High-throughput screening of iPSC derived motor neurons on Qube and QPatch

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Introduction

Human induced pluripotent stem cells (hiPSCs) can be differentiated into many cell types, including neurons and cardiomyocytes, and therefore constitute a novel way to model human diseases for drug testing *in vitro*[1]. Ion channels represent attractive therapeutic targets in the nervous and cardiovascular systems[2] rendering electrophysiological studies of hiPSCs interesting for their usage in drug discovery. However, such studies have traditionally been limited by the labour-intensive and low throughput nature of patch clamp electrophysiology[2].

Here we present the electrophysiological characterization of hiPSC-derived motor neurons using our automated patch clamp (APC) platforms, Qube 384 and QPatch. Our results include a measure of channel expression versus time in culture, the pharmacological dissection of endogenous ion channels (e.g. voltage-gated Na^+ (Na_V) and voltage-gated K^+ (K_V) channels), identification of ligand-gated receptors and recordings of action potentials using current clamp.

The major challenge when investigating neurons using APC platforms is the requirement to dissociate the cells from their neuronal network while maintaining cell viability and membrane integrity[3]. By optimization of the harvest- and whole-cell protocols we have overcome this obstacle resulting in success rates of up to 60% using our 384-well APC system.

Utilizing the high throughput nature of our system we tested in parallel two disease models, using hiPSC neurons derived from Spinal Muscular Atrophy (SMA) or Amyotrophic Lateral Sclerosis (ALS) patients, together with control cells from healthy subjects and isogenic control cells. The results show a general overactivity of Na^+ channels in both cell lines, which could be rescued by a point mutation in the superoxide dismutase (SOD1) gene in the isogenic ALS cell line.

Materials and methods

Cells: Spinal motor neurons were generated from the following human iPSC lines: normal control (WC-30), SMA Type I (GM00232), ALS with D90A SOD1 mutation, and D90D SOD1 isogenic control (ND29149). The D90D iPSC line was established by correcting the D90A SOD1 mutation using TALEN technology[5]. Directed differentiation was performed as described[6]. After 5 – 11 days culturing according to BrainXell protocols, the culture medium was removed, and the cells were washed x2 in PBS and harvested using 2 mL Accumax™ per T25 cell flask (incubate 7 min at 37°C). Subsequently, 6 mL culture medium was added and the cells were spun down (4 min, 150 x g) and resuspended in extracellular solution to achieve the required cell density.

K_V - and Na_V channel IV relationship: ***Extracellular solution***: 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 4 mM KCl, 145 mM NaCl, 10 mM Glucose, pH = 7.4. ***Intracellular solution***: **Qube**: 120 mM KF, 20 mM KCl, 10 mM HEPES, 10 mM EGTA, pH = 7.2. **QPatch**: 4.3 mM $CaCl_2$, 1.8 mM $MgCl_2$, 25 mM KOH, 24 mM KF, 10 mM HEPES, 10 mM EGTA, 100 mM KCl, 3 mM Na_2-ATP. ***Voltage protocol***: The protocol consisted of a 700 ms pre-step at -120 mV followed by 200 ms voltage steps from -90 mV to + 60 mV (ΔV = +10 mV). ***Analysis***: Cells with membrane resistance R_{mem} < 200 MΩ and current amplitudes I < 150 pA were excluded from the analysis. The minimum Na_V peak current was extracted within a 2.5 ms time interval. The average K_V current was extracted within a 30 ms time interval (starting at 890 ms). The membrane conductance (G) was quantified by dividing the peak sodium current by the current driving force ($V_m – V_{ev}$) and was normalized to the conductance at -10 mV (G_{max}).

GABA-induced currents: ***Extracellular solution***: 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 4 mM KCl, 145 mM NaCl, 10 mM Glucose, pH = 7.4. ***Intracellular solution***: 90 mM KCl, 50 mM KF, 1 mM $MgCl_2$, 10 mM HEPES, 11 mM EGTA, pH = 7.35. ***Ligand addition***: 100 µM GABA was added at a voltage V = -90 mV.

Acknowledgements
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Section 1: Screening hiPSC – derived motor neurons using automated patch clamp (APC)

The major challenge when investigating neurons using APC platforms is the requirement to dissociate the cells from their neuronal network while maintaining cell viability and membrane integrity[3]. By optimization of the harvest- and whole-cell protocols we have overcome this obstacle resulting in success rates of up to 60% using our 384-well APC system Qube (Fig. 1). On our medium-throughput system QPatch, we obtained up to 20% success rates. Quantification of Na_V - and K_V channel current-voltage relationship yielded very similar results on the two systems (Fig. 2).

