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Life & Health Sciences

Functional phenotypic characterization of iPSC-neurons from Alzheimer's disease patients carrying PS-1 mutation in drug screening and disease modeling

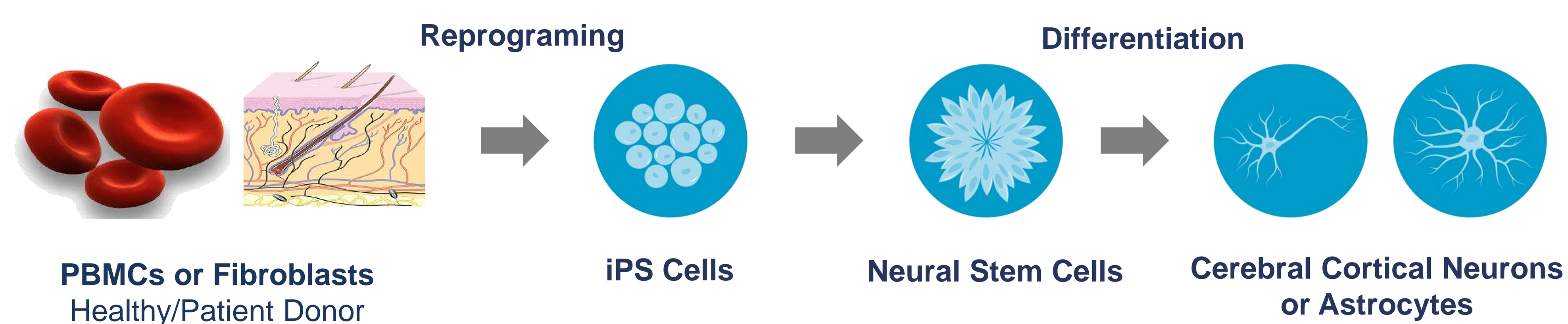
David Nagel[1], Naomi Wessel-Carpenter[2], Rhein Parri[1], Robert Sims[1], Julian George[3], Eric Hill[1], Maria Winkler[4], Priyanka Dutta-Passecker[2], Olaf Schröder[4], Benjamin M. Bader[4]

[1]Life and Health Sciences, Aston University, Birmingham, B4 7ET e.j.hill@aston.ac.uk, [2]Axol Bioscience Ltd., Suite 3, Science Village, Chesterford Research Park, Little Chesterford, Cambridgeshire, CB10 1XL p.dutta@axolbio.com, [3]Institute of Biomedical science, University of Oxford, Department of Engineering Science Old Road Campus Research Building, University of Oxford, OX3 7DQ, [4]NeuroProof GmbH, Rostock, Germany benjamin.bader@neuroproof.com

Introduction

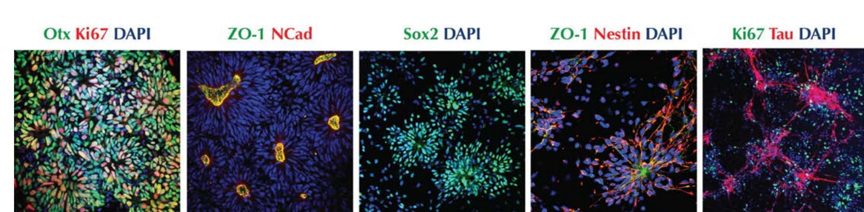
Human induced pluripotent stem cell (iPSC)-derived neural cells provide a powerful tool that can be used to model neuronal behaviour and disease pathology. The increased use of these cells in drug discovery promises to help accelerate current drug screening processes and reduce the use of *in vivo* models used at the earliest stages of testing. Importantly, the production of specific populations, such as cortical neurons, has allowed researchers to investigate the activity of neural networks from particular regions of the brain. Adult cells from human individuals carrying disease-associated gene mutations can be reprogrammed into iPSCs and can then be differentiated into a variety of cell types including human neural stem cells (hNSCs) and cerebral cortical neurons (hCCNs). Our aim was to phenotypically investigate patient iPSC-derived neurons carrying the presenilin-1 (PS-1) mutation (L286V) and to compare them with cells from healthy controls.

