Characterization of axoCells™ iPSC-derived cortical inhibitory interneurons

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Abstract

Interneurons are the neuronal cells responsible for regulating the relay of electrical impulses between sensory neurons, motor neurons and the Central Nervous System (CNS). Interneurons synchronise neural network activity due to their capability to form highly specific, gap-junction mediated electrical synapses.

Using a small molecule monolayer differentiation technique, Induced Pluripotent Stem Cells (iPSCs) can be differentiated into interneuron progenitors, which can then be matured to functional activity regulating interneurons.

Using immunocytochemistry, RNA sequencing, multi-electrode array and neuronal activity assay profiling, axoCells™ cortical inhibitory interneurons were characterized to be used as an unlimited source of such cells for drug discovery and modelling neurodegenerative diseases.

In addition, we are also able to demonstrate that putting these cells in co-culture models with cortical excitatory neurons and astrocytes provides a powerful humanized model for pre-clinical drug discovery in the neuroscience field.

The axoCells iPSC-derived cortical inhibitory interneurons are a mixed population expressing a multiple of different subtypes such as Parvalbumin, Somatostatin, Tyrosine Hydroxylase and NKX2.1, the key transcription factor determining interneuron cell fate.

The interneurons are also GABAergic in nature, displaying markers for GAD2 and gamma-Aminobutyric acid (GABA).

Interneuron progenitor cells are provided cryopreserved. These cells can be differentiated and matured with Axol's custom media and can also be used alongside our neural stem cell to cortical neuron differentiation.

Methods

Cell culture:

Cell culture of iPSC progenitor cells to mature cell types was performed using Axol Bioscience user protocols and media. The maturation of interneurons from progenitor cells takes place over 20 days from thawing using our *Enriched Cerebral Cortical Neuron* User Guide.

Immunocytochemistry:

Cells were matured until day 20 before fixation. Immunostaining was conducted using standard PFA fixing, 0.3% Triton, and commercially available antibodies.

RNA sequencing:

Cells were matured to day 20 before lysing.

MEA Recording:

Extracellular field potentials were acquired at 37°C, 5% CO_2 using the Axion Maestro Pro high-throughput MEA platform. 48-wells plates with a 350µm separation between each well-floor mounted electrode were used, enabling simultaneous recording of extracellular potentials from 16 electrodes per well at a sampling rate of 20kHz/channel. Neural stem cells and interneuron progenitors were then plated and matured *in situ*.

Spontaneous Neuronal Activity (SNA) Recording:

Monocultures of interneurons and cortical neurons and a co-culture of 10:1 cortical neurons and interneurons were transfected at day 7 post-thaw with Incucyte® Neuroburst Orange Lentivirus, encoding a calcium reporter driven from the synapsin promoter. Spontaneous activity was recorded using Incucyte® Neuronal Activity Analysis Software on an Incucyte® S3.

Results

Expression of axoCells interneuron markers by immunocytochemistry: healthy control cell line (ax0667)

Parvalbumin-expressing interneurons make up the largest subtype of interneurons expressed in the neocortex at around 40% of the population and are responsible for generating network oscillations, targeting excitatory neurons, and receiving excitatory inputs from the thalamus and cortex. Somatostatin-expressing interneurons, the other major subtype which makes up around 30% of all interneurons, facilitate input from excitatory cells and target dendritic tufts in an inhibitory manner.

Axol interneurons are also GABAergic in nature, releasing gamma-aminobutyric acid (GABA), a neurotransmitter which acts as an inhibitor of synaptic activity. Through control of Cl- intake and K+ release, the action potential of the targeted cell is modulated. Therefore GABA, along with GAD2 which encodes its protein catalyst, is a key marker for functionality.

