

Complex human iPSC-derived neurodegeneration and neuroinflammation disease models for drug discovery

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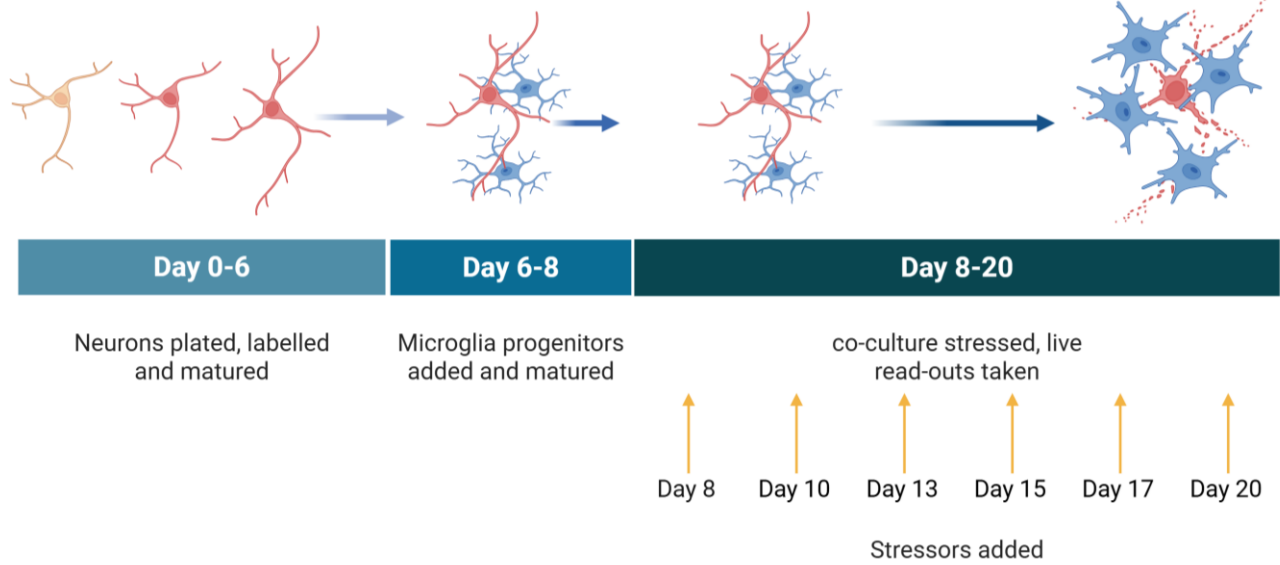
Introduction

Standard neuronal mono-culture models fail to capture the complex cellular environment and intricate non-cell autonomous processes preceding neurodegeneration and neuroinflammation. Therefore, more complex human neuronal models that also include non-neuronal cells (e.g., microglia and astrocytes) serve as more relevant platforms for investigation of disease mechanisms and screening of therapeutic molecules for efficacy potential. To this end we have established human iPSC-derived neuronal co-culture models with microglia and/or astrocytes where the response of each cell type to several disease-relevant stimuli and therapeutic agents is being evaluated, using a combination of bespoke assays such as immunoassays, single-cell transcriptomics and high-content imaging combined with unbiased *in silico* approaches.

Co-culture model (Neurons + Microglia)

