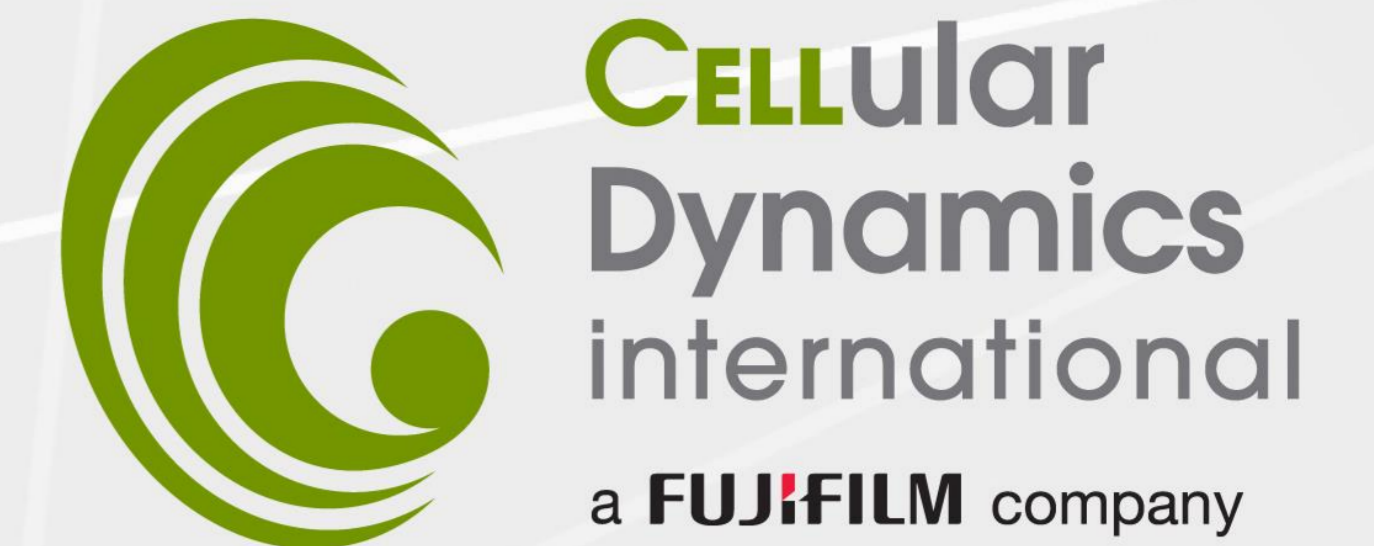


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# Multiparametric Assessment of Networked Electrical Activity Using Induced Pluripotent Stem Cell-derived Glutamatergic Neurons

