Human iPSC-derived neurons for functional assessment of in vitro neurotoxicity and seizure liability

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BACKGROUND

In vitro pharmacology profiling of new chemical entities during early phases of drug discovery has recently become an essential tool to predict clinical adverse effects. While for cardiac safety testing high technology platforms are available, specific in vitro neurotoxic panels are not, and in vivo models are used instead. However, correlations between animal and human data are often weak; in addition, animal studies are expensive, ethically questionable and require large amounts of chemical compounds.

We have developed assays to assess in vitro neurotoxicity in a human system based on two different types of human induced pluripotent stem cell (iPSC)-derived cells and multiwell microelectrode array (MEA) technology.

Peri.4U™ are iPSC-derived peripheral neurons that reveal clear detectable burst-like activity after 3-4 day culture on MEA chips, indicating the presence and establishment of a functional neuronal network. Reference compounds with a known neurotoxic potential, such as neuroleptica, antidepressiva, neurotransmitter blockers, pesticides or plant toxins, were analyzed for their effect on neuronal network behavior. Peri.4U™ showed at least similar, if not higher sensitivity to reference compounds in this validation study. This demonstrates the potency of this in vitro system for reliable detection and quantification of neurotoxic compound actions.

CNS.4U™ are a highly physiological iPSC-based co-culture model of neurons (glutamatergic, GABAergic and dopaminergic) and astrocytes, obtained through simultaneous generation from neural precursor cells. CNS.4U™ rapidly form neuronal networks in culture and show synchronous network activity assessed by MEA technology over c. 5 weeks in culture. Here, we provide proof-of-concept results that reveal the suitability of CNS.4U™ for seizure liability assays based on dose-dependent reactivity to compounds that are known to affect seizure.

METHODS

- Peri.4U™ and CNS.4U™ were plated in PEI coated 48-wells MEA chips from Axion Biosystems (Maestro System)
- 7.2 x 10^4 cells were seeded per well in 3 µl droplets
- After 3-4 days in culture, both neuron types are spontaneously active
- MEA recordings were performed with cells that had been cultured for up to 3 weeks (Peri.4U™) or up to 8 weeks (CNS.4U™)
- Drugs were diluted in medium and applied as single dose or cumulatively in increasing concentrations. During drug application, 10% of the bath solution was replaced with a 10-fold concentrated drug solution

