Functional phenotypic screening of patient iPSC-derived motor neurons – in vitro HTS disease modeling with micro electrode arrays coupled with multi-variate analysis methods.

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Objectives

One of the major challenges of drug discovery is decreasing attrition rates which requires developing more predictive pre-clinical in vitro models. Human induced pluripotent stem cell-derived (hiPSC) neuronal cultures promise higher physiological relevance and thus, better translation to the in vivo situation. Patient-derived iPSC models have been designed for various indications. We focused on investigating motor neuron diseases (MND) such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), which cause the loss of motor neurons. **Our aims** were to 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 to enabled compound testing to rescue the disease phenotypes.

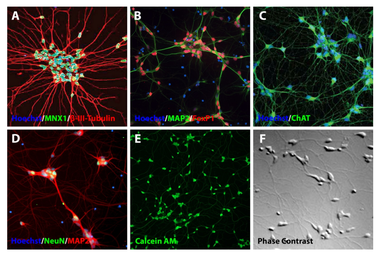
Methods

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.
MEA Data analysis: Acquired spike train data was analysed multi-parametrically (NPWaveX software, NeuroProof). "Effect Score" calculation: Projection of multiple parameters into a single parameter based on Z' factor. For more information on Z'-factor, see Kuemmel et al. 2010. Biomol Screen 15(1):95-101 and Kozak et al. 2010.

Results

Characterization of wild type human motor neurons

1



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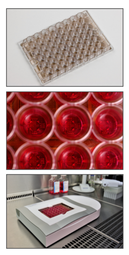

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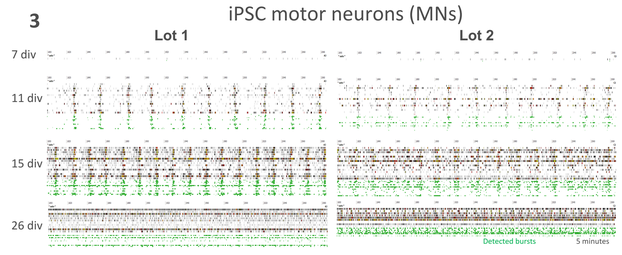

1) Human iPSC-derived motor-neurons express neuronal and motor neuron-specific marker proteins.
2) Cryo-conserved motor neurons were cultured on 48-well MEAs by spotting 10 µl onto PEI-coated electrode fields.
3) Spontaneous functional activity develops over time showing synchronized activity between 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.

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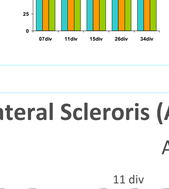

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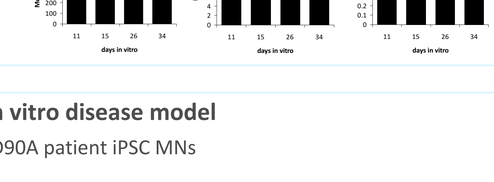
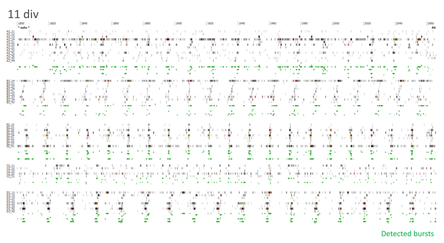
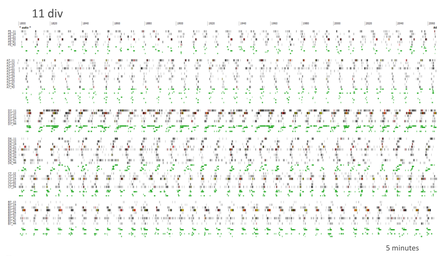


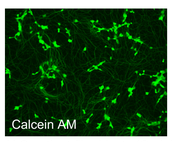
Amyotrophic Lateral Scleroris (ALS) in vitro disease model

6 Gene-corrected SOD-90D control MNs



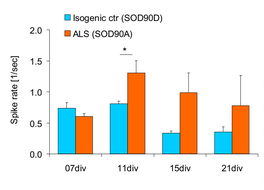
ALS SOD90A patient iPSC MNs



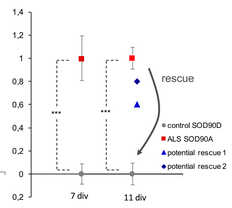


ALS-patient fibroblasts (Coriell) carrying SDO90A mutation were reprogrammed into iPSC, gene corrected and both diseased and corrected clones differentiated into motor neurons (MNs).
6) Control and diseased SOD90A MNs show different activity patterns at 11 div.
7) General activity (here: spike rate) is elevated at 11 div indicating a hyper-activity state.
8) Effect score calculation (see 11 for more information) for ALS vs. control at 7 and 11 div shows a significant phenotypic difference as the basis for disease modelling. N ctr = 16 (7 div), 5 (11 div), n ALS = 16 (7 div), 13 (11 div).
9) Preliminary results of treatment with ALS drug edaravone suggests a decrease of the hyperactive state (study ongoing).

