Development of a Fully Defined, Synaptically Competent iPSC-Derived Neuronal Platform for Drug Discovery

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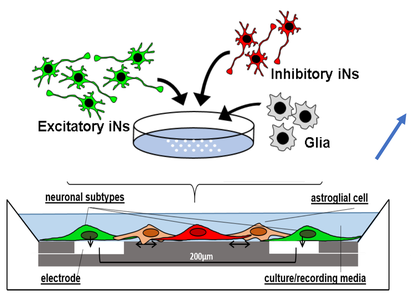
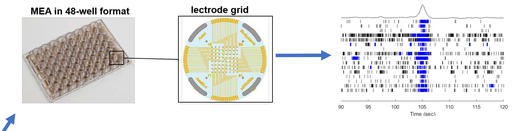
1. Abstract

NeuCyte's SynFire® technology is based on direct reprogramming of induced pluripotent stem cells (iPSCs) into pure highly functional neurons of various defined subtypes (induced neurons, iNs). Here we present a human *in vitro* neuronal/glial co-culture platform capable of comprehensive neuronal activity measurements using multi-electrode arrays (MEAs). Due to parallel recording of multiple electrophysiological parameters, this platform allows identification and detailed characterization of neurotoxic and neuroactive effects of chemical compounds.
For a first proof-of-concept study, we tested a set of compounds (i.e. $GABA_AR$ modulators and pyrethroids) for their capacity to modulate neuronal network activity. We further showed that NeuCyte's platform is suitable to quantitatively assess chemically-induced seizure-like activity in a semi high-throughput manner. Finally, we tested a set of anti-epileptic drugs (AEDs) for their potential to serve as single antidotes against chemically-induced seizures, as modeled by the potent $GABA_AR$ blockers picrotoxin (PTX) and Bicuculline (BIC).

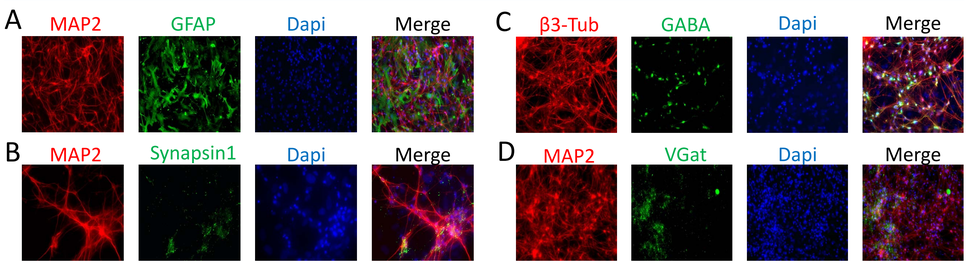
2. SynFire® iPSC-Derived Neural Co-Culture Platform





We combined our iN technology with human primary glial supporter cells on 48-well MEA plates to develop a pure human neural co-culture system consisting of glutamatergic excitatory neurons (140K cells/well), GABAergic inhibitory neurons (60K cells/well), and astrocytes (70K cells/well). Reproducible formation of spontaneous synchronized neuronal network activity was detected between 3-4 weeks after plating.

