Using iPSC-derived neural stem cells as a CNS model to study neuronal behaviour in development and neurodegeneration

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Introduction

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 and dopaminergic neurons, has allowed researchers to investigate the activity of neural networks from particular regions of the brain. We developed a number of endpoint assays using human iPSC-derived neural stem cells (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. We have also manipulated the cells using Lentivirus and have demonstrated long-term expression of over 9 months. The methods developed offer a platform to facilitate our understanding of normal physiological functions and the causes of central nervous system (CNS) pathology.

Results

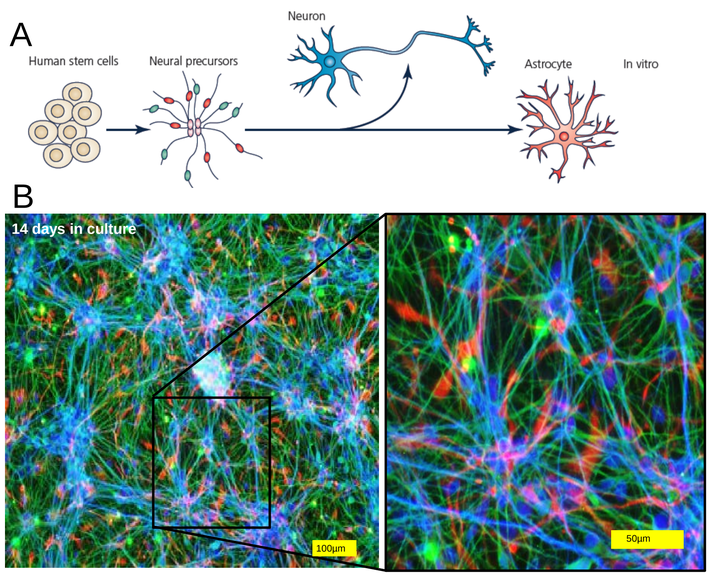

Fig 1. Cortical differentiation of hiPSC cells. A) Schematic of neuronal differentiation. B) Immunofluorescent image of 14 day old cortical neurons and astrocytes B-tubulin (green)/ Neurofilament (Blue)/ GFAP (red).