Co-cultures successfully established

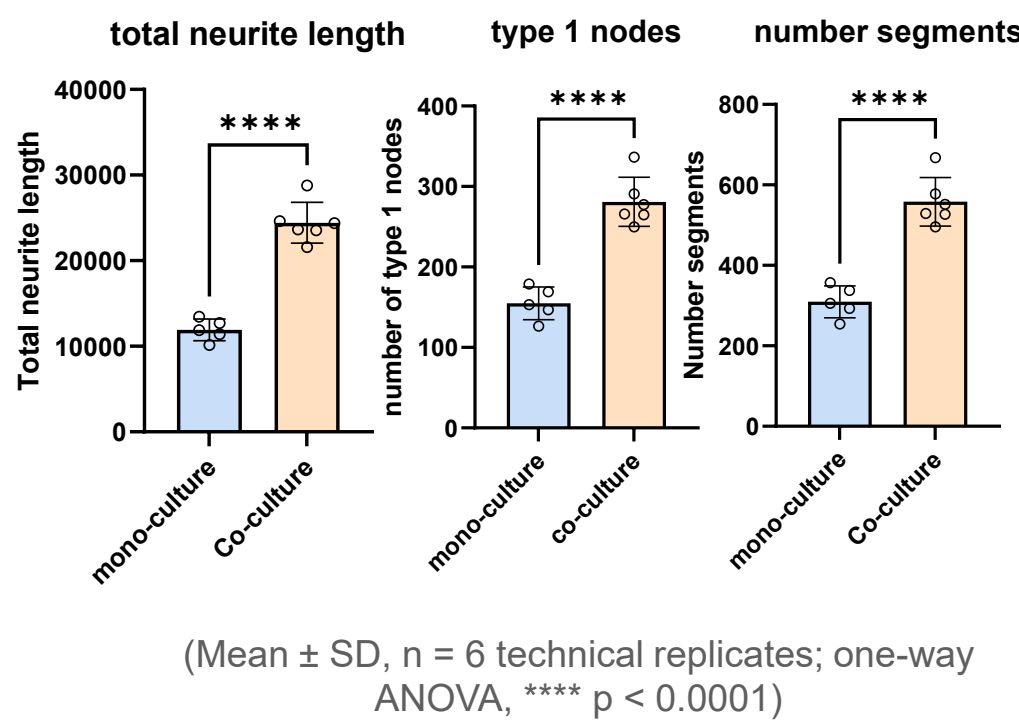
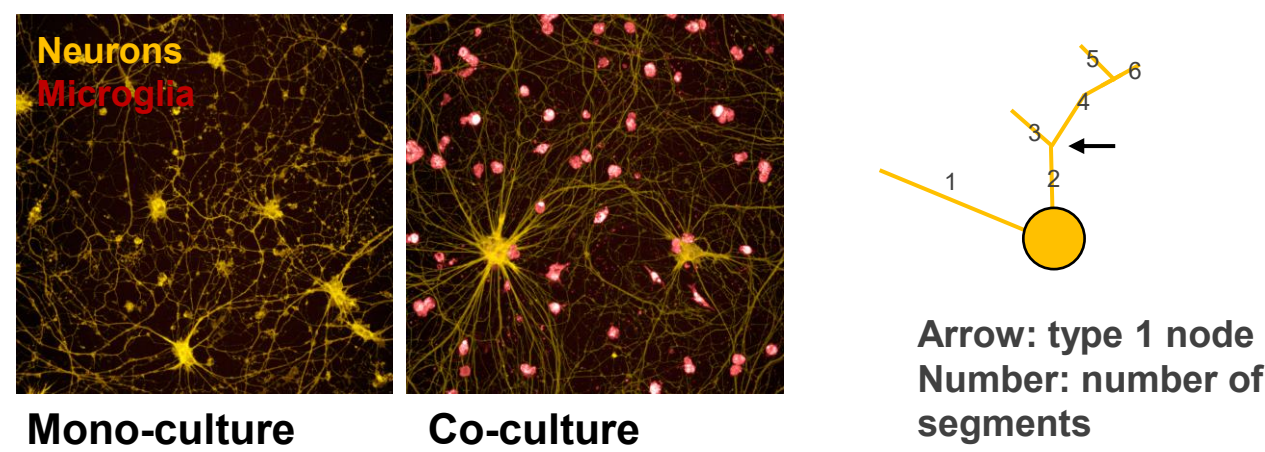
Co-cultures of iPSC-derived cells were established by addition of microglia to neuronal cultures six days after plating and subsequent treatments two days later. Microglia were differentiated from RBi001-A iPSC line purchased from EBiSC using a protocol adapted from Haenseler et al. (Stem Cell Rep, 2017). Motor neurons were purchased from FujiFilm Cellular Dynamics (01279). During culture, cells were monitored by Incucyte and endpoint images taken using the Opera Phenix.



