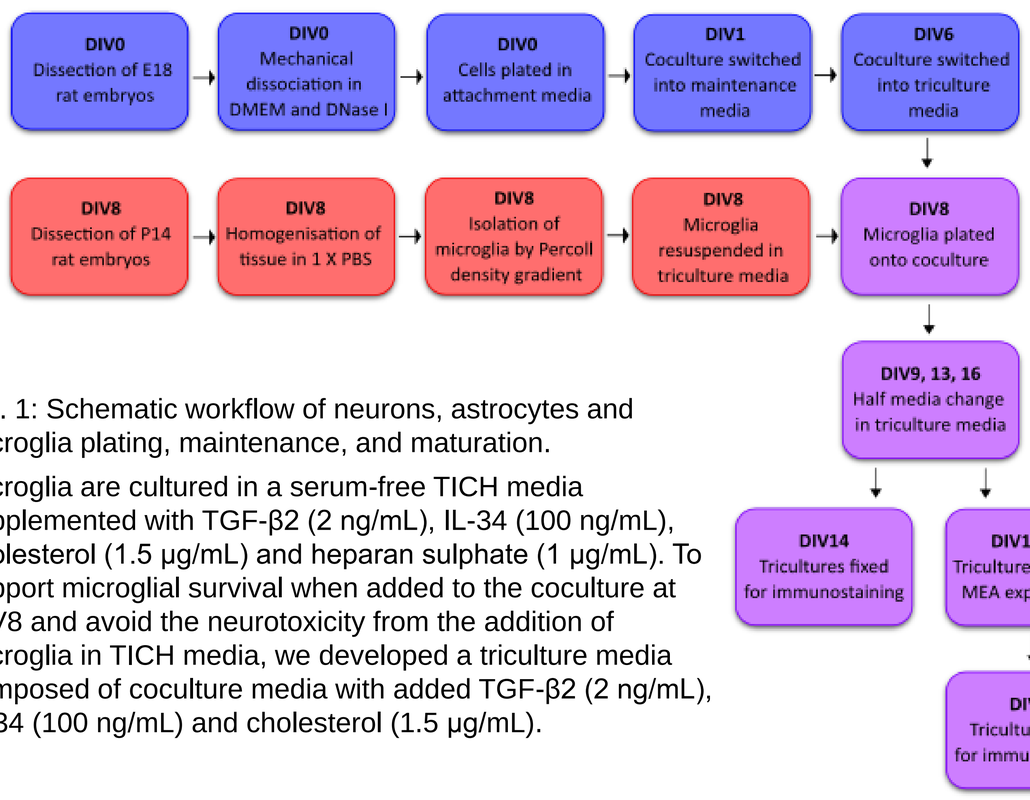


Fig. 1: Schematic workflow of neurons, astrocytes and microglia plating, maintenance, and maturation.

Microglia are cultured in a serum-free TICH media supplemented with TGF-β2 (2 ng/mL), IL-34 (100 ng/mL), cholesterol (1.5 µg/mL) and heparan sulphate (1 µg/mL). To support microglial survival when added to the coculture at DIV8 and avoid the neurotoxicity from the addition of microglia in TICH media, we developed a triculture media composed of coculture media with added TGF-β2 (2 ng/mL), IL-34 (100 ng/mL) and cholesterol (1.5 µg/mL).

Quantification of cell numbers.

