iPSC-derived Glutamatergic Neurons: Something to Get Excited About!
Cyprotex Overview

Contract Research Organisation providing ADME-Tox, DMPK & Biosciences services

Focused on support for pharmaceutical industry, agrochemical industry, tobacco industry, cosmetics and personal care industry and chemical industry

• Founded in 1999
• Acquired in 2016, wholly owned subsidiary of Evotec AG
  – Cyprotex laboratories in Alderley Park (UK), Kalamazoo and Boston (US)
  – Over 1,600 customers worldwide with exceptional client retention rate
  – Focus on ADME-Tox/PK services
    ▪ In vitro ADME-Tox screening
    ▪ In vitro regulatory ADME-Tox services
    ▪ Internal PBPK modelling expertise
Cyprotex is now part of Evotec AG

Evotec’s worldwide operations
Cyprotex Capabilities

What we do ...

- **100% focus on pre-clinical ADME-Tox/DMPK** (absorption, distribution, metabolism, excretion, toxicity, pharmacokinetics) and **bioscience models**
  - *In vitro, in silico* and *in vivo*

- **Highly automated** screening platform
  - Cost-effective, fast turnaround (5–10 days), reduced human error
  - Robust, industry-validated protocols following regulatory guidelines

- **Support for later stage IND/NDA enabling studies**
  - Full *in vitro* DDI package according to FDA/EMA guidelines
  - Metabolite profiling and structural elucidation using high resolution accurate mass spectrometry
  - *In vitro* GLP genotoxicity studies

- **Dedicated Project Managers**
  - ADME-Tox experts from big and small pharma, academia, CROs
  - Scientist-to-scientist communication, commentary, advice, support, guidance, discussion

- **Over 1,600 customers worldwide**, with exceptional client retention rate
Expanded Capabilities through Evotec
Stand-Alone and Integrated Drug Discovery
Cyprotex Capabilities

Our Differentiators

- **Consultative** interactive approach for small biotech and start-ups – guidance
- **Combination of *in vitro* data and *in silico* modelling** to maximise value of data and predict clinical outcome – not just a data provider
- **Responsive**, agile, focused on delivery and quality
  - Response to enquiries within 2 hours
  - Full study proposal provided within 24 hours
  - Fast turnaround times
- **Knowledge sharing and research**
  - Internal R&D, publications and posters
  - Development of novel approaches and solutions in conjunction with clients
    - **Unique Technologies ...**
  - Cyprotex free educational guides (ADME, Tox, DDI and Chemical and Cosmetics Testing guides) [www.cyprotex.com/guides](http://www.cyprotex.com/guides)
  - Presentations, webinars, training sessions
eCiphrNeuro: Predicting Neurotoxicity

- Assay for neurotoxicity developed using primary rat cortical neurons
  - Capable of predicting seizure inducing compounds as well as other neuroactives and neurotoxins
- The primary rat cortical neurons have sufficient baseline activity, can network with each other, and are inducible with test agents
  - Able to measure spontaneous action potentials in neurons and determine what changes a compound may cause
  - Same distribution of cells and neurochemistry as found in native brain.
  - Cells isolated with a native dissociation so the cell distribution is not altered
  - In addition to neurons, you have astrocytes (glia) and microglia also present
Maestro Platform

The Maestro
Axion’s 768 channel system
- 768 stimulating and recording channels
- SBS-Compliant Multiwell MEA plates
- Accommodates 12, 48 and 96 wells
- Fast MEA insertion with auto MEA-plate identification
- Ultra-thin, transparent, & affordable MEA plates
- Fully integrated heater with software controls
- Automated electrode characterization & diagnostics

48-Well
- Higher throughput 48-well configuration
- 16 low-noise microelectrodes per well
- 4 integrated ground electrodes per well
- Polymer (Kapton) insulation
- Nano-textured Gold electrodes
- ANSI compliant well plates
- Evaporation-reducing lid
Characteristics of Axion Biosystem’s Array

- Grid of tightly spaced electrodes, and each electrode is capable of simultaneously monitoring and stimulating the activity of >12 cells
- The arrangement of multiple electrodes in a grid extends the recording range across a relatively large area, providing concurrent access to both single cell and network-level activity
- The control and monitoring of this cellular activity is made possible by the electronics, which impart multiple functions to each electrode

Axion Biosystem’s Array (electrodes are 30 µm in diameter and are spaced 200 µm apart; this image is representative of a 12-well system)
Cells growing on MEA
Criteria for monitoring of neural activity
The advantages of MEA

- Label-free, non-invasive operation to observe natural cell function
- Preservation of cellular interconnectivity
- Real-time measurement of the phenotypic signal: voltage
- Sufficient resolution to capture action potentials
- Multiple recording sites to assay action potential propagation
Burst Endpoint Characterization

- Irregular burst duration and organization
- Irregular interburst intervals
- Individual spikes
- Longer burst durations
- Regular interburst intervals
- Increased burst organization
- Increase in burst duration regularity
- Most spikes occur within bursts

Picrotoxin Post-Dose
Synchrony Endpoint

Changes in synchronous activity are observed below

Picrotoxin Post-Dose
eCiphrNeuro: Predicting Neurotoxicity
Can we make a human eCiphrNeuro?

