Multielectrode array assay for seizurogenic potential evaluation using human pluripotent stem cells derived neurons

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo
This method makes use of	Human derived cells / tissues / organs
Specify the type of cells/tissues/organs	Human pluripotent stem cell derived neurons, human pluripotent stem cell derived astrocytes

DESCRIPTION

Method keywords

Stem cell

multi electrode array

electophysiology
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 human induced pluripotent stem cells supplemented with hiPSC derived astrocytes and their reagents can be obtained commercially and cultured as per manufacturer's instructions. 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□□g/ml) (Sigma) was added to each 48-well plate which was then incubated for 1h□at 37□°C. Thereafter neurons were cultured at 37□°C, 5% CO2, 95% air atmosphere, in the hiPSC derived neurons media provided by the manufacturers for the hiPSC derived neurons and astrocytes (e.g. NCardia, CNS4U®). 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 AxIs 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□□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□±□S.E.M.

Lab equipment

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

Method status

Still in development
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Early (fast) evaluation of seizurogenic potential using human cells.

Challenges

- Maturation of the human iPSC derived neuronal cells remains debate ;
- Cell-layer don't reflect 3D-complexity of a (human) brain.

Modifications

- Use of more mature cells (due to improved culture conditions ?) ;
- Use of more diverse types of neuronal cells (glia cells, oligodendrocytes ...).

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Associated documents

Role of Kv7.2Kv7.3 and M 1 muscarinic receptors in the regulation of neuronal excitability in hiPSC-derived neurons.pdf

PARTNERS AND COLLABORATIONS

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