“Resting” microglia support neuronal health

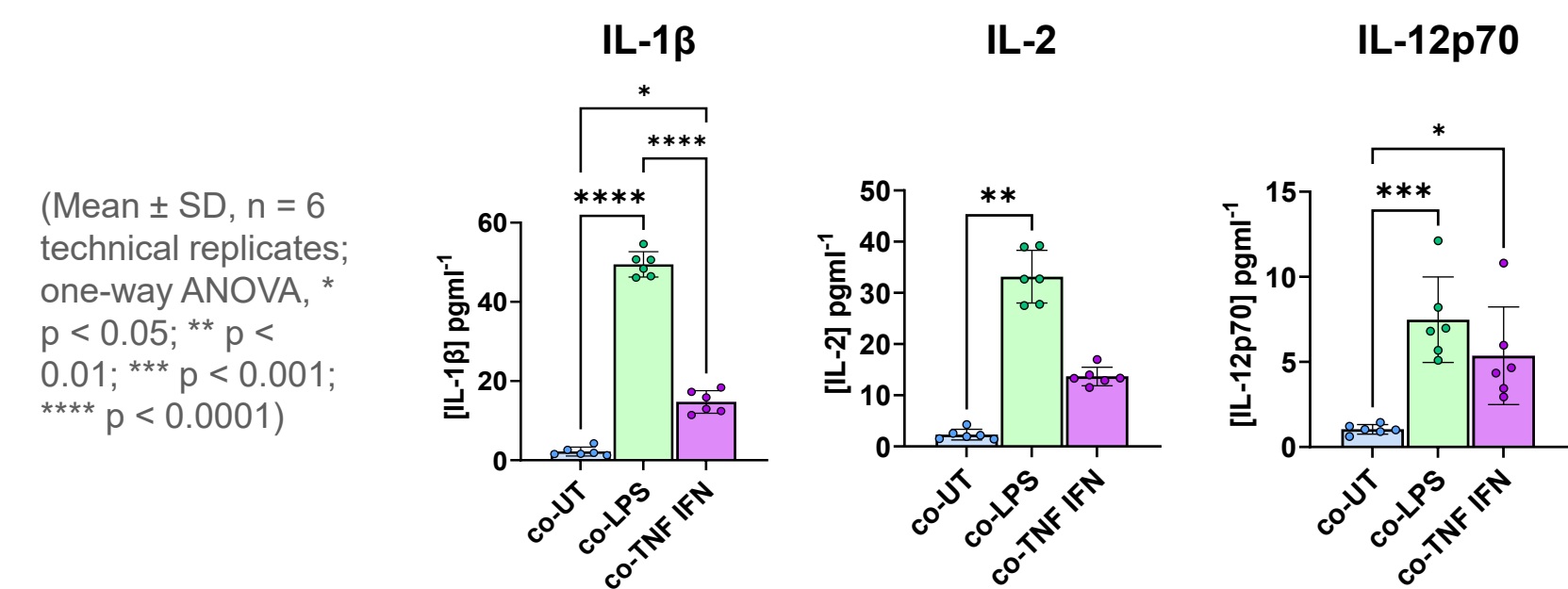
Fluorescently-labelled microglia and motor neuron co-cultures were monitored to observe the effect of microglia on neuronal health and function. Neuronal health was assessed by determining neurite morphology and complexity.

Neurites were quantified after 17 days *in vitro*. Co-culture with microglia resulted in