

Multielectrode array assay for seizurogenic potential evaluation using primary neurons

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Organisation

Name of the organisation Janssen Pharma of JNJ

Department Global safety pharmacology

Country Belgium

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Regulatory use - Routine production, Translational - Applied Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	rodent
Type of cells/tissues/organs	Cortical neurons

DESCRIPTION

Method keywords

Multi-electrode arrays

Neurons

Scientific area keywords

Safety Pharmacology

Side effects

medium-high throughput assay
Neuronal toxicity/ seizure derisking
regulatory Safety Pharmacology
medium-high throughput assay
regulatory

Method description

Experiments were conducted using cortical cells that contains glutamatergic and gabaergic neurons and glia. Primary neurons were freshly dissociated from embryonic E18-19 rat cortices as described previously and plated onto 48-well MEA plates (Maestro system, Axion Biosystems). One day before plating the cells, each 48-well MEA plate was pre-coated with a polyethyleneimine (PEI) (0.1%) solution (Sigma), washed four times with sterile distilled water and then allowed to dry overnight. On the day of plating, Laminin (20 μ g/ml) (Sigma) was added to each 48-well plate which was then incubated for 1h at 37 $^{\circ}$ C. Thereafter both types of neurons were cultured at 37 $^{\circ}$ C, 5% CO₂, 95% air atmosphere, in Neurobasal medium (Thermofisher cat. No. 21103-049) supplemented with 0.5 mM L-Glutamine (Thermofisher cat No 25030149) and 2% B27 (Thermofisher cat. No 17504044) for the rat cortical neurons. At DIV28, spontaneous neuronal activity obtained for 40 min in culture solution was defined as baseline. Compounds are then added at a single dose per well (n = 8 per dose).

Data analysis was performed using Axls software (Axion Biosystems Inc.) and GraphPad Prism (version 7.00; GraphPad Software Inc., San Diego, CA). Active electrodes, AEs, (16 electrodes per well) were defined as an electrode having an average of more than 6 spikes per min (0.1 Hz). An active well should have more than 15% active electrodes. All wells below this threshold were discarded upon these quality criteria. The threshold for the spike detection was 5.3x the standard deviation of the rms (root mean square) noise.

Statistical analysis consisted of expressing the treatment ratio of exposed wells (percentage change between the baseline and the treatment) normalized to the treatment ratio of 100% in control experiments. Normalized treatment ratios of n = 8 wells were averaged per condition. Each well of the MEA served as its own control, and the changes in electrical activity elicited by the treatments were expressed as percent of that control activity and normalized to the wells treated with the vehicle

control DMSO. The final concentration of DMSO added to each well was 0.1% (1 μ l/ml), which did not alter the pH or the ionic concentration of the medium. Differences were determined using one-way ANOVA with Dunnett's correction; p values below 0.05 were considered significant. Data are expressed as means \pm S.E.M.

Lab equipment

- Laminar flow hood ;
- Cell Incubator ;
- Axion Maestro Multielectrode arrays ;
- Analysis software.

Method status

Internally validated

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Early (fast) evaluation of seizurogenic potential using rat cortical cells.

Challenges

Cell-layer don't reflect 3D-complexity of a (human) brain.

Future & Other applications

Multielectrode array method can potentially be applied for measuring activity of other cell types as well.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Associated documents

[Do in vitro assays in rat primary neurons predict drug-induced seizure liability in humans.pdf](#)

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