- Although rat cortical neurons are highly predictive of neurotoxicity, there is a desire in the industry to have a human model for prediction.
- With the advent of stem cell derived neurons, this became a possibility although early hiPSC derived neuronal models lacked complex burst organization, making electrophysiological neurotoxic prediction challenging when utilizing an MEA platform.
- Recent advancements in hiPSC neuronal models have addressed these disadvantages with the introduction of multiple neuronal cell types.
- CDI has created iCell Glutaneurons, iPS cell-derived human cortical neurons consisting primarily of 90% glutamatergic (excitatory) neurons.
- We will show here our initial evaluation of these cells, both alone as well as with iCell Astrocytes and iCell Neurons.
Methods

- iCell GlutaNeurons were plated at 120,000 cells per well directly over the electrode grid in a 48-well MEA plate pre-coated with PEI and laminin.

- iCell GlutaNeurons were also plated in combination with iCell Astrocytes (13%) and with iCell Astrocytes (14%) / iCell Neurons (14%) for a total of 120,000 cells per well for each plating condition.

- Cells were maintained for ~14 days by changing 50% medium 3 times a week.

- Recordings were acquired on the Axion Biosystems’ Maestro periodically throughout the maintenance period to document the maturation process.

- Recordings were also acquired immediately before compound treatment (baseline) and 1 hour post treatment.

- Custom MATLAB scripts were used to analyze the spike trains. Endpoints reported include: firing rate, burst rate, number of spikes in bursts, percent isolated spikes, ISI CV, normalized IQR burst duration, burst duration, interburst interval, IQR/median ISI, skewness ISI, median/mean ISI and median ISI.

- Raster plots were generated with Axion BioSystems’ Neural Metric Tool.
Maturation: iCell GlutaNeurons

Day 4, 12 and 15
Maturation: iCell GlutaNeurons / iCell Astrocytes

Day 6, 9 and 15

[Graph and data analysis]
Maturation: iCell GlutaNeurons / iCell Astrocytes / iCell Neurons

Day 5, 9 and 13
iCell Glutaneurons show great promise as a human iPS cells with good burst organization and network organization

- They start out with significant firing activity almost from plating with no real network organization
- After 9 days you start to see some bursting
- Soon after this networks start to form and you see some degree of synchrony

Addition of other cell types such as astrocytes appears to help to speed up the maturation process

- Firing patterns appear to be more mature at same time point
- Firing rate may be a little lower but in our experience organization of the firing is the real key

Analysis of data on MEA

- Due to the significant firing rates and the long bursting phenotype, adjustments need to be made to analysis
- Need to set the spike threshold to a fixed amplitude because the long bursts tend to cause the adaptive threshold to slide up because it begins to see them as noise
- We have had to focus on identifying some new endpoints as our original endpoints developed for rat neurons are not always effective with the patterns generated with iCell Glutaneurons
Pharmacology: Neurotoxicity Prediction
Pharmacology: iCell GlutaNeurons
0.2% DMSO (vehicle)
Pharmacology: iCell GlutaNeurons
4-aminopyridine

Baseline

50µM 4-aminopyridine
Pharmacology: iCell GlutaNeurons
SNC80
Pharmacology: iCell GlutaNeurons

Strychnine
Pharmacology: iCell GlutaNeurons
Gabazine and Picrotoxin
Pharmacology: iCell GlutaNeurons / iCell Astrocytes / iCell Neurons
4-aminopyridine

Baseline

50μM 4-aminopyridine
Pharmacology: iCell GlutaNeurons / iCell Astrocytes / iCell Neurons
SNC80

Baseline

10µM SNC80
Pharmacology: iCell GlutaNeurons / iCell Astrocytes / iCell Neurons

Strychnine

![Graphs showing the effect of Strychnine on GlutaNeurons, Astrocytes, and Neurons.]
Pharmacology: iCell GlutaNeurons / iCell Astrocytes / iCell Neurons
Gabazine and Picrotoxin
Pharmacology: iCell GlutaNeurons / iCell Astrocytes

4-aminopyridine
Pharmacology: iCell GlutaNeurons / iCell Astrocytes

SNC80

Baseline

5μM SNC80
Pharmacology: iCell GlutaNeurons
Pilocarpine

Baseline

100μM Pilocarpine

Baseline

12.5μM Pilocarpine
Pharmacology summary

- iCell Glutaneurons have significant and robust responses to many compounds with seizure liability

- GABA A antagonists do not seem to have the same robust response that we see in rat cortical neurons
  - More work will be done to try and tease this out as there may be some changes
  - May need to adjust cell content or possibly timing of the experiments

- For other non GABA A antagonist targets, we get robust responses with all combinations of cell types tested.
  - Astrocytes appear to enhance the response to the compounds
  - iCell neurons do not interfere with responses but also may not enhance them
  - Different compounds give different firing phenotypes. It will be interesting to see if these phenotypes correspond to a target that is engaged

- Steps moving forward
  - Narrow in on iCell Glutaneurons with iCell Astrocyte combination
  - Test more compounds
  - Try and determine if there are some changes that can be made to pick up GABA antagonists more robustly
  - Modify our analysis software to make sure we catch all of the finer details of the responses to compounds
Acknowledgements

- Cyprotex
  - Jenifer Bradley

- CDI
  - Blake Anson
  - Susan DeLaura
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