NeuroProof

Assessing functional phenotypic complexity of stem cell-derived neuronal culture network activity in relation to brain region-specific primary cultures

A.Voss*, K. Jügelt, C. Ehnert, A.-M. Pielka, A. Podßun, B.M. Bader, O. H.-U. Schroeder
NeuroProof GmbH, Germany.
***Corresponding author: alexandra.voss@neuroproof.com**

Introduction

Neuronal cell cultures derived from murine and human stem cells are in the focus of international research now. Primary neuronal cultures have a long tradition and are well characterized and validated. There is a plethora of literature data documenting their physiological relevance in research and drug discovery.
The option of using human stem cells and even personalized patient cultures will bridge *in vitro* assays closer to man. Yet, one of the most important issues is their physiological relevance. This question can not be answered in general, but a lot of empirical data contribute to a more and more comprehensive picture.

We aim to compare the difference of electrical functional activity patterns from primary murine neuronal cell cultures and those cultures derived from human induced pluripotent stem cells cultivated on micro-electrode arrays (MEA). As a result of their phenotypic receptor and neuron type composition, primary neuronal cultures show very specific and complex activity patterns after four weeks *in vitro*. This complexity results from a high level of organization in network cultures, which is present in primary cultures but missing from those derived from stem cells. Thus, we are able to compare but also classify the complexity of stem cell-derived activity patterns in comparison to the current standard of primary cells.

Results

Distinguishable Brain Region-Specific Cell Cultures with Unique Network Activity Patterns

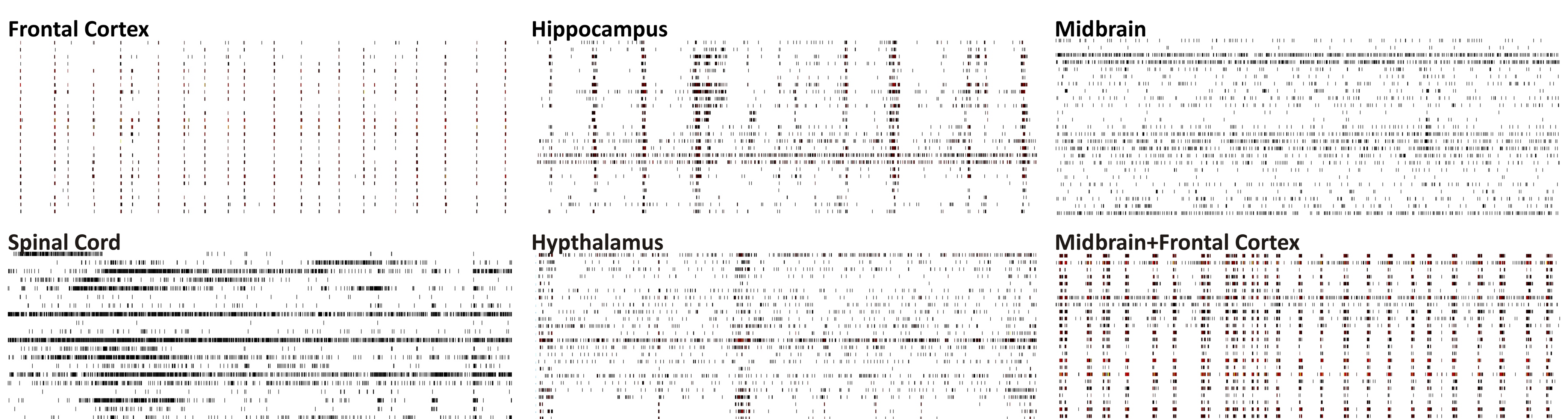
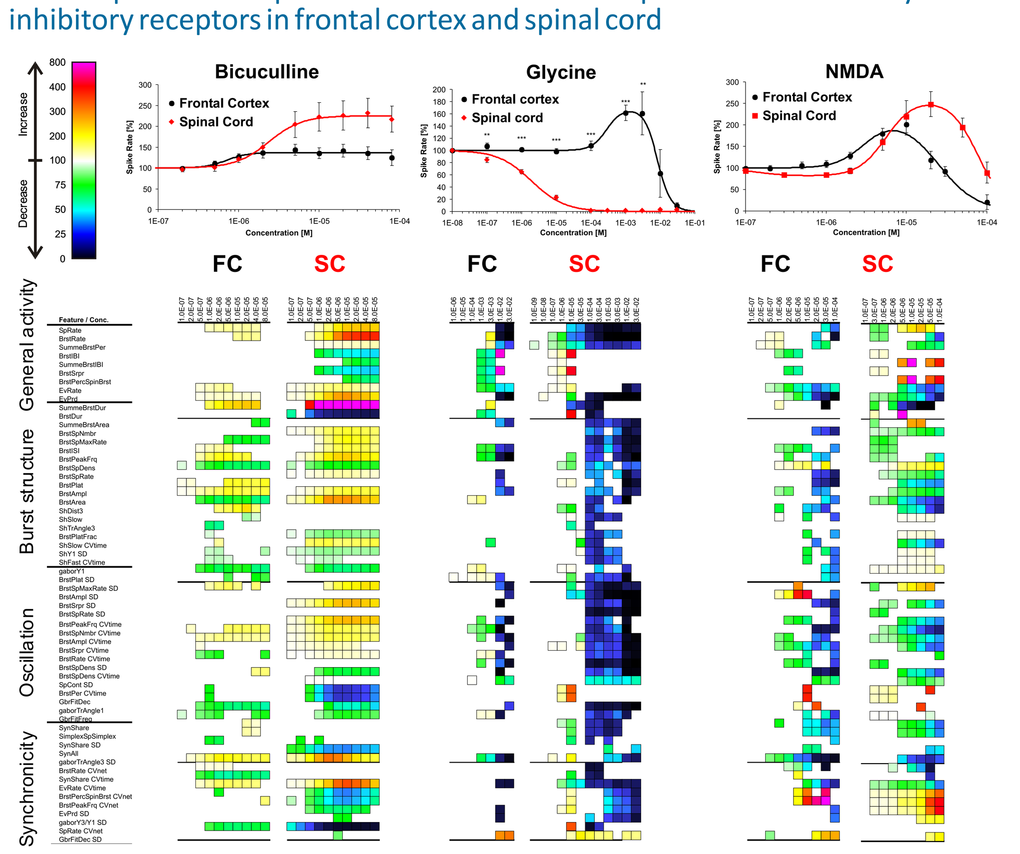