Operetta CLS High Content Analysis

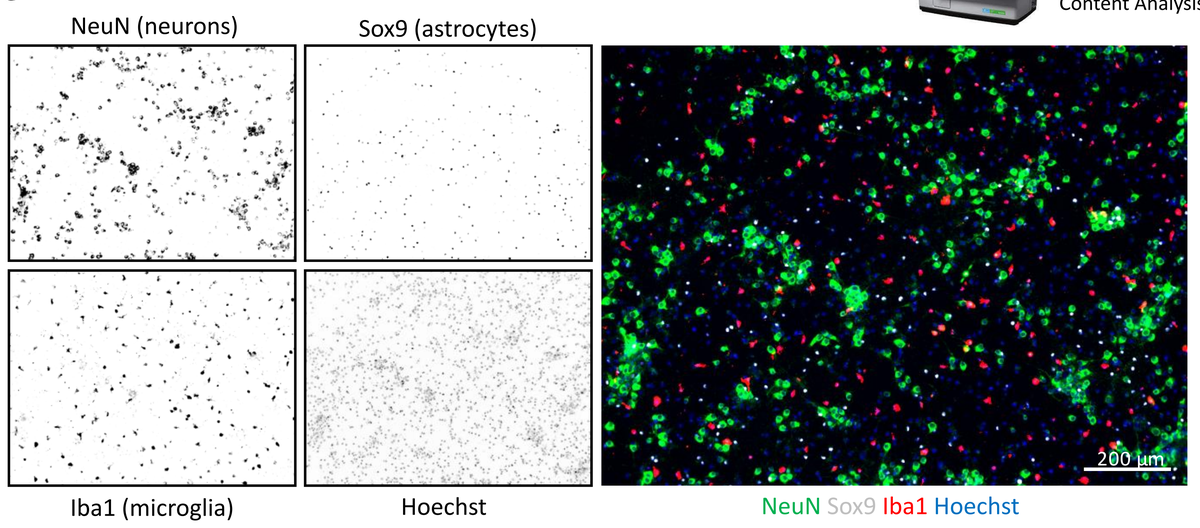
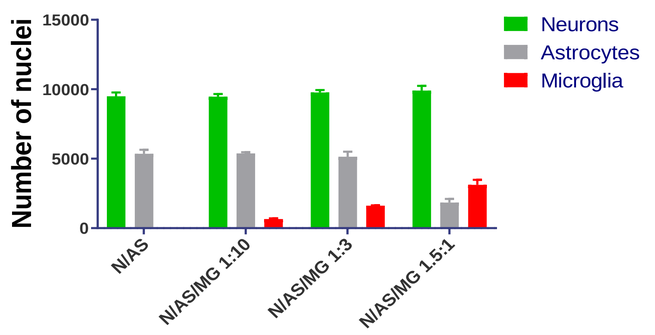


Fig. 2: Representative image at DIV14 in triculture N/AS/MG 1:3 (above). The ratios of microglia : neurons/astrocytes are 1:10, 1:3 and 1.5:1. The number of NeuN-positive cells is unaffected by addition of microglia at all ratios, but the number of Sox9-positive cells is reduced by the highest ratio of microglia (right).



Cocultures and tricultures are cultured on MEA plates to record neuronal activity.

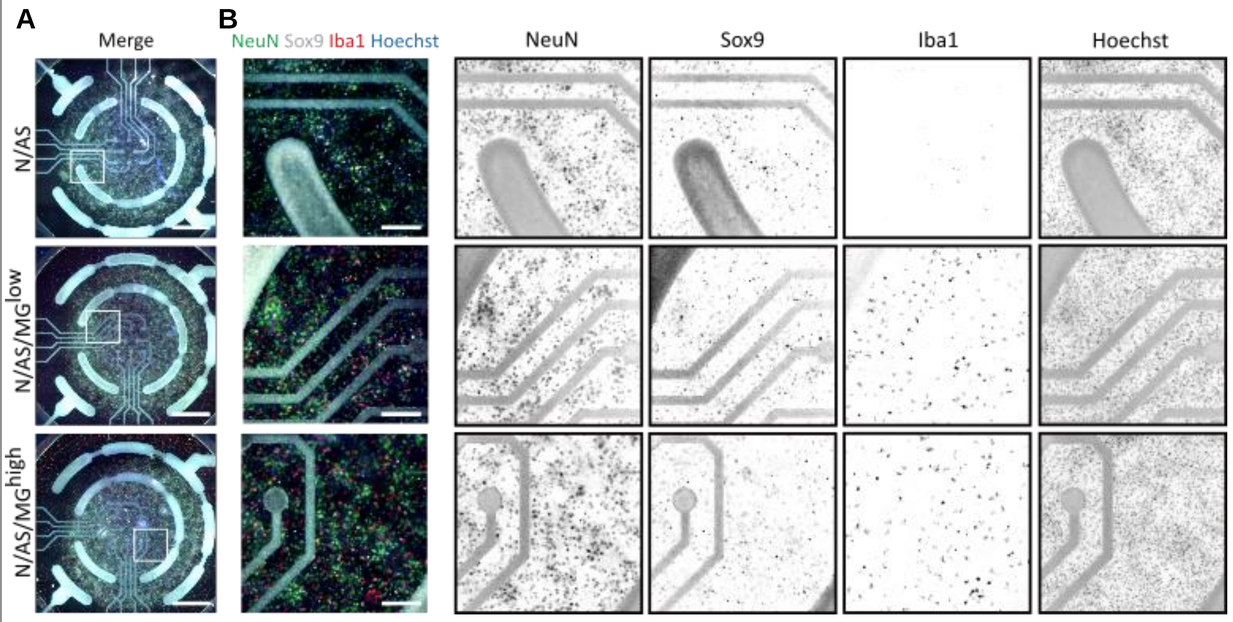


Fig. 3: A) Representative images showing cultures used for recordings in MEA plates at DIV17. Tricultures 1:3 and 1.5:1 were chosen for experimentation and are referred to as N/AS/MG^{low} and N/AS/MG^{high}. White frame = field of view of image (B)

