Functional phenotypic characterization of novel human iPSC-derived neuronal cell lines to validate and increase their physiological relevance

Introduction
One of the most important concerns is physiological relevance of human iPSC cell models needed for disease modeling e.g. for Parkinson’s disease. This question cannot be answered in general, but a lot of empirical data contribute to a more and more comprehensive picture. We aim to understand and compare the differences between multiple iPSC neuronal cultures by comparing them to a well-known reference: the robust electrical functional activity patterns from primary murine neuronal cultures recorded with multwell micro-electrode arrays.

Methods
Spikes train data sets from hiPSC neurons were compared with hundreds of data sets from primary mouse neuron/glia cultures from 4-different brain tissue cultures grown on multi-electrode arrays (MEAs).

Primary culture: primary mouse tissue cultures from embryos (NMRI) were cultured on MEAs for 4 weeks.

hiPSC culture: We cultured human iPSC Neurons (all Axiongena AG, Germany) on 12 and 48-well MEAs (Axion Biosystems) for at least 4 weeks.

Data analysis: multi-parametric data analysis of more than 200 spike train parameters and classification analysis were performed using NeuroProof software tools NIPyBelt and PatternExpert.

Conclusions
We show that human neuronal cell lines exhibit specific phenotypic similarity profile when compared to the primary culture reference database, e.g. to hippocampus or midbrain or mixed similarities. Moreover, the similarity profiles can be changed by compound addition.

In conclusion, we provide a functional tool to characterize neuronal phenotypes from hiPSC neurons to either adapt their differentiation protocols or mixing neuron-specific cell lines to reach a more relevant phenotype, needed for disease-relevant in vitro modeling.

Results

Human iPSC-derived Neurons:
Dopa.4U

CNS.4U

Peri.4U

Brain Region-Specific Cell Cultures with Unique Network Activity Patterns

Comparing phenotypes from human iPSC-neurons to primary neurons

Phenotypic similarity

MPP+ affects functional activity development of Dopa.4U neurons

Functional phenotype can be shifted and used as a readout for disease modeling

NeuroProof Technology

Phenotypic Screening with MEA-Neurochips

Neuronal Cell Culture

Multichannel Recording

Multiparametric Data Analysis

Pattern Recognition

MAESTRO Recording System

Human neurons: Dopa.4U

Multiparametric Characterization of Neuronal Network Activity

Read-out:
- Extracellular action potentials on a single neuron and network activity level
- Spatio-temporal activity changes as well as synchronicity and oscillation in time scales of 100 ms and bursts

Each specific spike train is described by 200 parameters in 4 categories:

1 General Activity
- spike rate, burst rate, burst period, percent of spikes in burst

2 Burst Structure
- e.g. number, frequency and ISI of spikes in bursts; burst duration, amplitude, area, plateau position, plateau duration

3 Oscillation
- Variance over time as an indicator for the strength of the oscillation; in addition e.g. Gamma function parameters fitted to autocorrelation diagrams

4 Synchronization
- Variance within the network as an indicator for the strength of the synchronization; in addition e.g. simplified synchronicity, percent of units in synchronized burst

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