Detection of drug-induced seizure-like activities using MEA system in cultured human iPSC-derived neurons: Report from multi-site pilot study of the HESI NeuTox Committee in collaboration with CSAHi and iNCENS

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Introduction

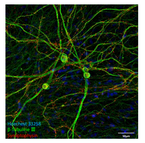
Micro-electrode array (MEA) systems have recently attracted attention for use in predicting the seizure risk of new drugs. MEA subteam of The Translational Biomarkers of Neurotoxicity (NeuTox) Committee in Health and Environmental Science Institute (HESI) have started the pilot study for the prediction of seizure liability of drugs. We are also attempting the prediction of seizure liability using MEA in iNCENS (iPSC Non-Clinical Experiments for Nervous System) project and CSAHi (Consortium for Safety Assessment using Human iPS Cells). Here, we report novel analysis methods that distinguish the responses to convulsants from that to non-convulsants. Human iPSC-derived cortical neurons (Axol) and astrocytes (Axol) were cultured on 24-wells MEA plate for extracellular recording using MED64 Presto. HESI twelve compounds (pentylenetetrazole, picrotoxin, 4-aminopyridine, linopyridine, amoxapine, strychnine, pilocarpine, amoxicillin, chlorpromazine, enoxacin, phenytoin, and acetaminophen) and dimethyl sulfoxide (DMSO) were tested at 5 concentrations for each compound (n>10).

Material & Methods

Human iPSC-derived cortical neurons [AXOL Bioscience]

Human iPSC-derived neural stem cells (ax0019, Axol Bioscience) were cultured 8.0×10^5 cells/cm^2 on the MEA. After 14 days culture, Human iPSC-derived mature astrocyte (ax0084, Axol Bioscience) were added 2.0×10^4 cells/well.

Twelve compounds (pentylenetetrazole, picrotoxin, 4-aminopyridine, linopyridine, amoxapine, strychnine, pilocarpine, amoxicillin, chlorpromazine, enoxacin, phenytoin, and acetaminophen) and dimethyl sulfoxide (DMSO) were added to neurons co-cultured with astrocytes at 5 concentrations for each compound. Each compound was cumulative administered in 31 weeks culture samples.



High-sensitivity MEA system [Alpha med scientific]

To record the electrophysiological responses to HESI 12 compounds plus DMSO, we used a planar MEA measurement system (Presto, Alpha Med scientific, Japan). The MEA chips contain 384 electrodes across 24 well plate with low impedance and high S/N ratio. Spontaneous firings in cumulative administration were recorded for 10 min per each. Spike detection were performed using Presto software (Alpha MED Scientific) . Synchronized burst firings (SBFs), major seizure-like activities, were detected using our '4-step method' (Matsuda et. al., BBRC, 2018) that can accurately detect the number of SBFs and the duration in a SBF.

Conclusion

■ As a result of analyzing the response to HESI 12 compound in human iPSC-derived cortical neurons with 10 parameters, the parameters capable of detecting dose-responses varied depending on drug type.

⇒ These results indicate that it is difficult to detect the responses to convulsive drugs with different mechanisms of action with a single parameter.

■Principal component analysis with parameter set of TS, IBI, MF, and CV of IMFI resulted in a dose - dependent response in convulsants but not in negative control drugs.

⇒ The principal component analysis using multiple parameters (TS, IBI, MF, and CV of IMFI) is effective for the detection of responses to convulsive positive compounds

■HESI 12 compounds were classified into 6 clusters. In addition, Picrotoxin and PTZ, 4-AP and linopyridine were classified into the same cluster respectively.

⇒It was suggested that TS, IBI, MF and CV of IMFI parameter set are effective for the detection of convulsion toxicity and the prediction of mechanism of action.

Results① Electrophysiological responses to HESI 12 compounds in human iPSC-derived cortical neurons

Raster plots

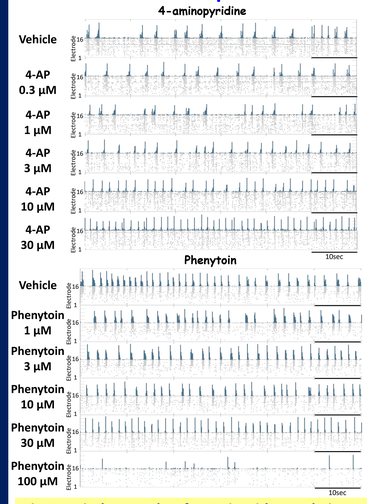
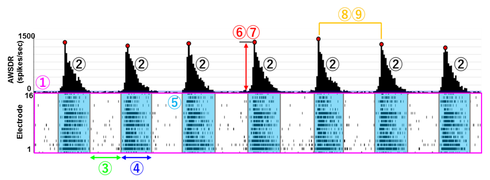