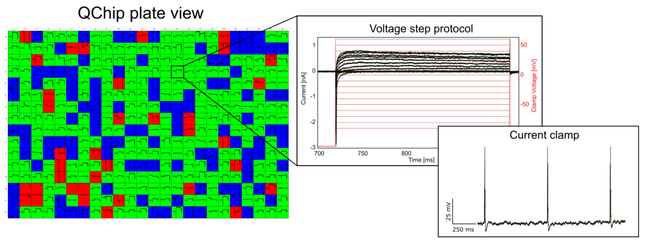

Fig. 1: **High throughput measurements of hiPSC-derived motor neurons on Qube**
Overview of the 384-well QChip illustrating the high throughput nature of Qube measurements yielding up to 60% success rates (cell resistance > 200 MΩ). The system allows us to perform voltage- and paced current clamp protocols within the same experiment.

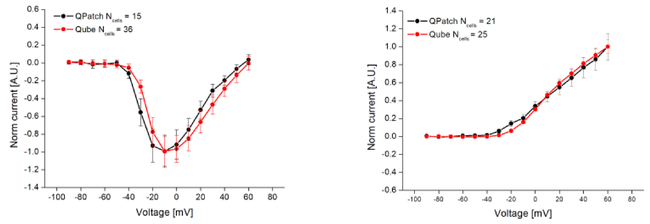

Fig. 2: **QPatch and Qube measurements on hiPSC-derived motor neurons yielded similar current-voltage (IV) relationship of Na_V and K_V channels**
The IV relationships of Na_V and K_V channels were quantified by stepping from -90 mV to +60 mV (ΔV = +10 mV) on QPatch (black) and Qube (red). A: Average Na_V current (normalized to the current recorded at -10 mV) versus step voltage. B: Average K_V current (normalized to the current recorded at +60 mV). All error bars are SEM.

Summary

Our results demonstrate the feasibility of conducting electrophysiological characterization and screening of hiPSC-derived neurons on APC platforms like Qube 384 and QPatch, thus paving the way for high throughput ion channel-targeted screening of drugs for neurological disorders.

References

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Section 2: Electrophysiological characterization of hiPSC-derived motor neurons

Characterization of hiPSC-derived motor neurons included Na_V - and K_V - channel IV-relationship (Fig. 2), addition of channel blockers (Fig. 3) and recordings of currents induced by γ-aminobutyric acid (GABA) (Fig. 4). For all experiments, neurons with a whole-cell resistance (R_{mem}) below 200 MΩ were excluded from the data analysis. Of these cells (N = 645 cells) 98% ± 2% expressed K_V channels and 85% ± 3% expressed Na_V channels. The IV curves revealed that the cells expressed different fractions of delayed rectifier and A-type K_V channels (Fig. 3A). Upon thawing a vial of BrainXell cryopreserved hiPSC neural progenitors they require a week maturation in culture. During this time, the cells are differentiated into functional post-mitotic motor neurons, creating a neuronal dendritic network (Fig. 5A). Quantifying Na_V -, K_V - and GABA - channel currents as a function of culturing days in vitro (Fig. 5B) revealed that the expression level of these channels increases over time. However, the simultaneous development of the dendritic network challenges the harvest procedure thereby lowering overall success rate (Fig. 5C).

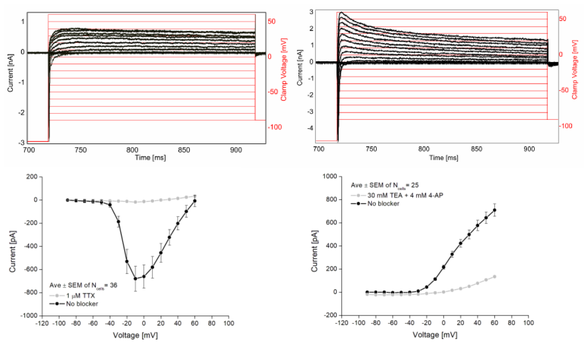

Fig. 3: Current voltage (IV) relationship of Na_V and K_V channels in hiPSC-derived motor neurons
A: Representative current responses (black) to a voltage step protocol (red) when stepping from -90 mV to + 60 mV (ΔV = +10 mV), showing that the cells expressed Na_V channels and a mixture of delayed rectifier and A-type K_V channels. **B**: Average Na_V current versus step voltage. The current was fully blocked by subsequent addition of 1 µM TTX. **C**: Average K_V current versus step voltage. Addition of 30 mM TEA and 4 mM 4-AP resulted in a ~85% block at +60 mV. Error bars are SEM.