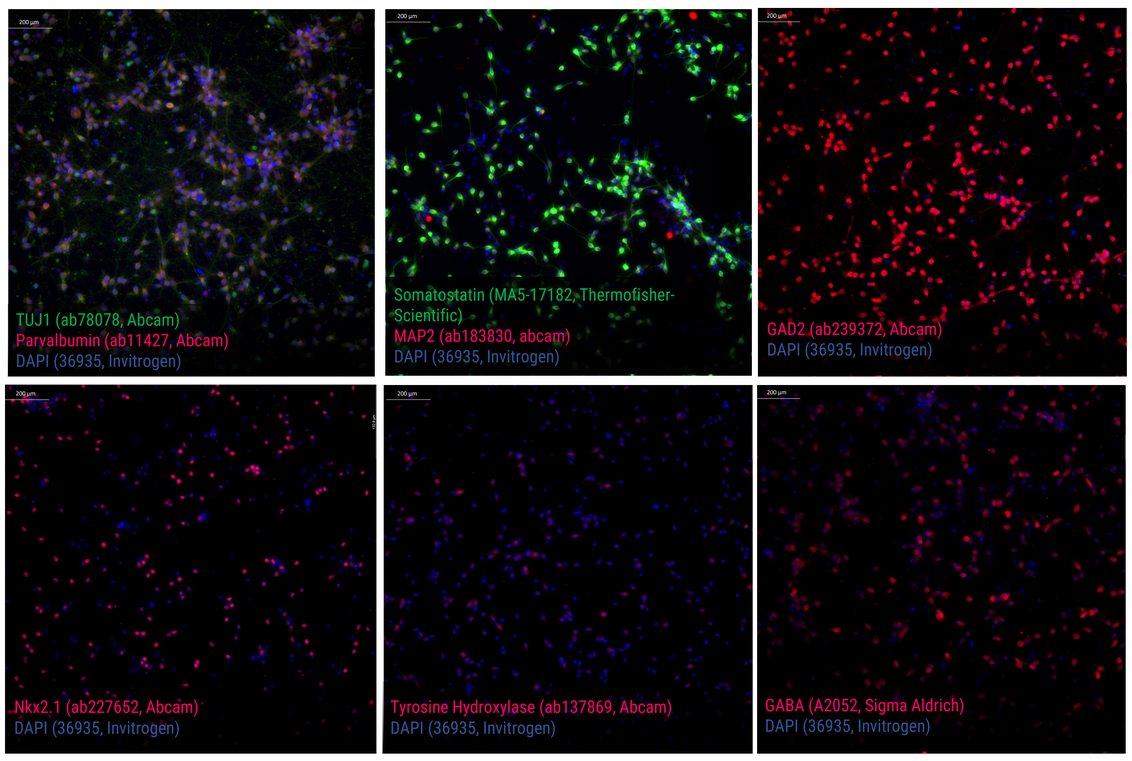


Figure 1. Immunocytochemistry using interneuron markers on axoCells interneurons ax0667 and ax0662 after 20 days of maturation

