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High-Throughput Seizure Assessment Using Human iPSC-Derived Neural Cells in a 96-Well Plate Format

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Background

- Seizurogenicity is one of the major reasons for the termination of drug development. Therefore, it is crucial to accurately assess seizurogenicity during non-clinical studies. Considering the difficulty and effort required to evaluate seizures induced by test compounds in animal studies, in vitro assessment of seizurogenicity at earlier stages of drug discovery is valuable. Recently, several in vitro models to assess seizurogenicity using hiPSC-derived neural cells by detecting extracellular field potentials have been reported.

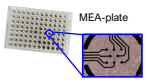
Purpose of research

- In this study, we established an in vitro seizurogenic model using a co-culture of iPSC-derived neural cells in a 96-well plate format, designed to accommodate high-throughput screening of a large number of compounds in the early stages of drug discovery. Additionally, we evaluated 23 different small-molecule compounds.

Materials and Methods

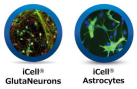
Measuring equipment

✓MEA：Maestro Pro (Axion BioSystems)
✓MEA plate: Cytoview 96 well plate (Axion BioSystems)

Reagents

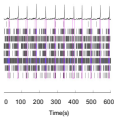
✓Cell：iCell® GlutaNeurons, iCell® Astrocytes (FUJIFILM Cellular Dynamics, Inc.)
✓Coating agent：0.1% polyethylenimine
✓Media：BrainPhys Neural Medium with N2, iCell Neural Supplement B, iCell Nervous System Supplement, and Penisilln-Spreptomycin



AssayProtocol

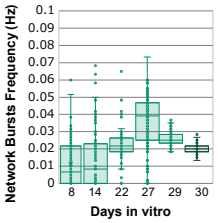
Data analysis

✓Raster plots and network burst Frequency (NBF) data were obtained using Neural Metric Tool (Axion BioSystems).
✓For each well, the change rate of NBF was calculated relative to the baseline value measured before treatment. The average was calculated with n = 4.
✓Positive or negative responses were determined at each cumulative dose based on the vehicle control's mean ± 2 standard deviations (SD) of NBF change rate. (>±2 SD: positive, <±2 SD: negative)



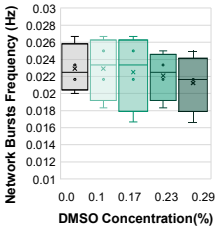
Result

Time-dependent changes of NBF in the 96-well plate



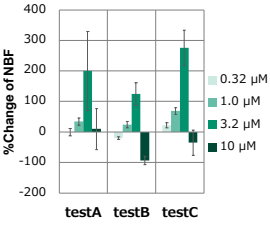
At the early stage of culture, NBF varied significantly between wells. As the culture progressed, this variability gradually converged. On the measurement day, the mean value and coefficient of variation (CV) were 0.020 and 0.12, respectively, indicating no significant differences across all wells in the 96-well plate.

Changes in the vehicle control during cumulative addition



The concentration of the vehicle changed from 0.1% to 0.28% due to cumulative addition, and coefficient of variation (CV) in NBF ranged were from 0.13 to 0.20. No significant differences were observed with respect to vehicle concentration or cumulative exposure time.

Consistency of the positive control compound



The positive control compound (Amoxapine) was evaluated using three independent plates. According to the positive criteria, all test runs showed positive identification within comparable concentration ranges.

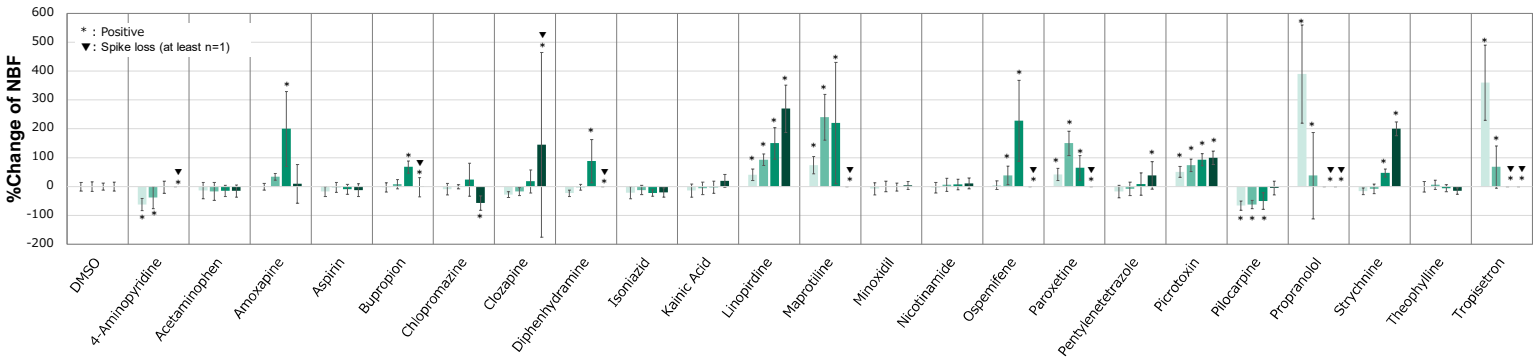
Results of Compound Evaluation Using a 96-Well MEA Plate

a Seizurogenicity
A: Clinical evidence available
B: Non-clinical evidence available
C: No information available

b Positive/Negative determination
P: Positive
N: Negative
X: Positive due to spike loss

