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

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Introduction

One of the most important concerns is physiological relevance of human iPS 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 hiPSC neuronal cultures by comparing them to a well-known reference: the robust electrical functional activity patterns from primary murine neuronal cell cultures recorded with multiwell micro-electrode arrays.

Methods

Spike 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 tissued cultures from embryos (NMRI) were cultured on MEAs for 4 weeks.

hiPSC culture: We cultured human iPSC Neurons (all Axiogenesis 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 NPWaveX 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:

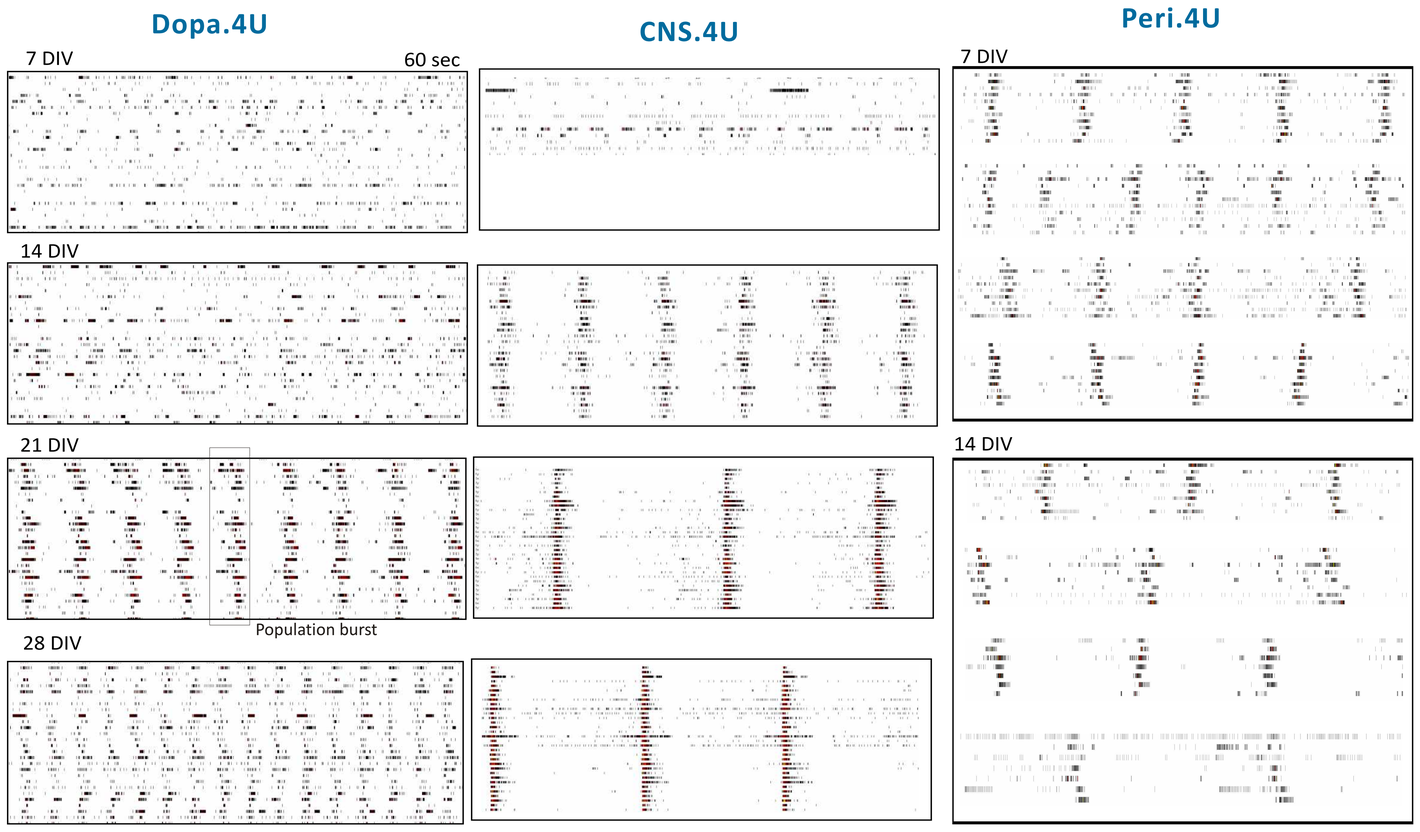
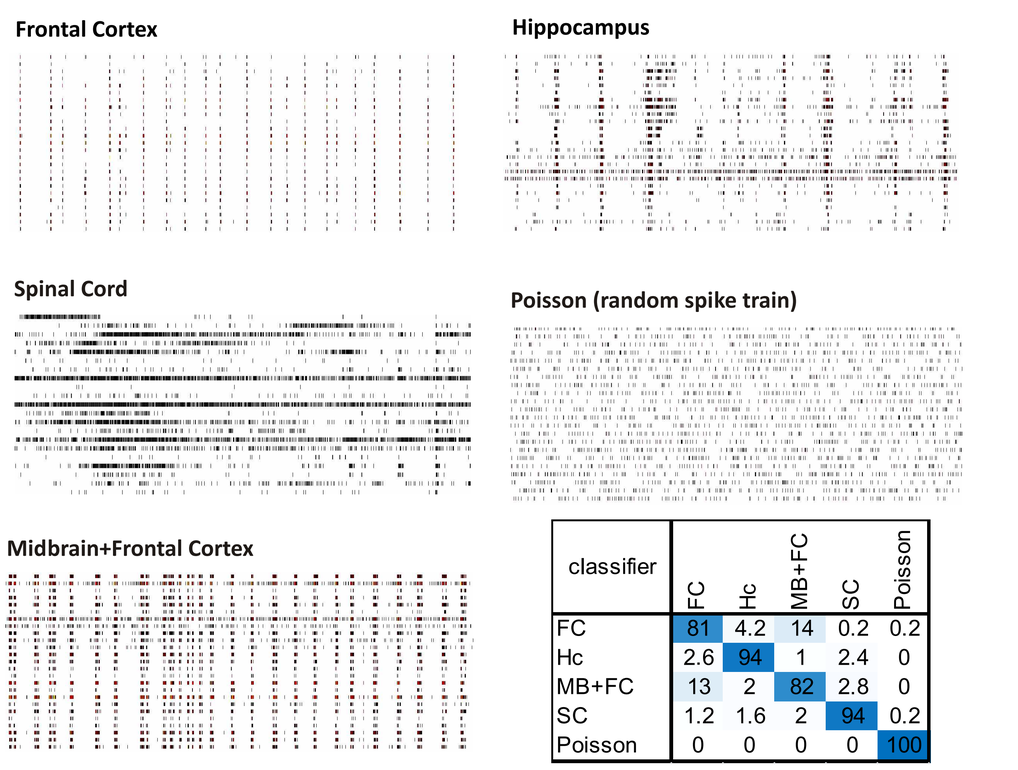


