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MEA assay with human iPSC-derived neurons generated by a rapid differentiation method

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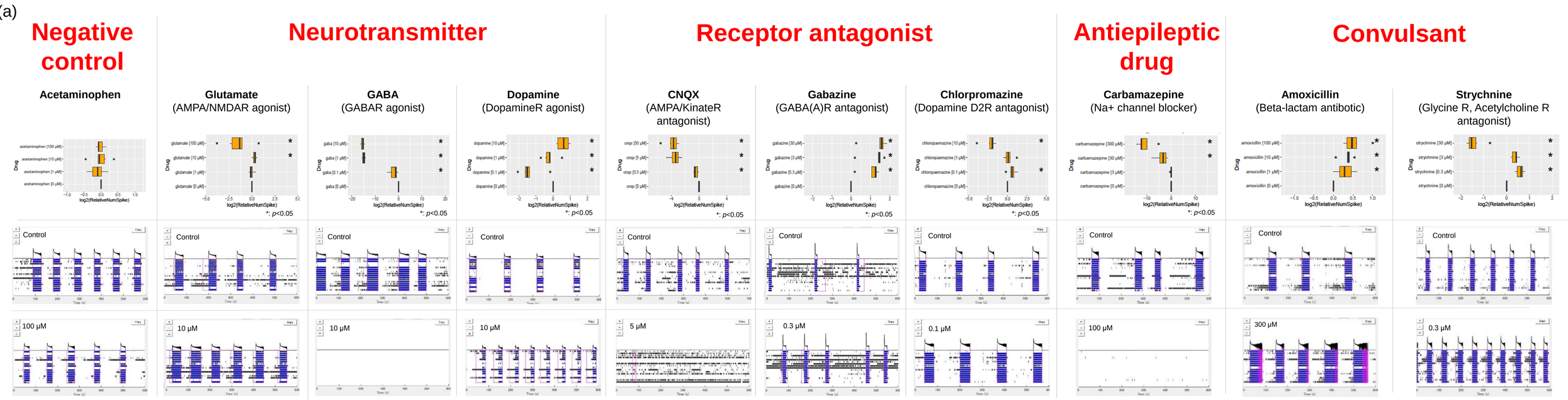
Introduction

Neurotoxicity is a major cause of failures in drug development. Animal models have been insufficient for precise prediction of neurotoxicity because of the species difference. To overcome this difficulty human neurons generated from induced pluripotent stem cell (iPSC) have been extensively tested. A major assay system for this purpose is neuronal networks cultured on microelectrode arrays (MEAs). In this paper we investigated whether Quick-Neuron™ Excitatory (EX-SeV-CW50065) generated with the recently developed quick differentiation method (Quick-Tissue™ technology) are suited to neurotoxicological assays. In addition, we optimized the fabrication and culturing to enable transportation of live-cell MEAs.

Results

1. Reliable pharmacological responses of Quick-Neuron™ Excitatory

(a)

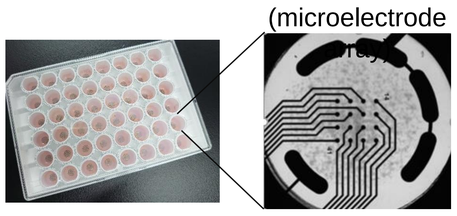


(b)