iPSC Technology



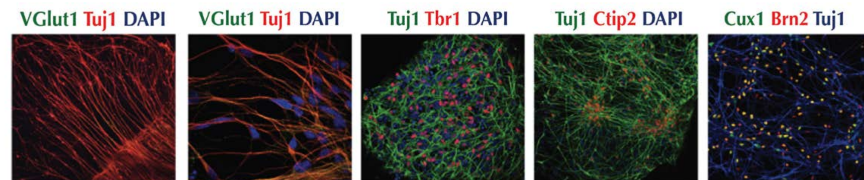
Characterization of Human Neural Stem Cells & Cerebral Cortical Neurons

Human Neural Stem Cells



Axol hNSCs form neural rosettes and express markers typically observed in neural precursor cells as seen by immunocytochemistry.

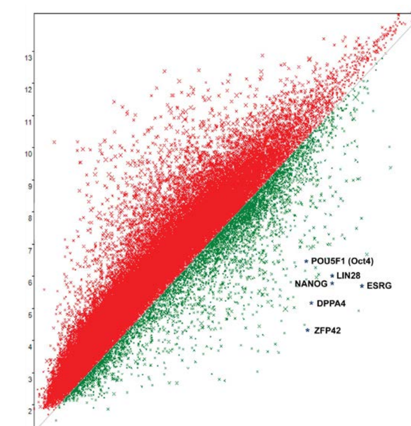
Human Cerebral Cortical Neurons



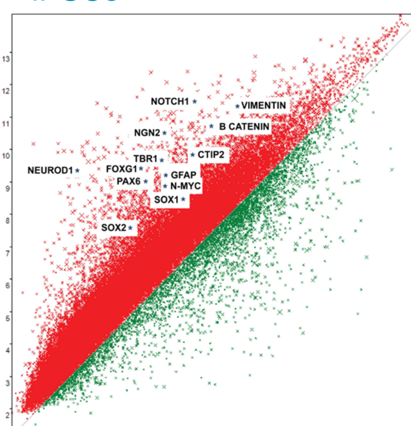
Differentiation of hNSCs generates hCCNs that express neuronal markers observed using immunocytochemistry. These neurons increase in maturity over time in culture.