Compounds	Mechanism of Action	Seizuro-genicity[a]	Test concentration (μmol/L)							
			Dose1	P/N[b]	Dose2	P/N[b]	Dose3	P/N[b]	Dose4	P/N[b]
DMSO	Vehicle	C	0.10%	-	0.17%	-	0.23%	-	0.29%	-
4-Aminopyridine	K^+-channel blocker	$B^{1,2}$	32	P	100	P	320	N	1000	X
Acetaminophen	COX-1, COX-2, and COX-3 inhibitor	C	3.2	N	10	N	32	N	100	N
Amoxapine	5-HT/D2 receptor antagonist	$A^{3,4}$	0.32	N	1	N	3.2	P	10	N
Aspirin	COX-1 and COX-2 inhibitor	C	32	N	100	N	320	N	1000	N
Bupropion	Norepinephrine and dopamine reuptake inhibitor	A^4	3.2	N	10	N	32	P	100	X
Chlopromazine	D1, D2, D3 and D4 receptors antagonist	A^5	0.32	N	1	N	3.2	N	10	P
Clozapine	D2 and 5-HT2A receptors antagonist	A^6	0.32	N	1	N	3.2	N	10	X
Diphenhydramine	Histamine H1 receptor antagonist	A^7	0.95	N	3	N	9.5	P	30	X
Isoniazid	Mycolic acids synthesis inhibitor	A^8	32	N	100	N	320	N	1000	N
Kainic Acid	Kainate receptor agonist	$B^{1,2}$	0.032	N	0.1	N	0.32	N	1	N
Linopirdine	KCNQ2-KCNQ3 proteins antagonist	B^2	0.32	P	1	P	3.2	P	10	P

Compounds	Mechanism of Action	Seizuro-genicity*	Test concentration (μmol/L)							
			Dose1	P/N[b]	Dose2	P/N[b]	Dose3	P/N[b]	Dose4	P/N[b]
Maprotiline	Norepinephrine reuptake inhibitor	A^4	0.32	P	1	P	3.2	P	10	X
Minoxidil	Histamine H1 receptor antagonist	C	0.95	N	3	N	9.5	N	30	N
Nicotinamide	GPR109A receptor activator	C	32	N	100	N	320	N	1000	N
Ospemifene	Estrogen receptor modulator	C	3.2	N	10	P	32	X	100	X
Paroxetine	5-HT receptor inhibitor	A^9	0.32	P	1	P	3.2	P	10	X
Pentylenetetrazole	GABAA receptor antagonist	$B^{1,2}$	158	N	500	N	1581	N	5000	P
Picrotoxin	GABAA receptor antagonist	$B^{1,2}$	3.2	P	10	P	32	P	100	P
Pilocarpine	Muscarinic M3 receptor agonist	A^{10}	95	P	300	P	949	P	3000	N
Propranolol	Non-selective β adrenergic antagonist	A^{11} neuropsychiatric adverse events	3.2	P	10	P	32	X	100	X
Strychnine	Glycine agonist, AchR antagonist	$B^{1,2}$	0.32	N	1	N	3.2	P	10	P
Theophylline	Adenosine receptors antagonist	A^{12}	3.2	N	10	N	32	N	100	N
Tropisetron	Serotonin 5HT-3 receptor antagonist	C	3.2	P	10	P	32	X	100	X



Discussion & Conclusion

- **We established a seizure model using iPS cell-derived neurons in a 96-well format.**
- **After approximately four weeks of culture, NBF was consistently observed in all wells, and we characterized the variability of NBF upon cumulative addition of vehicle. Reproducibility of positive control compounds was confirmed. Furthermore, 23 low-molecular compounds were tested by cumulative addition, yielding an accuracy of 78%.**
- Among seizure-inducing compounds, no change in NBF was detected for isoniazid, kainic acid, and theophylline. Conversely, among without seizure-inducing compounds, ospemifene and tropisetron caused changes in NBF.
- Isoniazid is known to induce seizures through its metabolites rather than the parent compound, which likely explains the negative result in this assay system[13]. Kainic acid has shown variable seizure-inducing results in similar assay systems[14], suggesting technical factors may contribute to this discrepancy. Theophylline, which is reported to induce seizures by acting on adenosine receptors, requires further investigation to clarify its mechanism in this model.
- Ospemifene is suggested to affect neuronal cells, although its mechanism remains unclear; neuroprotective effects and promotion of myelin differentiation have been reported[15, 16]. Tropisetron acts as a partial agonist of the α7 nicotinic acetylcholine receptor[17] and may cause neuronal hyperactivity by increasing intracellular calcium levels.

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