Mode	Receptors / Ion channels	Chemicals	Testing density	Changes of spike number	Ref: IC50
Negative control	-	Acetaminophen	1-100 µM	No change	
Neuro Transmitter	Glutamate receptors	Glutamate	1-100 µM	↗ ↘	2.3 µM
	GABA receptors	GABA	0.1-10 µM	↘	2.8 µM
	Dopamine receptors	Dopamine	0.1-10 µM	↘ ↗	0.122 µM（D1) 2.76 µM (D2) 1.66 µM (D5)
	Muscarine receptors	Pilocarpine(HCl)	0.1-10 µM	↗	18 µM(M1,3) 4.5 µM(M2)
	Histamine receptors	Histamine	0.3-30 µM	↗	24 µM
Receptor Antagonist	AMPA, Kainate receptors	CNQX	0.5-50 µM	↘	0.92/6.1µM
	GABA(A) receptors	Gabazine	0.3-30 µM	↗	0.2 µM
	Dopamine D2 receptors	Chlorpromazine	0.1-10 µM	↗ ↘	0.363 µM
	NMDA receptors	D-AP5	0.5-50 µM	No change	4.1 µM
	GABA(A) receptors	Picrotoxin	0.1-10 µM	↗	2.4 µM
	Histamine H1 receptors	Ketotifen	0.1-10 µM	↗ ↘	-
Antiepileptic Drug	Sodium channels	Carbamazepine	3-300 µM	↘	131 µM
Convulsant	beta-lactam antibotic	Amoxicillin	1-100 µM	↗	100 µM- 10 mM※
	Glycine receptors, Acetylcholine receptors	Strychnine	0.3-30 µM	↗ ↘	17-40 nM
	Potassium channels	4-AP	0.3-30 µM	↗	147/117 µM

※Since the IC50 value of amoxicillin is unknown, the IC50 value for other GABA receptors of β-lactams is described.

Device : Maestro Cytoview 48 well plate, Axion Biosystems
Neurons : Quick-Neuron™ Excitatory, Elixirgen Scientific
Astrocytes : Human astrocytes, ThermoFisher
Medium : Neurobasal plus based medium
Culture : DIV51
Evaluation : Relative change of number of spikes



Human iPSC-derived neuron generated with transcription-factor-induced differentiation of iPSCs were co-cultured with human astrocytes. The cells were plated onto MEA plates (48 wells, 16 electrodes/well, Axion BioSystems). (a) (Top) The spike numbers generated in response to the indicated compounds (three concentrations) were divided by that generated in response to the control compound (0.1% DMSO). (Middle and bottom) Raster plots. (b) Summary of the results.

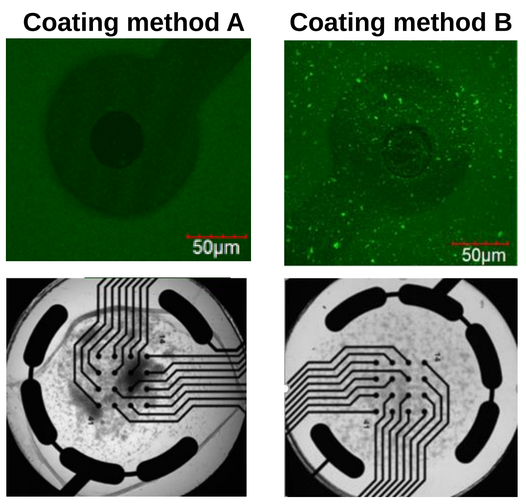
Quick Tissue Neurons show expected responses to neurotransmitters, receptor antagonists, channel blockers, antiepileptic drugs, and convulsant.

2. Optimization of culture stability

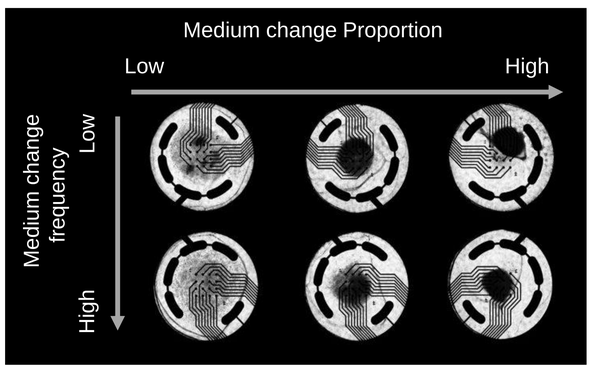
Shinnosuke Koshizuka, Momoko Shionoiri (RICOH).

