Comparison of Acute Effects of Neurotoxic Compounds on Network Activity in Human and Rodent Neural Cultures

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1. Abstract

Assessment of neuroactive effects of chemicals in cell-based assays remains challenging as complex functional tissue is required for biologically relevant readouts. Recent *in vitro* models using rodent primary neural cultures grown on multielectrode arrays (MEAs) allow quantitative measurements of neural network activity and have been demonstrated to be suitable for neurotoxicity screening. However, robust systems for testing effects on network function in human neural networks are still lacking. The increasing number of differentiation protocols for generating neurons from induced pluripotent stem cells (iPSCs) holds great potential to overcome the unavailability of human primary tissue and expedite human cell-based assays. Yet, the variability in neuronal activity, prolonged ontogeny and rather immature stage of most neuronal cells derived by standard differentiation techniques greatly limit their utility for screening neurotoxic effects on neuronal networks. Here, we used excitatory and inhibitory neurons that were separately generated by direct conversion from human iPSCs together with primary human astroglial cells to establish highly functional neural cultures. Such neuron/glia co-cultures showed pronounced neuronal activity and robust formation of synchronized network activity on MEAs, albeit with noticeable delay vs primary rat cortical cultures. We further investigated the effects of neurotoxic test compounds, including 4 $GABA_A$ receptor antagonists, an organotin, and 4 pyrethroid insecticides, as well as 3 negative control compounds on network activity in these human neuron/glia co-cultures. Importantly, we observed largely corresponding dose-dependent alterations in firing, burst and synchrony metrics of neuronal network activity in iPSC-derived human neuron/glia and rat primary cortical cultures. These results demonstrate the utility of this direct-differentiated human model for neurotoxicity screening using MEAs.

2. SynFire iPSC-Derived Neuronal (iN) Cell Technology

Glutamatergic excitatory and GABAergic inhibitory neurons were generated separately from human iPSCs through direct reprogramming using neurogenic transcription factors. Co-culture conditions with primary human astrocytes were optimized to a total composition of 52% excitatory iNs, 22% inhibitory iNs, and 26% astrocytes. iN/glial co-cultures showed morphology and marker expression of mature neurons, including presence of synapses, and exhibited an even distribution of the different cell types. iN/glial co-cultures were grown on MEA plates for quantitative assessment of neuronal firing and network activity.

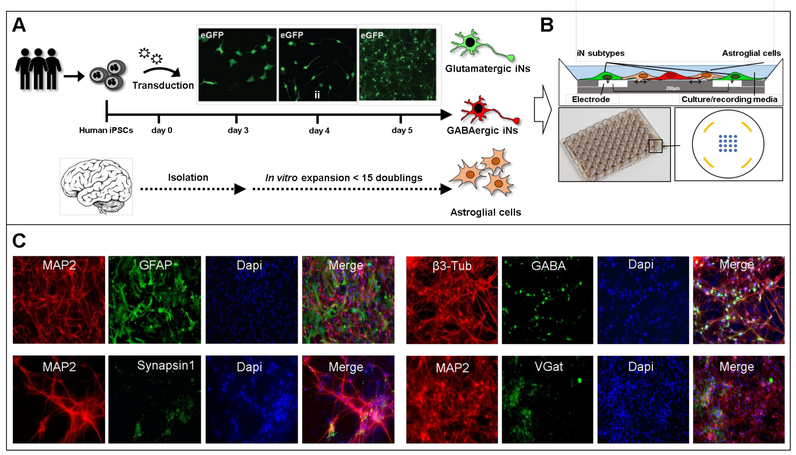


Fig. 1: Human iPSC-derived neuronal/glial co-cultures. (A) Combination of direct reprogramming of iPSC to generate specific induced neurons (iNs) with human primary astrocytes. (B) Co-cultures of glutamatergic/GABAergic iNs with astrocytes on MEAs for electrophysiological readouts. (C) Characterization of iN/glial co-cultures by immunocytochemistry staining. (i) Pan-neuronal marker MAP2 / astroglial marker GFAP / nuclear staining Dapi. (ii) Pan-neuronal marker β3-Tub (TuJ1) / inhibitory neurotransmitter GABA / nuclear staining Dapi. (iii) Pan-neuronal marker MAP2 / synaptic marker Synapsin1 / nuclear staining Dapi. (iv) Pan-neuronal marker MAP2 / vesicular GABA transporter VGat/ nuclear staining Dapi.

