Functional phenotypic in vitro screening of patient iPSC-derived motor neurons used for in vitro HCS disease screening with AI-based analysis of microelectrode array data

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Introduction

Patient-derived iPSC models have been designed for various indications promising higher physiological relevance and thus, better translation to the in vivo situation. Their application eventually may decrease attrition rates in drug discovery and development. This assay is suited for campaigns with medium throughput by comprehensive, physiologically relevant data. We focused on investigating the motor neuron diseases (MND) amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), both causing loss of motor neurons and associated symptoms. We phenotypically describe the consequence of the genetic variation present in ALS and SMA patient iPSC-derived motor neurons on the functional activity and network connectivity. We further elucidated how functional ALS and SMA phenotypes separated from controls during network establishment. This difference is a prerequisite enabling compound testing to rescue the disease phenotypes.

Summary and Conclusions

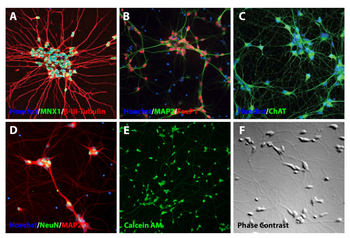
Our novel screening assays platform for motor neuron diseases shows a reproducible functional development of human iPSC-derived motor neurons with synchronous patterns when cultured on and recorded with multi-well MEAs and analyzed with proprietary data analysis methods.
We identified disease-specific functional phenotypes and showed how reference compounds can affect them, establishing the following drug screening assays applicable for medium/high throughput screening:

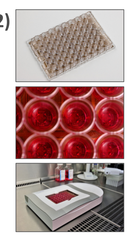
ALS screening assay:
- Robust functional phenotype of ALS patient iPSC-derived motor neurons compared to gene-corrected control motor neurons.
- Partial rescue with ALS drug edaravone.

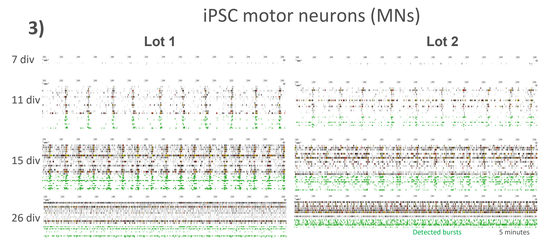
SMA screening assay:
- Robust functional phenotype of SMA patient iPSC-derived motor neurons compared to healthy control motor neurons.
- Concentration-dependent rescue with RG7800.

Results

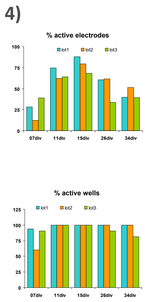
Characterization of wild type human motor neurons

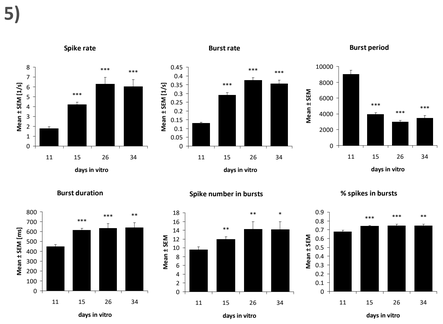
1) 

2) 

3) 

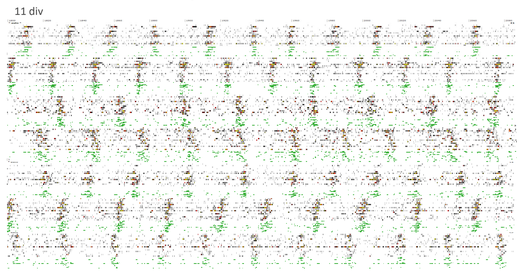
1) Human iPSC-derived motor-neurons express neuronal and motor neuron-specific marker proteins.
2) Cryo-conserved motor neurons were cultured on 48-well MEAs by spotting 10 µl onto PEI-coated electrode fields.
3) Spontaneous functional activity develops over time showing synchronized activity between 11 and 15 days in vitro (div).
4) Lot comparison shows reproducible high numbers of % active electrodes (>0.1 spikes per second) and % active wells (>1 electrode) with a peak of electrode numbers at 11-15 div. n > 10.
5) Multi-parametric spike train data analysis shows a continuous increase of activity until 26 div (spike and burst rate), some parameters (e.g. % spikes in bursts) reach the plateau at 15 div already. * Unpaired Student's t-test.

