Development of a Neural Co-culture MEA Assay for Seizurogenic Risk Assessment (ft. Human iPSC-derived Cells)

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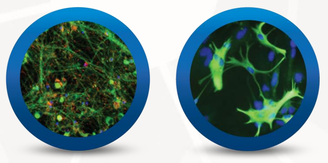

Introduction

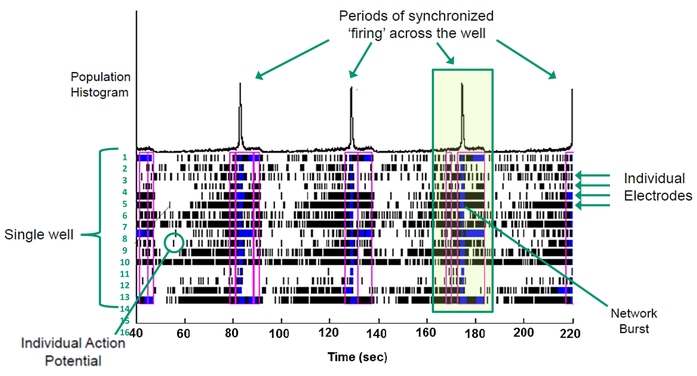

Figure 1. Architecture of a raster plot. Electrophysiological spiking activity recorded from cultured neurons in a single well on an MEA can be depicted in a time-sequenced raster plot. A vertical bar is drawn each time a neuron fires an action potential (**black** tick mark). If a burst of spikes are recorded on an individual electrode they are colored in **blue**. If the neurons display synchronous network activity, single-channel bursts align across all electrodes in the well simultaneously to form a network burst (**pink** boxes). Raster plots provide a simple method to visualize patterns of neural network activity.

Materials and Methods

Cells: Human iPSC-derived glutamatergic neurons (iCell® GlutaNeurons; Cat. #C1033) and human iPSC-derived astrocytes (iCell® Astrocytes; Cat. #R1092) were from FUJIFILM Cellular Dynamics (FCDI).

Media: BrainPhys™ Neuronal Medium (STEMCELL Technologies, Cat. #05790) was further supplemented with iCell GlutaNeurons Media Kit (FCDI, Cat. #R1149), N-2 Supplement (Thermo Fisher, Cat. #17502-48), and Laminin Solution (FUJIFILM Wako Pure Chemicals, Cat. #120-05751). This is the "complete" medium formulation recommended for assay.

Compounds: 4-AP, Chlorpromazine (CPZ), Picrotoxin (PTX), and SR-95531 (GABAzine; GBZ) were all from FUJIFILM Wako Pure Chemicals. AP5 and NBQX were from Tocris. Amoxapine, Bicuculline, Isoguvacine, Muscimol, and Strychnine were from Sigma-Aldrich.

MEA system: Maestro Pro from Axion Biosystems with 48w and 96w Cytoview or Biocircuit MEA plates.

Protocol: The data featured in this poster were generated using the materials and following the steps outlined in this App Protocol found at https://www.fujifilmcdi.com/wp/wp-content/uploads/2021/07/FCDI_iCellGNC_ASC_MEA-Maestro_AP-GNCMEA120721.pdf. Data was analyzed using Neural Metrics Tool software from Axion.