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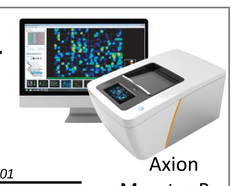
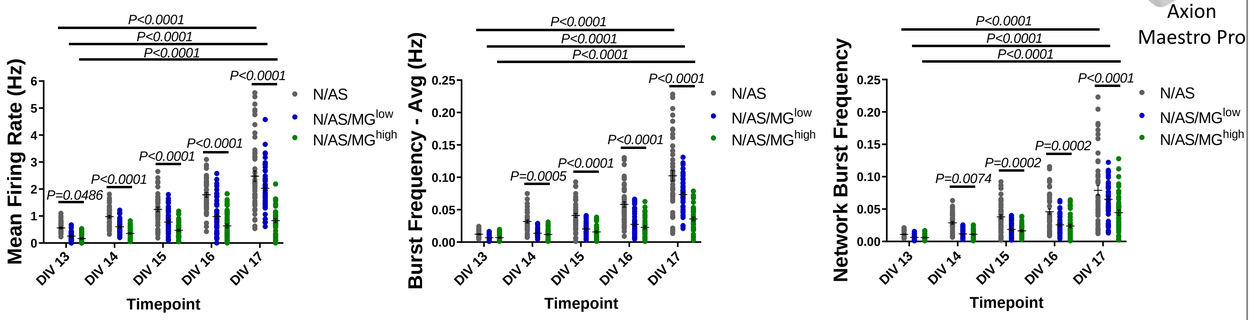
Microglia suppress spontaneous neuronal activity in a dose-dependent manner.



Axion Maestro Pro



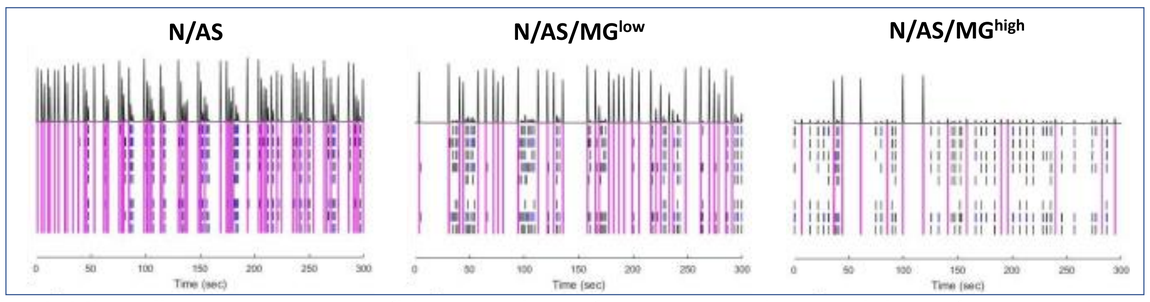
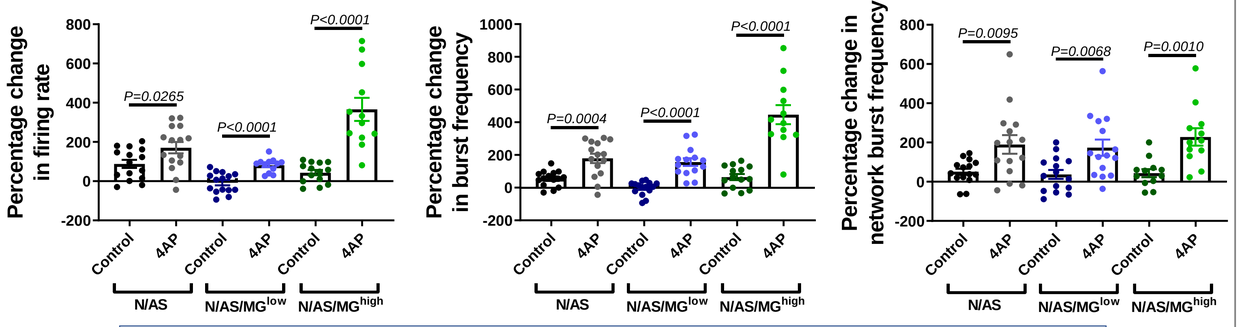


Fig. 4: Microglia suppress mean firing rate, burst frequency and network burst frequency in a dose-dependent manner. Data shown as mean ± SEM and is representative of 5 separate experiments. Representative activity traces at DIV17 are shown below.

Treatment with 4-AP increases cortical neuronal activity in cocultures and tricultures.