4) 

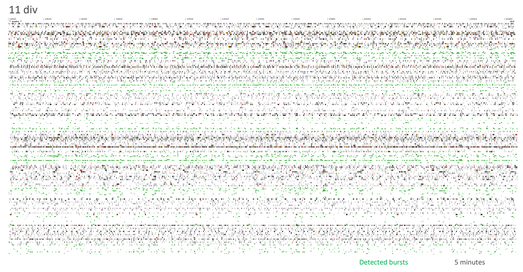
5) 

Spinal Muscular Atrophy (SMA) in vitro disease model

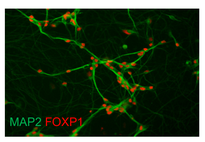
10) Healthy control iPSC MNs



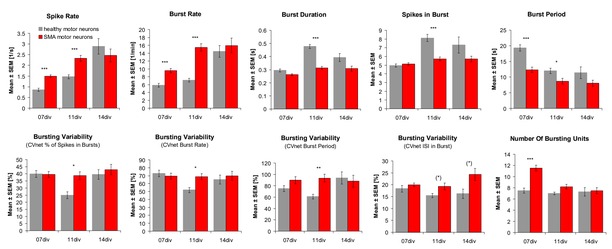
SMA323 patient MNs



11) FoxP1 staining

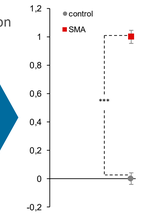


12) Multi-parametric disease phenotype



integration

13) Effect Score

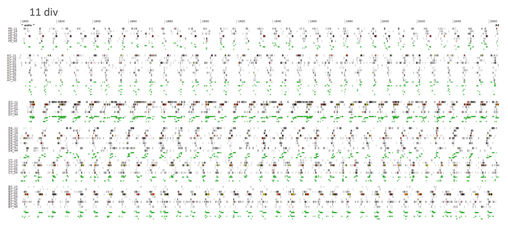


Fibroblasts from SMA-patient and healthy control were reprogrammed into iPSC and differentiated into motor neurons (MNs).
10) Control and diseased MNs show dramatically different activity patterns at 11 div.
11) MN-specific forkhead homeobox 1 protein FoxP1 staining in SMA motor neurons. MAP2 = microtubule-associated protein 2;
12) Multi-parametric spike train data analysis shows a hyper-active state of SMA MNs at 7-11 div (spike and burst rate, shorter burst period, number of bursting units), weaker bursts (reduced # spikes in bursts) and a significantly reduced synchronicity (CVnet increased at 11 div). * unpaired student's t-test with Bonferroni correction. N ctr 7/11/14div > 10, N SMA 7/11/14div > 10.
13) Linear projection of 20 functional parameters into the NeuroProof Effect Score by z' factor-based selection. This disease-specific parameter score shows a significant phenotypic difference between control and SMA neurons at 11 div. This readout allows ranking of rescue efficacies after applying test compounds.

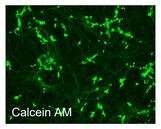
Amyotrophic Lateral Sclerosis (ALS) in vitro disease model

6) Gene-corrected SOD-90D control MNs

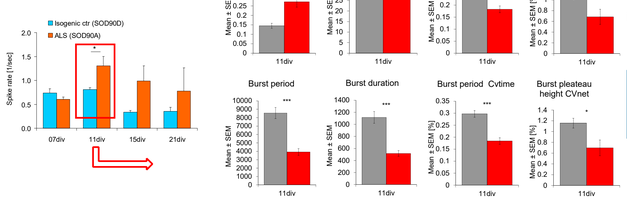
ALS SOD90A patient iPSC MNs



7) Calcein staining

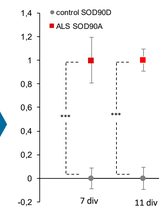


8) Disease phenotype



integration

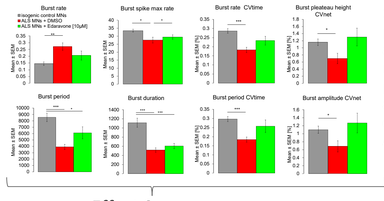
9) Effect Score



ALS-patient fibroblasts (Coriell) carrying SDO90A mutation have been reprogrammed into iPSC, gene-corrected and both diseased and corrected clones differentiated into motor neurons (MNs).
6) Control and diseased SOD90A MNs show different activity patterns at 11 div.
7) MN-specific calcein staining in ALS MNs.
8) General activity (here: spike rate) is elevated at 11 div indicating a hyper-activity state.
9) Effect score calculation for ALS vs. control at 7 and 11 div shows a significant phenotypic difference as the basis for disease modeling.