Figure 1: Activity patterns of brain region specific neuronal cell cultures. Network spike train patterns of brain-region specific primary cell cultures derived from embryonic murine tissue of the frontal cortex (FC), spinal cord (SC, with dorsal root ganglia), hippocampus (Hc), and midbrain (Mb; co-cultured with frontal cortex: Mb+FC). Plotted are 60 s of 25 neurons of spontaneous network activity at 28 days *in vitro*.

Compound- and Brain Region-Specific Changes in Network Activity Patterns Reflecting *in vivo* like Behaviour

Tissue-specific composition of functional response of excitatory and inhibitory receptors in frontal cortex and spinal cord



Specific functional responses of the $GABA_A$ receptor alpha subunit in frontal cortex and hippocampus

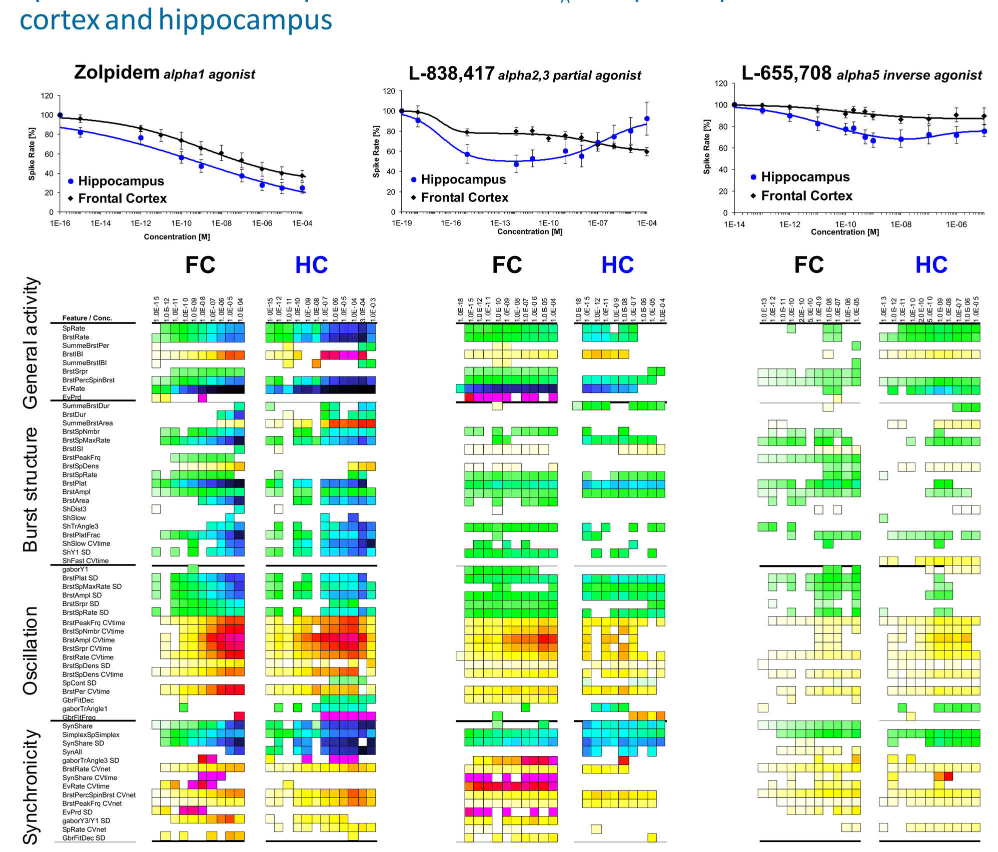


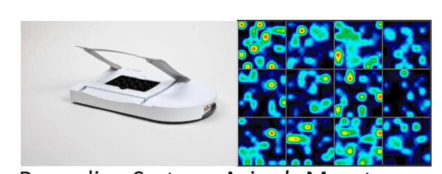
Figure 2: Comparison of brain region-specific activity profiles (frontal cortex: FC; hippocampus: Hc; spinal cord: SC). **Top**: Concentration-response curves of the network spike rate for various compounds, illustrating the compound- and tissue specificity. **Bottom**: Heat maps with significant changes on the 60 most representative parameters for each concentration. The activity parameters characterize the substance-specific activity changes in the 4 main categories: general activity, burst structure, oscillatory behavior and synchronization for treatment of accumulating concentrations. The color codes changes in activity parameters according to their percent changes (100: no change. Only statistically significant effects are shown (p 0.05).

Conclusion

We compared primary neuron/glia cultures from different brain tissues such as frontal cortex, hippocampus, hypothalamus, midbrain, a midbrain/frontal cortex co-culture, spinal cord with dorsal root ganglia, and human stem cell derived neuronal dopaminergic networks grown on MEAs *in vitro* and a randomly generated spike train pattern generated with a Poisson process. We show, that the biological network activities of those primary and human networks are stable, reproducible, and brain region-specific which can be clearly distinguished by pattern recognition methods, revealed by their correct self-recognition of 44 - 100 %. We show that the pattern complexity from human dopaninergic stem cell-derived cultures is sorted between those generated by primary hypothalamus, midbrain and midbrain-cortical co-cultures. Thus, we provide a tool to optimize the hiPSC culture condition towards a higher functional network complexity.

Development of human stem cell based dopaminergic network activity

- **Dopa.4U neuronal networks show similar activity patterns compared to primary networks once at 28 div**
- **First coordinated network activity after 14 div**