more complex dendritic arbours with longer neurites, more branching and more segments.



Cytokine response to pro-inflammatory stimulation

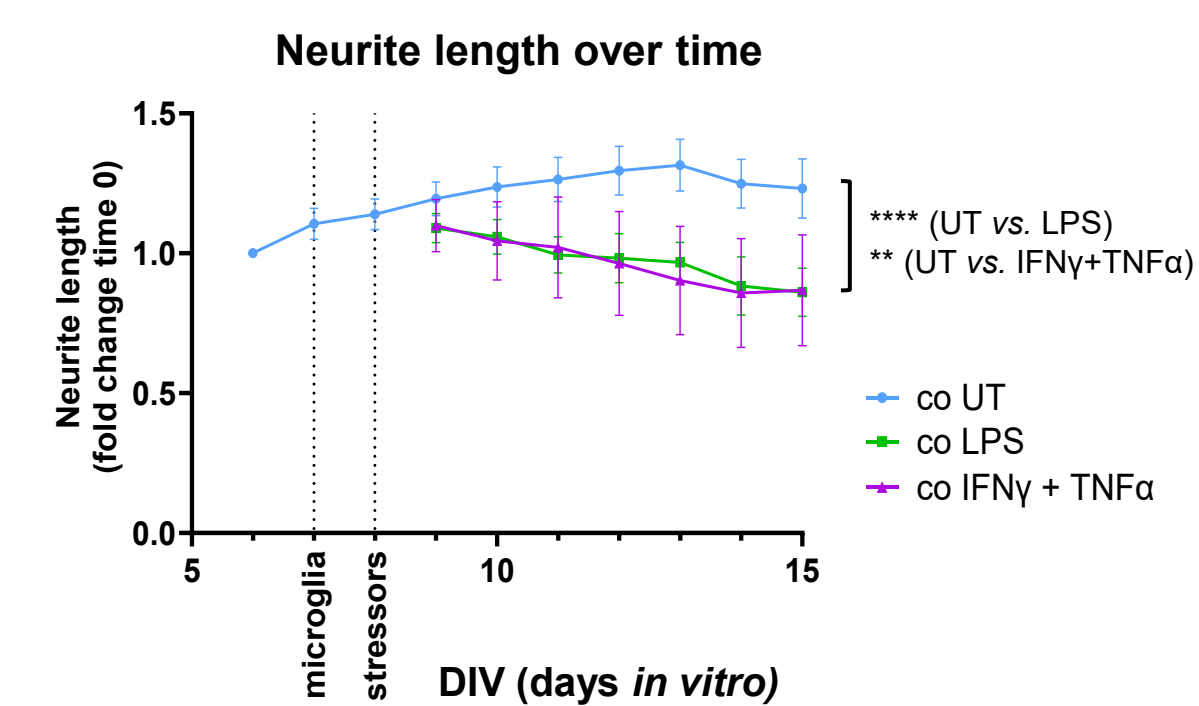
Inflammatory response of co-cultures assessed after 24 hours treatment with lipopolysaccharide (LPS) or TNFα+IFNγ vs. untreated (UT) by MSD 10-plex assay. IL-1β, IL-2, IL-12p70 quantification provided as examples.



