Functional characterization of iPSC-derived neurons grown on micro electrode arrays (MEA) and their application to phenotypic modeling of disease models and neurotoxicity assessment in comparison to primary mouse cultures.

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Introduction

Primary cultures are widely used for testing drug candidates in phenotypic in vitro models. Moreover, they serve as the gold standard and are used to evaluate human induced pluripotent stem cell-derived (hiPSC) neuronal cultures to transfer current models into the human background. The goal is to increase predictability, sensitivity and specificity.
We cultured different hiPSC-derived CNS neurons including TH+/dopaminergic hiPSC neurons on MEAs and recorded the spontaneous electrical network activity over weeks in culture using micro electrode arrays (MEAs).

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 Dopa.4U Neurons (Axiogenesis AG, Germany) on 12-well MEAs (Axion Biosystems) for 3-4 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

Primary cell cultures show brain region-specific activity pattern which can be clearly distinguished by pattern recognition methods. We show that the pattern complexity from hiPSC dopa neuron cultures is most similar to primary mouse ventral midbrain|cortex co-cultures and that this phenotype can be shifted and restored thereby providing a means for in vitro disease modeling. In conclusion, well-characterized functional human iPSC-derived neuronal in vitro systems and comparison to known primary models increase the predictive value for disease modeling, neurotoxicity assessment and compound screening.

Results

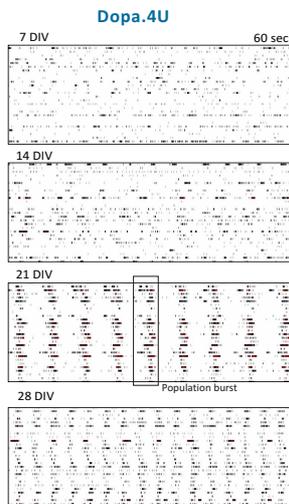### Human iPSC-derived Neurons:

Dopa.4U

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.
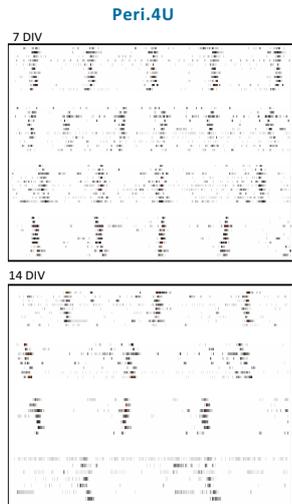
Peri.4U

Figure 2: 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.
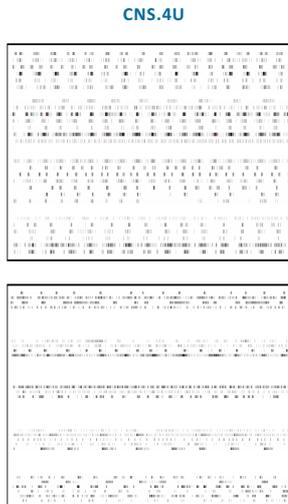
CNS.4U

Figure 3: Example MEA spike trains of CNS.4U neurons at 7 and 14 days in vitro after thawing. Cultured on 48-well MEAs with 16 electrodes each. Synchronized bursting is observed at 7 days in vitro (div) shown by strong populations bursts.

Brain Region-Specific Cell Cultures with Unique Network Activity Patterns
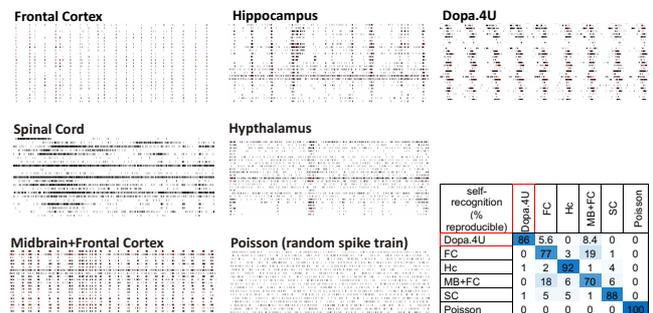

