Introduction

Alterations in synaptic transmission are associated with a number of psychiatric and neurological disorders, suggesting that approaches directly targeting synaptic function represent an attractive strategy for CNS drug discovery. We previously described the development of a high-throughput screening technology, termed the MANTRA™ (Multiwell Automated Neuronal Transmission Assay) system, for identifying modulators of synaptic function (Hempel CM et al., 2011) in rodent primary neuronal cultures. We are employing the MANTRA system in an integrated drug discovery platform that targets synaptic transmission at multiple levels.

The MANTRA system can be applied first to define synaptic functional alterations in CNS disease model systems and then to perform screening campaigns to identify compounds that restore normal synaptic function. In addition to neuronal cultures from genetic mouse models, neurons derived from human-iPSC represent a valuable cellular model system for measuring neurotransmission abnormalities in a human disease-relevant context.

Use of human neurons for neurotransmission screening applications requires that cultures achieve a sufficient degree of synaptic maturation to yield a measurable proportion of synapses with pre- and post-synaptic functionality. Here, we show that cultures of human neurons derived from induced pluripotent stem cells (iPSCs) can be utilized in the MANTRA system for synaptic functional assays.

Materials & Methods

Cell Culture: Pericytes human neurons derived from iPSCs (“iCell® Neural”). Cell Culture. iCell Neural neuronal cultures were derived from human iPSCs (Lonza) cultured with a 1:1 ratio of laminin-521 (Sigma) and poly-L-lysine (Sigma) before addition of supplemented DMEM/F12 media (Lonza). Cells were maintained in an incubator at 37ºC, 5% CO2. Image 1: Image of growth media used in all experiments. iCell® Neural neuronal cultures were derived from iPSCs and maintained by glia-free neural differentiation media. iCell® Neural media contained 4% FBS, 1% B27, 10 ng/ml bFGF (Peprotech) and 10 ng/ml EGF (Peprotech). All media used were purchased from Lonza. Glia-free differentiation media was employed for all experiments.

Reporter Cell Transduction: For analysis of synaptic function, cultures were infected with an aden-associated virus (AAV) of mixed 1/2 serotype to deliver synaptophysin to neuronal cultures. Synaptophysin was chosen as the reporter for the MANTRA system. An adeno-associated virus (AAV) of mixed 1/2 serotype was used to deliver synaptophysin to neuronal cultures. Synaptophysin expression was driven by the human synapsin promoter (hSyn-synphysy AAV).

Rat Neuron Cultures: Rat neuronal cultures were derived from adult rat brains. Rat neuronal cultures were maintained in serum-free media (Lonza), Neurobasal (Invitrogen) supplemented with 2% B27, 1% G418 (100 µg/ml), and 10 ng/ml EGF (Peprotech). Cultures were maintained at 37ºC, 5% CO2.

Rat and Human Neuron Cultures: Rat and human neuron cultures were grown on PDL-coated black glass-bottomed 96-well plates. Cultures were maintained in serum-free media (Lonza), Neurobasal (Invitrogen) supplemented with 2% B27, 1% G418 (100 µg/ml), and 10 ng/ml EGF (Peprotech). Cultures were maintained at 37ºC, 5% CO2.

Effect of Glia on MANTRA Activity

(a) Triple immunofluorescence of Cell nuclei grown for 6 weeks in absence or presence of rat glia. Similar results were obtained with human glia (not shown).

(b) Synaptophysin immunofluorescence at higher magnification shows punctate staining in axons and less staining in the cell bodies of human neurons in presence of rat or human (not shown) glia.

Conclusions

- Transduced iCell neuronal cultures display measurable levels of evoked presynaptic activity after 6 weeks in culture.
- Enables application of human neurons to high throughput (compound A) validation in CNS drug discovery.
- Further optimization required for HTS in human neurons.

Applications of MANTRA for New Functional Phenotypic Assays in iPSC-derived Neurons

1. The high-throughput capacity of the MANTRA system provides a unique capability to test multiple conditions in parallel to generate human iPSC-derived neurons with optimal synaptic functionality.
2. Ultimately, the MANTRA system can be used to characterize synaptic abnormalities in neurons derived from patients and to screen for compounds that restore normal synaptic transmission.

Galenea is interested in developing these 2 approaches via collaborations. If your group is interested please contact us at plaing@galenea.com.

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Optimization of Neuronal Cultures Derived from Human Induced Pluripotent Stem Cells for High Throughput Assays of Synaptic Function

Pascal Laeng, Chris M. Hempel, James J. Mann, Jeffrey R. Cottrell and David J. Gerber, Galenea Corp, Cambridge MA 02139