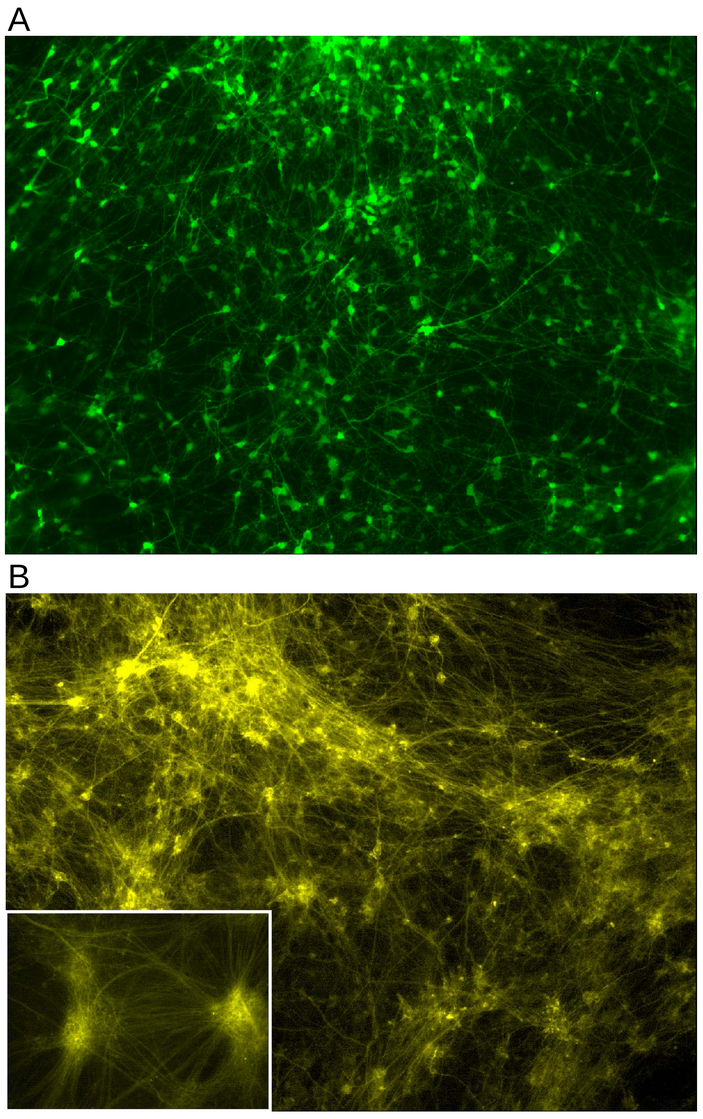

Fig 2. Lentiviral transduction of differentiated cultures for long term expression. A) GFP expression in neurons and astrocytes 100 days post transduction with lentivirus encoding GFP under the control of the CMV promoter (MOI 5). B) Neuronal expression of the channelrhodopsin (ChR2-E123T-T159C-EYFP) under the control of the CamKii promoter in 50 day old differentiated cultures, 9 days post transduction and inset in 180 day old differentiated cultures 80 days post transduction (MOI 5).

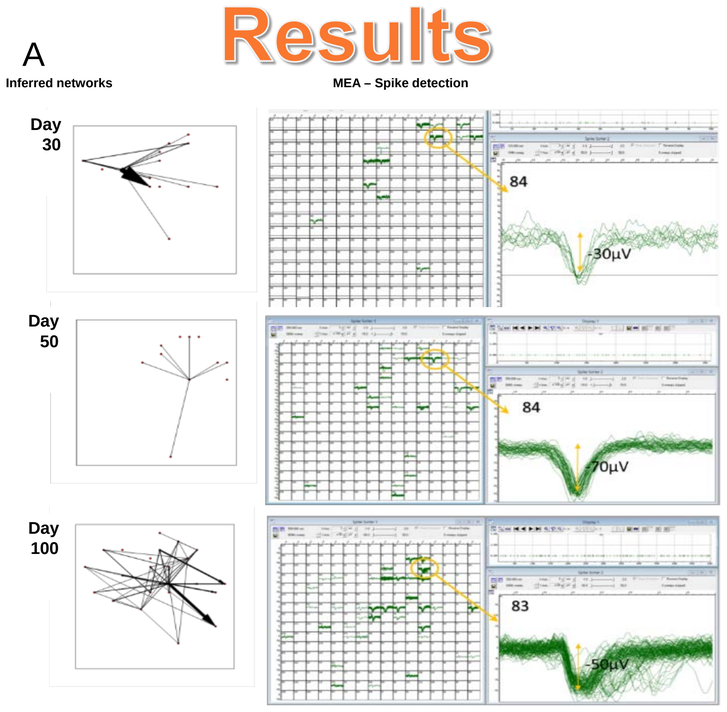

Fig 3. Network properties of iPSC-derived neurons and astrocytes. Electrical activity in maturing networks recorded on MEAs. Changes in the rate of depolarisation spikes increase overtime. These changes can be quantified as network measures of maturity, and can be used to generate entropy-based connectivity maps between areas of the networks.