7 Disease phenotype



8 Effect Score



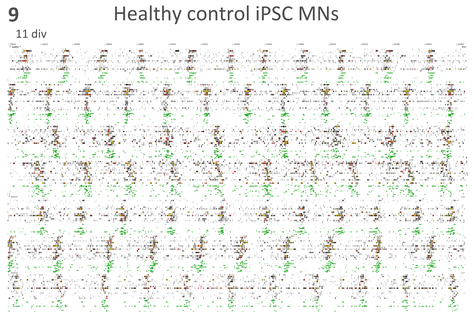
9 Treatment

+ Edaravone *preliminary*

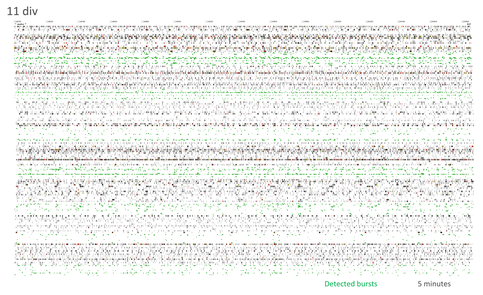
ALS SOD90A patient MNs + Edaravone 10 µM

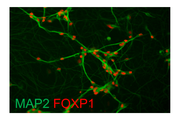
Spinal Muscular Atrophy (SMA) in vitro disease model

9 Healthy control iPSC MNs



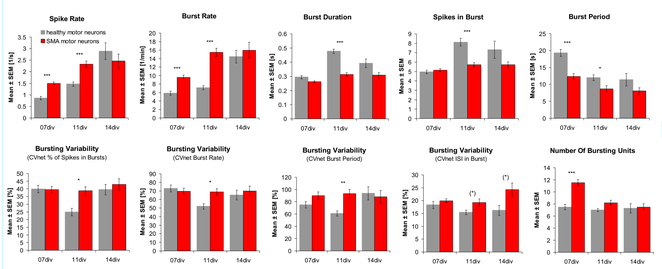
SMA323 patient MNs





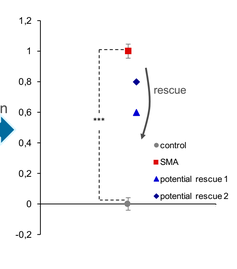
Fibroblasts from SMA-patient and healthy control were reprogrammed into iPSC and differentiated into motor neurons (MNs).
9) Control and diseased MNs show dramatically different activity patterns at 11 div.
10) Multi-parametric spike train data analysis shows a hyper-active state of SMA MNs at 7-11 div (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.
11) 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.

10 Multi-parametric disease phenotype



integration

11 Effect Score

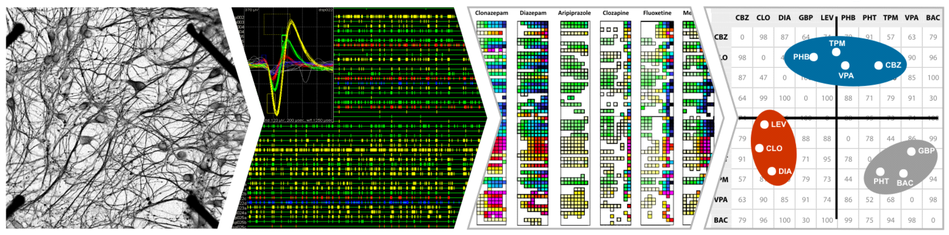


Summary and Conclusions

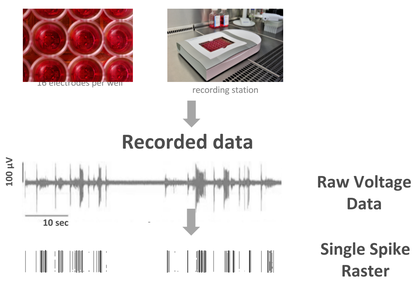
Our results show reproducible spontaneously active motor neuron networks with synchronized activity on the majority of the electrodes. We identified disease-specific functional phenotypes and show how reference compounds can affect them. In conclusion, we show that hiPSC-derived motor neurons are able to produce meaningful functional in vitro phenotypes and that these phenotypes can be associated with known motor neuron diseases. By using artificial intelligence-based multivariate MEA data analyses combined with reproducible physiologically relevant iPSC neuron models we provide a functional phenotypic assay platform for high throughput compound screening.

NeuroProof Technology

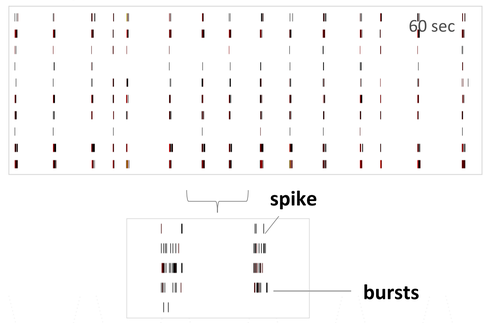
Neuronal Cell Culture | **Phenotypic Multichannel Recording** | **Multiparametric Data Analysis** | **Pattern Recognition**



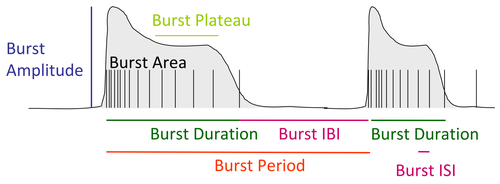
Spike Detection



Well Spike Raster



Multi-parametric analysis



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