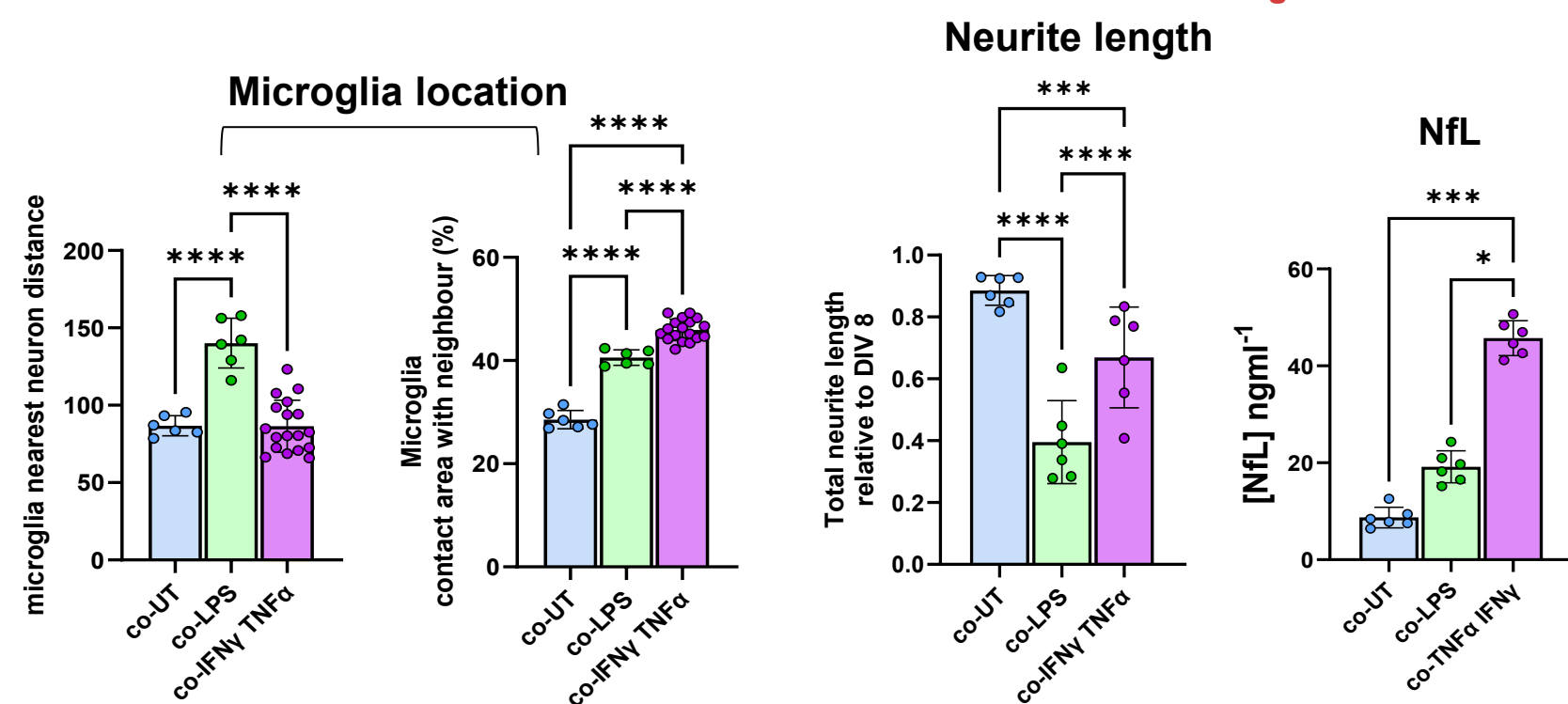
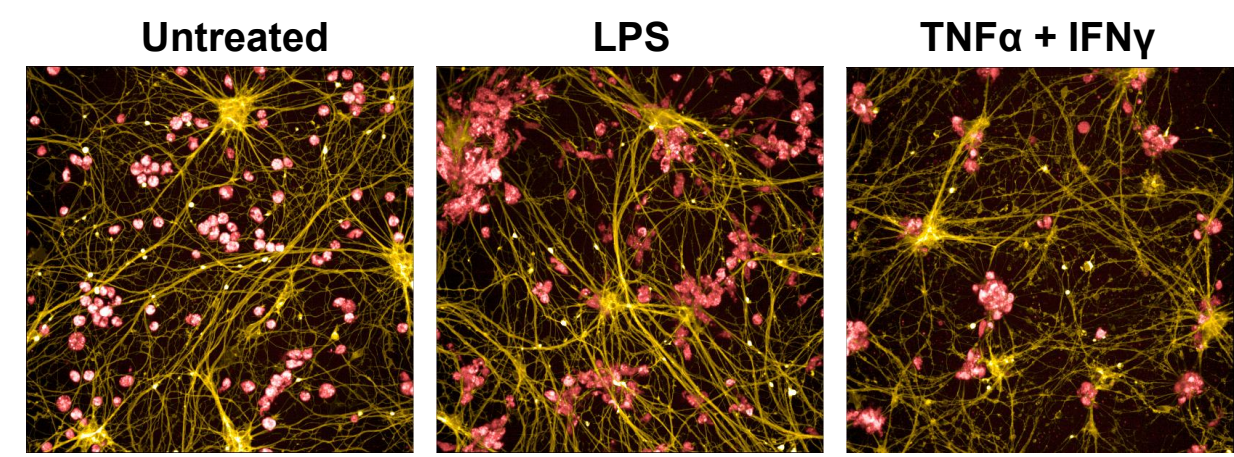
Activated microglia are detrimental to neuronal health

