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 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 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 antitoxins against chemically-induced seizures, as modeled by the potent GABA_A blockers picrotoxin (PTX) and Bicuculline (BIC).

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

We combined our in vitro 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 immunostaining. (A) Pan-neuronal marker MAP2 / astroglial marker GFAP / nuclear staining Dapi. (B) Pan-neuronal (3-Tubb) / inhibitory neurotransmitter GABA / nuclear staining Dapi. Double immunostainings of human iPSC-derived neurons confirmed efficient neuronal/glial co-culture system (3-Tubb / GABA and 3-Tubb / GFAP). (C) Pan-neuronal (TuJ1) / inhibitory neurotransmitter GABA / nuclear staining Dapi.

4. Results

4a. Human iNs Responses to Environmental Toxicants

After seeding, cultures were allowed to mature for 47 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.

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

Exposure to Bicuculline (1 µM) increased network synchrony compared to solvent control (DMSO 0.01%) when exposed at 22 days after plating. In contrast, Bicuculline did not further increase synchrony 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 synchrony 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 equilibrium 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 3h.

5. Summary

- Upon exposure to GABA_A blockers BIC or BIC + PTX, neuronal activity was reduced upon co-application of well-established AEDs. The presence of phenytoin, carbamazepine, and gabapentin reversed the BIC blocker-induced increase in activity in a dose-dependent manner.