3. Neural Network Activity

Development of neural network formation was compared between rat primary cortical cultures and human iPSC-derived iN/glial co-cultures on MEAs. Both rat and human neural cultures developed synchronized network activity. However, human cultures show a considerable delay in network ontogeny as determined by multiple spiking and bursting parameters. After 37 days, neural network activity in human cultures resembles mature rat cultures at day post plating (DPP) 21. Inhibition of gap junctions in human cultures further demonstrated network synchronization to be predominantly mediated by synaptic signaling.

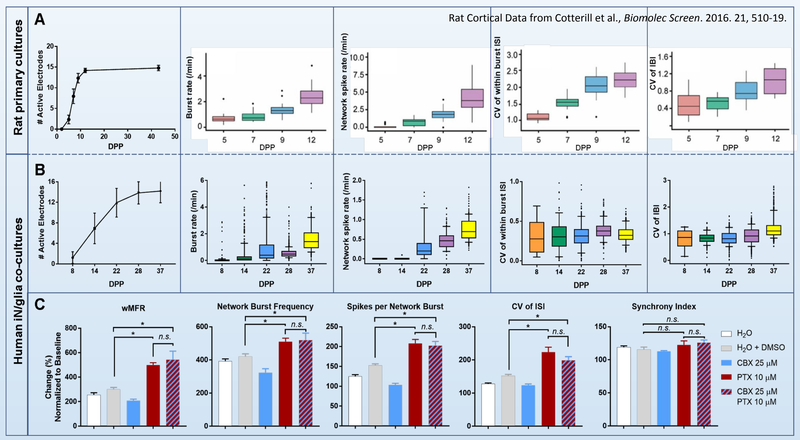


Fig. 3: Comparison of neuronal network ontogeny in rat primary cortical cultures and human iN/glia co-cultures. (A) Development of neural network activity in rat primary cortical cultures assessed by previously identified descriptive parameters (Cotterill *et al.*, 2016). (B) Development of neural network activity in human iPSC-derived neural cultures. (C) Blocking of gap junction by application of Carbenoxolone (CBX) to isolate electrical connectivity mediated by synapses.

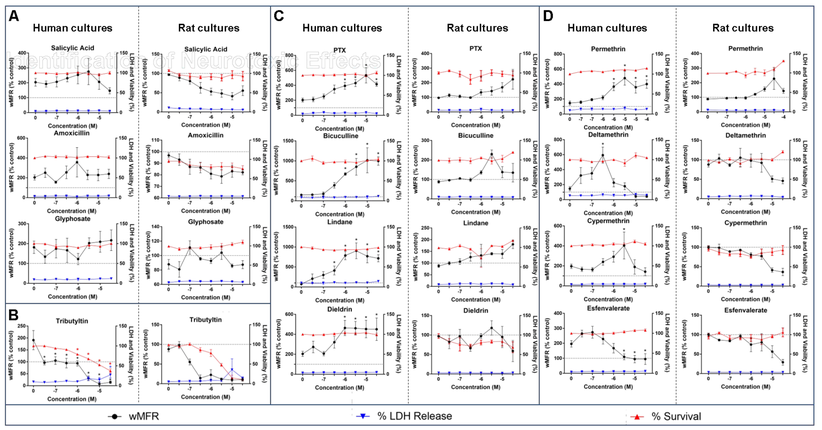
5. Comparative Testing of Neurotoxic Chemicals

Human iPSC-derived iN/glia co-cultures were evaluated for their applicability to identify acute neuroactive effects of chemical compounds through alteration of network activity measured on MEAs. We tested a set of 9 compounds of with well-established neurotoxic effects and 3 controls for impairment of cell viability and alteration of neural network activity in rat primary cortical cultures and human iPSC-derived neural cultures.

Table 1: Chemical compounds used for neurotoxicity testing on MEAs:

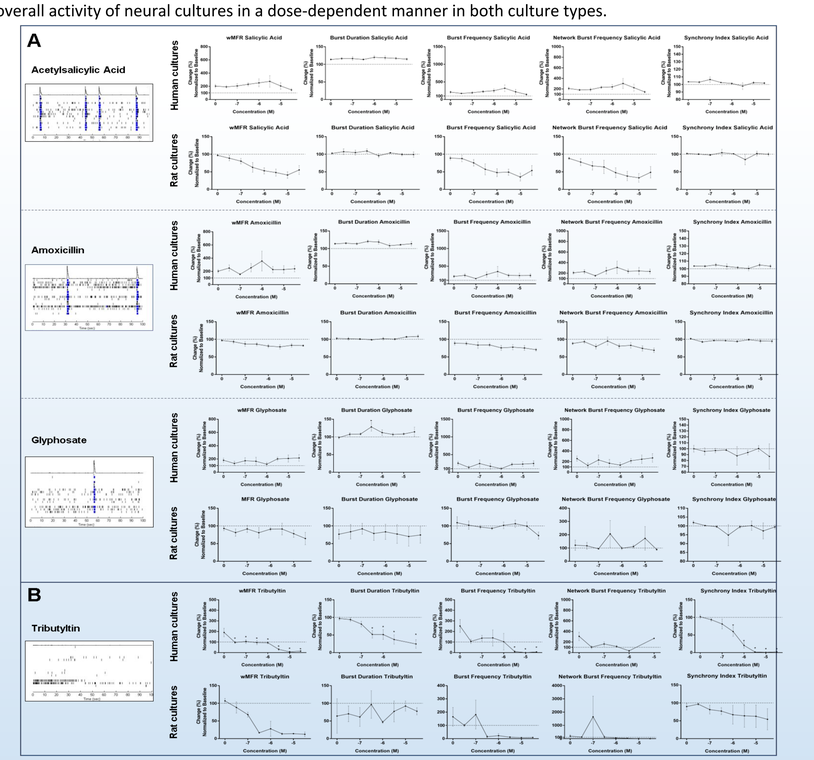
Compound	Class	Effect	Solvent
Amoxicillin	penicillin-class antibiotic	negative control	DMSO
Acetylsalicylic acid	non-steroidal anti-inflammatory drug	negative control	DMSO
Glyphosate	organophosphorus herbicide	negative control	water
Bicuculline	isoquinoline alkaloid	$GABA_A$ antagonists	DMSO/ethanol
Picrotoxin	convulsant alkaloid	$GABA_A$ antagonists	DMSO
Lindane	organochloride insecticide	$GABA_A$ antagonists	ethanol
Dieldrin	organochloride insecticide	$GABA_A$ antagonists	DMSO
Permethrin	type I pyrethroid insecticide	modulation of Voltage-sensitive sodium channel (VSSCs) kinetics	DMSO/ethanol
Deltamethrin	type II pyrethroid insecticide	prolonged modulation of VSSCs kinetics	DMSO/ethanol
Cypermethrin	type II pyrethroid insecticide	prolonged modulation of VSSCs kinetics	DMSO/ethanol
Esfenvalerate	Type I/II pyrethroid insecticide	intermediate modulation of VSSCs kinetics	DMSO/ethanol
Tributyltin	organotin biocide	unknown	DMSO/ethanol

Overall cell viability of human and rat neural cultures was quantified by LDH release and the CytoTox 96® Assay for each MEA well immediately after recording. All negative control compounds and the vast majority of neurotoxicants showed no impairment of cell viability in both culture types at any tested concentration. In contrast, tributyltin exhibited neuronal cytotoxicity in a dose-dependent manner which was equally detected in rat and human cultures.



4. Alteration of Neural Network Activity

Dosing with the negative controls amoxicillin and glyphosate showed no significant effects on neural network activity in both rat and human neural cultures. However, salicylic acid decreased firing and burst frequencies only in rat primary cortical cultures. In concordance with it's neuronal cytotoxic effects, tributyltin decreased overall activity of neural cultures in a dose-dependent manner in both culture types.



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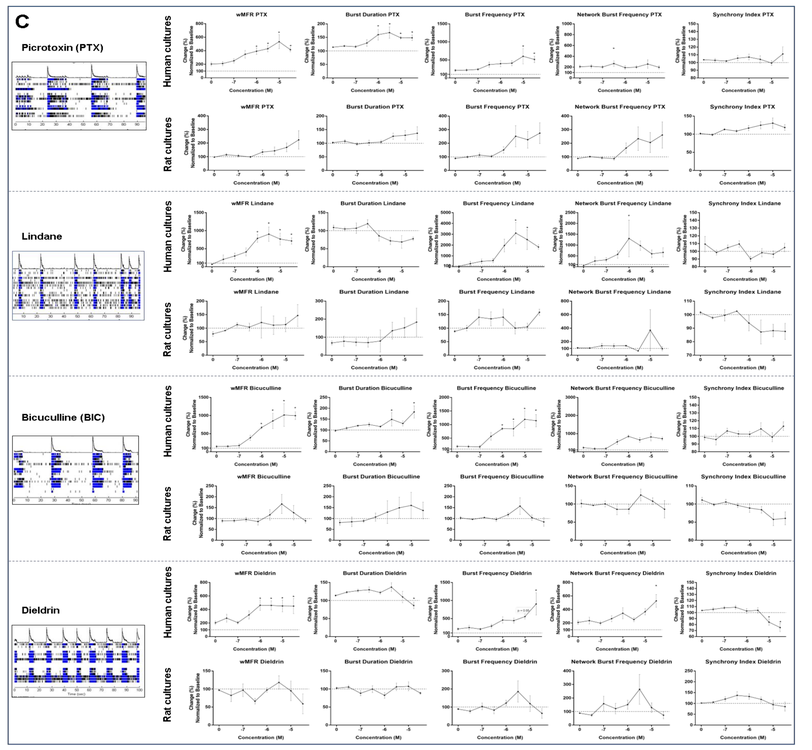
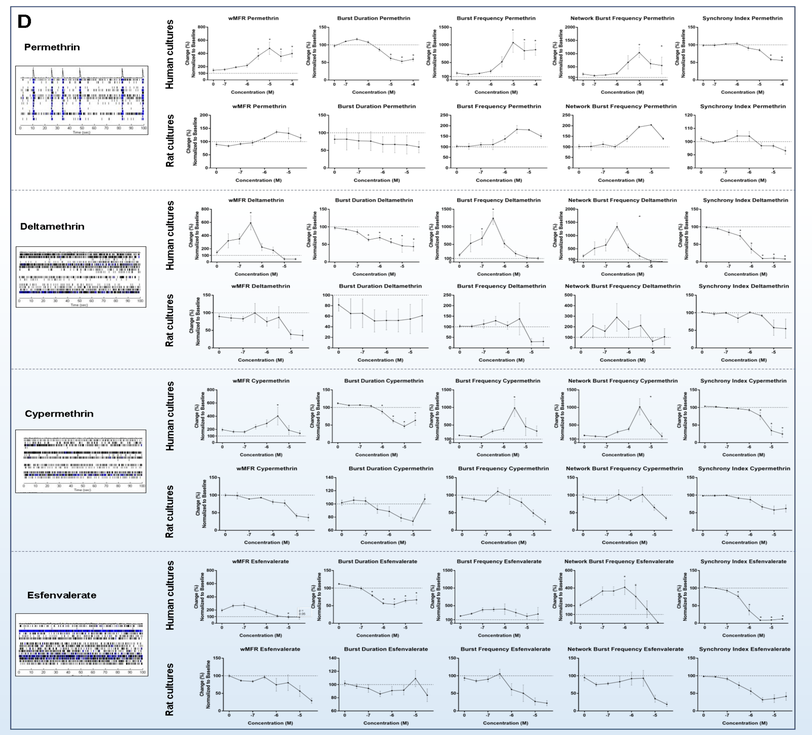
Dosing with neurotoxic chemicals that inhibit function of $GABA_A$ receptors exhibited a mostly dose-dependent increase in neuronal firing and variable alteration of analyzed network metrics with high concordance between rat and human cultures. Responses of human iN/glia co-cultures showed a striking correspondence to rat cultures for all compounds in the vast majority of analyzed parameters.



Dosing with neurotoxic pyrethroids showed drastic and diverse effects on neuronal activity. The type I pyrethroid permethrin showed an overall increase in spiking and bursting metrics while largely maintaining the network bursting structure. In contrast, the type II pyrethroids deltamethrin and cypermethrin, as well as the type I/II pyrethroid esfenvalerate showed a complete disruption of network organization representing the different mechanisms of action for these chemicals on neuronal cells. Importantly, responses of neural network activity recorded from of human iN/glia co-cultures showed a striking correspondence to rat cultures for all compounds in the vast majority of analyzed parameters.



6. Conclusions

- **Co-cultures of primary astrocytes and iPSC-derived induced neurons form functional neural tissue**
- **Specific alteration of neural network activity measured by in vitro assays on MEAs is useful to identify adverse neuroactive effects of chemicals toxicants beyond neural cytotoxicity**
- **Alteration of network activity through application of neurotoxic compounds shows high concordance between human and rat cultures**
- **Human iPSC-derived neural cultures represent an alternative for neurotoxicity testing**