Figure 1: Example MEA spike trains of Dopa.4U neurons during 4 weeks in vitro after thawing. Cultured on 12-well MEAs with 64 electrodes each. A high level of synchronization occurs between 14 and 21 days in vitro (div) shown by strong populations bursts.

Figure 2: Example MEA spike trains of CNS.4U neurons at 7 -28 days in vitro after thawing. Cultured on 48-well MEAs with 16 electrodes each. Synchronized bursting is observed at14 days in vitro (div) shown by strong populations bursts. Unit separation per elexctrode performed by Splitter Software NeuroProof.

Figure 3: Example MEA spike trains of Peri.4U neurons at 7 and 14 days in vitro after thawing. Cultured on 48-well MEAs with 16 electrodes each. A high level of synchronization is already seen at 7 and 14 days in vitro (div) shown by strong populations bursts.

Brain Region-Specific Cell Cultures with Unique Network Activity Patterns



classifier	FC	Hc	MB+FC	SC	Poisson
FC	81	4.2	14	0.2	0.2
Hc	2.6	94	1	2.4	0
MB+FC	13	2	82	2.8	0
SC	1.2	1.6	2	94	0.2
Poisson	0	0	0	0	100

Figure 4: Brain region-specific neuronal cell cultures from mice and human (Dopa.4U). Network spike train patterns of brain-region specific primary cell cultures derived from embryonic mouse tissue of frontal cortex (FC), spinal cord (SC, with dorsal root ganglia), hippocampus (Hc), and midbrain co-cultured with frontal cortex (Mb+FC). Plotted are 60 s of 25 neurons of spontaneous network activity at 28 days *in vitro*.

Figure 5: Cross validation shows that activity patterns are unique (high % self-recognition) and thus, also highly reproducible. Average values of 5 classification rounds using the combination of more than 200 parameters.

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

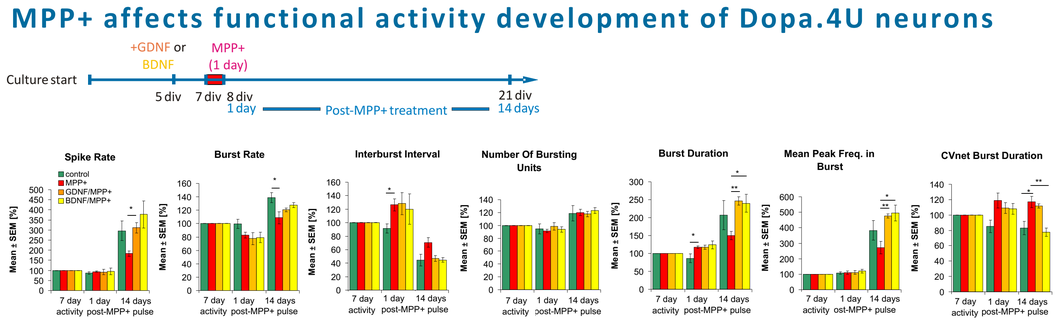
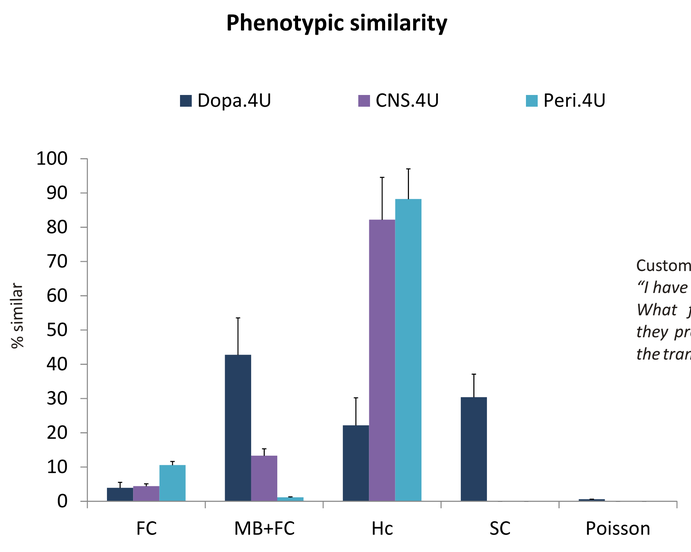


Figure 6. Growth factors prevent functional MPP+ effects on Dopa.4U network development. 6 selected functional parameters show inital reduction of activity and strong effects on burst structure as well as regularity. Network activity is most affected 14 days post-MPP+ treatment. Pre-treatment with GDNF (orange) and BDNF (yellow) prevents functional effects shown by multiple functional parameters.

Comparing phenotypes from human iPSC-neurons to primary neurons



Customer question:
"I have a hiPSC neuronal cell line. What functional phenotype do they produce and is it related to the transcriptional identity?"

Figure 9: classification of activity patterns from three different human iPSC derived neurons after 3 weeks in culture shows a cell line specific phenotype (=fingerprint showing a distribution of similarities compared to primary mouse neuronal cultures). Dopa.4U neurons show the highest similarity to ventral midbrain cultures mixed with cortex. CNS.4U and Peri.4U are most similar to primary hippcampus cultures.

