Grow Your Own Brain Cells: Neuronal Differentiation as a Tool for Therapeutic Development

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Introduction

Neurological conditions contribute significantly to global disease burden, with prevalence set to rise alongside ageing populations. Not only do these diseases account for 9 million deaths a year globally, but those suffering from such conditions deal with severe impacts on their daily lives. When studying these diseases for therapeutic development, it is vital to have access to physiologically relevant systems amenable to assay development. This allows for investigation of potential leads, and for discarding of inappropriate compounds, in a disease-appropriate model as early as possible. However, this approach can be challenging when considering neurological diseases, as neuronal cells are non-proliferative and challenging to handle.

Neurons can be obtained as primary cells, which can be costly, or through the differentiation of iPSCs (induced pluripotent stem cells) into neurons across multiple weeks. The latter method, whilst challenging, is a useful option when developing assays for compound screening, as it can provide a stock of proliferative progenitor cells from which to continually produce differentiated neuronal cells. At Charnwood Discovery, we have experience of working with iPSC-derived neural stem cells, differentiating them into mature neurons for the development of various assays. This is just one of our many capabilities within our fully integrated drug discovery service, that allows us to bring huge value to our clients' early-stage drug discovery projects.

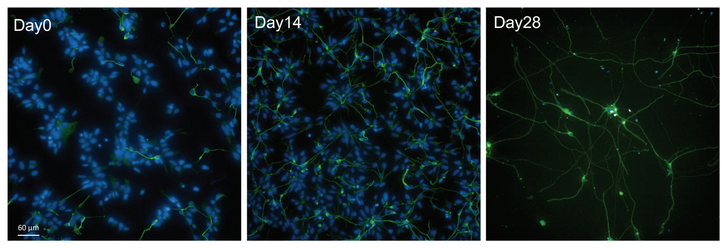
Target Identification & Validation → **Assay Development** → **Hit Identification** → **Hit to Lead** → **Lead Optimization** → **Development & Process Chemistry**

Our neuronal capabilities include:

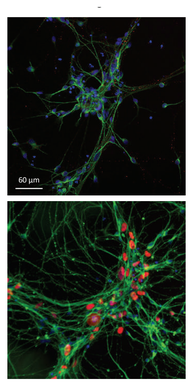
- High Content Imaging and In-Cell Western Assays
- FLIPR Calcium Assays
- Flow Cytometry
- Capillary-based Immunoblotting (Bio-Techne Jess)
- qPCR
- Other plate-based assays

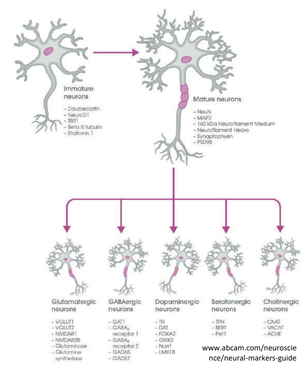
Differentiation of Neurons from iPSC-derived Neural Stem Cells

iPSC-derived neural stem cells are differentiated into neurons across 28 days. After seeding at day 0, cells were cultured in differentiation medium for 14 days, before the addition of Ara-C (a compound toxic to dividing cells), to remove any remaining undifferentiated cells (Ara-C removed on day 19). Neurons were then further matured in differentiation medium until at least day 28.



iPSC-derived neural stem cells differentiated over 28 days, stained with the neuronal-specific marker ß-iii-Tubulin (green) and Hoechst (blue).



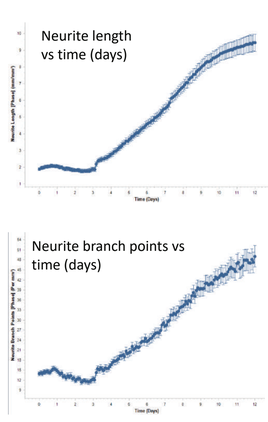


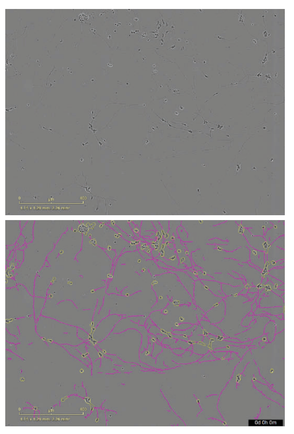
28-day differentiated neurons. Top image labelled with MAP2 and vGLUT1; Lower with ß-iii-Tubulin and FOXA2.

Characterization of neurons can be achieved through flow cytometry and high content imaging, staining for mature neuronal markers (such as Microtubule associated protein 2 – MAP2) and neuronal lineage specific markers (such as the vesicular glutamate transporter vGLUT1 for glutamatergic neurons, or transcription factor FOXA2 for dopaminergic neurons).

Tracking Neuronal Differentiation by Live-cell Imaging

At Charnwood Discovery, we can monitor the differentiation process in real time using the IncuCyte SX5 live cell imaging system, tracking metrics such as neurite length, branch points and cell body area.