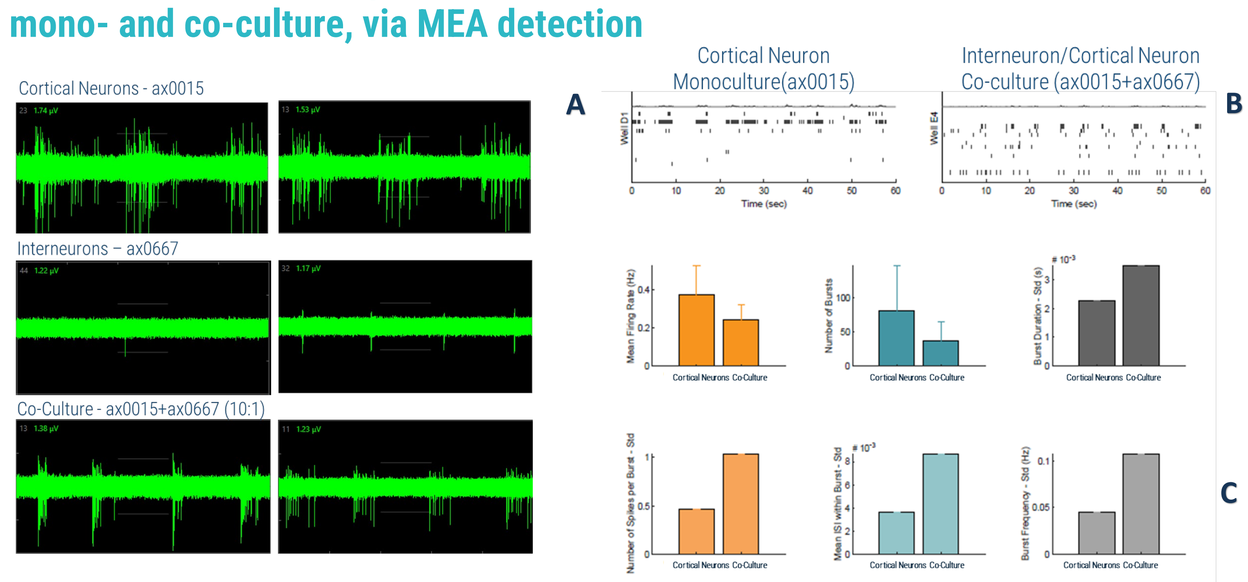
RNA sequencing data showing relative gene counts for RNA from iPSCs, neural stem cells, interneuron progenitors and mature interneurons

Gene	Cell Line 5 (iPSC)	AX0015 (NSC) D3	ax0667 (INs) D3	ax0667 (INs) D20
NKX2-1				
GAD2				
FOXG1				
MAP2				
GABARAPL2				
SST				
PVALB				
SOX1				
TH				

Key: Low / High

Figure 2. RNA sequencing data for iPSC line 5, neural stem cells (ax0015), and interneuron line ax0667 at Days 3 and 20 of maturation, showing increased relative expression of interneuron markers NKX2.1 in the interneuron progenitors compared to the neural stem cells, and increased GABARAPL2, GAD2, Tyrosine Hydroxylase, Somatostatin, and Parvalbumin in the mature cells.

Measurement of activity of axoCells™ interneurons and cortical neurons in mono- and co-culture, via MEA detection



Burst Duration	Average time from first spike to last spike in a single electrode burst
Mean Firing Rate	Total number of bursts/Duration of analysis
Number of Bursts	Total number of bursts over the duration of the analysis
Number of Spikes per Burst	The average number of spikes in a single electrode burst
Mean ISI within Burst	Mean time between spikes in a single electrode burst
Burst Frequency	Total number of single electrode bursts/Duration of analysis

Figure 3. Interneuron characterisation by electrophysiology.
A) MEA recordings on Day 30 of maturation post thaw from axoCells cortical neuron (ax0015) monoculture plated at 150,000 cells per cm^2, interneuron (ax0667) monoculture plated at 150.000 cells per cm^2, and interneuron and cortical neuron co-culture, with cortical neurons plated at 96.000 cells per cm^2, and interneurons plated at 9600 cells per cm^2. B) Analysis of MEA recording for cortical neuron and cortical/interneuron co-culture C) Table showing endpoint definitions for the graphs in B.

Measurement of axoCells™ inhibitory interneurons and cortical neurons activity in mono- and co-culture, via Neuroburst Orange® and Incucyte S3®