A reduction in neurite length accompanied by increased extracellular NfL (MSD) was observed with five days LPS or TNFα + IFNγ stimulation of co-cultures but not in neuron mono-cultures. Microglia were closer in proximity to each other and the neurons, indicating their activation in response to stimulation.

Using Incucyte to profile motor neurons co-cultured with microglia enables longitudinal, quantitative assessment of both cell-autonomous and non-cell-autonomous neurodegeneration *in vitro*.



(Incucyte longitudinal assessments)
(Mean ± SD, n = 6 technical replicates; two-way ANOVA with Geisser-Greenhouse correction, ** p < 0.01; **** p < 0.0001)



(Opera Phenix endpoint assessments)
(Mean ± SD, n = 6 technical replicates, one-way ANOVA, * p < 0.05; *** p < 0.001; **** p < 0.0001)

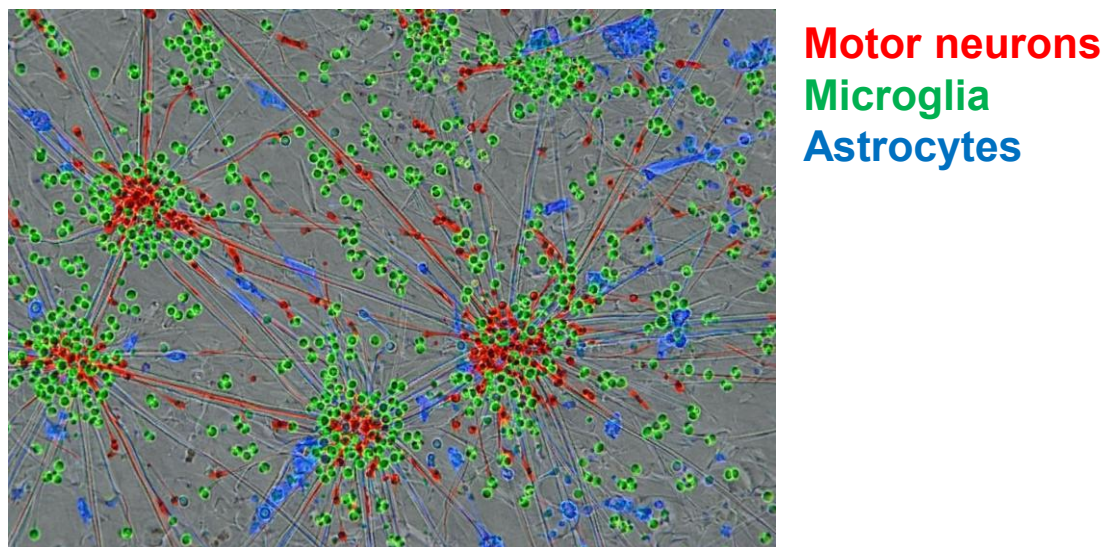
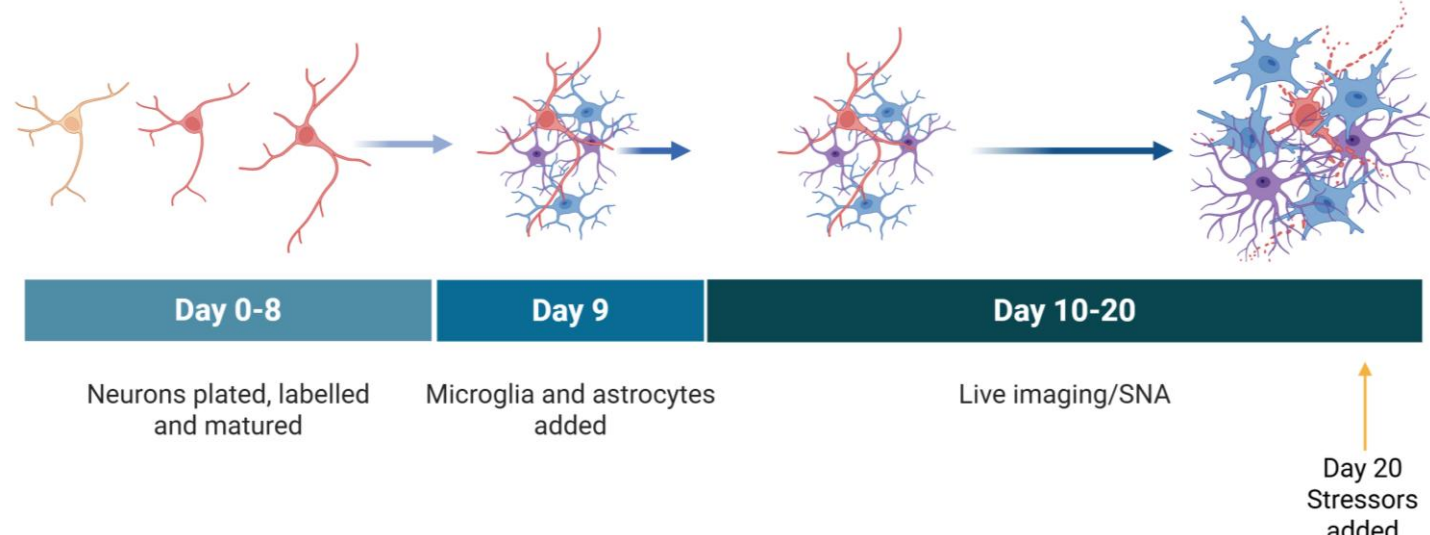
Overview of our iPSC-platform

We have successfully established human iPSC-derived co-culture models with two or three different brain cell types (neurons, microglia, astrocytes). In these models, disease-relevant stimuli modify established neuronal and glial disease markers such as neurofilament light chain (NfL) and inflammatory cytokines. Readouts for processes such as neuronal function, autophagy-lysosomal activity, and seeding of aggregation-prone proteins are also being established. We are also applying machine learning methods to imaging data using cell-specific markers to enable unbiased cell-specific phenotyping and scRNAseq methodologies to further characterise cellular responses to stressors and therapeutic agents.

Tri-culture model (Neurons + Microglia + Astrocytes)