3. Human iN/Glia Cell Characterization

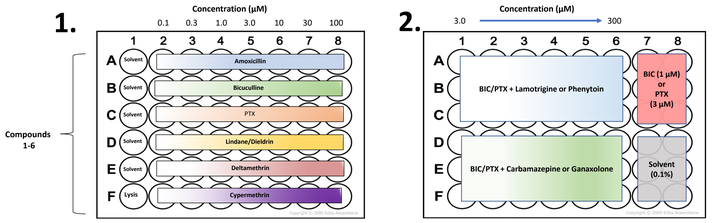


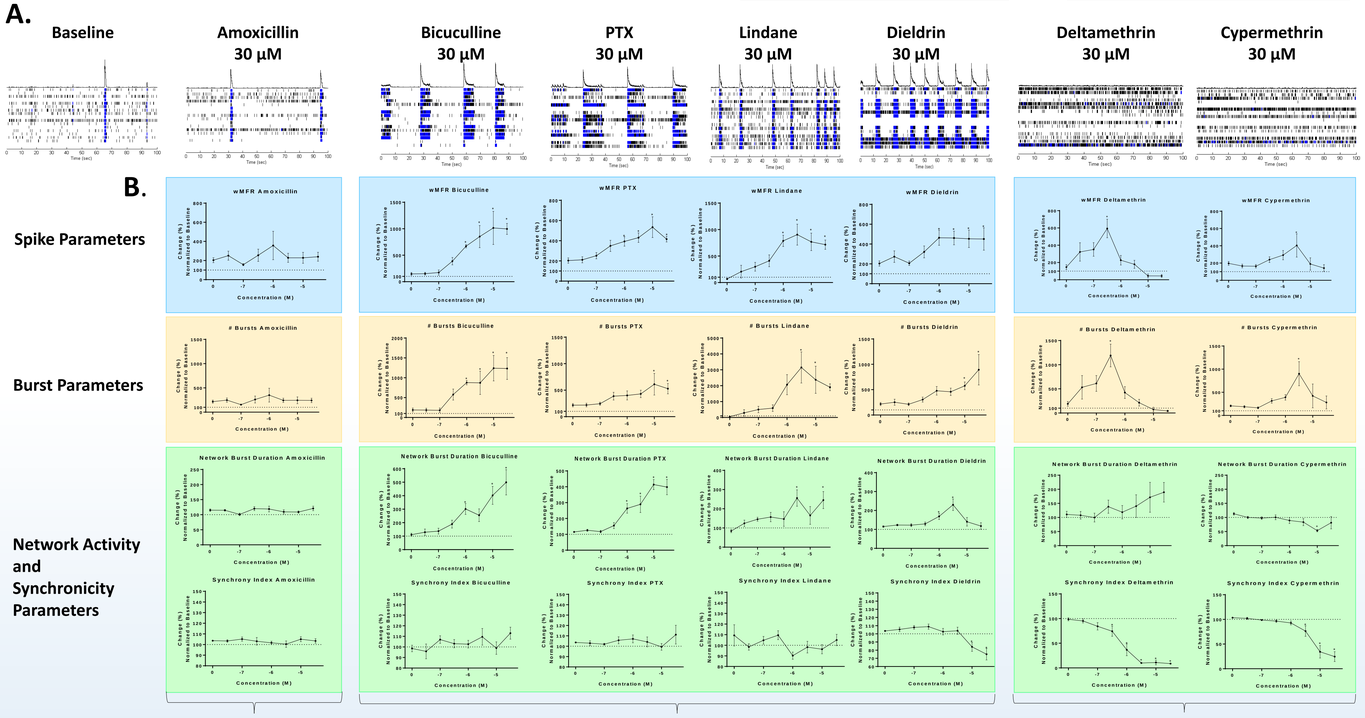
Characterization of our human induced neurons by immuno-staining. (A) Pan-neuronal marker MAP2 / astroglial marker GFAP / nuclear staining Dapi. (B) Pan-neuronal marker MAP2 / synaptic marker Synapsin1 / nuclear staining Dapi. (C) Pan-neuronal marker β3-Tubb (TuJ1) / inhibitory neurotransmitter GABA / nuclear staining Dapi. (D) Pan-neuronal marker MAP2 / vesicular GABA transporter VGat/ nuclear staining Dapi.

4. Results

4a. Human iNs Responses to Environmental Toxicants

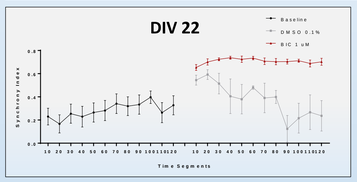
After seeding, cultures were allowed to mature for 37 days. Spontaneous neuronal activity was recorded using the Axion Maestro system as follows: Baseline activity was recorded for 40 min after an equilibration period of 20 min. Then, test compounds were added to individual wells at increasing concentrations (see plate layout 1), and neuronal responses were recorded for 40 min.





(A) Raster plots of MEAs measurements after dosing with amoxicillin, $GABA_AR$ blockers bicuculline, picrotoxin (PTX), lindane, and dieldrin, or two pyrethroids. (B) $GABA_AR$ blockers increased neuronal firing (wMFR), # bursts, and network burst duration in a dose-dependent manner. Dosing with control compound amoxicillin showed no change in neuronal activity. Exposure to deltamethrin and cypermethrin increased neuronal firing at specific concentrations but caused a significant disruption in network synchronicity. Parameters are plotted as percentage of the untreated baseline value in each well. Data are shown as means ± SEM. One-Way ANOVA and the Dunnett's test for multiple comparisons were used for detecting significant differences between the mean of each compound concentration and the mean of its solvent (concentration 0) (* $P \leq 0.05$).