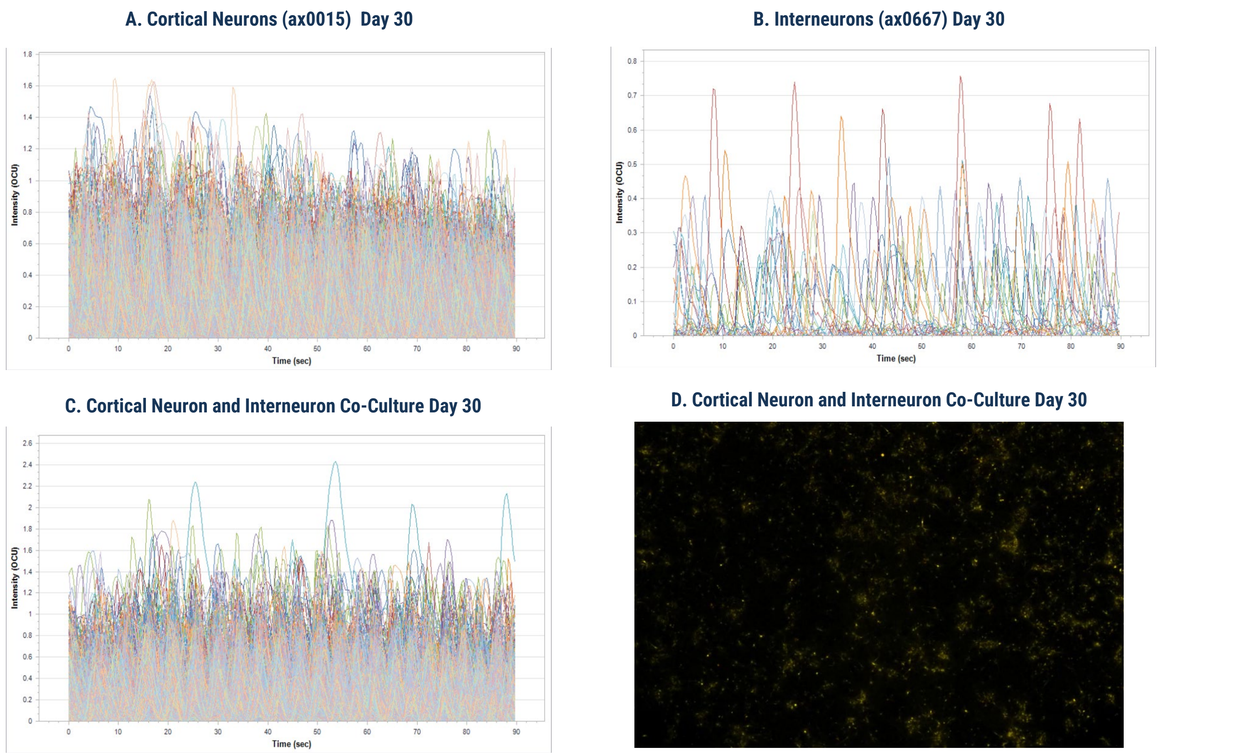


Figure 4. Analysis from recording of spontaneous neural activity.
Incucyte® Neuroburst Orange lentiviral reagent was used to image neuronal firing after 30 days of maturation post-thaw. A) axoCells cortical neuron (ax0015) monoculture plated at 150.000 cells per cm^2 B) axoCells interneurons (ax0667) monoculture plated at 150.000 cells per cm^2 C) Interneuron and cortical neuron co-culture with cortical neurons plated at 96.000 cells per cm^2 and interneurons plated at 9600 cells per cm^2. D) Representative image from recording of spontaneous neural activity for the interneuron and cortical neuron co-culture (4x). These images show small scale spontaneous activity from the interneurons and more intense bursting from the cortical neurons and co-culture, with the co-culture being more synchronous.

Conclusion

In conclusion, cortical inhibitory interneurons differentiated from iPSCs according to Axol's small molecule monolayer differentiation technique display key interneuron markers such as NKX2.1, Parvalbumin, Somatostatin, and Tyrosine Hydroxylase, as shown through immunocytochemistry and RNA sequencing.

axoCells cortical inhibitory interneurons were characterized using multi-electrode array and the Incucyte® Neuroburst Orange Lentivirus, showing functionality through the development of synchronous, spontaneous firing in the cortical neuron and interneuron co-culture at day 30 of maturation, in comparison to the less regulated cortical neuron monoculture. The co-culture showed bursting of less frequency but greater duration then the cortical neuron monoculture, as shown in figure 3, displaying the inhibitory effect of the GABAergic interneurons.

References

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