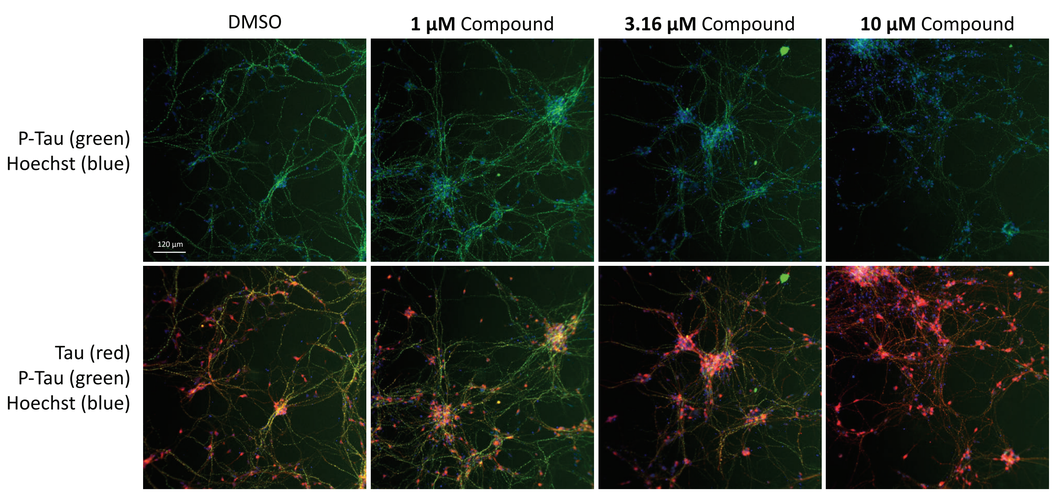


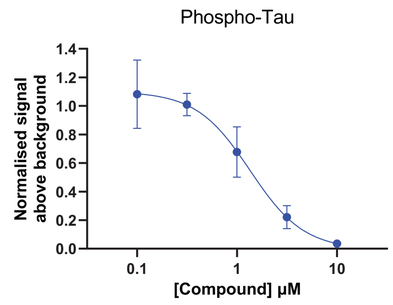
Images and associated graphs of neurons captured on the IncuCyte SX5, displayed with and without overlayed analysis mask - neurites in pink and cell bodies in yellow.

Scan the QR code to watch our video of neurons differentiating in real time.

Investigating Neurological Diseases Through High Content Imaging and Immunoblotting

Pathological deposits of hyperphosphorylated Tau is a hallmark of neurodegenerative tauopathies, including Alzheimer's disease. Here we use high content imaging to track phosphorylation levels of Tau in neurons treated with different concentrations of a kinase inhibitor. This powerful technique can provide an insight into how compounds interact with targets at the subcellular level within cell-based, disease-relevant assays.

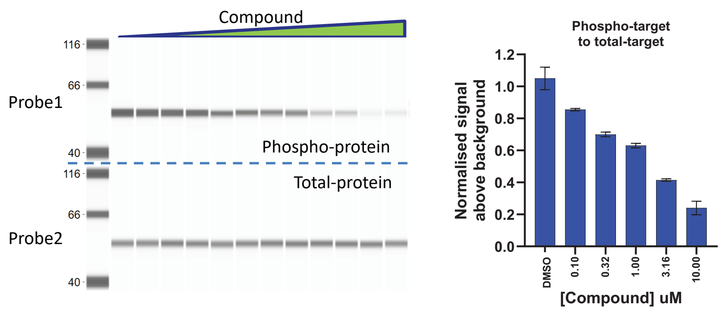




Representative images of differentiated neurons stained with P-Tau (green), Tau (red) and Hoechst (blue), dosed with a kinase inhibitor. Graph shows data for full dose response.

As an orthogonal assay for tracking phospho-protein levels, we perform immunoblotting using the capillary-based Bio-techne Jess system. Targets can be multiplexed or probed by Replex (the equivalent of stripping and re-probing), allowing comparison of Phosphoprotein to total protein levels.

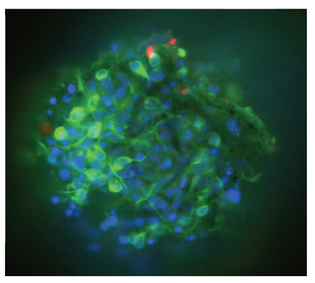
Here we show the reduction in levels of a phosphorylated protein across a compound dose response with probe 1, normalized to the total levels of the protein with probe 2 after stripping of probe 1.



Pseudo-blot of neuronal lysates probed for phospho- and total levels of a target protein. Graph shows phospho-signal over background normalized to total.

Differentiation of Neurons in 3-D

Another exciting area in the drive for physiological relevance is the ability to grow cells as 3-dimensional systems. At Charnwood Discovery, we can do this either in ultra-low attachment plates or using the CelVivo ClinoStar rotating incubator. Cells can be grown as spheroids or attached to magnetic scaffolds for easy manipulation.



Z-slice of iPSC-derived neural stem cells, differentiated for 2-weeks on a 400 µM diameter, magnetic scaffold and stained with with ß-iii-tubulin (green) and Hoechst (blue). Cells can be seen growing around the darker strands of the scaffold.

Summary and Conclusion

At Charnwood Discovery we have successfully developed a suite of assays to screen compounds against neuronal cells differentiated in-house from iPSC-derived neural stem cells.

With this and our many other services and capabilities across bioscience, chemistry and DMPK/ADME, we can progress your pre-clinical drug discovery project with robust, disease-relevant data.