Tri-culture model successfully established

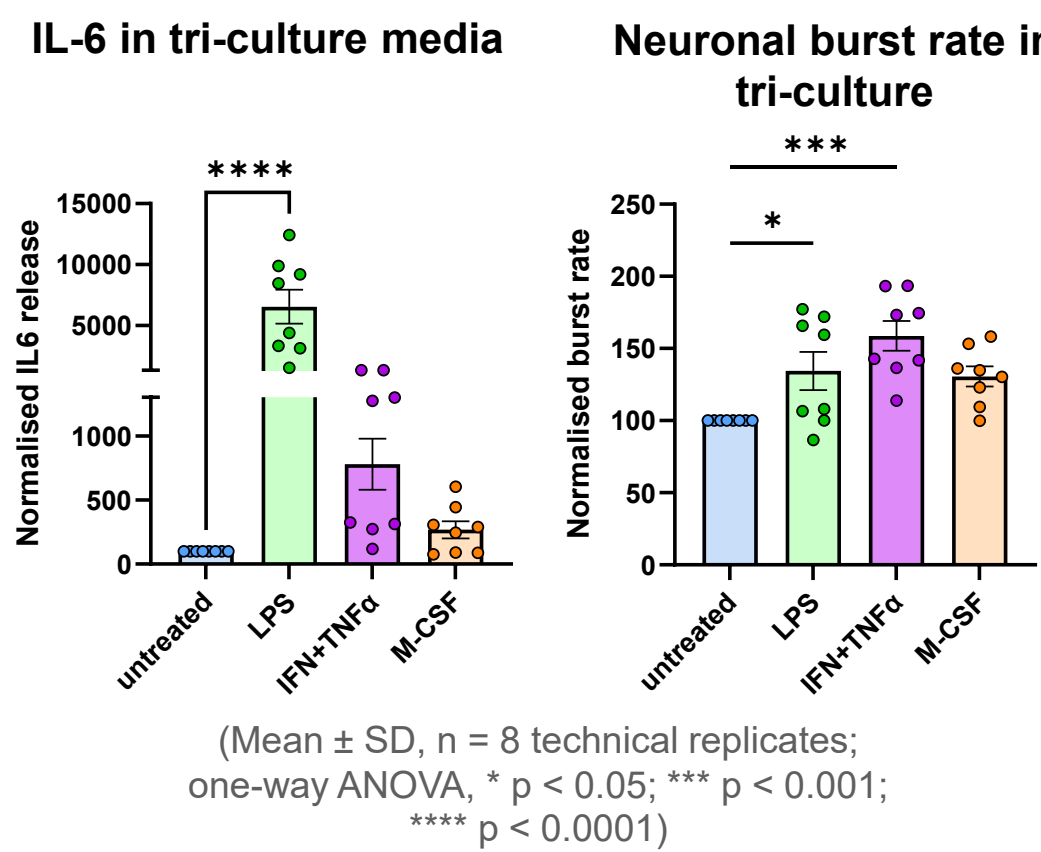
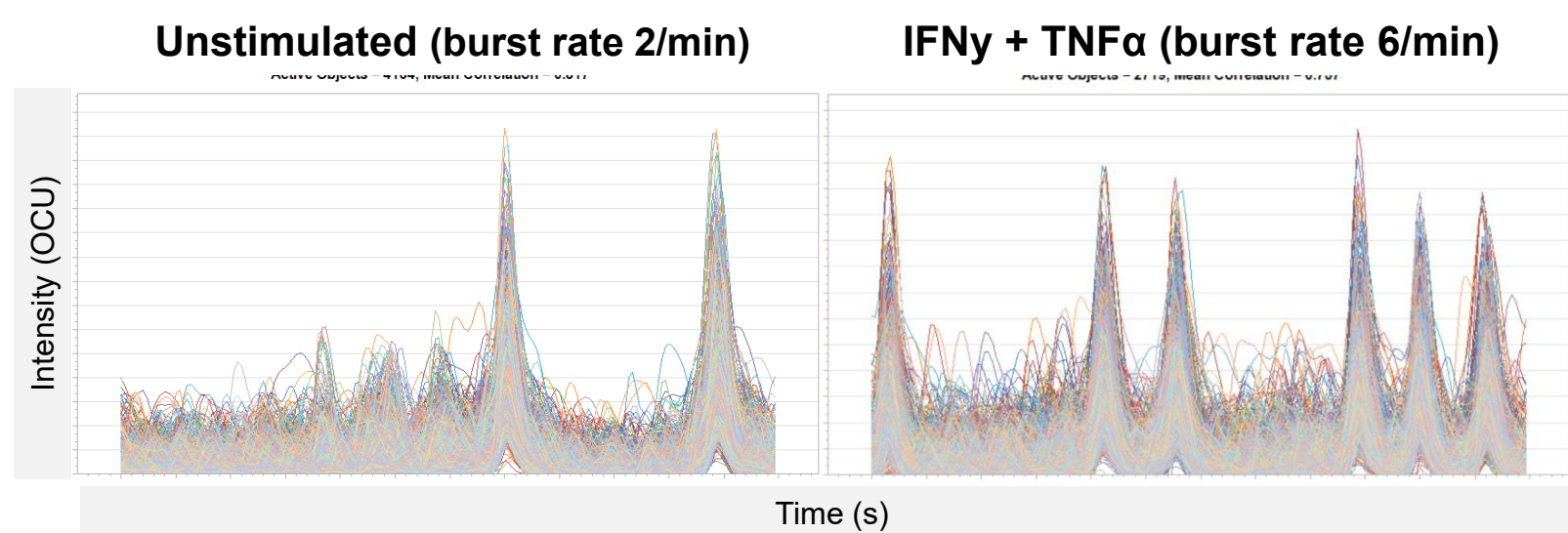
Tri-culture were generated from iPSCs (CENSOi4E) differentiated to microglia, astrocytes and motor neurons by Axol Bioscience. Neuronal function was assessed using Incucyte spontaneous neuronal activity (SNA) Ca²⁺ flux assay and NfL measurement in media (Bio-Techne). Activation of glia was monitored by measuring IL-6 by HTRF (Renvity).



Glia activation associates with neuronal hyperexcitability

Incucyte SNA was used to screen for microglia stimulants that induce neuronal hyperexcitability. Several different glia-activating stimuli impacted neuronal firing rate. Further work was focused on three stimulants.

Incubation of tri-cultures with IFNγ + TNFα, LPS, or M-CSF at day 20 for 48 hours induced inflammatory response (IL-6) and increased neuronal network burst rate (SNA), suggesting detriments to neuronal health.



(Mean ± SD, n = 8 technical replicates; one-way ANOVA, * p < 0.05; *** p < 0.001; **** p < 0.0001)

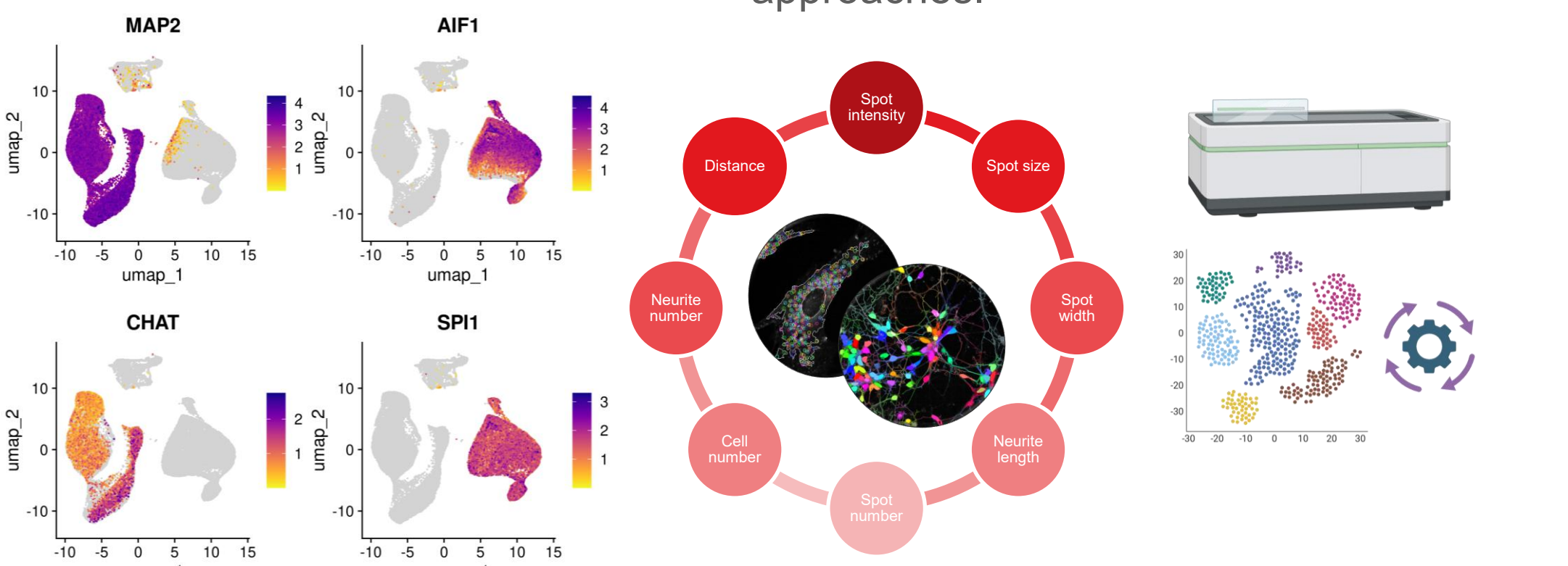
Unbiased phenotyping of co-cultures

Single-cell transcriptomic profiling

Clustering of microglia and motor neuron populations using cell type-specific marker genes.

Imaging-based multidimensional profiling

Unbiased morphological profiling of co-cultures has been performed using the Opera Phenix high content imaging system combined with machine learning approaches.



E.g. top-ranked features by variance between untreated vs. treated clusters (ANOVA F-value > 10):

- Total neurite length
- Neurite segment length
- Cell roundness

Gao et al. (Glia, 2026)

GLIA

Conclusions

We have developed a complex human iPSC-derived platform and established its phenotypes and responses to various external stimuli relevant in the context of neurodegeneration and neuroinflammation. This is expected to provide us with a valuable tool for discovery and development of novel therapeutic molecules, with improved predictive value of human efficacy over mono-culture models.

Acknowledgements

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