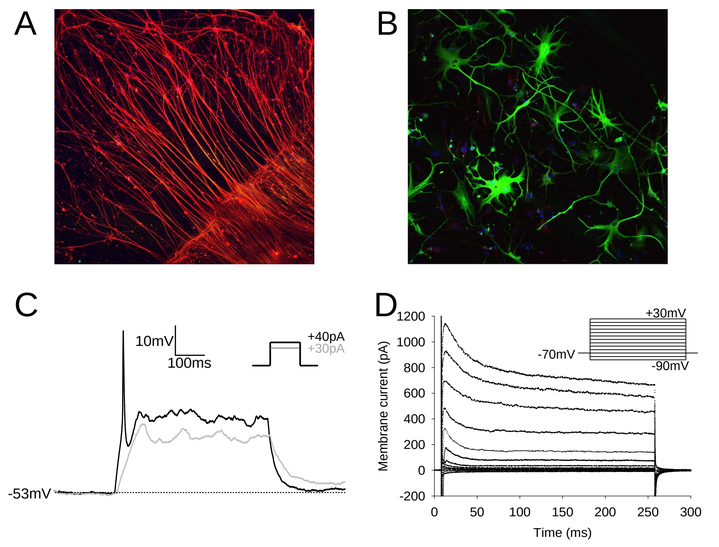

Fig 4. Properties of iPSC-derived neurons and astrocytes. A) Immunofluorescent image of cortical neurons. B) Immunofluorescent image of pure astrocytes C) Voltage responses elicited by current steps in a patch clamped neuron. D) Current responses in an astrocyte elicited by voltage steps.

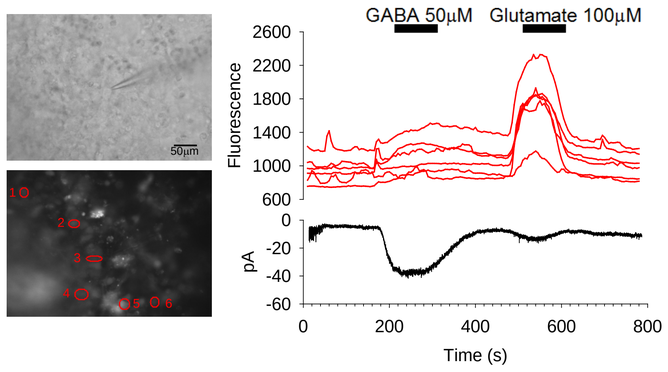

Fig 5. A) Field of hNPCs in culture showing recording pipette. B) Fluorescence image showing the Fluo-4 loaded neurons. C) Traces of fluorescence over time (from neurons circled in B) showing responses to GABA and glutamate. Trace below displays current from single neuron recorded from pipette shown in A.