Fig. 1 Typical raster plots for 1 min with cumulative administration of 4-AP and phenytoin
Upper: 4-AP (Vehicle, 0.3, 1, 3, 10, 30 μM)
Lower: Phenytoin (Vehicle, 0.3, 1, 3, 10, 30 μM)

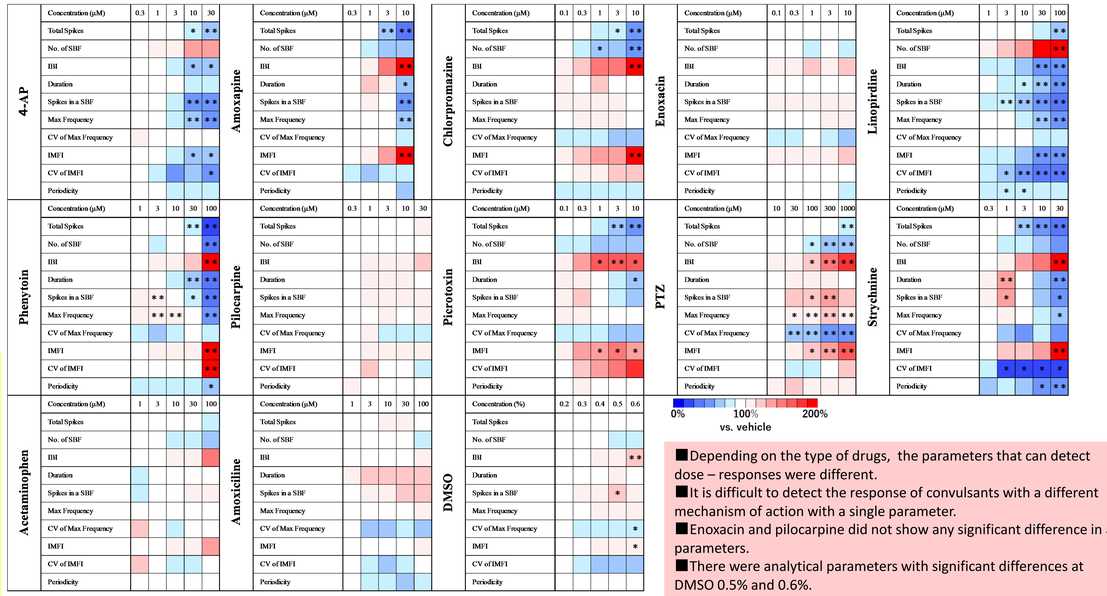
Analysis Parameters



① Total Spikes (TS)
② No. of SBF
③ Inter Burst Interval (IBI)
④ Duration of SBF (Duration)
⑤ Spikes in a SBF
⑥ Max Frequency (MF)
⑦ CV of MF
⑧ Inter MF Interval (IMFI)
⑨ CV of IMFI
⑩ Periodicity

Fig. 2 10 analysis parameters and the analysis results of HESI 12 compounds
(A) Schematic drawing of 10 parameters. CV of MF: Coefficient of variation of max frequency in a SBF, CV of IMFI: Coefficient of variation of inter max frequency interval.
(B) Analysis results of 12 compounds plus DMSO. Spontaneous activities in cumulative administration for 10 minutes were analyzed. The increase or decrease of each parameter is indicated by color. Red and blue indicate the increase or decrease, respectively. 4-AP (n = 18 wells), Amoxapine (n = 11), Chlorpromazine (n = 13), Enoxacin (n = 11), Linopirdine (n = 10), Phenytoin (n = 26), Pilocarpine (n = 12), Picrotoxin (n = 17), PTZ (n = 25), Strychnine (n = 16), Acetaminophen (n = 13), Amoxiciline (n = 13), DMSO (n = 39) . one-way ANOVA and post hoc Dunnett's test, vs. Vehicle, *p < 0.05, **p < 0.01.)

Analysis results of 12 compounds plus DMSO in 10 parameters



■Depending on the type of drugs, the parameters that can detect dose – responses were different.
■It is difficult to detect the response of convulsants with a different mechanism of action with a single parameter.
■Enoxacin and pilocarpine did not show any significant difference in all parameters.
■There were analytical parameters with significant differences at DMSO 0.5% and 0.6%.

