Automated patch-clamp measurements of action potentials for functional classification and evaluation of hiPSC-derived neurological disease models

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Introduction

Induced pluripotent stem cells (iPSCs) show great potential for the generation and characterization of neuronal subtypes as well as the investigation of neurological disease models. However, in practice the intercellular variability in a population of iPSC-derived neurons in combination with the low-throughput nature of manual electrophysiological experiments, have made such studies challenging.

Here, we use automated patch clamp (APC) for high-throughput characterization and comparison of commercially available healthy (WT) and frontotemporal dementia (FTD; genetically engineered granulin R493X heterozygous knockout)

iPSC-derived excitatory neurons. The results include an optimization of the cell suspension for APC and an analysis of voltage-gated (K_V and Na_V) and ligand-gated (AMPA) ion channel currents. We also analyze action potential parameters (such as spike frequency, spike threshold and action potential amplitude) in WT and FTD model iPSC-derived excitatory neurons. We see a clear correlation between the functionality of Na_V channels and the ability to fire action potentials, with the FTD neurons showing more immature properties.

Conclusion

The study demonstrates the usability of high-throughput APC for the electrophysiological characterization of iPSC-derived neurons and statistically robust identification of disease cell phenotypes with voltage-gated, ligand-gated and step current assays.

References:
1. Lin H-C et al., **Stem Cell Reports**, Vol 16, 2118-2127, **2021**

Materials and methods

Cellular Dynamics Fujifilm kindly provided the hiPSC-excitatory neurons. Dissociated hiPSC neurons, in a pure, homogenous suspension with a cell density of (1 – 2) mio/mL, were added to the APC platform of choice (QPatch or Qube 384). The minimum number of cells required is about 0.2 mio cells for 48 sites. Experiments were performed 16, 23, 30 and 35 days in vitro (DIV).

Voltage protocols: The voltage step protocol consisted of a 200 ms pre-step at -120 mV followed by 300 ms voltage steps from -90 mV to + 60 mV (ΔV = 10 mV).

Solutions: Please contact us for further details (info@sophion.com).

All the analysis and figures were prepared in the Sophion Analyzer and Prism 9.3.1 (GraphPad Software, Inc, La Jolla, CA, USA). Data in graphs are depicted as avg ± sem. Statistical significances are depicted as (*) = $p < 0.05$, (**) = $p < 0.01$, (***) = $p < 0.0001$.

Development and characterization of iPSC-derived excitatory neurons of high purity and consistent gene expression
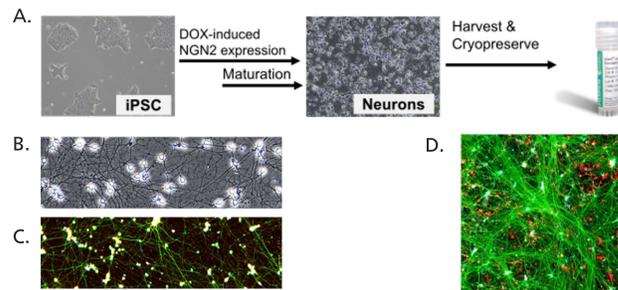

Fig. 1: Characterization of iCell hiPSC-derived Excitatory Neurons. A: Human iPSC cell-lines were genetically engineered to contain a doxycycline (DOX)-inducible NGN2 construct at the AAVS1 safe harbor site. iCell Induced Excitatory Neurons differentiation protocol was optimized for highly consistent and reproducible neuronal cultures, the ability to cryopreserve the cells, and result in a product that yields functionally mature excitatory neurons upon thaw and reanimation. **B**: Brightfield image of the cells on Day 3 post-thaw shows robust neural cultures extensive neurite outgrowth. **C**: Immunostaining for VGLUT2 (green) and synapsin 1/2 (red) on Day 7 post-thaw highlights glutamatergic synapses containing VGLUT2 transporters. **D**: Immunostaining of induced excitatory neurons (Tau; green) in co-culture with iCell Astrocytes 2.0 (GFAP; red). **E**: Post-thaw gene expression analysis across four lots of iCell Induced Excitatory Neurons reveals a primarily excitatory glutamatergic neuronal population and high lot-to-lot consistency, similar to published results from NGN2-induced neurons[1].