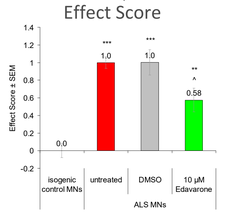
In vitro drug screening assay for genetic motor neuron diseases

Amyotrophic Lateral Sclerosis (ALS)



15) Functional activity parameters showing a snapshot of ALS-mutation-induced phenotype and status after treatment with **edaravone** (approved ALS drug). N=7-34.

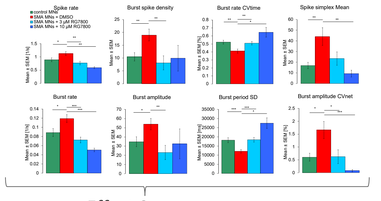
Effect Score



16) Treatment with the FDA approved ALS drug edaravone rescues the ALS genotype-specific functional phenotype significantly.

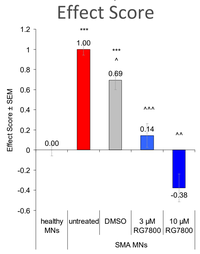
* vs. control, ^ vs. untreated.

Spinal Muscular Atrophy (SMA)



17) Functional activity parameters showing a snapshot of SMA-mutation-induced phenotype and conc.-dependent rescue after treatment with SMA-clinical phase compound **RG7800**. N=3-29.

Effect Score



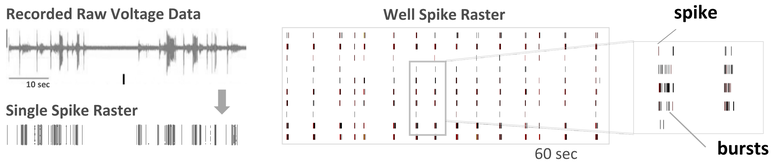
18) Treatment with the clinical phase tested SMA compounds RG7800 rescues the SMA genotype-specific functional phenotype significantly and concentration-dependently.

* vs. healthy control, ^ vs. untreated.

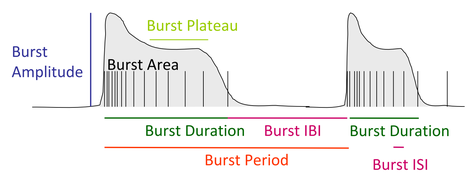
Methods

hiPSC culture: human iPSC-derived motor neurons from healthy control (lot 1-3), ALS patient and gene-corrected isogenic control and spinal muscular atrophy patient (SMA) (BrainXell) were cultured on 48 well MEAs (Axion Biosystems, USA).
MEA recording: MEAs were recorded on the MAESTRO recording station (Axion) at different days in vitro (div) during functional development. Per data point, 60 minutes were recorded at 37°C und stable pH condition. 30 minutes of stable activity was analyzed.
MEA Data analysis: Acquired spike train data was analyzed and more than 200 functional parameters were generated to enable optimal detection and highest resolution of phenotypic differences (NPWaveX software, NeuroProof). "Effect Score" calculation: Projection of multiple parameters into a single parameter based on Z' factor. For more information on Effect Score see Bader BM *et al.* Frontiers Pharmacology 2018.

Spike Detection



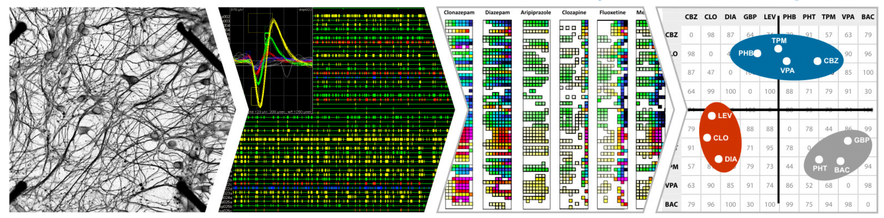
Multi-parametric analysis



Parameter sorted in 4 categories:
- General activity
- Burst structure
- Regularity/oscillation
- Synchronicity/connectivity

NeuroProof Technology

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



Maestro recording station | 48 well MEA | 16 electrodes per well | neurons on electrodes (12 well MEA) | Activity heat map (12 well MEA)