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Electrophysiological characterization of iPSC-derived excitatory neurons, including a comparison to genetically modified Frontotemporal Dementia neuron model, using automated patch clamp.

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Abstract

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 apparently healthy normal (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 and how they develop over time. We also compare action potential parameters (such as spike frequency, spike threshold and action potential amplitude) in WT and FTD model iPSC-derived excitatory neurons.

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 paced action potential assays.

References:

1. Lin H-C et al., Stem Cell Reports, Vol 16, 2118-2127, 2021
2. Ghovanloo M-R et al., Cell Reports Methods, Vol 3, 100385, 2023

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

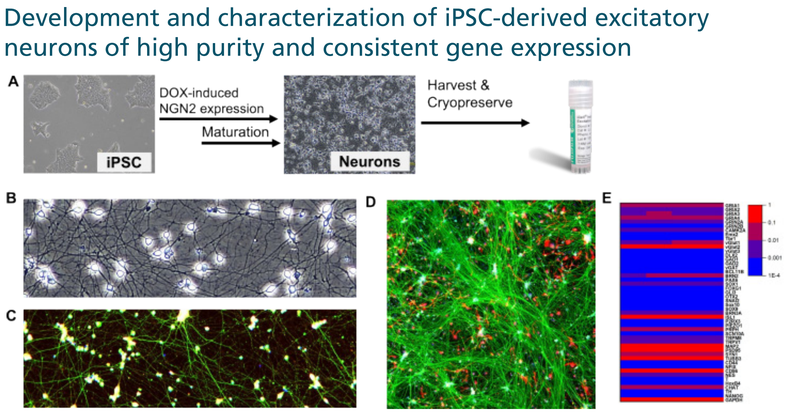

Fig. 1: **Characterization of iCell induced excitatory neurons**. **A**: Induced excitatory neurons were generated from human iPSC lines were genetically engineered to contain a doxycycline (DOX)-inducible NGN2 construct at the AAVS1 safe harbor site. iCell Induced Excitatory Neurons differentiation 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 4 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 (FTD) neuron model

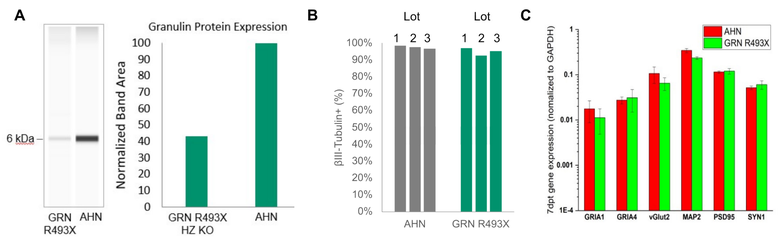

Fig. 2: **Characterization of iCell Induced Excitatory Neurons Containing GRN R493X HZ KO. A:** Cells were analyzed by Western blot for granulin protein expression, and the cleaved GRN fragments of ~6 kDa were much more lightly expressed in the GRN R493X HZ KO (FTD) neurons as compared to AHN (WT) control. **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 were 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), neuronal markers (MAP2) was observed.

Automated Patch Clamp (APC) experiment setup

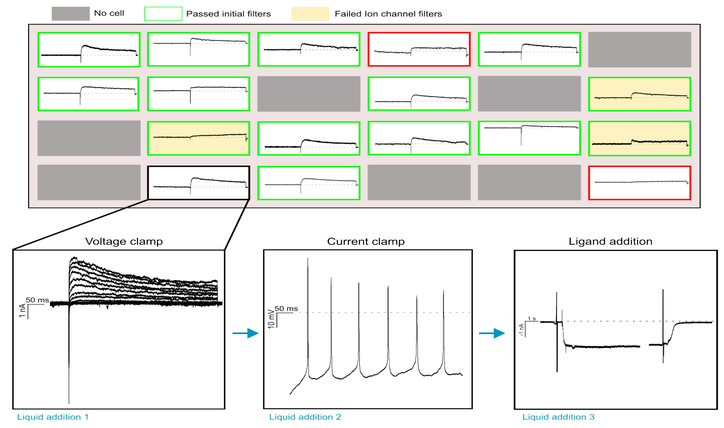

Fig. 3: **APC setup for measurements of ion channel expression and neuron excitability. Top:** Partial plate view of QPlate consumable (70% success rate) showing 24 out of 48 sites. Each site contains an individual experiment and is colored according to success, grey: no cell attached, green/red border: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