Comparison of WT and Frontotemporal Dementia neuron model

Fig. 2: Characterization of iCell Induced Excitatory Neurons Containing GRN R493X HZ KO. A: Cells were analyzed by Western blot for granulin protein expression, and the cleaved GRN fragments of ~6 kDa were much more lightly expressed in the HZ GRN neurons as compared to AHN control. **B**: Percent purity was evaluated across 3 lots of AHN and GRN R493X HZ KO iCell Induced Excitatory Neurons. Similar percentage of BIII-Tubulin-positive cells are recorded across lots and between cell lines. **C**: Comparison of post-thaw qPCR gene expression analysis of AHN and GRN R493X HZ KO induced neurons revealed a consistent and primarily excitatory glutamatergic neuronal population. Cells showed high expression of vesicular glutamate transporters (VGLUT2), and AMPA receptor subunits (GRIA1, 4). In addition, high expression of synaptic genes (SYN1 and PSD95) and neuronal markers (MAP2) was observed.

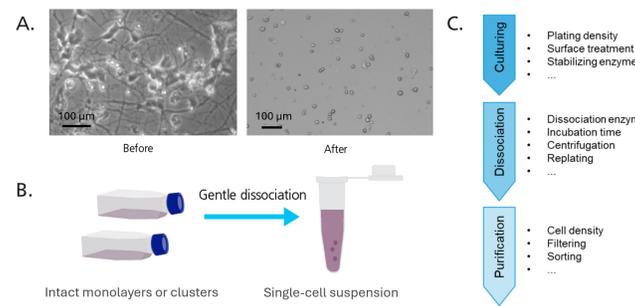## Automated Patch Clamp experiments setup

Fig. 3: Ensuring a pure, viable, homogenous cell suspension is essential for APC. A: Brightfield images of iCell hiPSC-derived Excitatory Neurons in culture before (left) and after (right) dissociation and purification, showing the purity and homogeneity of cell suspension needed for APC measurements. **B**: Cells must be dissociated gently but efficiently from their monolayers into a viable suspension that consists of single cells. **C**: The quality of the final cell suspension is affected by multiple factors including culturing conditions, dissociation methods, and purification steps.
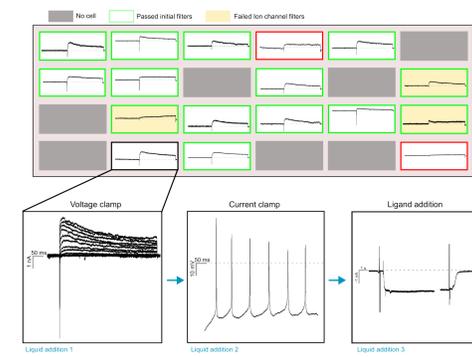

Fig. 4: APC setup for measurements of ion channel expression and neuron excitability. Top: Partial plate view of QPlate consumable (70% success rate) showing 24 out of 48 sites. Each site contains an individual experiment and is colored according to success, grey: no cell attached, green/red border: passed/failed initial QC filters, yellow: failed ion channel filters. **Bottom**: Current traces from subsequent applications of voltage- and current-clamp protocols. Liquid addition 1: voltage gated Na_V/K_V current. Liquid addition 2: Current clamp measurement of action potentials. Liquid addition 3: Ligand gated current in response to 150 μM AMPA in the presence of 100 uM of Cyclothiazide (CTZ), the positive allosteric modulator for AMPA receptor.