4b. Identifying the Optimal Assay Window: Maturation Time and Synchrony Level



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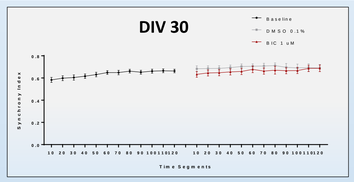
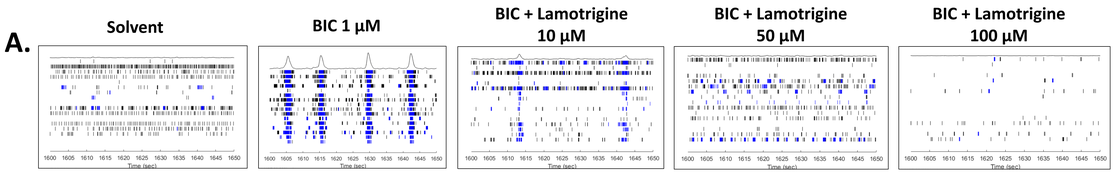
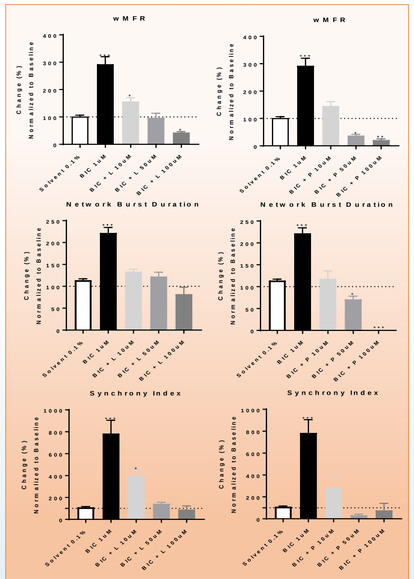


Exposure to bicuculline (BIC 1 µM) increased network synchronicity compared to solvent control (DMSO 0.1%) when exposed at 22 days after plating. In contrast, bicuculline did not further increase synchronicity when applied at 30 days after plating. Spontaneous neuronal baseline activity was recorded for 2h, followed by dosing and an additional recording period of 2h. Time-dependent changes in synchronicity were analyzed by binning the 2h periods into 10min windows.

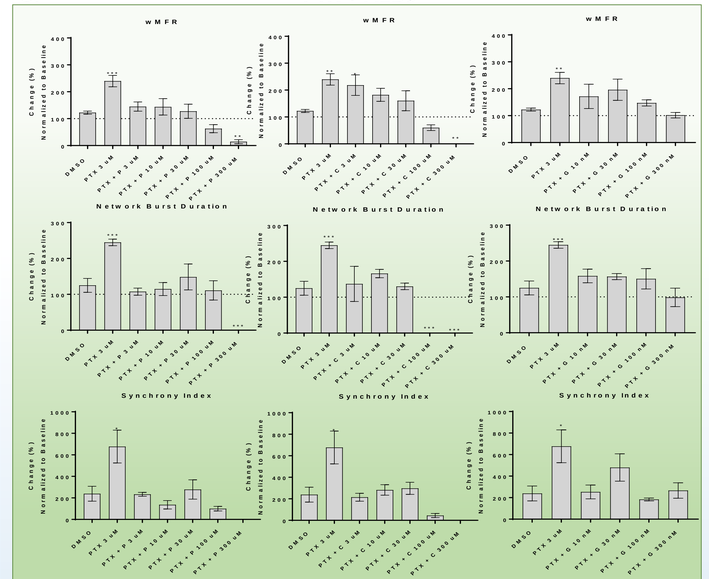
4c. Effects of AEDs on Chemically Induced Seizure-like Activity in Human Neural Co-Cultures

After seeding, cultures were allowed to mature for 21 days. Spontaneous neuronal activity was recorded using the Maestro system as follows: baseline activity was recorded for 30 min after an equilibration period of 20 min. Then, PTX/BIC alone or in combination with increasing doses of AEDs (see plate layout 2) were added to individual wells, and neuronal activity was recorded for 1h.





(A) Raster plots of MEA measurements after exposure to BIC or BIC plus increasing doses of lamotrigine. (B) Application of AEDs reversed bicuculline-induced seizure-like activity in a dose-dependent manner in our human iN/glia co-cultures (L: lamotrigine, P: phenytoin). Data are shown as means ± SEM. (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).



(C) Human iN/glia co-culture responses after exposure to PTX or PTX plus increasing doses of AEDs. Application of AEDs reversed picrotoxin-induced seizure-like activity in a dose-dependent manner (P: phenytoin, C: carbamazepine, G: ganaxolone). For each parameter, values are plotted as percentage of baseline measurement of the same well. Data are shown as means ± SEM. (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

5. Summary

- Upon exposure to $GABA_AR$-blocking chemicals such as PTX, lindane, or bicuculline, our neural co-culture system shows drastic increased in spike rates, burst number, and network burst duration, which resembled ictal discharges as they occur during status epilepticus. The changes in electrophysiological parameters were robust and reproducible across different wells showing a clear dose-dependency for every compound.
- Chemically-induced seizure-like neuronal activity was reduced upon co-application of well-established AEDs. The presence of phenytoin, lamotrigine, carbamazepine, and ganaxolone reversed the $GABA_AR$ blocker-induced increase in activity in a dose-dependent manner.