Identifying Seizures with MEA: Complementary Human and Rat Neuronal Models Enhance Predictivity

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Abstract

Predicting the seizurogenic and neurotoxic potential of test compounds using microelectrode array (MEA) platforms is an important tool for *in vitro* neurotoxicity screening. Much of the early work for this model involved isolated rat cortical neuron cultures. Responses to known seizurogenic and neurotoxic pharmacological agents targeting many different receptors have been robust and consistent using the rat cortical model. There are, however, certain targets that have not responded definitively or reliably, such as muscarinic receptor agonists. An alternative screening model using human iPSC-derived glutamatergic neurons co-cultured with human iPSC-derived astrocytes (FCDI) demonstrates a highly active and organized neural network that is also effective in identifying compounds with CNS liabilities. In most cases, this model overlaps with the rat cortical model in the successful identification of seizurogenic and neurotoxic compounds. Furthermore, the human model has also demonstrated the ability to identify chemical agents targeting the muscarinic receptor, which is not definitively identified in rat cortical neuron models. However, the human model fails to identify $GABA_A$ antagonists definitively, which is consistently identified by the rat cortical model. With further evaluation and comparison of each of these models and their predictive abilities, it was determined that the response patterns observed for both models are often complementary and when used together provide a robust and accurate *in vitro* model for predicting CNS liabilities.

Methods

1

Figure 1: Axion Biosystems Maestro High Throughput Microelectrode Array Platform

- 768 stimulating and recording channels
- SBS-Compliant Multiwell MEA plates
- Accommodates 12, 48 and 96 wells
- Fully integrated heater with software controls
- Automated electrode characterization & diagnostics

Rat Cortical Neurons

- 48-well MEA plates were pre-coated with a 0.1% PEI solution and allowed to dry overnight. One hour before plating cells, the plates were treated with a laminin solution by dispensing a 15 µL dot directly over the electrode grid and incubating at 37°C.
- Cryopreserved rat cortical neurons (QBM Cell Science) were rapidly thawed and slowly diluted with Neurobasal medium supplemented with B27, L-glutamine and penicillin-streptomycin.
- After a gentle centrifugation step, the cells were resuspended at the appropriate density to achieve 75,000 cells per well.
- The laminin droplet was aspirated and replaced with the same volume of the cell suspension at the appropriate density.
- The cells were incubated, humidified at 37°C in 5% CO_2 for 2 hours before medium was added to the wells.
- Cells were maintained for 14-18 days by changing 60% medium 3 times a week.
- Microelectrode array recordings were acquired on the Axion BioSystems' Maestro immediately before compound treatment (baseline) and 1 hour post treatment.
- Custom MATLAB scripts were used to analyze the spike trains for data generated for each recording.
- Raster plots were generated with Axion BioSystems' Neural Metric Tool.

iPSC-derived glutamatergic neurons co-cultured with human iPSC-derived astrocytes (FCDI)

- 48-well MEA plates were pre-coated with a 0.1% PEI solution and allowed to dry overnight.
- iCell GlutaNeurons and iCell Astrocytes were rapidly thawed and slowly diluted (to avoid osmotic shock) with complete BrainPhys Neuronal Medium supplemented with iCell Neural Supplement B, iCell Nervous System Supplement, N2 supplement, laminin and penicillin-streptomycin.
- After a gentle centrifugation step, the cells were resuspended at the appropriate density with cell dotting medium (complete BrainPhys Medium supplemented with additional laminin).
- A 10 µL droplet of 120,000 iCell GlutaNeurons with 20,000 iCell Astrocytes was dispensed over the electrode grid.
- The cells were incubated, humidified at 37°C in 5% CO_2 for 2 hours.
- 500 µL of complete BrainPhys medium was slowly added to each well in a 2-step process to avoid detaching the cells.
- Cells were maintained for 14-15 days by changing 50% medium 3 times a week.
- Microelectrode array recordings were acquired on the Axion BioSystems' Maestro immediately before compound treatment (baseline) and 1 hour post treatment.
- Custom MATLAB scripts were used to analyze the spike trains for data generated for each recording.
- Raster plots were generated with Axion BioSystems' Neural Metric Tool.

Results: Microelectrode Array with Cryopreserved Rat Cortical Neurons and iPSC-derived Glutamatergic Neurons with Astrocytes

MEA based assay for identifying sub-cytotoxic responses and detecting neurotoxic liabilities using cryopreserved rat cortical neurons (eCiphr®Neuro)

- eCiphr®Neuro measures direct firing of neurons and their organization into burst structures.
- Changes in patterns of firing and bursting can be translated into specific *in vivo* responses.
- This assay can identify compounds that cause seizure like responses as well as neurotoxic responses.
- These responses are almost always well below the cytotoxic responses that these compounds have.
- The assay has been trained on numerous targets from the literature as well as a significant group of pharma compounds which have been run through this assay.
- There are consistent pattern changes which are observed over many different compound classes due to their seizure causing liabilities.
- Some preliminary work suggests that patterns can also identify target driven responses.

MEA based assay for identifying sub-cytotoxic responses and detecting neurotoxic liabilities using human iPSC-derived glutamatergic neurons and human iPSC-derived astrocytes (eCiphr®Neuro-Human)

- We have also developed a human MEA assay: eCiphr®Neuro-Human.
- This assay utilizes FujiFilms CDI iCell GlutaNeurons and iCell Astrocytes.
- We use the same type of analysis for the firing and burst organization as we do for the rat cortical neurons.
- This co-culture of neurons mature differently than the rat cortical neurons with spontaneous spike activity observed much earlier than the rat cortical neurons.
- The current lots of iCell GlutaNeurons are ~90% glutamatergic neurons.
- $GABA_A$ antagonists are one of the most well understood seizure causing agents:
  - Although there may be some minor change in activity when testing $GABA_A$ antagonists (such as Picrotoxin and Gabazine) with this human co-culture, they do not respond significantly enough to make a blinded prediction of a liability.
- Most other classes of seizurogenic and neurotoxic compounds are detectable with this assay.

Results Table: Seizurogenic and neurotoxic detection results for compounds tested in rat and human MEA assays

Compound	Cryopreserved Rat Cortical Nuerons	Co-culture iPSC-derived Glutamatergic Neurons with iPSC-derived Astrocytes (FCDI)
Domoic Acid	Yes	Yes
Linopirdine	Yes	Yes
Amoxapine	Yes	Yes
SNC80	Yes	Yes
Chlorpromazine	Yes	Yes
Strychnine	Yes	Yes
Domoic Acid	Yes	Yes
4-Aminopyridine	Yes	Yes
Gabazine	Yes	No
Picrotoxin	Yes	No
Pilocarpine	No	Yes

Table 1: MEA results for cryopreserved rat cortical neurons vs. co-culture of human iPSC-derived glutamatergic neurons with iPSC-derived astrocytes for detection of seizurogenic or neurotoxic liabilities after one hour exposure to known positive control compounds

Results: Microelectrode Array with Cryopreserved Rat Cortical Neurons and iPSC-derived Glutamatergic Neurons with Astrocytes

2 **Chlorpromazine**

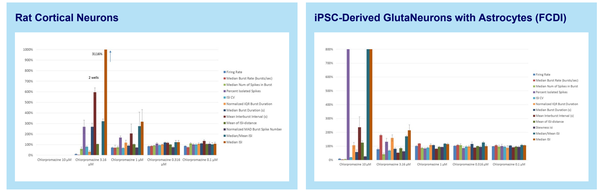

Figure 2: Graphical representation of MEA data for chlorpromazine tested in both the rat cortical neurons and the co-culture of human iPSC-derived glutamatergic neurons and astrocytes. Results represent responses to chlorpromazine tested at 10, 3.16, 1, 0.316 and 0.1 µM for 1 hour. Chlorpromazine eliminated activity in the rat cortical neurons at a concentration of 10 µM. At 3.16 µM, there was a substantial reduction in spike and burst rates and a clear disruption in burst organization with most spikes occurring outside of bursts in the rat cortical neurons. This response attenuates in a dose response manner with no effects detected at the lower concentrations. The response in the human co-culture is similar with a substantial reduction in spike and burst rates at 10 µM and a complete disruption in burst organization. This response attenuates at 3.16 µM and has no effect at the lower concentrations.

3 **Linopirdine**

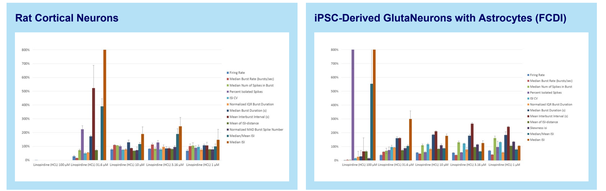

Figure 3: Graphical representation of MEA data for linopirdine tested in both the rat cortical neurons and the co-culture of human iPSC-derived glutamatergic neurons and astrocytes. Results represent responses to linopirdine tested at 100, 31.6, 10, 3.16 and 1 µM for 1 hour. Linopirdine eliminated activity in the rat cortical neurons at a concentration of 100 µM. At 31.6 µM, there was a substantial reduction in spike and burst rates and a clear disruption in burst organization with most spikes occurring outside of bursts in the rat cortical neurons. This response attenuates in a dose response manner with lesser effects detected at the lower concentrations although increases in synchrony and network bursting continue. The response in the human co-culture is similar with a substantial reduction in spike and burst rates at 100 µM and a complete disruption in burst organization. This response attenuates at the lower concentrations but continues to have an effect in all of the concentrations tested.

Results: Pilocarpine

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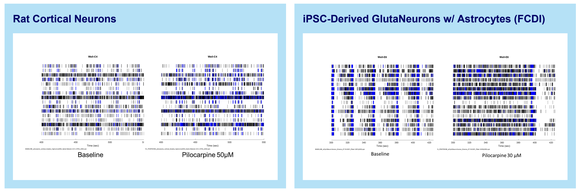

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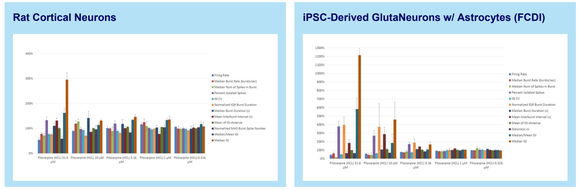

Figure 4: represents raster plots of spontaneous spike activity for cryopreserved rat cortical neurons and iPSC iCell GlutaNeurons and Astrocytes before and after treatment with pilocarpine at 50 and 30 µM, respectively. There are no substantial effects observed post-treatment for the rat neurons, while the human co-culture show substantial effects in activity, burst organization and network organization.

Figure 5: is a graphical representation of endpoint responses to pilocarpine treatment at 31.6, 10, 3.16, 1 and 0.316 µM. There are minimal effects in the rat cortical neurons for activity and burst organization at 31.6 µM, but these effects are not definitive enough to label this compound as a liability. The effects on the human co-culture, however, is substantial at the top two concentrations with changes in activity, burst organization and network organization. Clearly indicating a CNS liability.

Results: Picrotoxin

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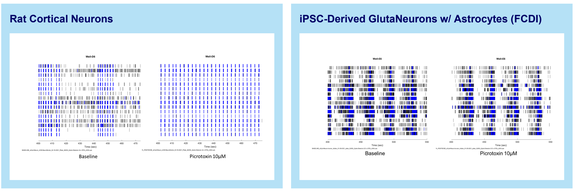

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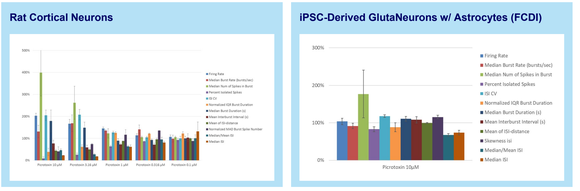

Figure 6: represents raster plots of spontaneous spike activity for cryopreserved rat cortical neurons and iPSC iCell GlutaNeurons and Astrocytes before and after treatment with picrotoxin at 10 µM. There are substantial effects observed with the rat cortical neurons which include an increase in spike and burst activity, an increase in burst organization and a change in network organization to one of complete synchrony. There are no substantial effects post-treatment for the human co-culture.

Figure 7: is a graphical representation of endpoint responses to picrotoxin treatment at 10, 3.16, 1, 0.316 and 0.1 µM for the rat cortical neurons and at 10 µM for the human co-culture (there were no effects for the human neurons at any concentration tested). The rat neurons show substantial effects at 10 and 3.16 µM with attenuating effects at 1 and 0.316 µM and no effects at 0.1 µM. The endpoint effects support the changes in raster plots with increases in activity, burst organization and network synchrony.

Conclusions

- The cryopreserved rat cortical neuron MEA assay. (eCiphr®Neuro) is a highly effective assay at identifying seizure causing compounds as well as neurotoxic compounds.
- The human MEA assay using FujiFilms CDI iCell GlutaNeurons and iCell Astrocytes (eCiphr®Neuro-Human) is also effective at identifying seizure causing compounds and neurotoxins.
- They are both capable of identifying liabilities at sub-cytotoxic concentrations.
- The rat cortical neuron assay fails to identify the muscarinic receptor agonist, pilocarpine when tested at with the normal conditions and time points (testing at 14-18 DIV).
- The human assay fails to identify $GABA_A$ antagonists when tested with the normal conditions and time points (14-15 DIV).
- The two assays together have identified every compound that we have tested. Where one assay fails, the other assay has been sensitive.

References

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