Transcriptome Analysis

Pluripotency markers are down-regulated in hNSCs vs. iPSCs

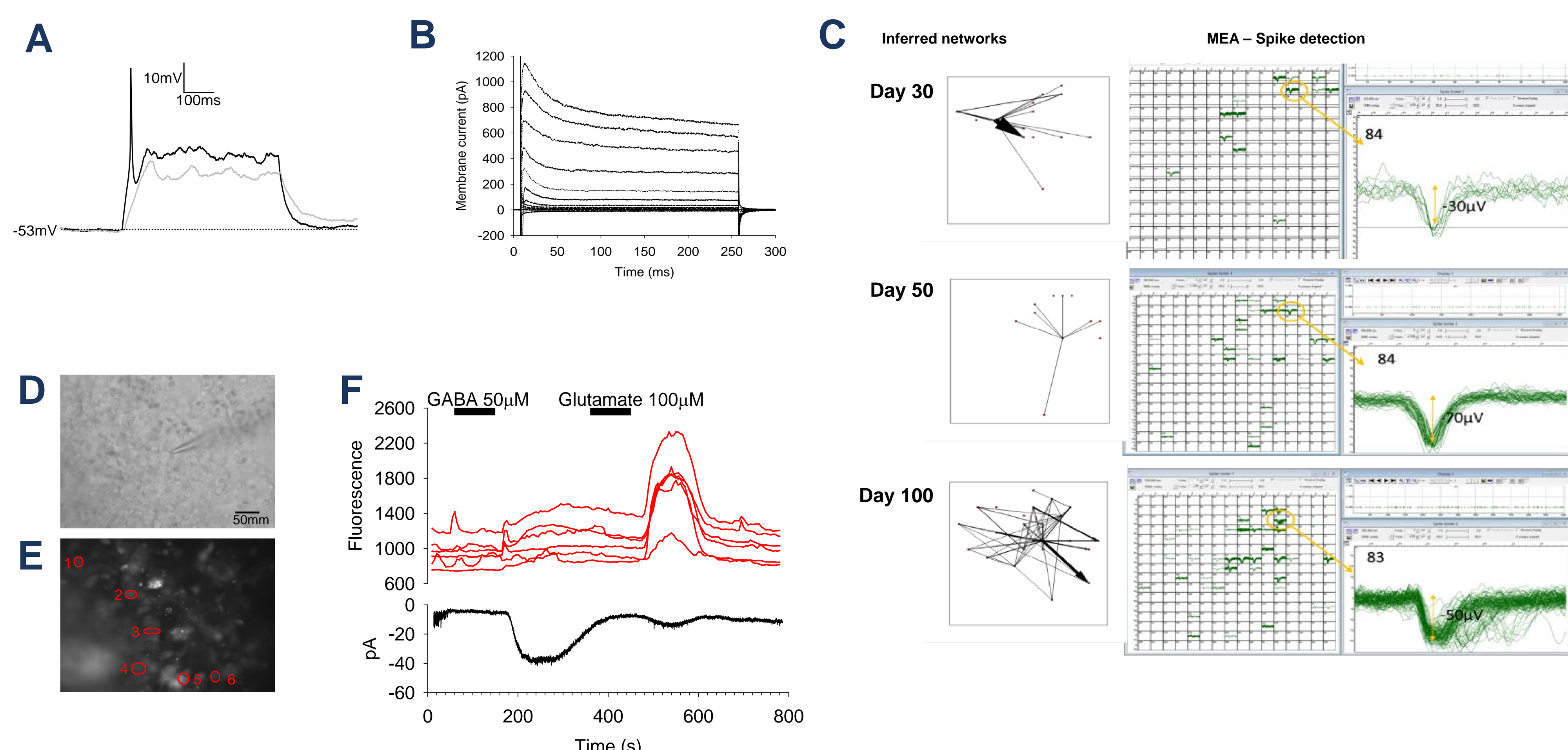


Neuronal markers are Up-regulated in hNSCs vs. iPSCs



Integration-free iPSCs were generated using an episomal vector and subsequently differentated into hNSCs using Axol's proprietary method.