Action Potential (AP) firing pattern classification divides neuronal population into functionally distinct types
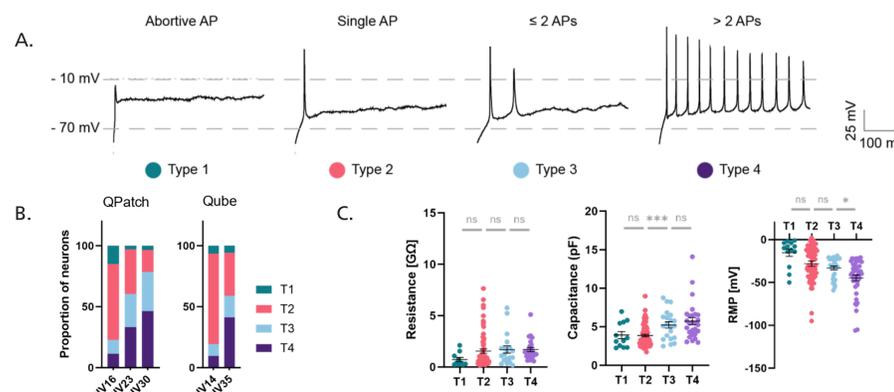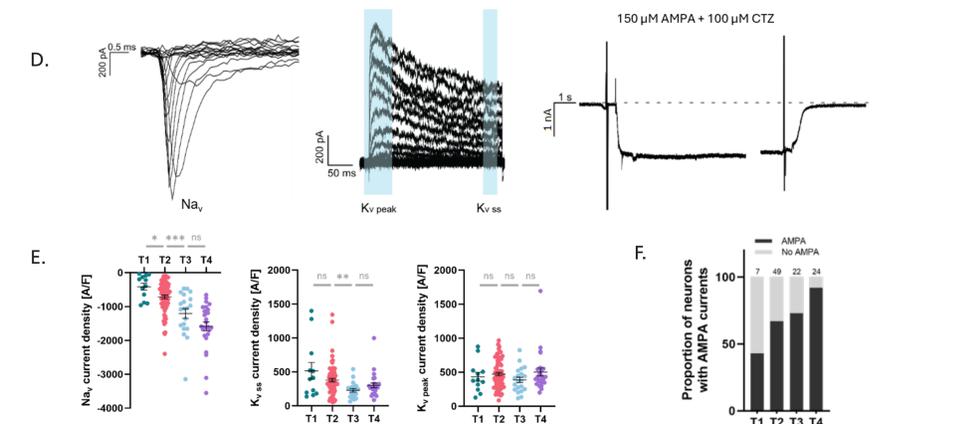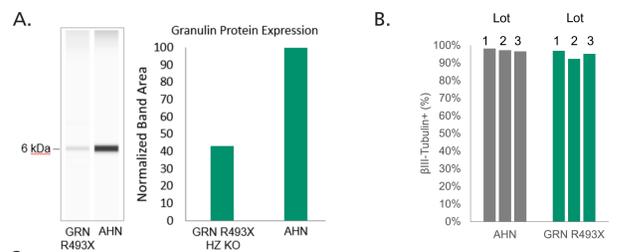

Fig. 5: Classification of AP types allows isolation of functionally comparable neurons. A: Wild type (WT) neurons were classified based on the number of APs fired. Type 1 (T1) neurons fired an abortive AP, Type 2 (T2) fired a single AP, Type 3 (T3) fired two APs, and Type 4 (T4) fired more than 2 APs. An AP was defined as having a peak potential above -10 mV and an action potential amplitude above 30 mV. **B**: Proportions of AP types changed with culturing time (days in vitro, DIV), with T4 neurons being more abundant at later time points. This was detected from measurement on both APC systems, the QPatch and the Qube. **C**: Membrane properties differed between AP types, with changes seen mainly in capacitance and resting membrane potential (RMP) measurements. Functionally mature (T4) neurons had highest capacitance and lowest RMP. **D**: Representative traces of Na_V, K_V, and AMPA currents measured using APC systems. **E**: Na_V and K_V current densities compared between AP types. More mature types showed larger sodium and smaller potassium steady state (SS) current densities. **F**: Neurons with detectable AMPA currents were more abundant in more mature AP types. N_{cells} = 12 for T1; 74 for T2; 21 for T3; 27 for T4.

