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

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.

Conclusions

The use of iPSC-derived neuronal models has enabled, in human relevant models, the study of neuronal development, and neuronal / neuronal network maturation. 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 neural maintenance media. Cells were maintained in culture for up to 12 months.

MEA Analysis: Cells grown on multi-electrode array dishes (Scientific) changes in the rate of depolarisation spikes over time were quantified used to generate entropy-based connectivity maps between areas of the networks.

Patch Clamp: Currents recorded using a Multiclamp700B amplifier.

Purification and Labelling of Recombinant Tau: Recombinant Tau was expressed in E. coli (strain 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 Maleimide (Sigma UK). For uptake experiments 1µM labelled Tau was added to the medium of differentiated cells.