Properties of iPSC-Derived Neurons & Astrocytes

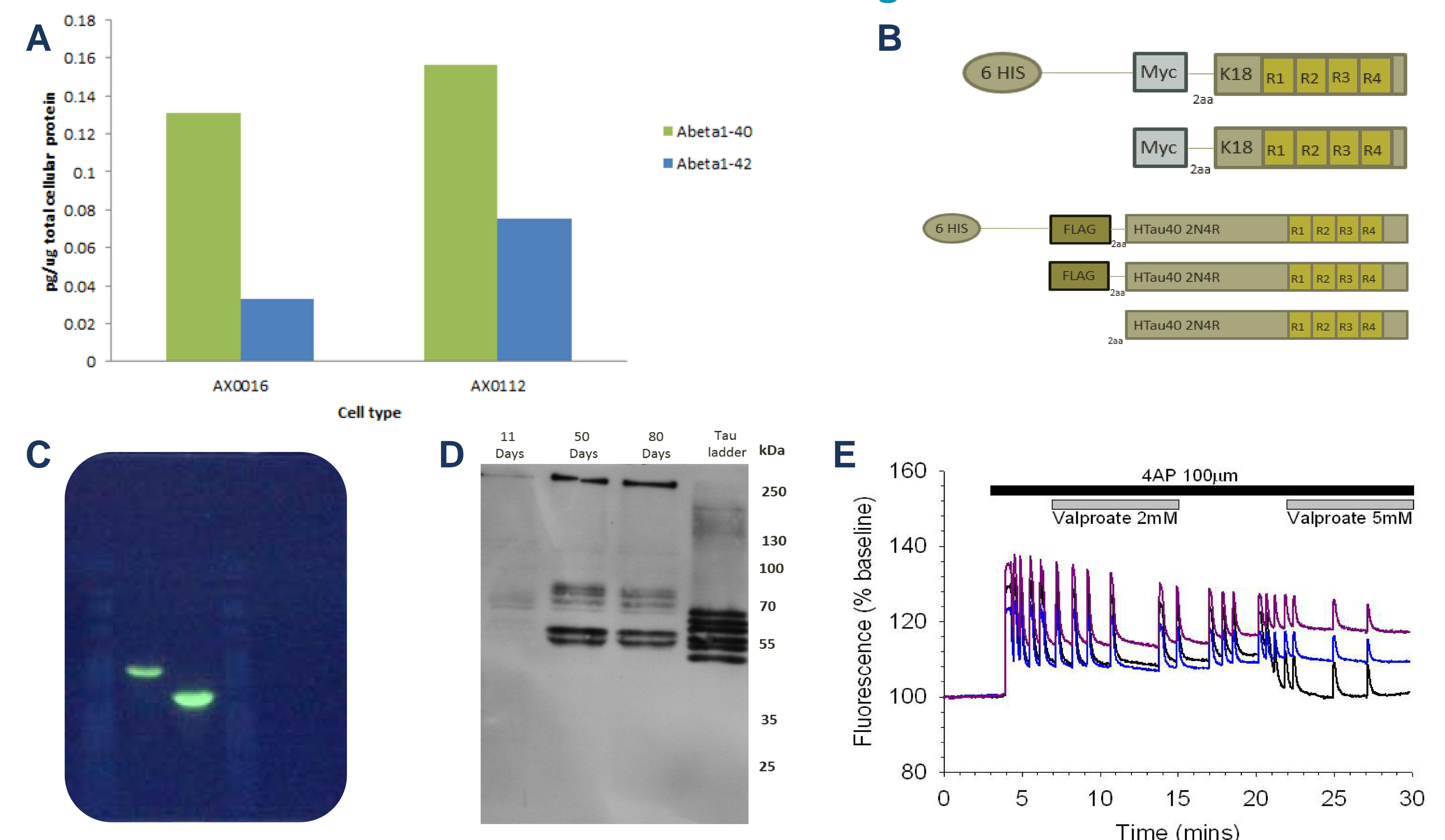


A) Voltage responses elicited by current steps in a patch clamped neuron, **B)** Current responses in an astrocyte elicited by voltage steps, **C)** Network properties of iPSC-derived neurons and astrocytes. Electrical activity in maturing networks recorded on MEAs. Changes in the rate of depolarization spikes increase overtime. These changes can be quantified as measures of network maturity, and can be used to generate entropy based connectivity maps between areas of the networks, **D)** Field of hNSC in culture showing recording pipette, **E)** Fluorescence image showing the Fluo-4 loaded neurons, **F)** Traces of fluorescence over time (from neurons circled in E) showing responses to GABA and glutamate. Trace below displays current from single neuron recorded from pipette shown in D.

