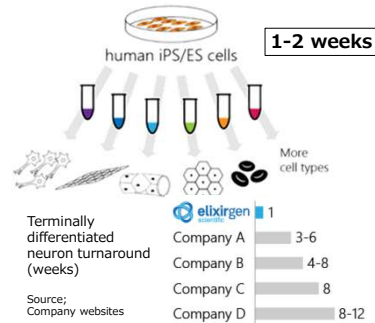


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Introduction

The Quick-Tissue™ technology is a transcription factor-based method for rapid differentiation of stem cells (iPSCs or ESCs) into desired cell types. The method generates pure populations of differentiated cells within 1-2 weeks.

To investigate the suitability of Quick-Tissue™ neurons to toxicological assays, we analyzed their pharmacological characteristics using Ca²⁺ imaging.

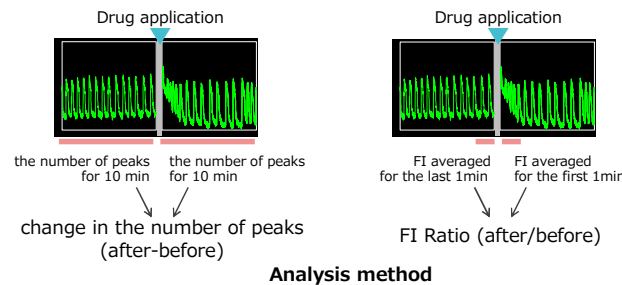


Materials and Methods

Cells: Excitatory neurons were generated using the Quick-Tissue™ technology from iPSCs originating from a healthy donor (Elixigen Scientific, Inc. #EX-SeV-CW50065). Primary human astrocytes were obtained from Thermo Fisher Scientific Inc.

Spheroid culture: 96-well plates were seeded with the neurons and pre-cultured astrocytes at the density of 8,000 cells/well. Plates were cultured in the BrainPhys medium (STEMCELL technologies Inc.) for 6 weeks.

Ca²⁺ imaging: Spheroids were loaded with a fluorescent Ca²⁺ reporter dye (MOLECULAR DEVICES, LLC.). Fluorescence intensity (FI) was measured with the FDSS6000 system (Hamamatsu Photonics K.K.). Drug responses were analyzed by determining the change in the number of peaks of Ca²⁺ oscillation and the ratio of FI before and after the drug application. Statistical significance of the comparison to 0.1 % DMSO was determined by the Wilcoxon rank sum test.