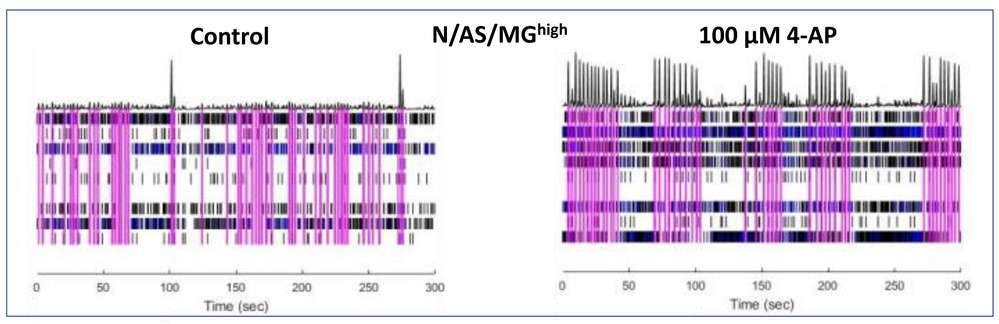
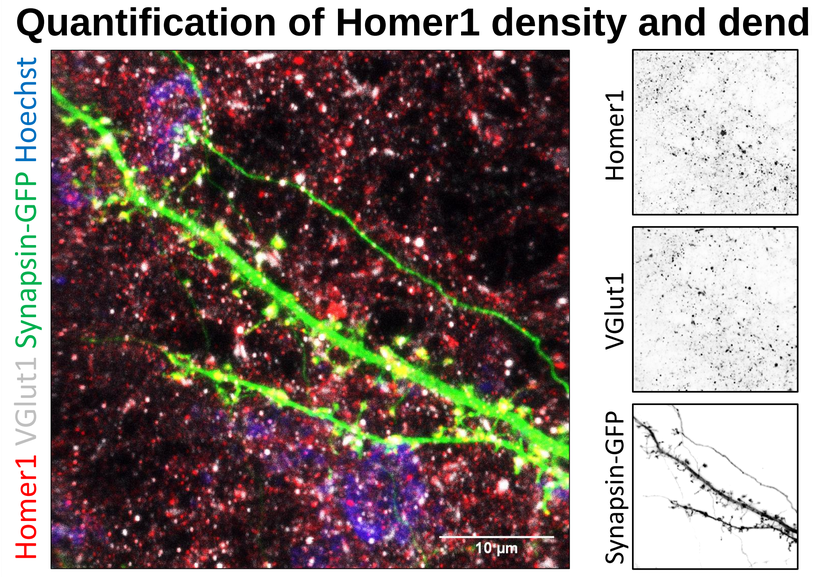


Fig. 5: Modulation of cortical neuronal activity with 100 µM 4-Aminopyridine (4-AP, a voltage-sensitive potassium channel blocker) indicates that neurons in tricultures remain functional. Data shown as mean ± SEM and is representative of 3 separate experiments. Representative activity traces from N/AS/MG^{high} at DIV17 are shown below.

Quantification of Homer1 density and dendritic spines.



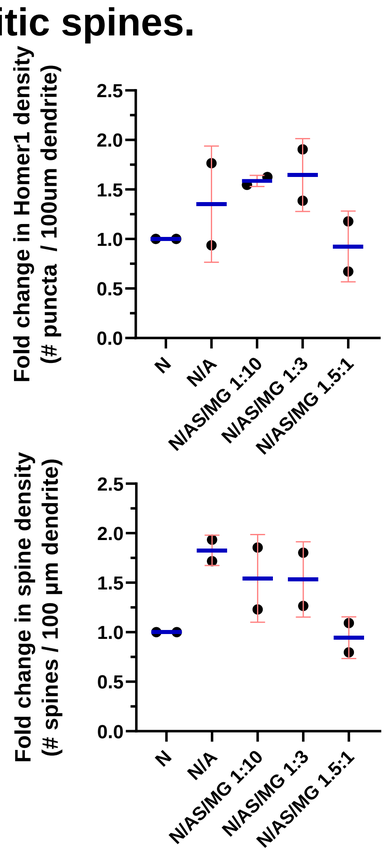


Fig. 6: Representative image at DIV17 in triculture N/AS/MG 1:3 (above). Homer1 is a postsynaptic marker and dendritic spines are visualised with synapsin-GFP. The number of Homer1 puncta and spines is increased in cocultures and tricultures 1:10 and 1:3 compared with neurons only. The presence of microglia in the tricultures 1.5:1 leads to a decrease similar to numbers in the neuron only culture (right). Data shown as mean ± SD and is representative of 2 independent experiments.

Conclusions

- Microglia suppress neuronal activity without affecting neuronal numbers
- Neurons in triculture model remain functional but high numbers of microglia reduce Homer1 and spine density

Future work

- Investigating the mechanisms responsible for microglial modulation of neuronal activity
- Introducing human iPSC-derived neurons/glia into the triculture model

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