Methods

We developed a number of endpoint assays using hNSCs (**Axol Bioscience**) to determine the functionality of these cells and their response to toxins or disease-relevant biomarkers in both Alzheimer's disease and epilepsy. **Cell Culture** hNSCs were obtained from **Axol Bioscience** and differentiated using neuronal maintenance media. Cells were maintained in culture for up to 12 months. **Patch Clamp** recordings made using pipettes (2–4 MΩ) with an internal solution of composition (in mM): $KMeSO_4$ 120, HEPES 10, EGTA 0.1, Na_2ATP 4, GTP 0.5. Currents recorded using a Multiclamp700B amplifier. **MEA Analysis** Cells grown on multi-electrode array dishes (Scientifica) changes in the rate of depolarization spikes overtime were quantified and used to generate entropy based connectivity maps between areas of the networks. To increase throughput of the analysis the use of multi-well MEA is feasible (Axion). **Purification & Labelling of Recombinant TAU** Recombinant TAU was expressed in *E. coli* BL21™ cells and purified using Ni affinity chromatography. Purification tags were removed by digestion with TEV protease and the purified protein labelled with the thiol reactive dye Atto 488 Malemide (Sigma UK). For uptake experiments 1µM labelled TAU was added to the medium of differentiated cells.

Disease Modeling



A) Release of amyloid peptides from patient ax0112 (presenilin-1 L286V) compared to 'normal' control (ax0016), **B)** Schematic representation of prokaryotic expression constructs used to obtain purified recombinant TAU labelled with Atto 488 Malemide visualized by SDS-PAGE **(C)**, **D)** Endogenous TAU production at different stages of neuronal differentiation, **E)** Fluorescent calcium imaging of epileptiform activity in 4AP treated iPSC-derived cortical cultures. Following treatment with 100µM 4AP, cells were treated with increasing doses of the anti-epileptic drug VPA (2mM and 5mM).

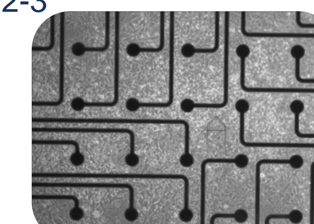
High-Throughput MEA Analysis

Simultaneous analysis of 12- or 48-well MEAs with 3 recording stations (Maestro, AxionBiosystems) allows high-throuput analysis of human iPSC cultures and test compound effects.

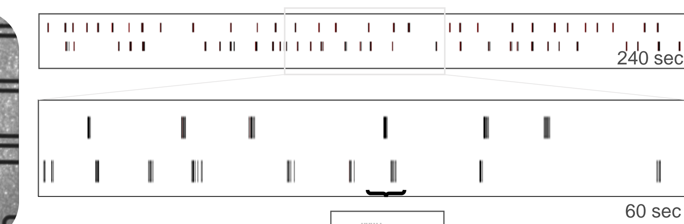
Axol iPSC Neural Stem Cells can be Differentiated into Neurons on MEAs

Example spike trains

ax0018 control
week 2-3



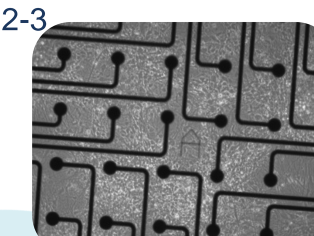
12 well MEA
with 64 electrodes



Bursts with complex burst structure

Lower frequency of compact bursts

ax0112 PS-1 mutant
week 2-3



High frequency of compact bursts

Spike train analysis

Parameters	ax0018 Control		ax0112 PS-1 mutant
Spike rate	23 s.p.m.	<	69 s.p.m.
Burst rate	3.4 b.p.m.	<	12 b.p.m.
% Spikes in bursts	63 %	<	75 %
Burst duration	200 ms	>	140 ms
Spikes in bursts	4.0	<	4.8
ISI in bursts	75 ms	>	54 ms
Frequency in bursts	28 Hz	<	116 Hz

ISI = inter-spike interval

Summary & Conclusions

The use of iPSC-derived neural cells has enabled the study of neuronal development and network maturation in physiologically relevant human models. Such models also enable the interrogation of mature cultures of neurons/astrocytes in both healthy models and those bearing disease-associated mutations. Utilizing these models obtained as neural stem cells, which routinely differentiate into functional neuronal networks, has expedited the generation of the preliminary data shown here. Carrying this research forward utilizing higher throughput MEA applications will undoubtedly provide the platform to rapidly accelerate our understanding of brain function and pathology.