iCell Astrocytes Improve Neural Network Development & Stability

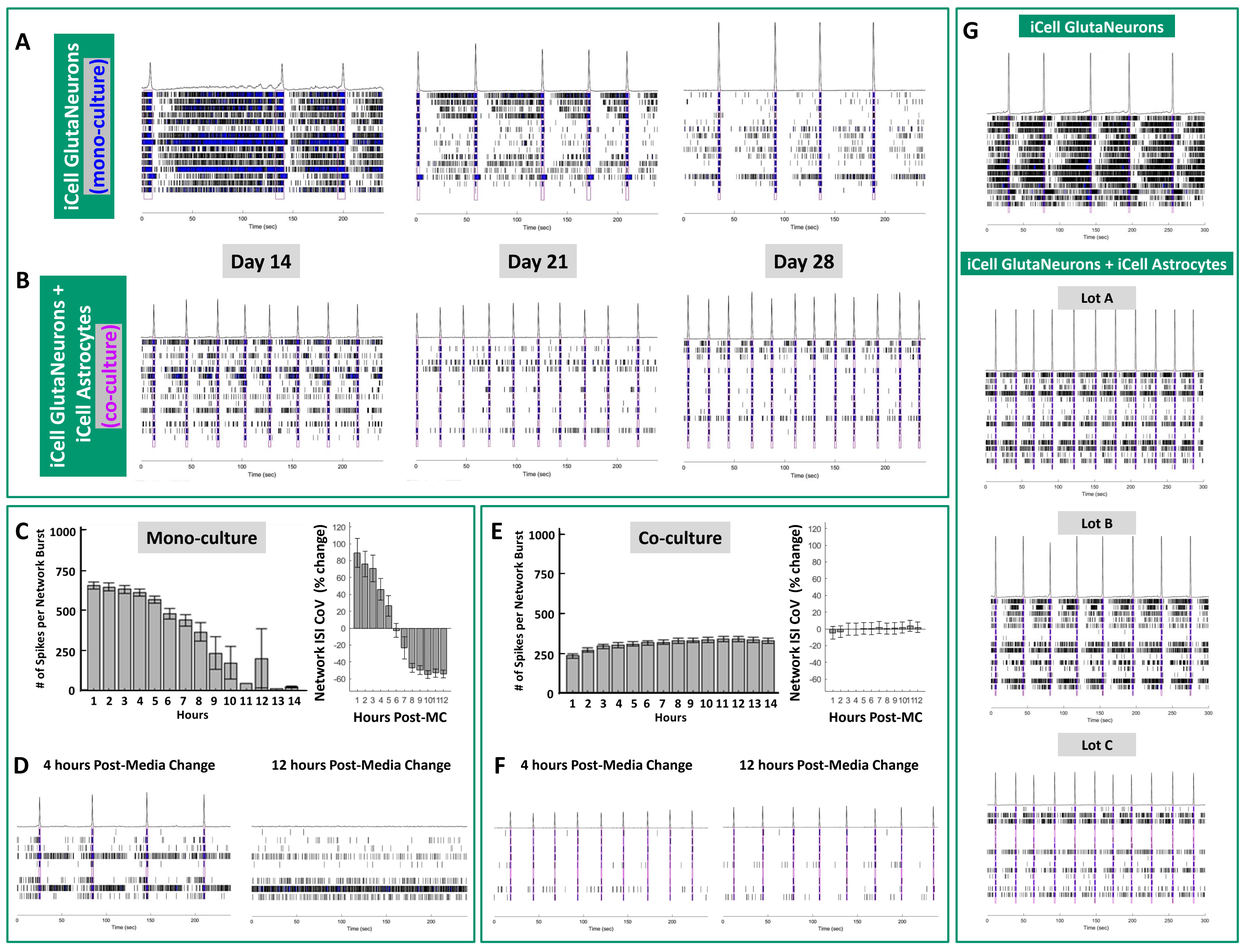

Figure 2. Mono- vs. co-culture. Series of raster plots for (**A**) iCell GlutaNeurons in mono-culture or (**B**) or in co-culture with iCell Astrocytes on a 48w CytoView MEA over time (Day 14, 21, 28) illustrate how astrocyte-neuron communication is important for stable and accelerated network formation. MEA recordings were performed every hour post-MC (media change) on the environmentally-controlled Axion Maestro Pro multi-well MEA system. Plotting the metrics *"# of Spikes per Network Burst"* and *"Network ISI CoV"* reveals that bursting behavior of iCell GlutaNeurons in mono-culture decreases / changes over time (**C**) as compared to co-cultures of iCell GlutaNeurons w/ iCell Astrocytes (**E**), which establish a more consistent and longer-lasting model of synchronous neural network activity. Raster plots at 4 h vs 12 h post-MC (**D**) and (**F**) further show that bursting remains more constant in co-culture. This observation stresses the importance of a regular schedule of MEA recordings for any neuronal culture post-MC. (**G**) The positive impact of iCell Astrocytes in co-culture was demonstrated across multiple different batches (or Lots A, B, and C) of iPSC-derived astrocytes.

Cell Culture Medium and Supplements Impact Network Phenotype

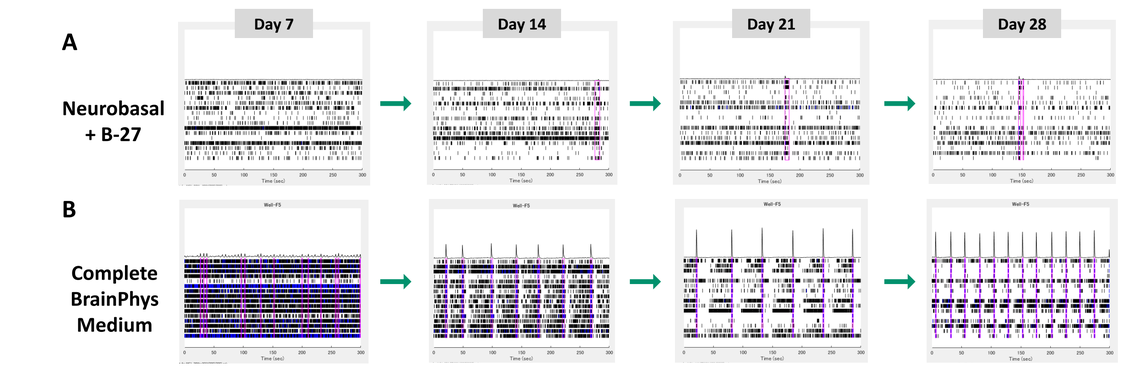

Figure 3. Media matters for MEA activity & proper network development. iCell GlutaNeurons + iCell Astrocytes were plated on a 48w CytoView MEA and then changed the next day into different media, incl. (**A**) Neurobasal™ + B-27 and (**B**) Complete BrainPhys™ Neuronal Medium. Representative raster plots from Days 7, 14, 21, and 28 emphasize the importance of basal medium formulation as well as the presence of the appropriate supplements. iCell Nervous System Supplement (Cat. #M1031) from FCDI is critical for network bursting.

Compound Pharmacology

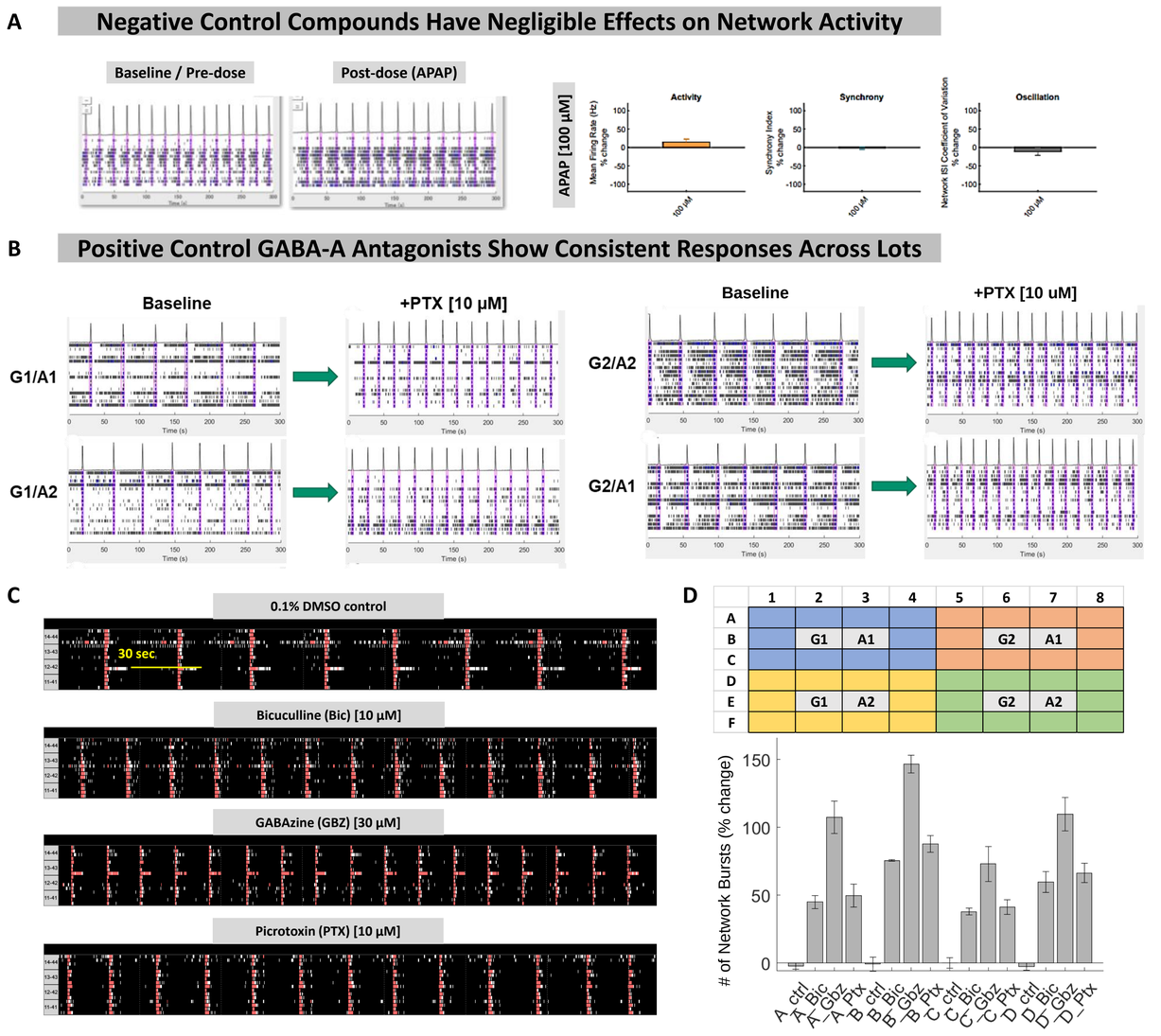

Figure 4. Control compound pharmacology. (**A**) Example raster plots of co-cultures pre- and post-dose treatment with the negative control compound acetaminophen (APAP). Activity, synchrony, and oscillation metrics remain unaffected by this compound. Similar data is obtained w/ 0.1% DMSO (not shown). **(B)** Various lot combinations of iCell GlutaNeurons + iCell Astrocytes (G1A1, G2A2, for example) were cultured in a 48w CytoView MEA plate and recorded on Day 21 before + after exposure to 10 µM PTX. Similar baseline measurements and consistent seizurogenic responses were observed across all conditions. (**C**) Raw data traces from co-cultures post-dose with the GABA-A antagonists (Bic + GBZ + PTX). A pro-seizurogenic response (e.g. high frequency network bursts) is observed visually and quantified in panel (**D**) where the *"# of Network Bursts"* increased significantly as compared to DMSO control wells (n=3 wells per dose).

Expanded Drug Set to Modulate Networks and Test Seizurogenic Activity

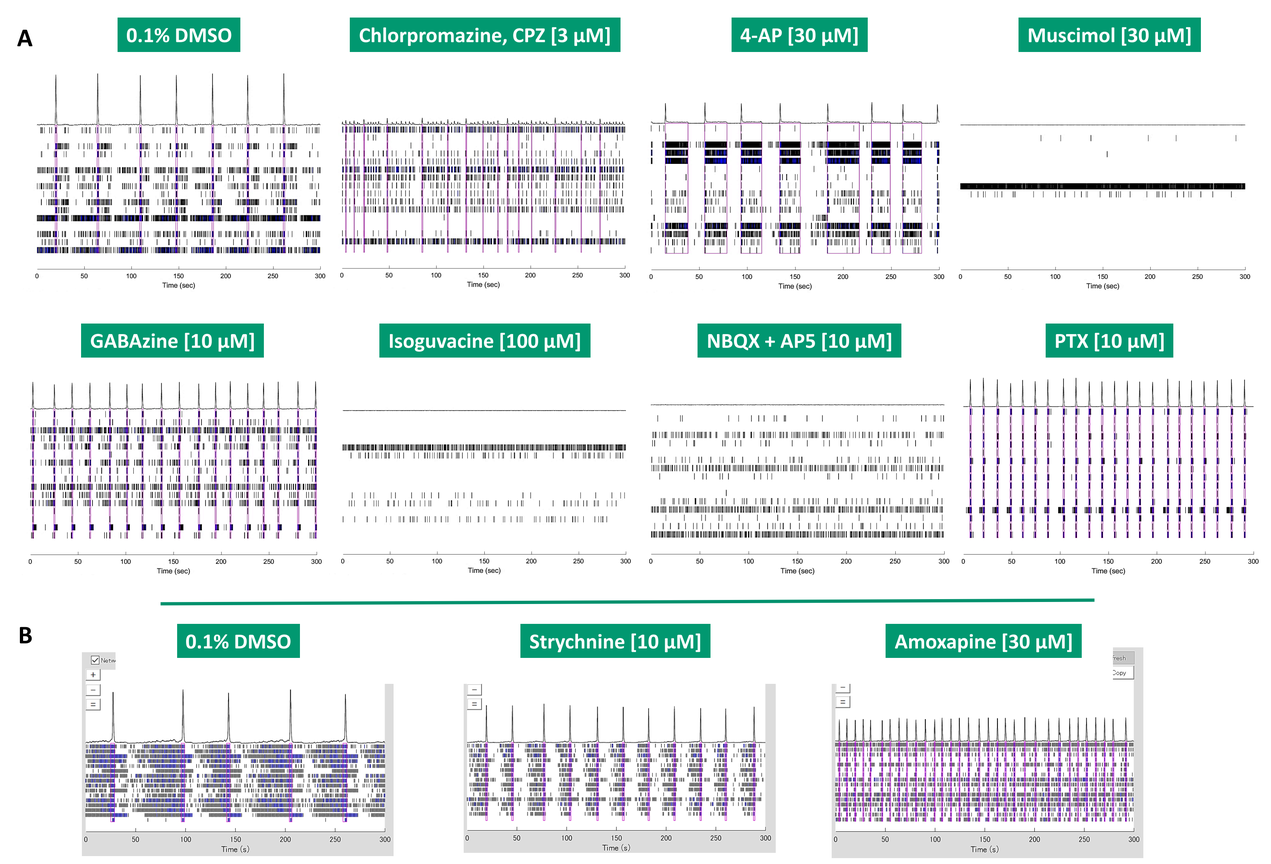

Figure 5. Expanded compound pharmacology panel. iCell GlutaNeurons + iCell Astrocytes were co-cultured as described in this poster on 48-well (**A**) CytoView MEA plates or (**B**) Classic MEA plates until Day 28. Representative raster plots of DMSO control wells are pictured next to a set of compounds that are either pro-seizurogenic (4-AP, Amoxapine, CPZ, GBZ, PTX or Strychnine), GABA-A receptor selective agonists (Isoguvacine or Muscimol) which result in inhibition of neuronal activity, or blockers of synaptically-drive excitatory activity (NBQX and AP5). Based on the mechanism of action of each of compounds, a combination of neural metrics for firing rate and network bursting activity can be used to quantify the effects.

Summary

iCell GlutaNeurons are a highly active population of human iPSC-derived neurons that can be used for numerous *in vitro* applications. Co-culture with iCell Astrocytes elevates the MEA assay performance by establishing a more consistent and longer-lasting model of neural network activity. After synchronous bursting is achieved, cells can be dosed with compounds to assess the impact on neural network activity and quantify pro-seizurogenic risk. Heavy presentation of raster plots here sets the expectation for what the data should "look like" when using iCell products and following recommended protocols from FCDI.

There are many possible applications with the neuron MEA assay beyond toxicity testing, including neurodegenerative disease modeling. Please visit our website at https://www.fujifilmcdi.com/ or contact one of the authors of this poster directly for more information.