Neurons: Quick-Neuron™ Excitatory, Elixirgen Scientific
Medium : Brainphys based medium

(c)



(d)

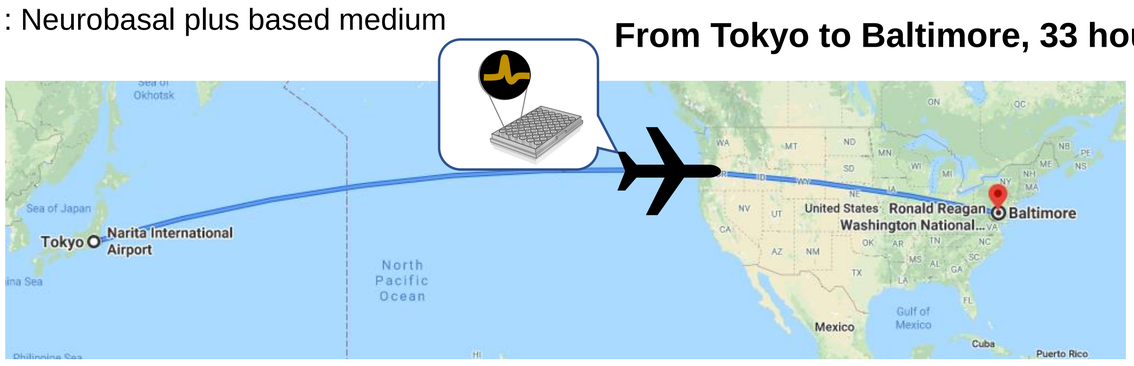


(c) Top: Immunostaining of laminin on the surface of MEAs. Bottom: Photographs of neuron cultures. Coating method B improved laminin adhesion and suppressed cell aggregation. (d) The effect of medium change on cell aggregation.

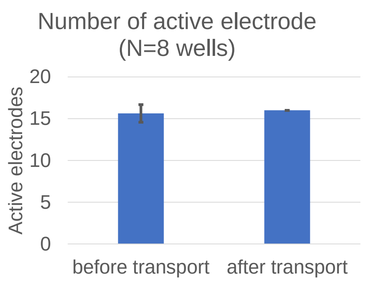
3. Overseas transportation of live-cell MEAs

Neurons : Quick-Neuron™ Excitatory, Elixirgen Scientific
Astrocytes : Human astrocytes, ThermoFisher
Culture : DIV49-52
Medium : Neurobasal plus based medium

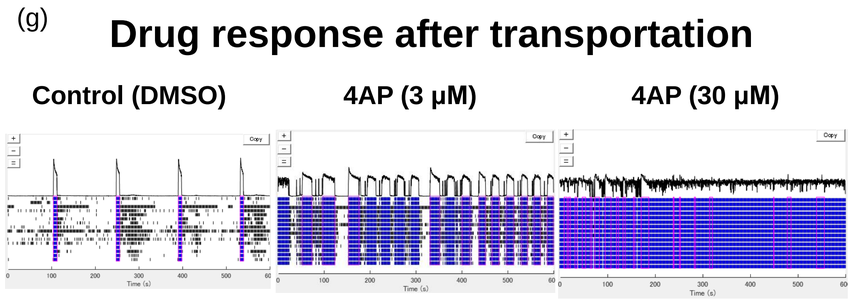
(e)



(f)



(g)



(e) The course of the transportation. (f) The number of active electrodes of MEAs before and after the transportation. (g) Response to 4-Aminopyridine (4-AP), a potassium channel blocker, after the transportation.

Conclusions and future works

Conclusions

- ✓ Quick-tissue neurons exhibit excellent pharmacological responses and are suited to neurotoxicological assays.
- ✓ Optimized culture conditions enable transportation of live-cell MEAs overseas.

Future work

- ✓ We will evaluate the cells with a larger number of compound.

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