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

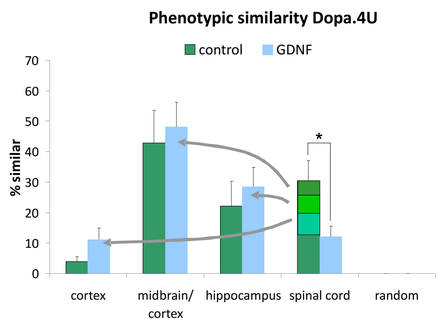


Figure 7. Similarity analysis of functional Dopa.4U phenotype at 8-21 div compared to primary neuronal networks from different tissue cultures (all 28 div). **Dopa.4U are most similar to primary mouse midbrain-cortex cultures.** GDNF changes this functional phenotype towards higher synchronicity and regularity shown by reduced similarity to spinal cord phenotype. This classification uses more than 200 parameters to define classes and similaries.

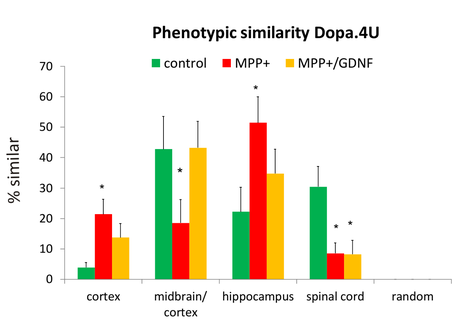
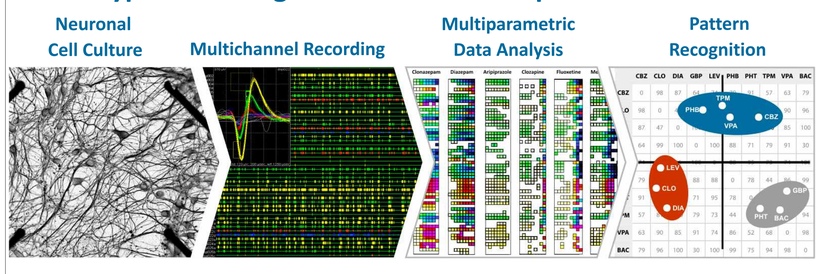


Figure 8: MPP+ treatment at day 7 leads to a decrease in similarity to midbrain-like activity which is rescued by GDNF pre-treatment. Thus, the original phenotype is almost completely restored, thereby phenotypically supporting the effects shown on the single parameter level (figure 7).

NeuroProof Technology

Phenotypic Screening with MEA-Neurochips



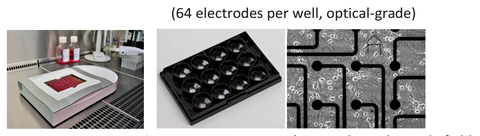
Primary murine cell culture:
- Frontal Cortex
- Hippocampus
- Midbrain
- Spinal Cord/DRG
Neuronal human Stem Cells

Network spike trains and single neuron action potential

Over 200 descriptors at baseline and drug treatment
- General activity
- Synchronization
- Oscillation
- Burst structure

Data base with functional fingerprints of over 100 basic and clinically compounds

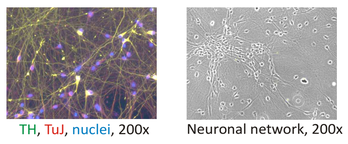
MAESTRO Recording System



Axion Maestro MEA recording Station

Neuronal network on electrode field

Human neurons: Dopa.4U



TH, TuJ, nuclei, 200x

Neuronal network, 200x

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 spikes and bursts

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

1 General Activity
e.g. 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
Variation over time as an indicator for the strength of the oscillation; in addition e.g. Gabor function parameters fitted to autocorrelograms

4 Synchronization
Variation within the network as an indicator for the strength of the synchronization; in addition e.g. simplex synchronization, percent of units in synchronized burst