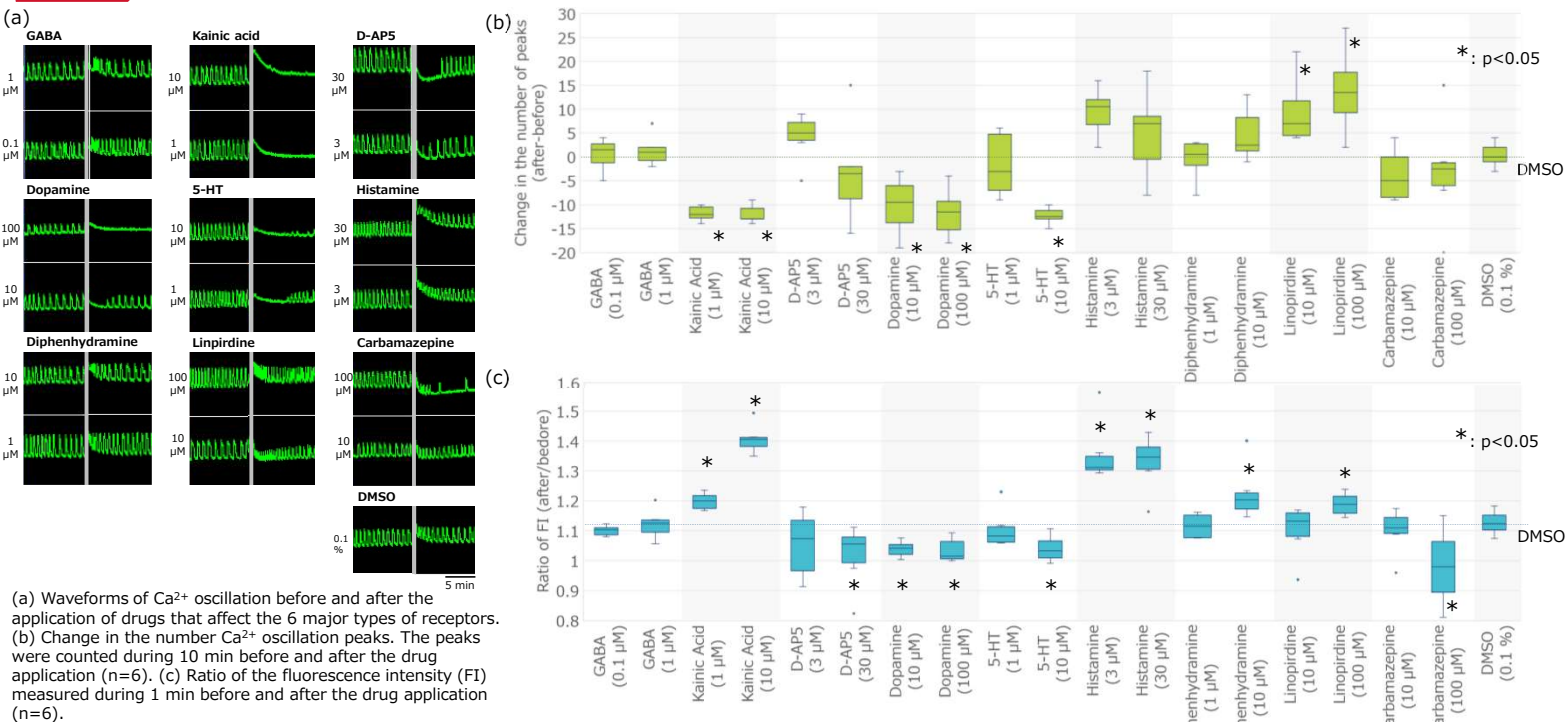


Summary of compounds

Compound	Function	Conc. (μM)
GABA	GABA agonist	0.1, 1
Bicuculline ¹		3, 30
Gabazine ¹		10, 100
Pentylenetetrazole ²	GABA(A) antagonist	100, 1000
Picrotoxin ¹		1, 10
Glutamate	AMPA/NMDA agonist	10, 100
Kainic Acid	Kainate agonist	1, 10
NMDA	NMDA agonist	10, 100
CNQX	AMPA/Kainate antagonist	10, 100
D-AP5	NMDA antagonist	3, 30
Dopamine	Dopamine agonist	10, 100
Chlorpromazine	Dopamine D2 antagonist	10, 100
Haloperidol		1, 10
5-HT	Serotonin agonist	1, 10
Amoxapine	Serotonin H1/2 agonist	3, 30
Buspiron	Mono amine reuptake blocker	3, 30
Bethanechol	Muscarine agonist	1, 10
Pilocarpine ²		1, 10
Atropine	Muscarine antagonist	1, 10
Strychnine ²	Nicotine/Gly antagonist	3, 30
Histamine	Histamine agonist	3, 30
Ketotifen ¹		1, 10
Diphenhydramine ¹	Histamine H1 antagonist	1, 10
4-Aminopiridine		3, 30
Linopirdine	Potassium channel blocker	10, 100
Carbamazepine ³		10, 100
Phenytoin ³	Sodium channel blocker	10, 100
Valproic acid ²		100, 1000
Enoxacin ²	Antibacterial agent	1, 10

1; Convulsant, 2; Epileptogenic, 3; Antiepileptic

Results Ca²⁺ response to major pharmacological compounds



Summary of drug response

Receptors	Drugs	Response
GABA	Agonist	all
	Antagonist	GABAA
Glutamate	Agonist	AMPA/NMDA
		Kainate
	Antagonist	AMPA/Kainate
		NMDA
Dopamine	Agonist	all
	Antagonist	D2
Serotonin	Agonist	all
	Reuptake blocker	
Cholin	Agonist	Muscarine
	Antagonist	Muscarine
		Nicotine/Gly
Histamine	Agonist	all
	Antagonist	H1
Ion channel blocker	Potassium	
	Sodium	

Functional receptors

Confirmed by the responses to the agonists; Glutamate, Dopamine, Serotonin, Cholin, and Histamine

Functional ion channels

Confirmed by the responses to the ion channel blockers; K⁺ and Na⁺

Functional Synapses

Confirmed by the responses to the antagonists; Glutamate, Serotonin, Cholin, and Histamine

*: p<0.05

Summary and Conclusions

- Pharmacological analyses of Quick-Tissue™ neurons suggest the presence of functional receptors for all the 6 major neurotransmitters and functional channels for two cations. The results also suggest synaptic connections for Glutamate, Serotonin, Cholin, and Histamine. The neurons also responded to convulsant, epileptogenic, and antiepileptic drugs.
- The fluorescent intensity (FI) and the number for Ca²⁺ oscillation peaks responded to drugs and thus likely constitute reliable drug response markers.
- These results suggest Quick-Tissue™ neurons are suited to neurotoxicity assays and drug screenings.

COI Disclosure Information

We have the following financial relationships to disclose. Research funds from: Elixigen Scientific, Inc.