Figure 5: Brain region-specific neuronal cell cultures from mice and human (Dopa.4U). Network spike train patterns of brain-region specific primary cell cultures derived from embryonic mouse tissue of frontal cortex (FC), spinal cord (SC, with dorsal root ganglia), hippocampus (Hc), and midbrain co-cultured with frontal cortex (Mb+FC). Plotted are 60 s of 25 neurons of spontaneous network activity at 28 days in vitro.

self-recognition (% reproducible)	Dopa.4U	FC	Hc	MB+FC	SC	Poisson
Dopa.4U	86	5.6	0	8.4	0	0
FC	0	77	3	19	1	0
Hc	1	2	92	1	4	0
MB+FC	0	18	6	70	6	0
SC	1	5	5	1	88	0
Poisson	0	0	0	0	0	100

Figure 6: Cross validation shows that Dopa.4U activity is 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 7. 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.

Acute functional response to receptor modulators is comparable between Dopa.4U and primay mouse cortex
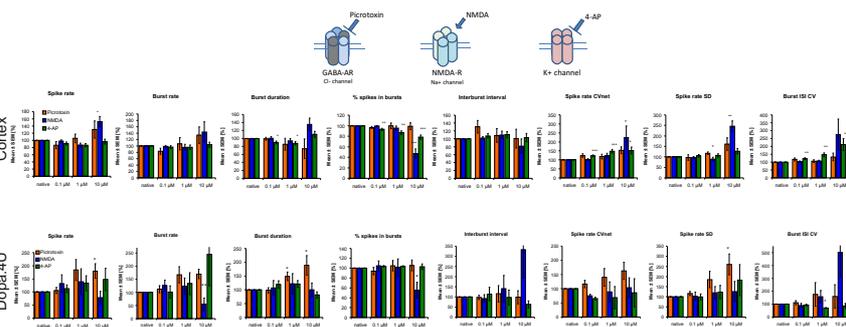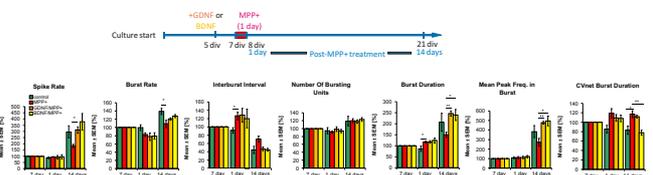

Figure 4. Dopa.4U pharmacologically respond to acute treatment with ion-channel modulators such as NMDA, Picrotoxin and 4-AP, indicating a complex ion channel constitution comparable to primary tissue cultures. Mouse frontal cortex and Dopa.4U neurons respond in the same qualitative manner to ion channel modulators. In some cases effects on Dopa.4U are more potent or cells respond more sensitively shown by excitotoxic-mediated loss of activity. Dopa.4U 14 div, mouse fronal cortex: 28 div.

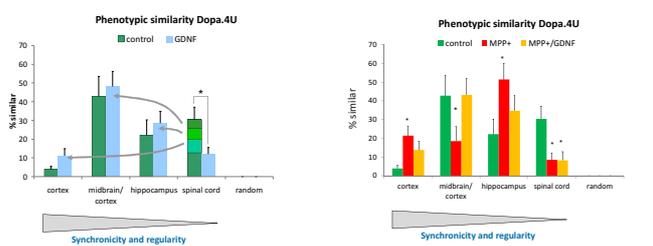### Functional phenotype can be shifted and used as a readout for disease modeling

Figure 8. 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 9: 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

Neuronal Cell Culture — **Phenotypic Multichannel Recording** — **Multiparametric Data Analysis** — **Pattern Recognition**

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

12-well MEA (64 electrodes per well, optical-grade)

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