Recording System: Axion's Maestro 768 channel system

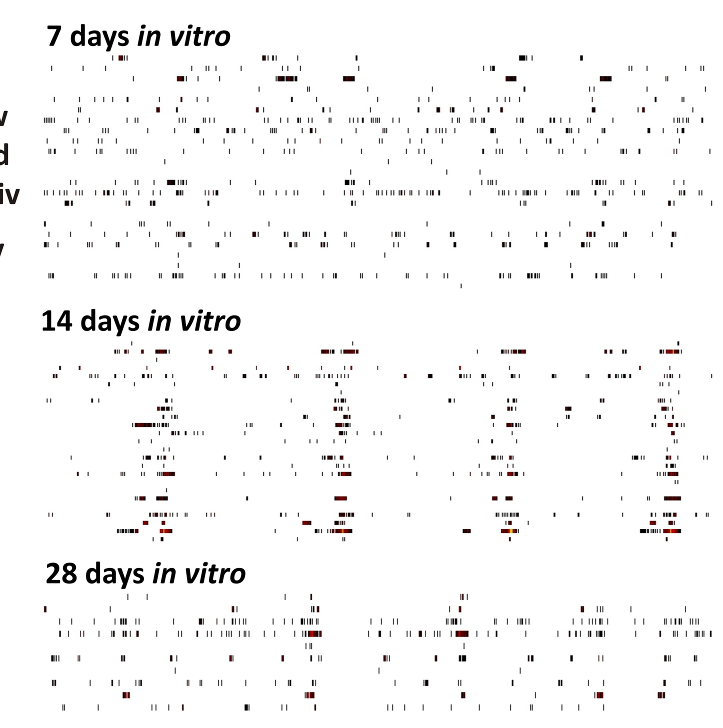


Figure 3: Development of neuronal network activtiy of human stem cell derived dopaminergic Dopa4U cell cultures. Plotted are 60 s of neurons of spontaneous network activity at 7, 14, and 28 days *in vitro*.

Human Stem Cell based Dopaminergic Network Activity: Classification into Brain Region-Specific Network Activity Patterns

Tissue culture	Dopa.4U	FC	Hc	HTh	MB	MB+FC	SC	Poisson
	predicted as							
Dopa.4U	100	0	0	0	0	0	0	0
FC	0	71	4	0	0	25	0	0
Hc	0	4	85	0	8	4	0	0
HTh	0	0	6	75	14	0	4	2
MB	2	2	8	19	44	8	17	0
MB+FC	0	14	4	0	10	68	6	0
SC	0	10	4	4	10	2	71	0
Poisson	0	0	2	0	0	0	0	98

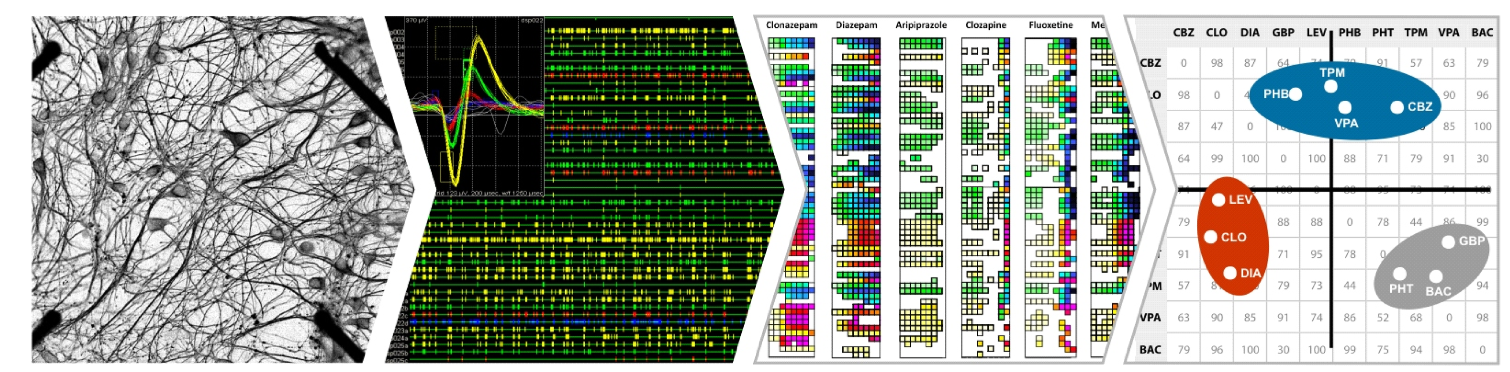
Table: Brain-Region-Specific distingueshable activity patterns % of datasets, n= 4 classification experiments with 20 data sets each. For the Poisson data set randomly and independent spike trains are created with exponential decreasing inter-spike interval histograms. Mean spike rates are identical per unit and defined according to mean SR of FC and SC network activtiy.

Dopaminergic neuronal cell cultures derived from induced pluripotent human stem cells generate:
- **stable and reproducible network activity pattern,**
- **with synchronized burst structure,**
- **a specific activity pattern, revealed by the self recognition of 100%,**
- **network activity that classifies as hypothalamus (36%), midbrain+frontal cortex (31%), midbrain (25%), and spinal cord (8%).**

NeuroProof Technology

Phenotypic Screening with MEA-Neurochips

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



Primary murine cell culture, e.g.:
- Frontal Cortex
- Hippocampus
- Midbrain
- Spinal Cord/DRG

Neuronal human stem cells

Network spike trains and single neuron action potential
Recording Systems:
- Axion Maestro 768 channel system
- Plexon 64 channel MAP system