Ion channel analysis

Voltage-gated Na^+ (Na_V) channels:

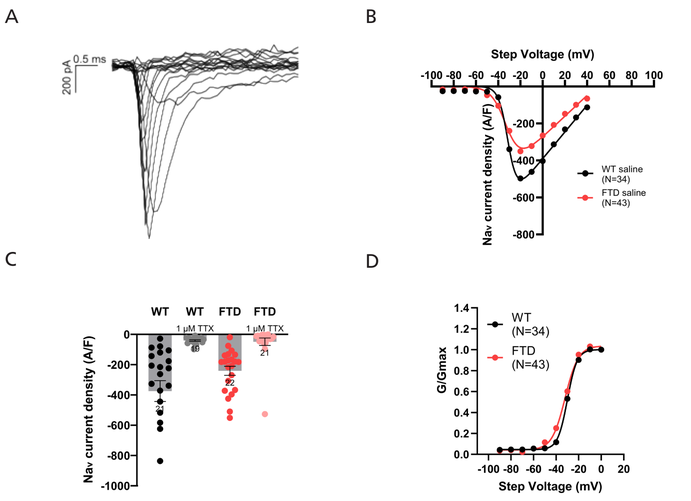

Fig. 4: **Na_V channel current evaluation. A:** Representative Na_V current. We recorded Na_V currents with $| I_{Nav,min} | > 200$ pA, in (60-80) % of the neurons. **B:** Average Na_V current density versus step voltage for WT neurons (black) and FTD neurons (red). Data points are avg ± sem of N_{cells} = 34 and 43, respectively. **C:** Na_V current density measured at 0 mV before (WT black and FTD red) and after (WT grey and FTD pink) addition of 1 µM TTX. The percentage of block was 89 % for WT and 80 % for FTD neurons and suggests that the majority of Na_V channels are TTX sensitive as expected for neuronal Na_V channels (i.e. $Na_V1.1$, $Na_V1.2$, $Na_V1.6$). **D**: Na_V conductance (G normalized to G_{max}) versus step voltage. $V_{0.5}$ of activation was extracted by fitting the Boltzmann equation to the data and was around -30 mV for both WT and FTD neurons. Data points are avg ± sem of N_{cells} = 34 and 43, respectively.

Voltage-gated K^+ (K_V) channels:

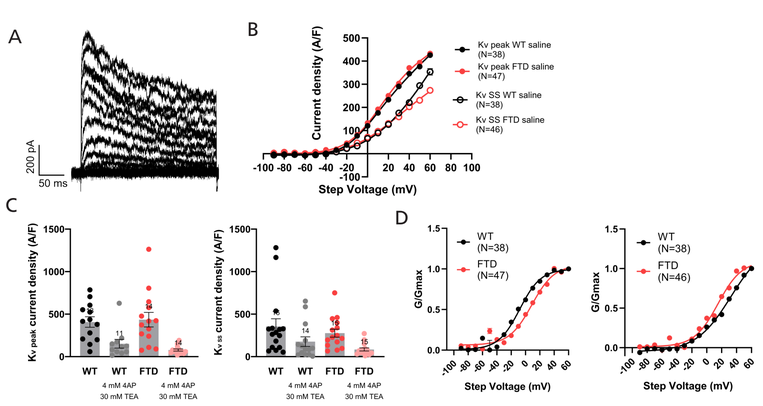

Fig. 5: **Kv channel current evaluation.** A: Representative K_V current. In agreement with literature the neurons expressed both delayed rectifier ($K_V1.1$, K_V2, K_V3, $K_V7.2/K_V7.3$) and A-type (K_V4) potassium channels. We recorded $K_{V,peak}$ and $K_{V,ss}$ currents with $| I_{Kv} | > 200$ pA, in (70-100) % of the neurons. **B**: Average $K_{V,peak}$ (solid points) and $K_{V,ss}$ (hollow points) current density versus step voltage for WT neurons (black) and FTD neurons (red). Data points are avg ± sem of N_{cells} = 38, 38, 47 and 46 respectively. **C**: K_{Vpeak} (left) and $K_{V,SS}$ (right) current density measured at 60 mV before (WT black and FTD red) and after (WT grey and FTD pink) addition of 4 mM 4-AP and 30 mM TEA. We observe about 60% and 80% block of the peak current and 50% and 70% block of the steady state current in WT and FTD neurons, respectively. **D**: K_V conductance (G normalized to G_{max}) versus step voltage. $V_{0.5}$ of activation was extracted by fitting the Boltzmann equation to the data. For the peak current $V_{0.5}$ of activation was about -8 mV and 8 mV for WT and FTD neurons, respectively. For the steady state current $V_{0.5}$ of activation was around 30 mV and 15 mV for WT and FTD neurons, respectively. Data points are avg ± sem of N_{cells} = 38, 38, 47 and 46 respectively.

AMPA receptor (AMPAR) channels:

Fig. 6: **AMPAR current evaluation. A:** Representative current evoked by the addition of 150 µM AMPA in the presence of 100 µM CTZ (PAM), followed by washout by saline. **B**: The percentage of cells with AMPAR currents ($| I_{AMPA} | > 30$ pA) were significantly higher in FTD than WT neurons at DIV16, however this difference disappeared at later DIVs. **C**: The rise time from 10 % - 90 % was calculated for WT (black) and FTD (red) current traces but did not display any significant difference.

Characterization of ion-channel expression during maturation reveals disease phenotypes

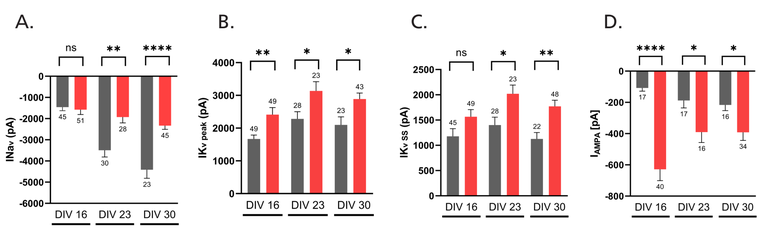

Fig. 7: **Evolvement of current amplitude during maturation measured in WT (black) and FTD (red) hiPSC cortical neurons. A:** Na_V current. **B**: $K_{V,peak}$ current and $K_{V,ss}$ current. *C*: AMPAR current. Data points are avg ± sem. N_{cells} is written above the bars. WT and FTD data were compared with students t-test with (*) = $p < 0.05$, (**) = $p < 0.01$, (****) = $p < 0.0001$. The Na_V and AMPA current amplitude changed significantly as the neurons matured, but in different manners for WT and FTD neurons. Na_V currents increased in WT neurons, while they did only increase slightly in FTD neurons. K_V currents did not change significantly over time but were in general lower in WT than FTD neurons. AMPAR currents decreased in FTD neurons but increased in WT neurons.