Differences in ion channel currents between WT and GRN T4 neurons correlate with action potential parameters and neuronal excitability

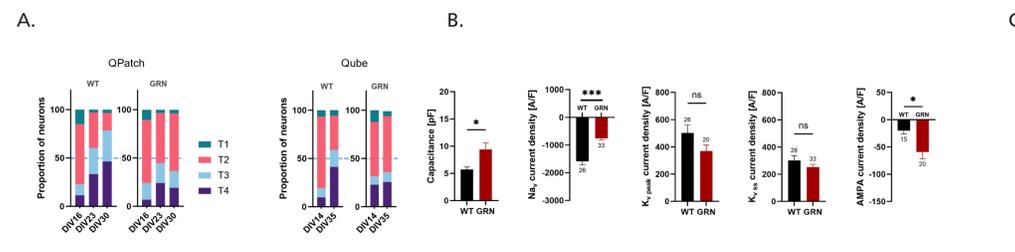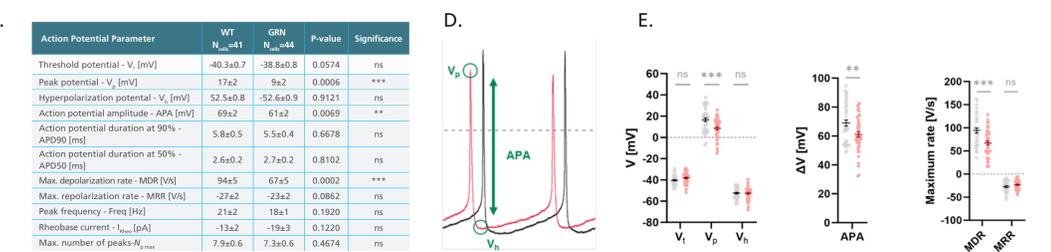| Action Potential Parameter	WT $N_{cells}=41$	GRN $N_{cells}=44$	P-value	Significance
Threshold potential - V_t [mV]	-40.3±0.7	-38.8±0.8	0.0574	ns
Peak potential - V_p [mV]	17±2	9±2	0.0006	***
Hyperpolarization potential - V_h [mV]	52.5±0.8	-52.6±0.9	0.9121	ns
Action potential amplitude - APA [mV]	69±2	61±2	0.0069	**
Action potential duration at 90% - APD90 [ms]	5.8±0.5	5.5±0.4	0.6678	ns
Action potential duration at 50% - APD50 [ms]	2.6±0.2	2.7±0.2	0.8102	ns
Max. depolarization rate - MDR [V/s]	94±5	67±5	0.0002	***
Max. repolarization rate - MRR [V/s]	-27±2	-23±2	0.0862	ns
Peak frequency - Freq [Hz]	21±2	18±1	0.1920	ns
Rheobase current - I_{rheo} [pA]	-13±2	-19±3	0.1120	ns
Max. number of peaks-N_{max}	7.9±0.6	7.3±0.6	0.4674	ns

Fig. 6: Functional properties of T4 neurons compared between WT and GRN populations. A: Proportions of AP types across all measured DIV timepoints compared between WT and GRN neuronal populations. A similar pattern was observed on both QPatch and Qube platforms, with fewer T4 neurons present within the GRN population at later timepoints. **B**: Capacitance and ion channel properties compared between WT and GNR T4 neurons. GRN T4 neurons had higher capacitances, lower sodium current densities and higher AMPA current densities. **C**: Table of AP parameters compared between WT and GRN T4 neurons. **D**: Depiction of AP parameters measured on representative traces from WT (black) and GRN (red) neurons. **E**: AP parameters with significant differences between the two groups. Peak potential (V_p) was lower in GRN neurons, as was action potential amplitude (APA) and maximum depolarization rate (MDR).