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

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

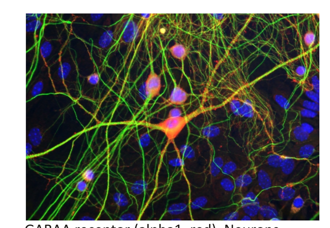
Disease related brain tissue culture
Acute and chronic compound induced disease rescue models

High resolution of spatio-temporal network activity patterns

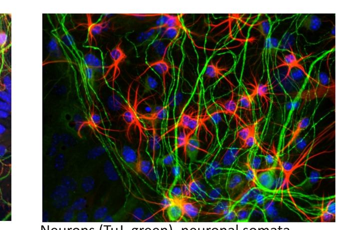
Specific in vitro assays with optimized compound or disease related parameter selection

Similarity and differentiation from known compound effects
Combination therapy

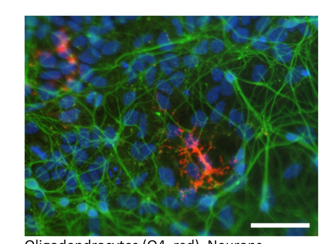
Primary Cortical Neuron/Glia Coculture



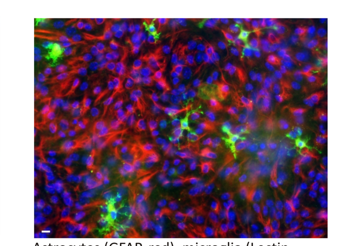
GABAA receptor (alpha1, red), Neurons (TuJ, green), nuclei (blue).



Neurons (TuJ, green), neuronal somata (HuCD, red, nuclei (blue).

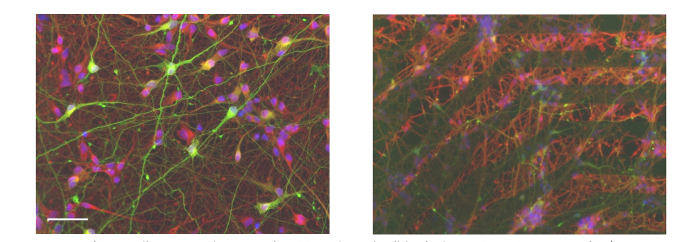


Oligodendrocytes (O4, red), Neurons (TuJ, green), nuclei (blue).



Astrocytes (GFAP, red), microglia (Lectin, green), nuclei (blue).

hiPSC derived Dopaminergic Culture



Neurons (TuJ, red), neuronal somata (HuCD, red, nuclei (blue), dopaminergic neurons (TH+, green).

iPSC-derived dopaminergic neurons and handling protocols are commercially available from Axiogenesis AG/Germany (Dopa4U; www.axiogenesis.com) and Cellular Dynamics International, CDI/USA (iCell Dopa; www.cellulardynamics.com).

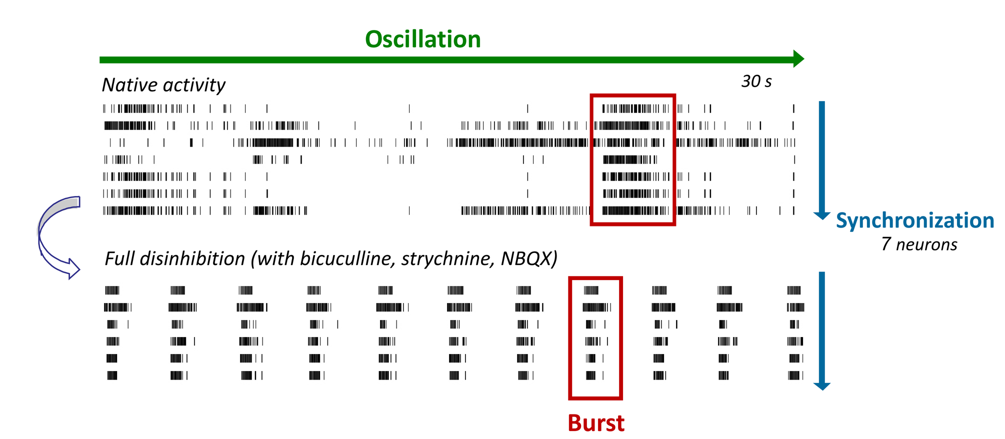
The Dopa4U cells for this study were kindly provided by Axiogenesis AG, Germany.

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 204 spike train parameters computed by in-house software NPWaveX 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