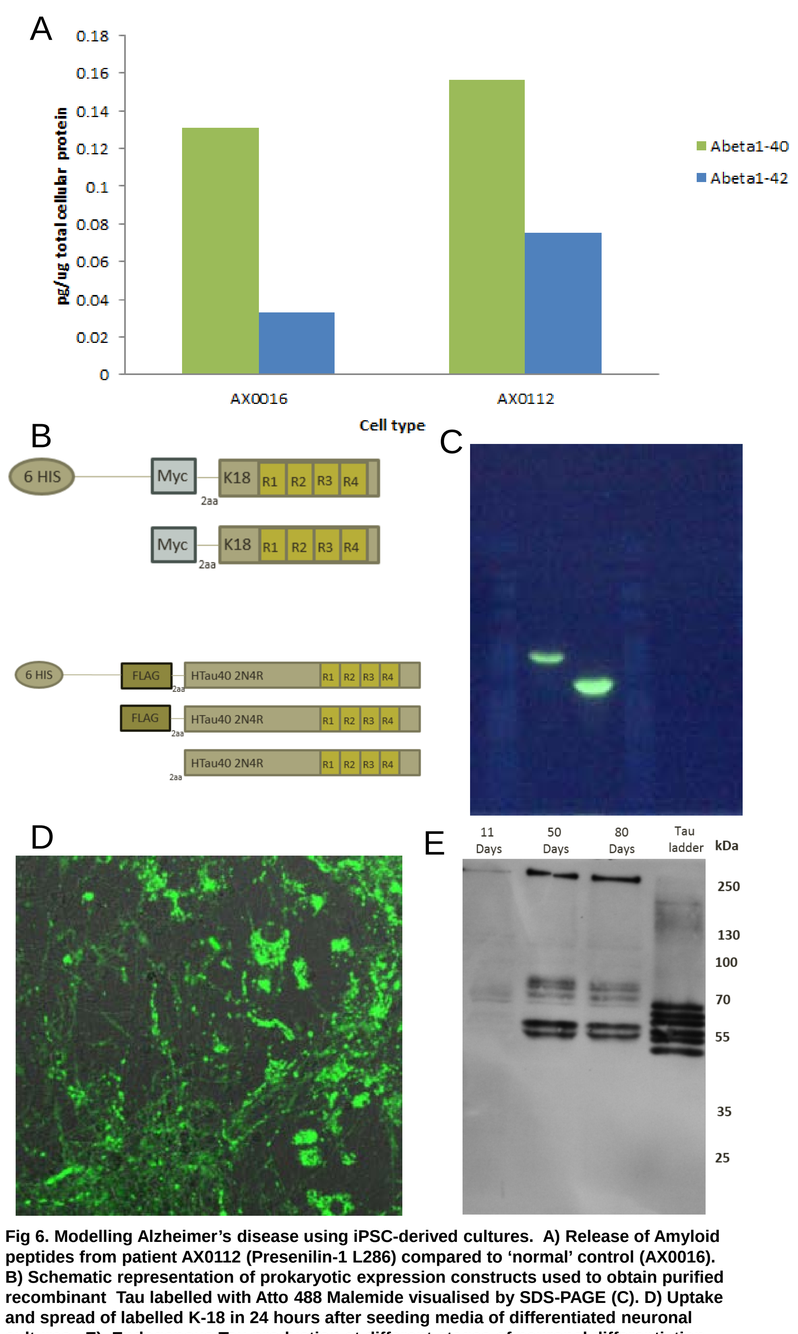

Fig 6. Modelling Alzheimer's disease using iPSC-derived cultures. A) Release of Amyloid peptides from patient AX0112 (Presenilin-1 L286) compared to 'normal' control (AX0016). B) Schematic representation of prokaryotic expression constructs used to obtain purified recombinant Tau labelled with Atto 488 Malemide visualised by SDS-PAGE (C). D) Uptake and spread of labelled K-18 in 24 hours after seeding media of differentiated neuronal cultures. E) Endogenous Tau production at different stages of neuronal differentiation.

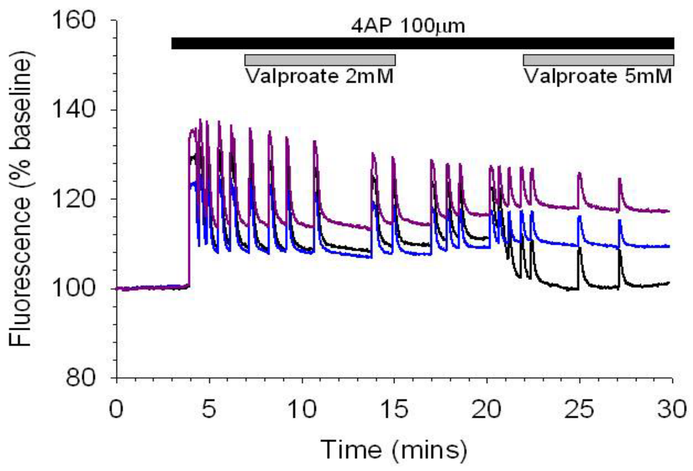

Fig 7. Fluorescent calcium imaging of epileptiform activity in 4AP treated iPSC-derived cortical cultures. Following treatment with 100µM 4AP cells were treated with increasing doses of the anti-epileptic drug VPA (2mM and 5mM).

Conclusions

The use of iPSC-derived neuronal models has enabled, in human relevant models, the study of neuronal development, and neuronal / neuronal network maturation. Such models also enable the interrogation of mature cultures of neurons/astrocytes in both healthy models and those bearing disease-associated mutations. Utilising these models obtained as neural precursor cells, which routinely differentiate into functional networks has expedited the generation of the preliminary data shown here. Allowing rapid testing and development of new techniques. The application of emerging technologies such as genome editing, next-generation sequencing and high-throughput image/activity analysis in conjunction with such models will undoubtedly provide the platform to rapidly accelerate our understanding of brain function and pathology.

Methods

Cell Culture: hNPC cells were obtained from Axol Biosciences (UK) and were 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 depolarisation spikes overtime were quantified used to generate entropy based connectivity maps between areas of the networks.
Lentiviral Transduction: Differentiated cultures were transduced by the addition of purified virus in PBS (at stated MOI's) directly to the media, after overnight incubation excess virus was removed by washing the cells in neural maintenance medium.
Purification and 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.