Results② Dose-responses by principal component analysis using 4 parameters (TS, IBI, MF, and CV of IMFI)

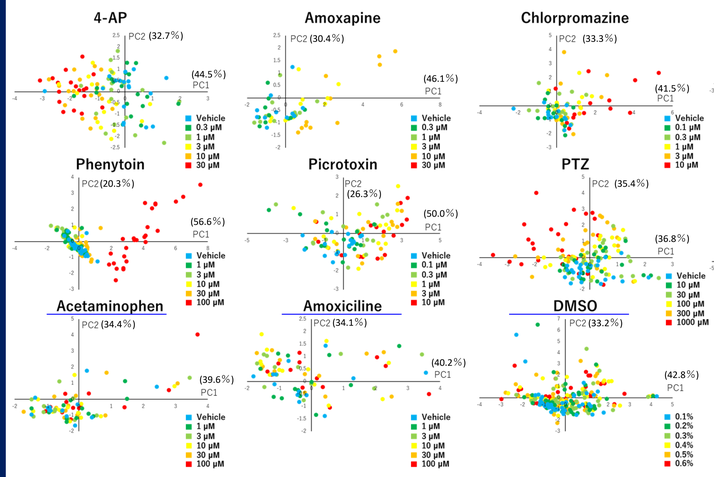


Fig. 3 Dose-responses by principal component analysis using the 4 parameters of TS, IBI, MF, and CV of IMFI.
Score plot of PC1-PC2 for 4-AP (n = 18), Amoxapine (n = 11), Chlorpromazine (n = 13), Linopirdine (n = 10), Phenytoin (n = 26), Picrotoxin (n = 17), PTZ (n = 25), Strychnine (n = 16), Acetaminophen (n = 13), Amoxiciline (n = 13) and DMSO (n = 39).

Table. 1 One-way MANOVA , vs. Vehicle

Drugs	*Concentration* (μM) / *p* value				
4 - AP	0.3 $p = 0.995$	1 $p = 0.726$	3 * $p = 0.0186$	10 ** $p < 0.01$	30 ** $p < 0.01$
Amoxapine	0.3 $p = 0.873$	1 $p = 0.936$	3 * $p = 0.0479$	10 ** $p < 0.01$	
Chlorpromazine	0.1 $p = 0.826$	0.3 * $p = 0.0351$	1 * $p = 0.0159$	3 * $p = 0.0162$	10 ** $p < 0.01$
Linopirdine	1 ** $p < 0.01$	3 ** $p < 0.01$	10 ** $p < 0.01$	30 ** $p < 0.01$	100 ** $p < 0.01$
Phenytoin	1 $p = 0.0627$	3 ** $p < 0.01$	10 * $p = 0.0357$	30 * $p = 0.0318$	100 ** $p < 0.01$
Picrotoxin	0.1 $p = 0.204$	0.3 * $p = 0.0468$	1 * $p = 0.0175$	3 ** $p < 0.01$	10 ** $p < 0.01$
PTZ	10 $p = 0.0657$	30 ** $p < 0.01$	100 ** $p < 0.01$	300 ** $p < 0.01$	1000 ** $p < 0.01$
Strychnine	0.3 $p = 0.847$	1 $p = 0.787$	3 * $p = 0.0371$	10 ** $p < 0.01$	30 ** $p < 0.01$
Acetaminophen	1 $p = 0.530$	3 $p = 0.999$	10 $p = 0.864$	30 $p = 0.816$	100 $p = 0.388$
Amoxiciline	1 $p = 0.936$	3 $p = 0.389$	10 $p = 0.227$	30 $p = 0.253$	100 $p = 0.426$
DMSO	0.2% $p = 0.717$	0.3% $p = 0.614$	0.4% $p = 0.390$	0.5% $p = 0.326$	0.6% $p = 0.218$

* p <0.05
** p <0.01

■Principal component analysis with parameter set of TS, IBI, MF, CV of IMFI detected the dose responses of convulsants.
■No dose-responses were detected in negative control drugs acetaminophen, amoxicilline, and DMSO.
■Dose-dependent changes were mainly detected by PC1.
■Principal component loading of 4-AP and linopiridine were characterized compared with other convulsants.

Results③ Hierarchical cluster analysis of drugs

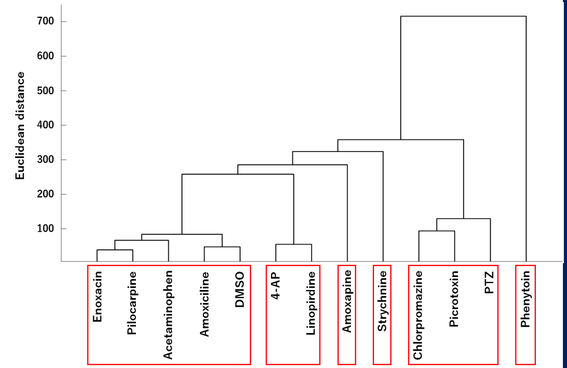


Fig. 4 Hierarchical cluster analysis of 12 compounds plus DMSO
Clustering was performed until the distance among all clusters exceeded 100.

■As a result of clustering by ward method using TS, IBI, MF, CV of IMFI, it was classified into 6 clusters.
■Enoxacin, Pilocarpine, in which no dose - dependent change was detected, was classified in the same cluster as the negative control drugs acetaminophen, amoxiciline, and DMSO.
■GABA-a receptor antagonists picrotoxin and PTZ were classified into the same cluster, and the K^+ channel antagonists 4-AP and linopyridine were also classified into the same cluster.