RESULTS

PERI.4U™ FOR NEUROTOXICITY ASSESSMENT

Fig. 1

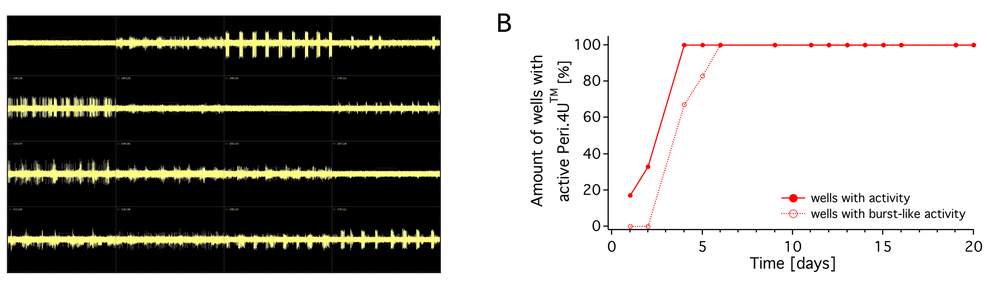
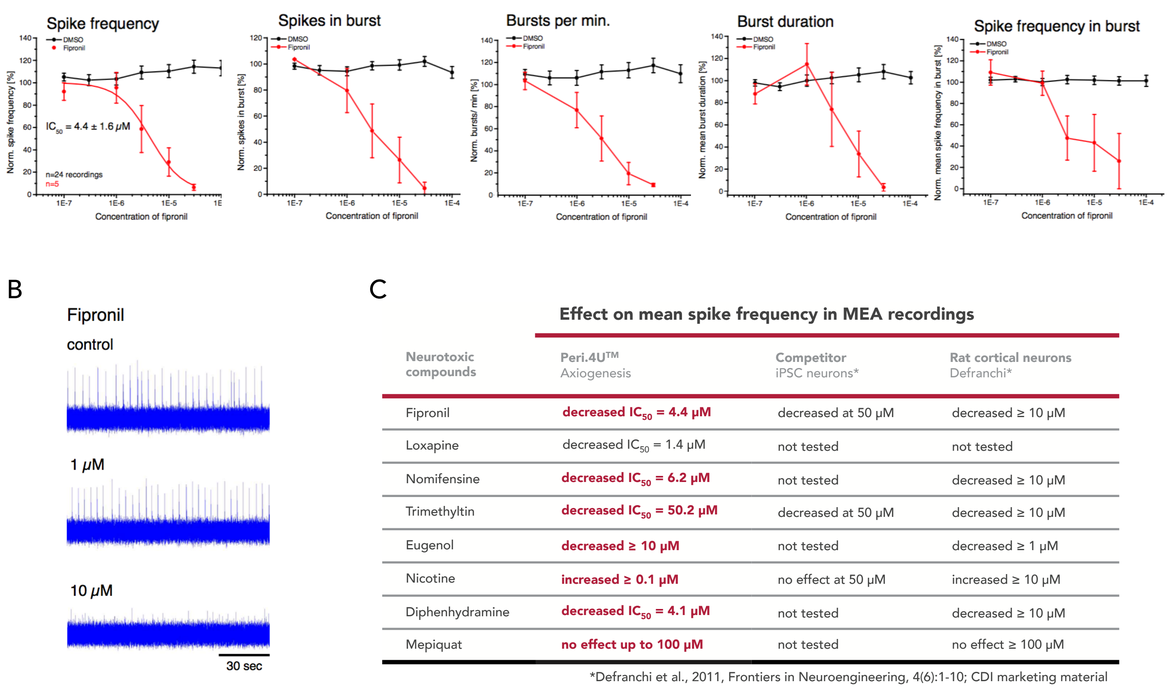


Fig. 2



C

Effect on mean spike frequency in MEA recordings

Neurotoxic compounds	Peri.4U™ Axiogenesis	Competitor iPSC neurons*	Rat cortical neurons Defranchi*
Fipronil	**decreased IC_{50} = 4.4 µM**	decreased at 50 µM	decreased ≥ 10 µM
Loxapine	decreased IC_{50} = 1.4 µM	not tested	not tested
Nomifensine	**decreased IC_{50} = 6.2 µM**	not tested	decreased ≥ 10 µM
Trimethyltin	**decreased IC_{50} = 50.2 µM**	decreased at 50 µM	decreased ≥ 10 µM
Eugenol	**decreased ≥ 10 µM**	not tested	decreased ≥ 1 µM
Nicotine	**increased ≥ 0.1 µM**	no effect at 50 µM	increased ≥ 10 µM
Diphenhydramine	**decreased IC_{50} = 4.1 µM**	not tested	decreased ≥ 10 µM
Mepiquat	**no effect up to 100 µM**	not tested	no effect ≥ 100 µM

*Defranchi et al., 2011, Frontiers in Neuroengineering, 4(6):1-10; CDI marketing material