Excitability of WT and FTD neurons

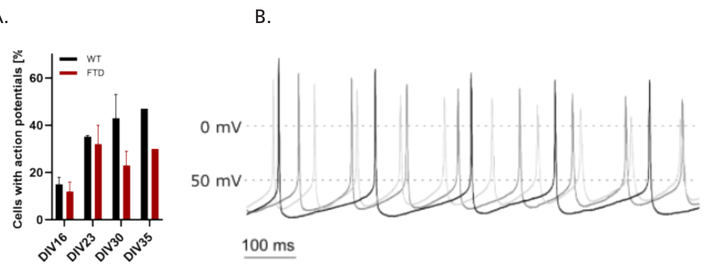

Fig. 8: **Representative action potential trace measured using current clamp feature. A:** The percentage of recorded WT (black) and FTD (red) neurons firing action potentials vs. DIV. Using the current clamp feature on Qube and QPatchII we recorded neuron action potentials (fig. 8A) in response to a step current protocol, (15 steps, ΔI = 10 pA starting from -50 pA) following a voltage step at -70 mV. Above 30 DIVs the percentage of neurons that fired action potentials were higher in WT than FTD neurons. **B**: Recorded single-cell action potentials in response to increasing current injections.

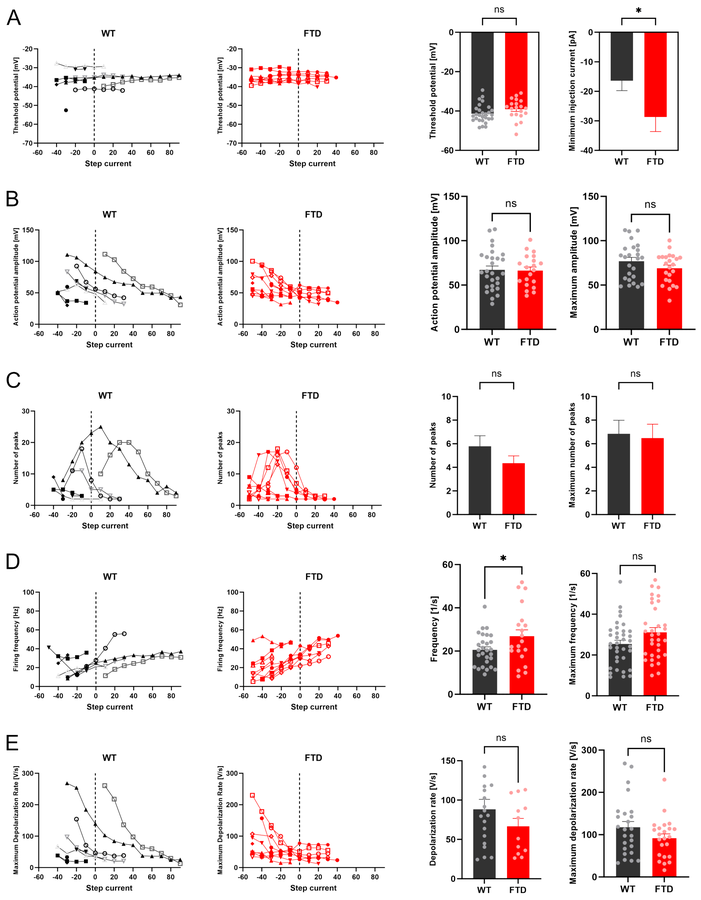

Fig. 9: **Comparison of action potential parameters in WT and FTD Neurons.** We extracted the average **A**: threshold potential (V_t), **B**: action potential amplitude (APA), **C**: number of peaks (n_p), **D**: firing frequency and **E**: maximum depolarization rate (MDR) and plotted as a function of input current as a function of input current for WT (9 cells) and FTD (11 cells). The third and fourth column display bar diagrams of the corresponding parameters recorded at 0 mV (left) and maximum or minimum values (right). The main observations from this initial data analysis are that FTD neurons start firing at lower input currents and at higher frequencies than WT.

Methods

FUJIFILM Cellular Dynamics kindly provided human iPSC-derived neurons: iCell induced Excitatory neurons (Cat # R1245) and iCell induced Excitatory Neurons GRN R493 HZ KO (Cat # R1247). Induced excitatory Neurons were cultured following manufacturer's User's Guide. 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 II 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).

A. B.

Sophion's automated patch clamp systems. A. **Qube 384** for high performance and high-throughput ion channel characterization and screening. B. **QPatch** - the benchmark solution for efficient, high-quality ion channel studies in physiological solutions.

Read more about our solutions here: