Human iPSC-derived cortical neurons and co-culture as translational platforms for modeling neurodegeneration

Sian Humphreys (sian.humphreys@axolbio.com), Ellie Bravington, David Wallbank, Sapna Vyas
Axol Bioscience, Roslin Innovation Centre, Charnock Bradley Building, Easter Bush Campus, Easter Bush, EH25 9RG, United Kingdom

Abstract

The FDA Modernization Act 2.0 invites alternatives to animal testing, to improve the translational success of clinical drug candidates. Human induced pluripotent stem cell (hiPSC)-derived models offer a more physiologically relevant background for pre-clinical assessment. Here, we investigate the activation state of microglia in response to LPS and assess inter batch and inter assay variability of control (ax0664) microglia.

Additionally, iPSC-derived cortical neurons from donors affected by sporadic Alzheimer's disease (sAD) were matured and characterized in terms of neurite outgrowth and response to stressors such as Tau PFFs[1,2]. Incorporating cells derived from donors with sAD into quad-culture alongside the use of physiologically relevant stressors provides a useful human cell platform to test therapies for neurodegeneration.

We have also developed a quad-culture model supporting function of iPSC-derived, isogenic, cortical excitatory and inhibitory neurons, microglia and astrocytes. Spontaneous neuronal activity (SNA) was monitored using IncuCyte® Neuroburst (Sartorius) and markers of each cell type examined by immunocytochemistry. Microglial addition increased neuronal activity and synchronisation. As clinical trials for dementia increasingly include drugs targeting neuroinflammation, co-culture of neurons and glia is a relevant model for *in vitro* compound screening.

Methods

Microglia cell culture and assays

Human iPSC lines were differentiated to microglia-like cells by mesoderm induction via embryoid-body formation[3]. Two batches of control microglia (ax0664) were matured in 96-well plates for 7 days before treating with various LPS concentrations. Following a 22 hours incubation, supernatant was collected and IL8, IL6 and TNFα concentrations were measured via HTRF (Revvity). TNFα concentrations from ax0664 control wells, treated with 300ng/ml LPS, were monitored over 24 experiments to measure inter-assay variability. Experiments outside +/- 3SD were rejected.

Neuronal monoculture cell culture and assays

iPSC-derived cortical neuron precursors[4] from unaffected donors (ax0004) and affected by Alzheimer's disease donors (ax0174 and ax0109) were matured in 96-well plates for 7 days. On day 7, 1µg/ml Tau (K18) PFFs (Stressmarq) were applied to neurons and neurite outgrowth measured by phase images on a Sartorius IncuCyte® S3 for 6 days.

Table 1. NSCs available from Axol, with ApoE genotype and background information. Lines used in this study are highlighted in bold. Other lines are available for differentiation into NSC.

Product code	ApoE genotype	Age	Sex	Diagnosis at donation
ax0004	E2/E3	40-50	M	Not affected
ax0109	E3/E3	80	M	sAD
ax0170	E3/E3	59	F	sAD
ax0174	E3/E4	60	M	sAD
ax0177	E3/E4	52	F	sAD
ax0108	E4/E4	69	F	sAD
ax0110	E4/E4	76	F	sAD

Isogenic quad cell culture and assays

iPSC-derived cortical neuron precursors and interneurons, from donors unaffected by Alzheimer's disease (ax0004 and ax0662) were thawed and plated together. Following culture iPSC-derived astrocytes and microglia (ax0704 and ax0664), from the same donor, were plated onto the neuronal cultures. Spontaneous neuronal activity was monitored on a Sartorius IncuCyte® S3. Immunocytochemistry was performed on day 33 quad-cultures, staining for IBA1, CD44, TUJ1, GABA and vGLUT1 to determine the identity of the cells in the quad-culture.

Results

Dose dependent cytokine release following stimulation

Concentration dependent release of IL8, TNFα and IL-6 from control microglia (ax0664) over two different batches demonstrated reproducible cytokine release when stimulated with LPS. Plotting the control 300ng/ml LPS stimulation across 24 experiments illustrated reproducibility of the assay and highlighted experiments where the control data was higher or lower than expected (+/- 3SD the mean).

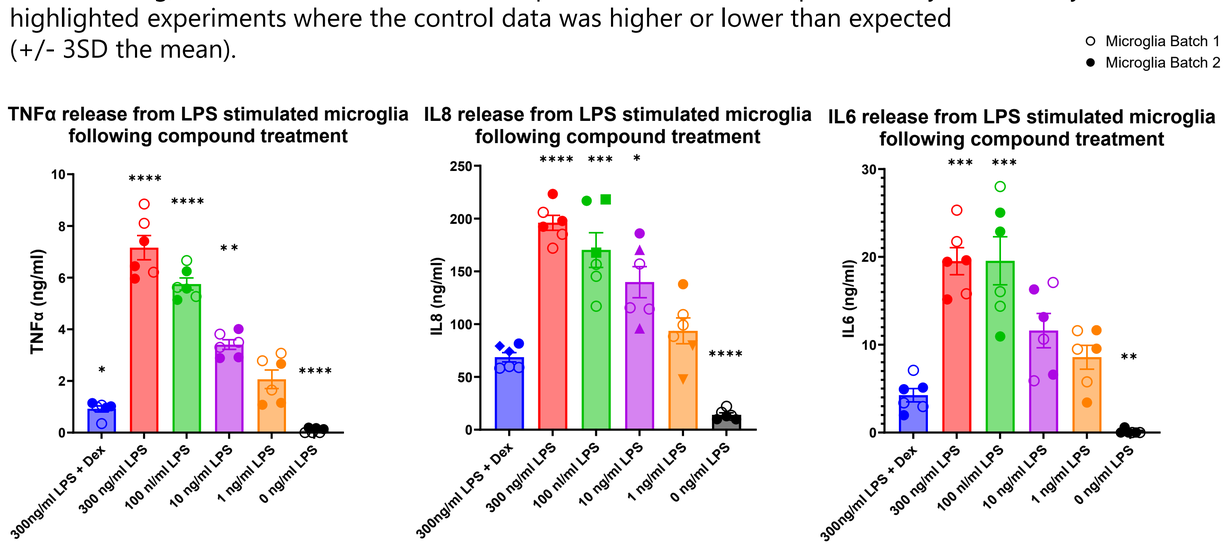


Figure 1. Two batches of wildtype microglia were stimulated over six experiments (each point is the mean of one experiment) with 0, 1, 10, 100 and 300ng/ml of LPS for 22 hours. A pretreatment with Dexamethasone (+Dex) was included to inhibit cytokine release. TNFα, IL6 and IL8 release was measured in the cell culture medium using HTRFs. Each point is the mean of one independent experiment, error bars = SEM. A one-way ANOVA with multiple comparisons was performed with each treatment being compared to the 1ng/ml LPS stimulation, * = P ≤ 0.05 , ** = P ≤ 0.01, *** = P ≤ 0.001 and **** = P ≤ 0.0001.

High reproducibility of a cytokine release assay

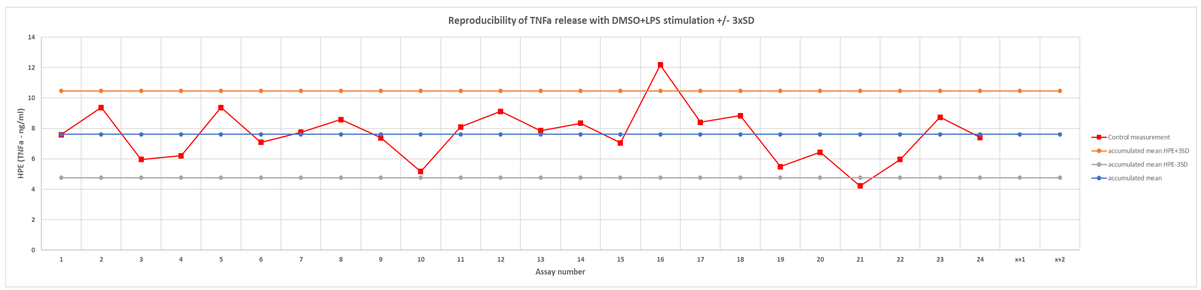


Figure 2. Levy-Jennings plot of twenty-four experiments measuring the TNFα concentrations in cell culture medium after a 21 hour, 300ng/ml LPS stimulation. If the average TNFα concentration of the experiment (red boxes) is larger than +/- 3SD (orange and grey lines) than the mean (blue line) then the experiment is rejected, and a repeat is performed.

Conclusion

Our iPSC-derived cell enable disease modeling in a physiologically relevant background from patient samples. Microglia demonstrate robust and reproducible responses to pro-inflammatory stimuli across multiple assays and differentiation batches. In addition, cortical neurons show a decrease in neurite outgrowth with a stressor relevant to neurodegeneration (Tau PFFs)[1,2]. Finaly, co-cultures of cortical excitatory neurons, interneurons, astrocytes and microglia demonstrate network activity and synchronization providing a more complex model for studying neuropathology than monocultures alone.

Neurite outgrowth of iPSC-derived cortical neurons from affected/unaffected doners by Alzheimer's disease (monoculture)

After 7 days of cultures, neurite outgrowth of cortical neurons from three donors was measured following treatment with 1µg/ml Tau (K18) PFFs (Stressmarq). After 6 days of treatment, all three donors exhibited decreased neurite length and branch points following normalisation to the pre-treatment measurement.

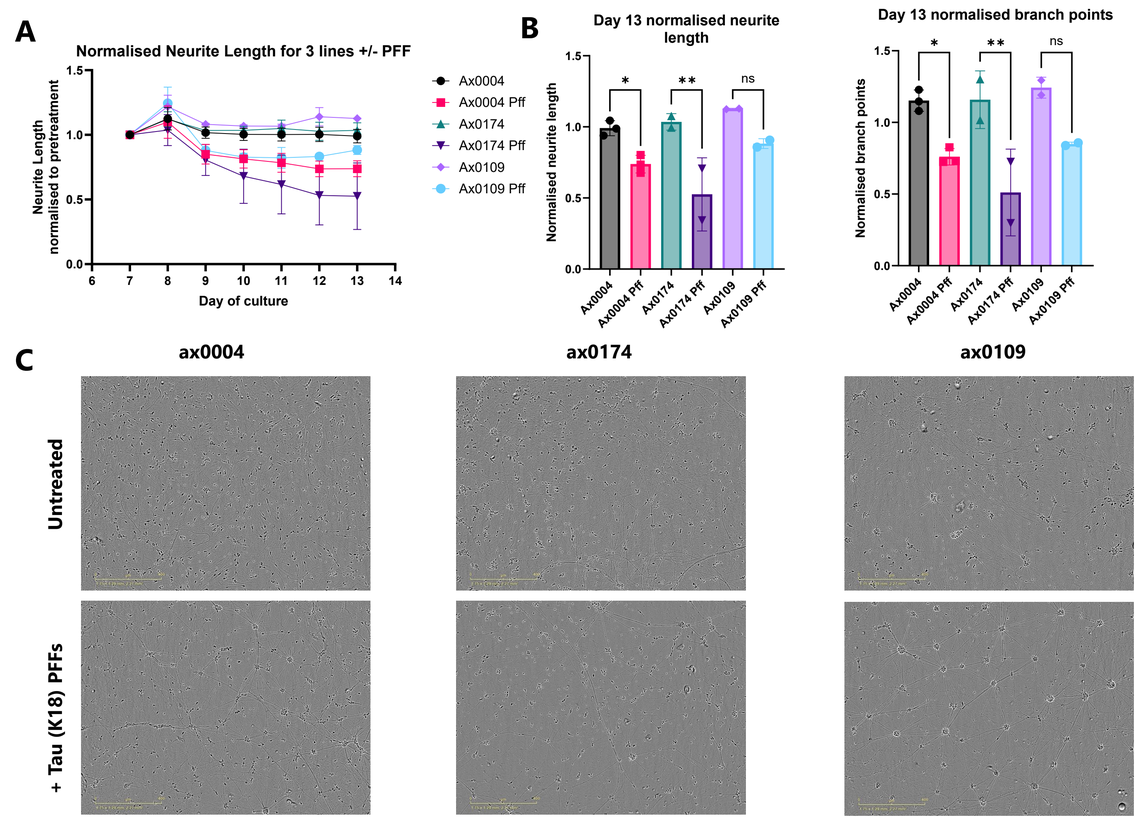


Figure 3. Neurite outgrowth of an unaffected donor (ax0004) and two sAD affected donors (ax0174 and ax0109) following treatment with 1µg/ml Tau PFFs. **A**) Time course of average neurite length normalised to the untreated timepoint (day 7) across three experiments (ax0004) and two experiments (ax0109 and ax0174). **B**) Average neurite length and branch points, normalised to the day 7 pretreatment on day 13 of cell culture. Average from three experiments (Ax0004) and two experiments (ax0109 and ax0174) Ordinary one-way ANOVA with multiple comparisons, * = P ≤ 0.05 , ** = P ≤ 0.01 – further experiments underway. **C**) Representative images from all three lines on day 13 +/- Tau PFF treatment.

Neurite outgrowth of iPSC-derived cortical neurons for toxicity

Neurite outgrowth can be used to monitor the health of unaffected or affected cortical neurons. Following an initial maturation, ax0004 cortical neurons treated with compound B showed a dose dependent decrease in cell health, measured as neurite length, after 7 days of treatment. This was not observed with compound A.

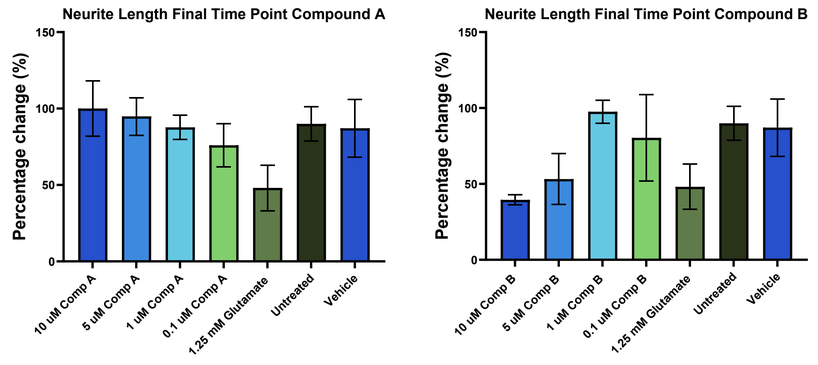


Figure 4 . Percent change of the final timepoint vs the pretreatment timepoint of neurite outgrowth of an unaffected donor (ax0004) following treatment with two compounds. Averages from one experiment.

Cortical, interneuron, astrocyte and microglia quad-culture

Activity of complex cultures, isogenic cells differentiated from the same donor (cortical NSCs (ax0004), interneuron NSC (ax0662), microglia (ax0664) and astrocytes (ax0704)) were monitored over 30+ days of maturation on an IncuCyte® S3. Network synchronization increased following day 20 with burst rate initially increasing before decreasing at the later timepoints. Day 33 staining reveled the presence of microglia (IBA1), cortical neurons (vGLUT1), interneurons (GABA) and astrocytes (CD44) in the cultures.

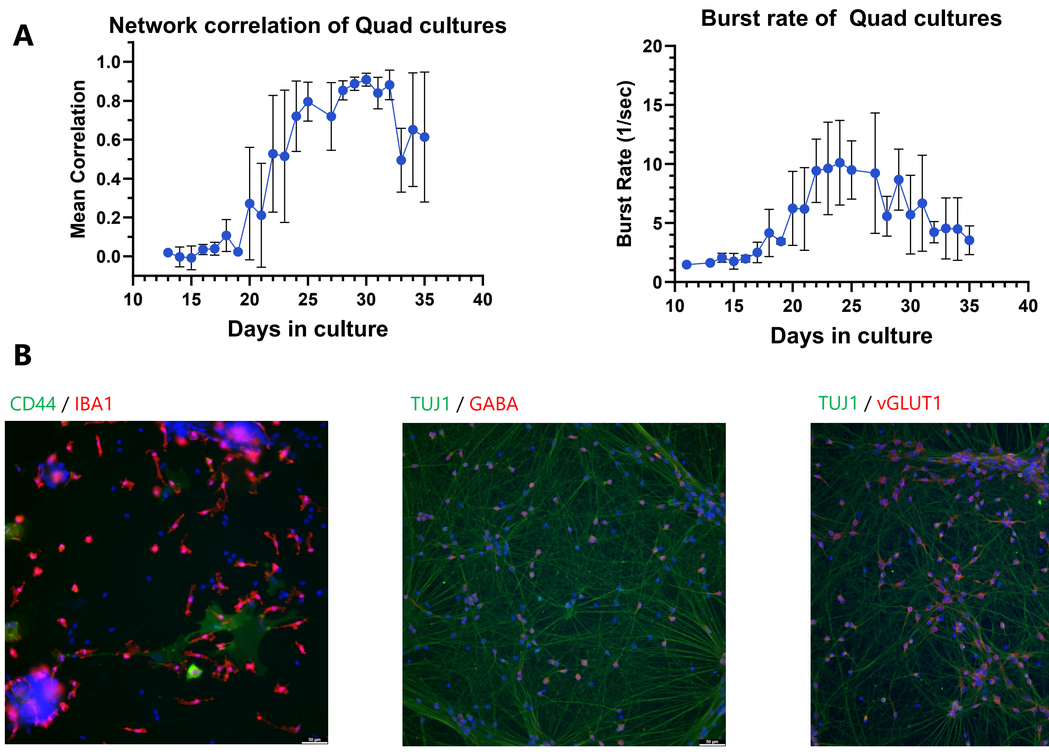


Figure 5. A) Average mean network correlation and burst rate of seven experiments with Quad culture. **B**) Immunocytochemistry of day 33 Quad cultures, showing the presence of microglia (IBA1), cortical excitatory neurons (vGLUT1), interneurons (GABA) and astrocytes (CD44).

References

1. Parra Bravo C, Giani A, Madero-Perez J, et al. (2024) Human iPSC 4R tauopathy model uncovers modifiers of tau propagation. Cell, 2024; 187, 2446-2464.e22
2. Tanabe H, Maeda S, Sano E, et al. (2025) Tau aggregation induces cell death in iPSC-derived neurons. Aging Brain. Apr 11;7:100136. doi: 10.1016/j.nbas.2025.100136.
3. van Wilgenburg B, Browne C, Vowles J, Cowley SA. (2013) Efficient, long term production of monocyte-derived macrophages from human pluripotent stem cells under partly-defined and fully-defined conditions. PLoS One. Aug 12;8(8):e71098. doi: 10.1371/journal.pone.0071098.
4. Shi Y, Kirwan P, Smith J, Robinson HP, Livesey FJ. (2012) Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses. Nat Neurosci. Feb 5;15(3):477-86, S1. doi: 10.1038/nn.3041.

www.axolbio.com | operations@axolbio.com

© 2026 Axol Bioscience Ltd. All rights reserved.