CNS.4U™ FOR SEIZURE LIABILITY

Fig. 1

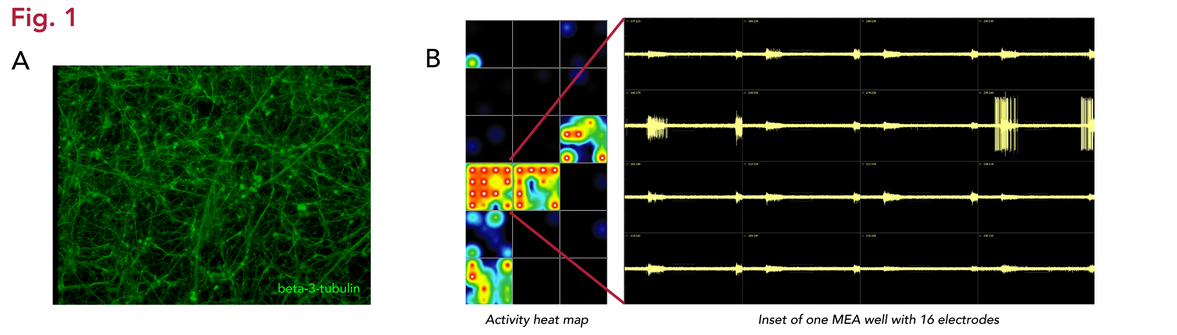
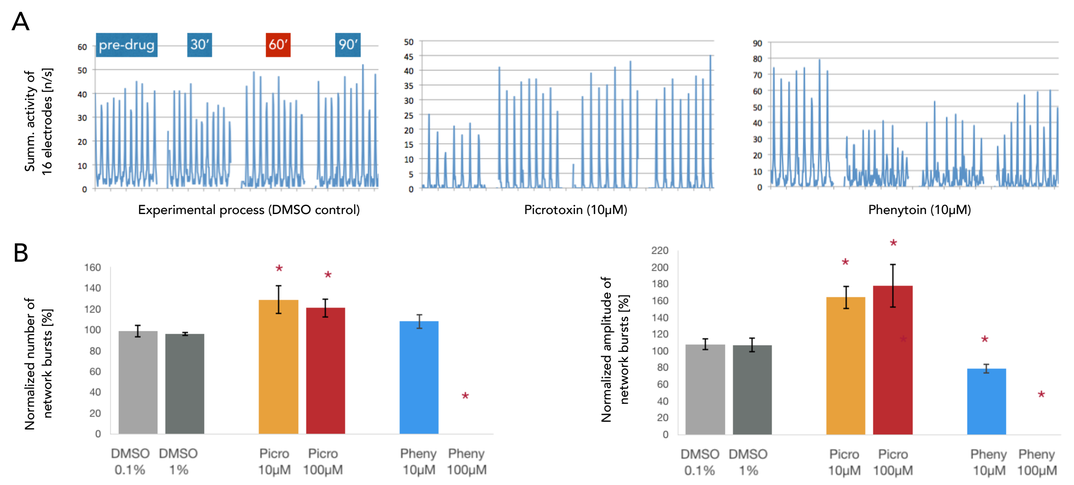


Fig. 2



Assay establishment on MEA (Fig. 1): Peri.4U™ exhibit long-term burst-like spontaneous activity. 16 electrodes of a 48well MEA measurement (A). 100% of wells reveal burst activity over c. 3 weeks (B). **Neurotoxicity validation study (Fig. 2):** Quantification of the fipronil effect on spontaneous activity of Peri.4U™ (red traces) in comparison to control (A). Representative recording of the effect of fipronil as exemplary compound (B). Comparison of compound effects on Peri.4U™ with published data. Peri.4U™ data is in good agreement with published data (indicated in red) (C).

Characteristics of CNS.4U™ (Fig. 1): CNS.4U™ form extensive neuronal networks (A) and exhibit long-term synchronous network activity assessed by MEA (B). CNS.4U™ exhibit burst-like spontaneous activity, synchronized over 16 electrodes of a 48well MEA measurement (C). Network bursts representing synchronous activity is illustrated in (D); this activity occurs after 3-4 weeks in culture (E). **Suitability of CNS.4U™ for seizure assays (Fig. 2):** Representative recording of vehicle, picrotoxin and phenytoin illustrated as summarized activity over time prior to and 30, 60, 90 minutes after drug application (A). Quantification of effects on number and amplitude of bursts at 60 min time point (B).

CONCLUSIONS

- MEA assays for two different types of iPSC-derived neuronal cell types have been established
- For both Peri.4U™ and CNS.4U™, burst-like activity can be measured on MEA with high reproducibility
- Peri.4U™ in combination with MEA technology comprise a suitable and predictive test system to assess neurotoxic compound effects
- Proof-of-concept experiments reveal suitability of CNS.4U™ for seizure liability assays given their long-term synchronous network activity and reactivity to seizure-active compounds

COOPERATION PARTNER

NMI TT Pharmaservices