Electrophysiological characterization of iPSC-derived excitatory neurons, including a comparison to genetically modified Frontotemporal Dementia neuron model, using automated patch clamp.

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Abstract

Induced pluripotent stem cell (iPSC) cultures show great potential for the generation and characterization of neuronal subtypes as well as for the investigation of neurological disease models. However, in practice the intercellular variability in a population of iPSC-derived neurons, in combination with the long-throughput nature of manual electrophysiological experiments, have made such studies challenging. In this study, we report an initial characterization of iPSC-derived excitatory neurons, focusing on cell action potential properties. Automated patch clamp (APC) was used for the electrophysiological characterization of iPSC-derived excitatory neurons. Results indicate that automated patch clamp is an efficient tool for high-throughput characterization of neuronal subtypes, including characterization of their time and voltage-dependent ion channel properties. The study demonstrates the usability of high-throughput APC for the electrophysiological characterization of iPSC-derived excitatory neurons.

Development and characterization of iPSC-derived excitatory neurons of high purity and consistent gene expression

Comparison of WT and Frontotemporal Dementia (FTD) neuron model

Automated Patch Clamp (APC) experiment setup

References:
1. Lin H et al., Stem Cell Reports, Vol 16, 2118-2127, 2021
2. Glovston et al., J Cell Reports, Vol. 10, 10385, 2023

Methods

Sophion’s automated patch clamp systems. A. Qube 384 for high performance and high-throughput ion channel characterization and screening. B. QPatch - the benchmark solution for efficient, high-quality ion channel studies in physiological solutions.

Solution: Please contact us for further details (info@sophion.com).

All the analysis and figures were prepared in the SOPHION Analyzer and Pream 8.2.1 (GraphPdB Software, Inc, La Jolla, CA, USA).

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Fig. 1. Characterization of iPSD-induced excitatory neurons. A. Induced excitatory neurons were generated from human iPSC lines were genetically engineered to contain a doxycycline response element. The neuronal differentiation protocol was optimized for highly consistent and reproducible neuronal cultures, the ability to cryopreserve the cells, and result in a product that yields functionally differentiated neurons of high purity and consistent gene expression and characterization of neuronal subtypes as well as the investigation of neurological disease models. B. Immunostaining of induced excitatory neurons (Tau; red) and glutamatergic neurons (vGlut2, green) in co-culture with CAT (astrocytes; blue). C. Induced neurons were recorded across 4 days of development (DIV) and high-to-low consistency, similar to published results from Nunez-induced neurons. 

Comparison of WT and FTD neuronal model

Fig. 2. Characterization of induced excitatory neurons containing GNR483 R493X KO. A. Cells were analysed by Western blot for granulin protein expression, and the cleared GNR483 R493X KO media were much more tightly expressed in the GNR483 R493X KO (FTD) neurons as compared to control (WT) cultures. B. Western blot analysis for granulin expression. 

Automated Patch Clamp (APC) experiment setup

Fig. 3. A. APC setup for measurements of ion channel expression and neuron excitability. Top: source plate versus intake plate (left) and AQMP preparation (middle). Bottom: The study demonstrates the usability of high-throughput APC for the electrophysiological characterization of iPSC-derived excitatory neurons. 

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