Human Induced Pluripotent Stem Cell-derived Glutamatergic Neurons: Evaluating Maturation and Neurotoxic Predictability in the Presence or Absence of GABAergic Neurons and Astrocytes Using a Microelectrode Array Platform

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Abstract

The prediction of seizurogenic and neurotoxic compounds using microelectrode array (MEA) technology and rodent neuronal models has proven to be a very powerful tool. Early hiPSC derived neuronal models lacked complex burst organization, making electrophysiological neurotoxic prediction challenging when utilizing an MEA platform. Advancements in hiPSC neuronal models have addressed these disadvantages. Glutamatergic neurons were plated on 48-well MEA plates and maintained for up to three weeks at different densities with and without various ratios of GABAergic neurons and astrocytes. When cultured alone, the glutamatergic neurons demonstrate robust firing rates, burst organization and network characteristics within 2 weeks of plating. Spike train analyses indicate a significant maturation rate with a 100% increase in firing rate when plated with astrocytes and GABAergic neurons, 1200% increase in spikes that occur within bursts, and 200-400% increase in other burst and network organization endpoints (i.e. ISI CV, ISI Skewness) when comparing ~5 days post plating to ~15 days. We also evaluated these combinations for their response to the neurotoxin domoic acid, negative control DMSO (0.2%) and seizurogenic agents, picrotoxin, gabazine, 4-aminopyridine, SCN80 and strychnine. As expected, 10μM domoic acid caused a complete loss in spike activity while 0.2% DMSO caused insignificant changes in activity. 4-aminopyridine decreased overall activity while increasing the burst length by 100% with an overall increase in burst organization. Both strychnine and SNC80 caused a unique dose response pattern with changes in spike/burst rates and a deleterious effect on burst and network organization. The $GABA_A$ antagonists, picrotoxin and gabazine, did not have robust responses in this study and require further evaluation. In conclusion, hiPS cell-derived glutamatergic neurons, alone, and in combination with hiPSC derived GABAergic neurons and astrocytes create a robust population of cells that are ideal for evaluating developmental patterns as well as responses to some neurotoxic/seizurogenic compounds when tested on a multi-well MEA platform.

Cellular Dynamics International's (CDI) GlutaNeurons, iCell Neurons, iCell Astrocytes

- iCell GlutaNeurons: iPS cell-derived human cortical neurons consisting primarily of 90% glutamatergic (excitatory) neurons.
- iCell Neurons: iPS cell-derived mixed populations of human cortical neurons consisting of 95% GABAergic (inhibitory) neurons.
- iCell Astrocytes: iPS cell-derived human astrocytes (>95% pure astrocytes).

Axion BioSystems' Maestro Microelectrode Array (MEA) Platform

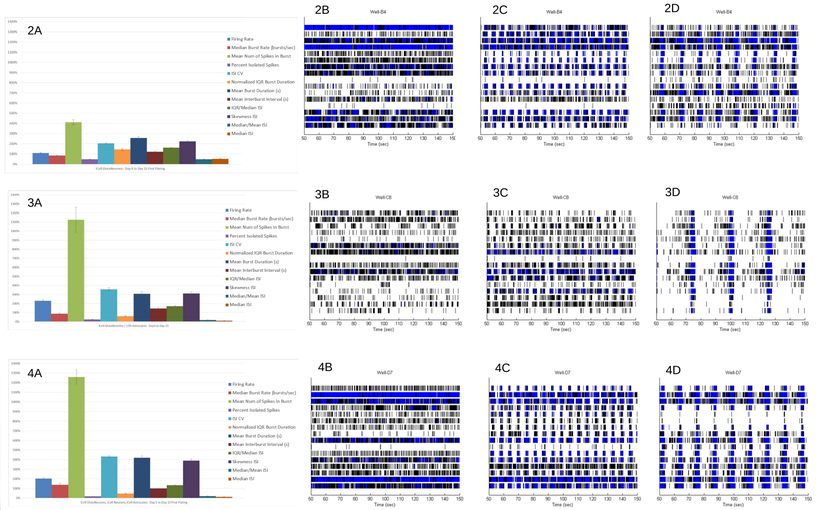
Figure 1A. The Maestro, Axion BioSystems. 768 recording channels with fully integrated heater and software controls. Accommodates 12, 48 and 96 well MEA plates. Figure 1B. 48 well configured MEA plate, Axion BioSystems. 16 microelectrodes per well, ANSI compliant, nano-textured gold electrodes with evaporation reducing lid.

All recordings were acquired on the Axion Maestro platform using 48-well configured MEA plates. The Axion ECmini was used to deliver pre-mixed CO_2 throughout the recordings. A Constant temperature of 37°C was maintained through the software controller.

Methods

- 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 10μL dot directly over the electrode grid and incubating at 37°C.
- iCell GlutaNeurons, iCell Neurons and/or iCell Astrocytes were rapidly thawed and slowly diluted (to avoid osmotic shock) with complete BrainPhys Neuronal Medium supplemented with iCell DopaNeurons Supplement, iCell Nervous System 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).
- The 10μL laminin solution pre-coating the electrode grid was aspirated and replaced with 10μL of the cell suspension.
- The cells were incubated, humidified at 37°C in 5% CO_2 for 2 hours.
- 500μL 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 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.

Results: Maturation

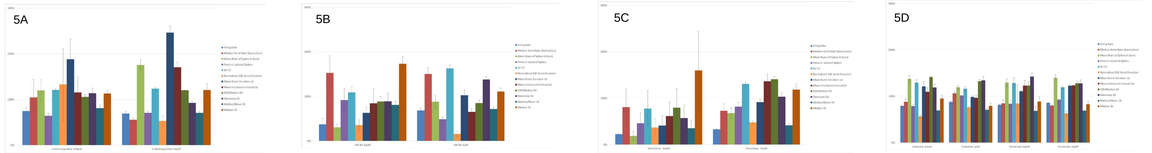


Figures 2A, B, C and D. iCell GlutaNeurons. Bar graph (2A) represents fold differences between recordings taken 4 days post plating and 15 days post plating. By day 4, spike rates were already very robust, therefore, this endpoint did not change significantly. Endpoints describing burst characteristics such as spikes in bursts, percent isolated spikes, ISI CV, normalized IQR burst duration and burst duration show an increase in bursting. An increase in the Skewness ISI endpoint indicates an increase in synchrony. Decreases in median/mean ISI and median ISI signify a move towards uniformity of spikes within bursts. Raster plots generated with Axion BioSystems' Neural Metric Tool represent spontaneous spike trains recorded on day 4 (2B), day 9 (2C) and day 15 (2D) post plating. Increases in burst organization and synchrony are observed over the course of the maintenance period.

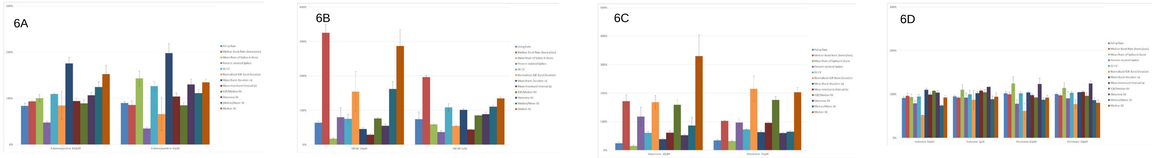
Figures 3A, B, C and D. iCell GlutaNeurons with 13% iCell Astrocytes. Bar graph (3A) represents fold difference between recordings taken 6 days post plating and 15 days post plating. By day 6, spike rates were already very robust, with an additional increase by day 15. Endpoints describing burst characteristics such as spikes in bursts, percent isolated spikes, ISI CV, normalized IQR burst duration and burst duration show an increase in bursting. An increase in the Skewness ISI endpoint indicates an increase in synchrony. Decreases in median/mean ISI and median ISI signify a move towards uniformity of spikes within bursts. Raster plots generated with Axion BioSystems' Neural Metric Tool represent spontaneous spike trains recorded on day 6 (3B), day 9 (3C) and day 15 (3D) post plating. Increases in burst organization and synchrony are observed over the course of the maintenance period.

Figures 4A, B, C and D. iCell GlutaNeurons with 14% iCell Astrocytes and 14% iCell Neurons. Bar graph (4A) represents fold difference between recordings taken 5 days post plating and 13 days post plating. By day 5, spike rates were already very robust, with an additional increase by day 13. Endpoints describing burst characteristics such as spikes in bursts, percent isolated spikes, ISI CV, normalized IQR burst duration and burst duration show an increase in bursting. An increase in the Skewness ISI endpoint indicates an increase in synchrony. Decreases in median/mean ISI and median ISI signify a move towards uniformity of spikes within bursts. Raster plots generated with Axion BioSystems' Neural Metric Tool represent spontaneous spike trains recorded on day 5 (4B), day 9 (4C) and day 13 (4D) post plating. Increases in burst organization and synchrony are observed over the course of the maintenance period.

Results: Pharmacology



Figures 5A, B, C and D. iCell GlutaNeurons. Responses to 4-Aminopyridine treated at 100 and 50μM (5A), SNC80 treated at 10 and 5μM (5B), Strychnine treated at 30 and 15 μM (5C), and Gabazine, 10 and 1μM and Picrotoxin, 50 and 10μM (5D). Data are fold over baseline normalized to 0.2 % DMSO (vehicle).



Figures 6A, B, C and D. iCell GlutaNeurons with 14% iCell Astrocytes and 14% iCell Neurons. Responses to 4-Aminopyridine treated at 100 and 50μM (6A), SNC80 treated at 10 and 5μM (6B), Strychnine treated at 30 and 15 μM (6C), and Gabazine, 10 and 1μM and Picrotoxin, 50 and 10μM (6D). Data are fold over baseline normalized to 0.2% DMSO (vehicle).

Results: Pharmacology Raster Plots and Discussion

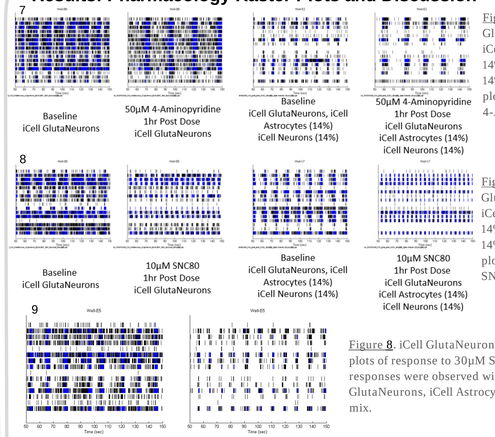


Figure 7. iCell GlutaNeurons alone and iCell GlutaNeurons with 14% iCell Astrocytes and 14% iCell Neurons. Raster plots of response to 50μM 4-Aminopyridine.

Figure 8. iCell GlutaNeurons alone and iCell GlutaNeurons with 14% iCell Astrocytes and 14% iCell Neurons. Raster plots of response to 10μM SNC80.

Figure 8. iCell GlutaNeurons alone. Raster plots of response to 30μM Strychnine. Similar responses were observed with the iCell GlutaNeurons, iCell Astrocytes, iCell Neurons mix.

- In response to 100 and 50μM 4-aminopyridine, a voltage-gated potassium channel blocker, iCell GlutaNeurons alone and in the presence of iCell Astrocytes and iCell Neurons demonstrated similar results. Although there was an overall decrease in spike an burst rates, there was a significant increase in spikes that occur within burst with a decrease in percent isolated spikes. The increase in ISI CV and decrease in normalized IQR burst duration indicate an increase in the "burstiness" of the spike trains. The increase in the burst duration is notable in both the raster plots and spike train analysis.
- In response to 10 and 5μM SNC80, a highly selective and potent non-peptide δ-opioid agonist, iCell GlutaNeurons alone and in the presence of iCell Astrocytes and iCell Neurons demonstrated similar results. There was an overall decrease in spike rate and an increase in burst rate. ISI CV and normalized IQR burst duration depict spike trains that do appear to move towards more bursts. At the higher doses, the bursts are shorter. This pattern tends to be conserved over doses and cell conditions.
- Strychnine, when treated at 30 and 15μM, causes a significant decrease in spike rate and burst rate with the iCell GlutaNeurons alone. Results are similar for iCell GlutaNeurons in the presence of iCell Astrocytes and iCell Neurons, although the burst rate increases at 30μM. Also, the pattern of responses for the mixture indicates a greater disruption of burst activity.

Conclusions

- Early hiPSC derived neuronal models lacked complex burst organization, making MEA-based electrophysiological neurotoxic prediction challenging.
- CDI's iCell GlutaNeurons demonstrate robust and early detectable spike activity with significant increases in burst organization and synchrony over time. Thus forming a complex neural network whose electrophysiological characteristics are easily detected and measured on MEA platforms.
- There is a distinct pattern of change observed in all plating conditions as the cells mature. iCell GlutaNeurons when plated alone may need longer to mature than when they are mixed with iCell Astrocytes and iCell Neurons (Figures 2A, 3A and 4A) as depicted by the significance of the changes in the bar graphs. The predictable and observable change in pattern lends itself to testing compounds that could interfere with developmental processes of neural networks.
- The response for $GABA_A$ antagonists is not as significant as in other models (i.e. rat cortical neurons). A pattern seems to be emerging with these compounds, but further evaluation is needed.
- Additional experiments with iCell GlutaNeurons and iCell Astrocytes mix will be performed with positive controls. Preliminary data suggest responses similar to the iCell GlutaNeurons / iCell Astrocytes / iCell Neurons mix.
- Once all conditions are optimized, a larger library of neurotoxic and seizurogenic compounds will be tested for evaluation in this system.
- Overall, iCell GlutaNeurons alone and in combination with iCell Astrocytes and iCell Neurons create a robust population of cells that are ideal for evaluating developmental and pharmacological responses when tested on a MEA platform.

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