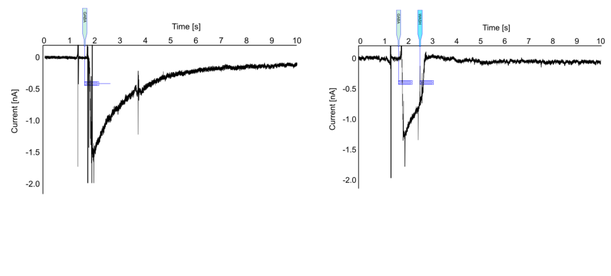

Fig. 4: **Recordings of currents induced by γ-aminobutyric acid (GABA)**
Current response recorded at a holding potential of -90 mV upon the addition of 100 µM GABA. **A**: Standard ligand application with washout after 30 s. **B**: Stacked ligand application, in which ligand and wash solutions are stacked in the same pipette, ensuring rapid washout after 0.8 s.

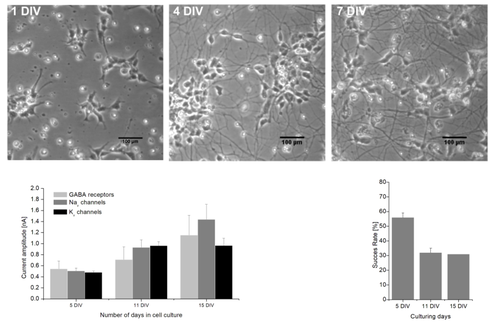

Fig. 5: **Expression level increases and success rate decreases as a function of culturing days in vitro (DIV)**
A: Light micrographs of hiPSC progenitor neurons differentiating into functional post-mitotic motor neurons, captured at 1 DIV, 4 DIV and 7 DIV. Scale bars are 100 µm. **B**: Current amplitudes recorded at 5 DIV, 11 DIV and 15 DIV for GABA receptor channels (light grey, V = -90 mV), Na_V channels (grey, V = 0 mV) and Kv channels (black, V = 40 mV). **C**: Percentage success rates (R > 200 MΩ) at 5 DIV, 11 DIV and 15 DIV. Error bars are SEM.

Section 3: Test of hiPSC motor neurons derived from Spinal Muscular Atrophy (SMA) or Amyotrophic Lateral Sclerosis (ALS) patients

To demonstrate the feasibility of Qube 384 for characterization and drug screening of central nervous system disorders we used hiPSCs from SMA4 or ALS5 patients and compared their electrophysiological properties to the control cell lines (Fig. 6 and 7). The K_V IV relationship revealed similar activity in all tested cell lines (Fig. 6A and 7A). However, the Na_V IV relationship of the SMA hiPSCs displayed, as previously shown[4], a trend towards increased Na_V current as compared to the control (Fig. 6B). In addition, we show that ALS disease cells (ALS D90A) exhibit a significantly increased Na_V current as compared to the control, which could be rescued by a single point mutation SOD1 gene of the isogenic cell line (ALS D90D)5 (Fig. 7B). No significant differences were recorded between the voltage at half-maximal activation (V0.5) between the cell lines (Fig 6C and 7C) indicating that the observed increase in Na_V amplitude is a result of elevated channel expression.

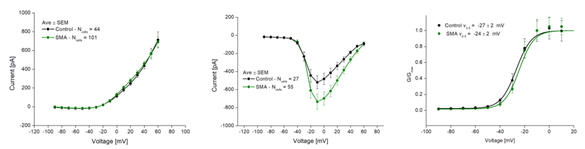

Fig. 6: **Current-voltage relationship of Na_V and K_V channels in SMA hiPSCs recorded at 8 DIV**
Parallel recordings of control (black) and disease (SMA, green) hiPSC-derived motor neurons. **A**: Average K_V current versus step voltage. **B**: Average Na_V current versus step voltage. **C**: Na_V conductance (G) (normalized to G_{max}) versus step voltage. Solid lines are Boltzmann fits to the data used to extract the voltage at half-maximal activation ($V_{0.5}$). Error bars are SEM.

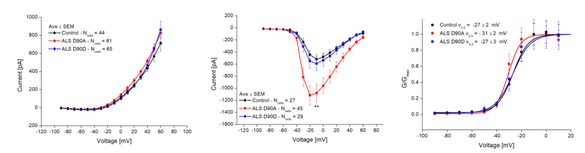

Fig. 7: **: Current-voltage relationship of Na_V and K_V channels in ALS iPSCs at 8 DIV**
Parallel recordings of control (black), disease (ALS D90A, red) and isogenic rescue (ALS D90D, blue) hiPSC-derived motor neurons. **A**: Average K_V current versus step voltage. **B**: Average Na_V current versus step voltage. The current amplitude at +10 mV is significantly larger in ALS D90A cells than control cells (students t-test, p < 0.01(**), 95% confidence interval). **C**: Na_V conductance (G) (normalized to G_{max}) versus step voltage. Solid lines are Boltzmann fits to the data used to extract the voltage at half-maximal activation ($V_{0.5}$). Error bars are SEM.