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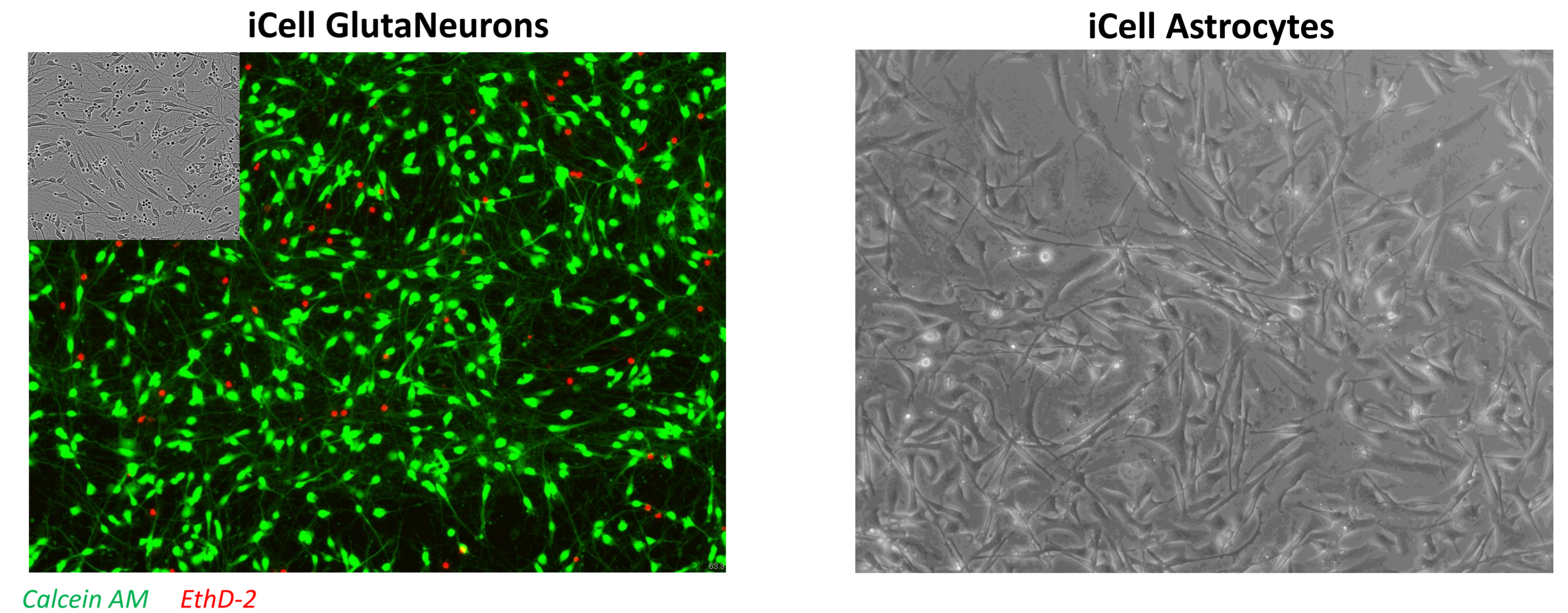
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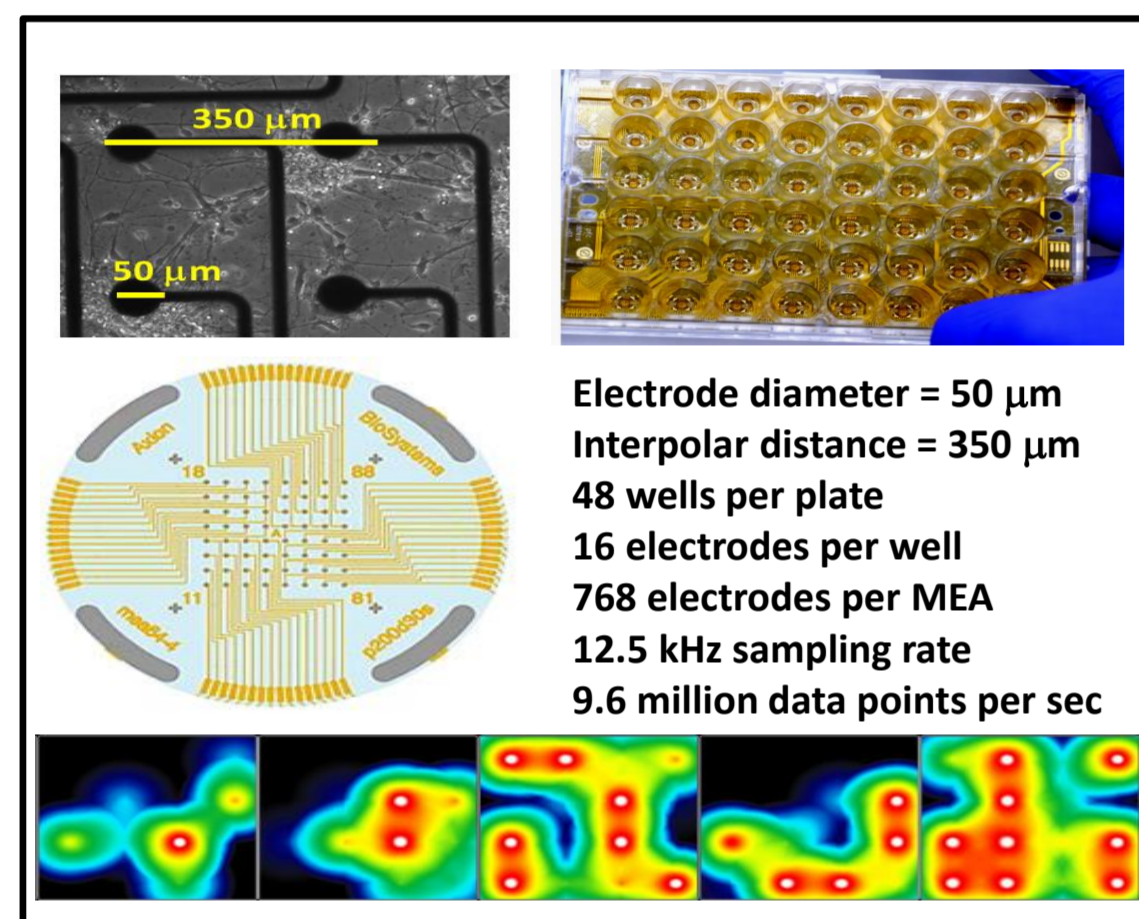
## Abstract

The lack of a predictable preclinical test system to identify CNS adverse effects greatly hinders the drug development process and contributes to high drug attrition rates. Here we develop a higher-throughput *in vitro* test system of human neuronal cultures with synchronized network electrical (bursting) for assessment of compound effects on neuronal function and network communication including seizurogenic liability. Excitatory human induced pluripotent stem cell (iPSC) derived neurons (i.e., iCell GlutaNeurons) were cultured for 23 days on multi-electrode array (MEA), alone or in combination with human astrocytes (i.e., iCell Astrocytes), and assessed for compound effects. Known excitatory compounds were measured for concentration-dependent effects, at clinically relevant concentrations, include bicuculline, picrotoxin, glutamate, pentyleneetetrazol, 4-aminopyridine, and chlorpromazine. Activity parameters displaying concentration-dependent changes with pharmacology include: mean firing rate, 'single-channel' burst rate, intensity and duration, 'network-level' burst rate, intensity and duration, and synchrony measures. The presented data demonstrate how iPSC technology coupled with MEA technology create a noninvasive human neuronal test system, previously limited to rodent models, and provide an unprecedented investigatory space for drug development. This method allows quantification of networked electrical activity, in human cortical neurons, which should be valuable to identify CNS liability and support preclinical toxicity programs.

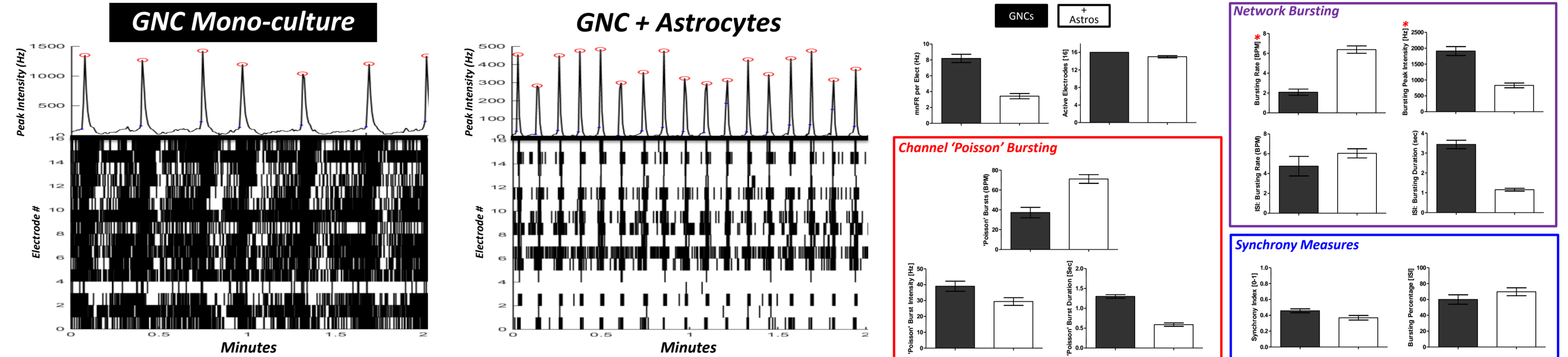
## iPSC-Derived Neuronal Cell Types



## Network-Level Synchronization detected via MEAs



Multi-Electrode Arrays (MEAs) were utilized to assess the activity levels and bursting behaviors of iCell GlutaNeuron & iCell Astrocyte co-cultures. iPSC-derived neuronal co-cultures develop synchronized bursting behaviors beginning near DIV11 and reach robust, re-producible and reliable bursting levels. Co-cultures display a slightly 1) more organized and 2) cleaner bursting phenotype compared to mono-cultures. Appropriately, co-culture conditions decrease mean Firing Rate but increase bursting behaviors both at a channel-level (Poisson) as well as at the network-level (ISI) resolution. We used Axion's Neural Metric analysis to assess bursting behaviors, along with an in-house all-point histogram (500 msec bins) algorithm (\*) that detects bursting peak time-points (o).



## Seizurogenic Assay Development

Seizurogenic Assay workflow, analysis and results are presented for all pharmacology tested, including vehicle, control, excitatory (i.e. seizurogenic) and anti-epileptic (AEDs) drugs. iCell GlutaNeurons and iCell Astrocytes were mixed upon thaw and dotted together onto 48-well MEA plates. Evaluation of seizurogenic pharmacology was performed on DIV 19 or 20 by adding 10  $\mu\text{L}$  (30X solutions) to wells containing 300  $\mu\text{L}$  of Brainphys Medium, assessing 6 different concentrations of each drug [0.003, 0.03, 0.3, 3, 30, 300  $\mu\text{M}$ ]. Each plate contained positive control (bicuculline [200  $\mu\text{M}$ ]) treated wells, as well as untreated wells. 'Before' and 'Treatment' 8-minute recordings were collected, with pre-incubation periods of 10 minutes preceding each recording. Spike Files (6 SD) were collected and processed for spike and bursting metrics via Axion Neural Metric analysis and by an all-points histogram burst-peak detection suite (CDI NeuroAnalyzer). Differences from baseline were normalized to vehicle control and are presented for all drug concentrations, for each metric. Far Right: Filled radar graphs (increasing concentration going clock-wise) for all pharmacology are presented depicting absolute value changes from baseline of all metrics. \*Note control and vehicle display no changes from baseline, while seizurogenic pharmacology alters spike and burst metrics >40 fold.

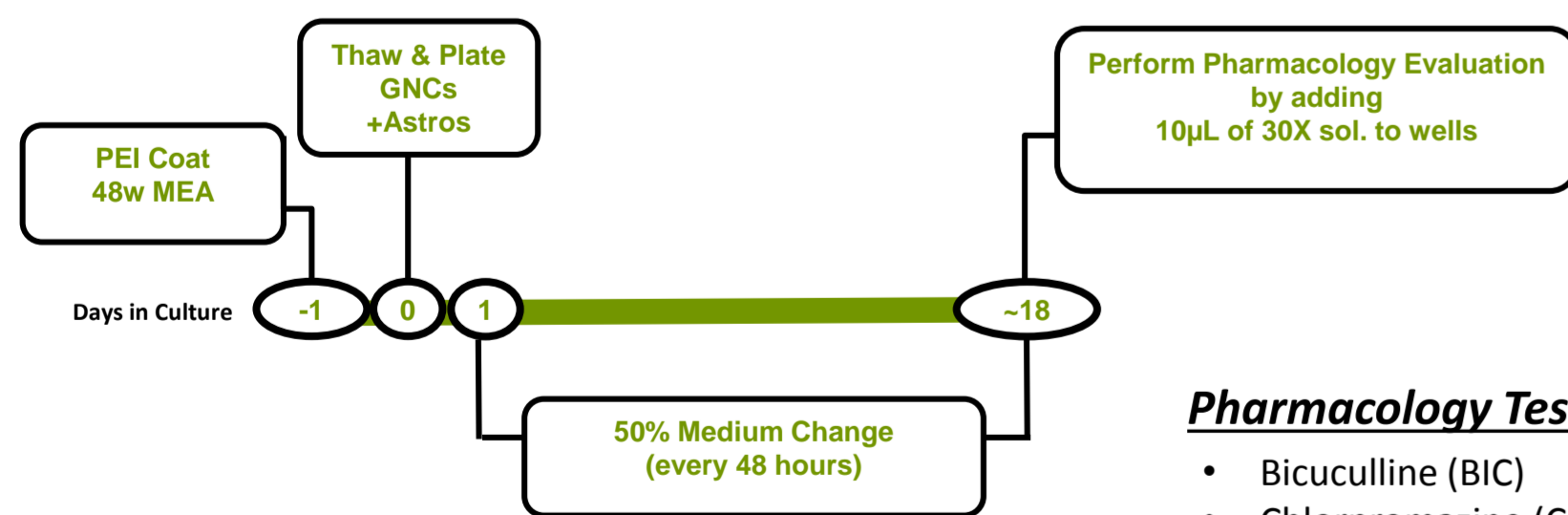


Plate Layout for ALL Plates ( $\mu\text{M}$ )

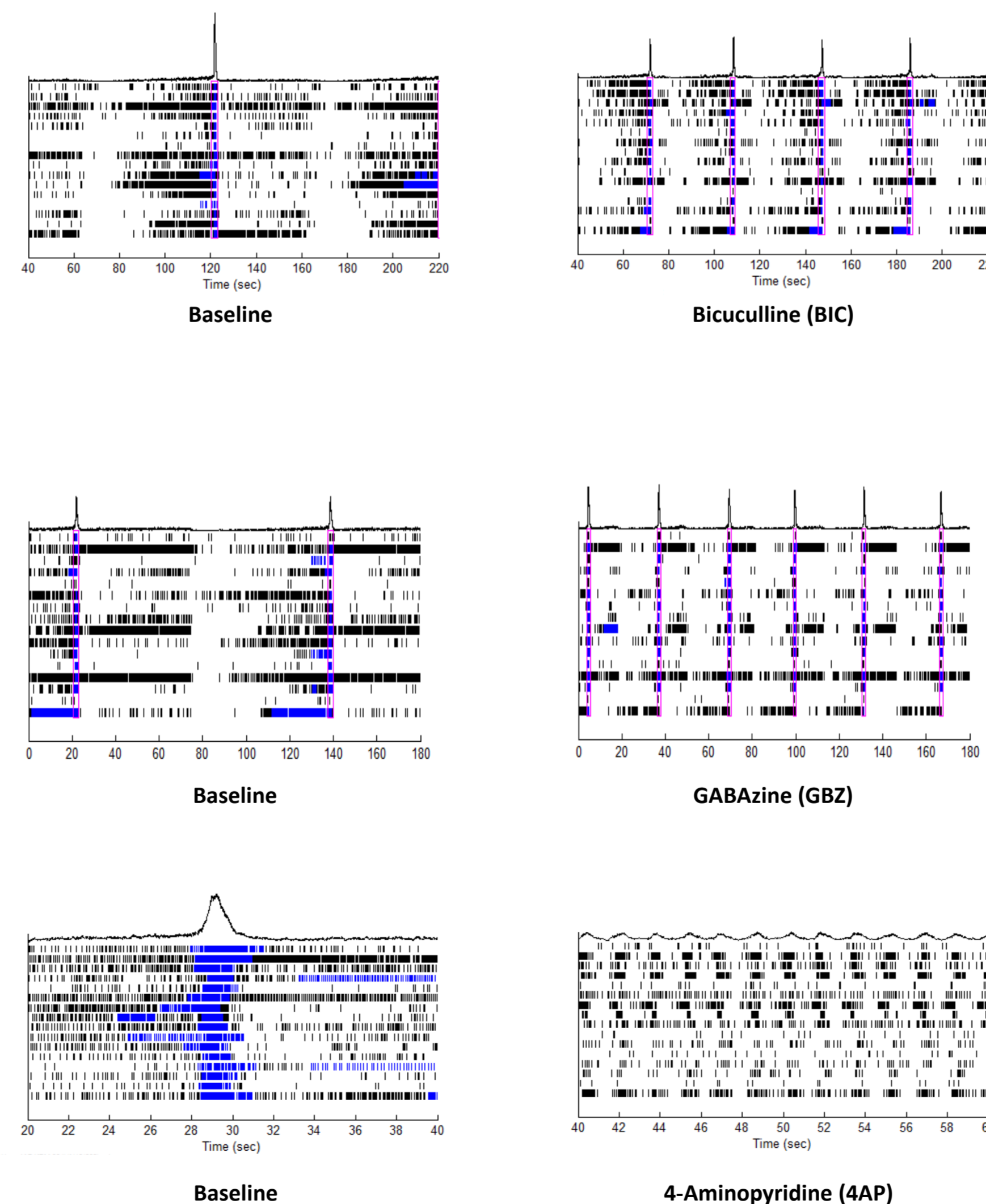
Row#	Column #	1	2	3	4	5	6	7	8	9	10	48-Well
1.A	0	300	30	3	0.3	0.03	0.003	uT				
2.B	0	300	30	3	0.3	0.03	0.003	uT				
3.C	0	300	30	3	0.3	0.03	0.003	uT				
4.D	0	300	30	3	0.3	0.03	0.003	uT				
5.E	0	300	30	3	0.3	0.03	0.003	uT				
6.F	BIC	uT	BIC	uT	BIC	uT	BIC	uT				

### Pharmacology Tested

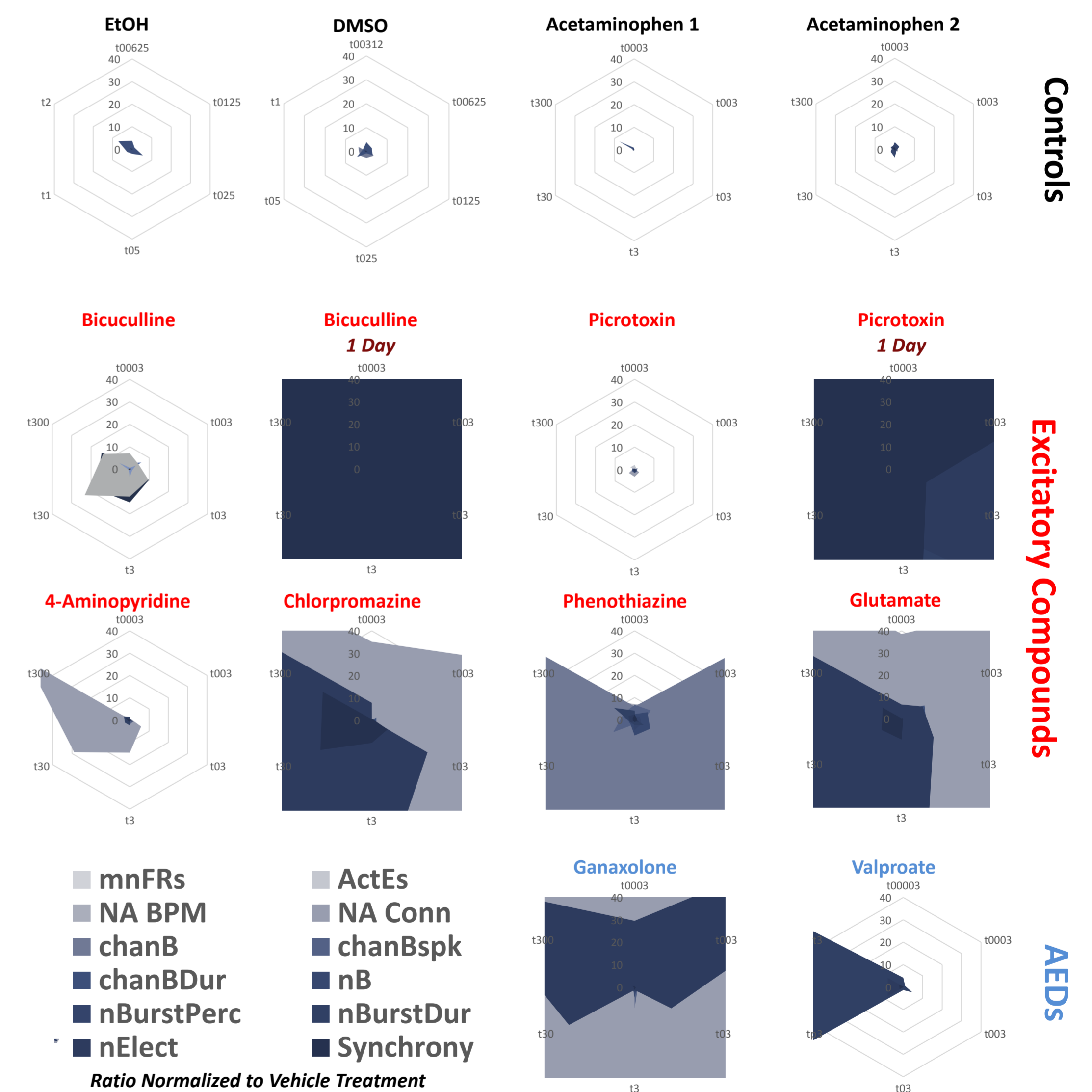
- Bicuculline (BIC)
- Chlorpromazine (CHL)
- Picrotoxin (PTX)
- Pentyleneetetrazol (PTZ)
- 4-Aminopyridine (4AP)
- Glutamic Acid (GA)

### Controls

- Acetaminophen (ACE)
- Ethanol (EtOH)
- Methanol (data not shown)
- DMSO



## Qualifying Compounds: Filled Radar Graphs



## Summary and Conclusions

- iCell GlutaNeurons and iCell Astrocytes can be mixed together to generate a purely human neuronal co-culture
- iCell GlutaNeurons and iCell Astrocytes co-cultures develop synchronized bursting cultures *in vitro*
- Co-cultures develop a robust, reproducible network-level bursting phenotype within 3 weeks

- Control and vehicle conditions do not alter co-culture synchronized bursting behaviors, while pharmacology that either stimulates or ameliorates excitatory pathways does alter bursting behaviors
- Co-cultures stimulated with excitatory pharmacology produce 'seizurogenic phenotypes'