High-throughput automated patch-clamp analysis reveals altered excitability and variation of ion channel activity in ALS iPSC-derived motor neurons

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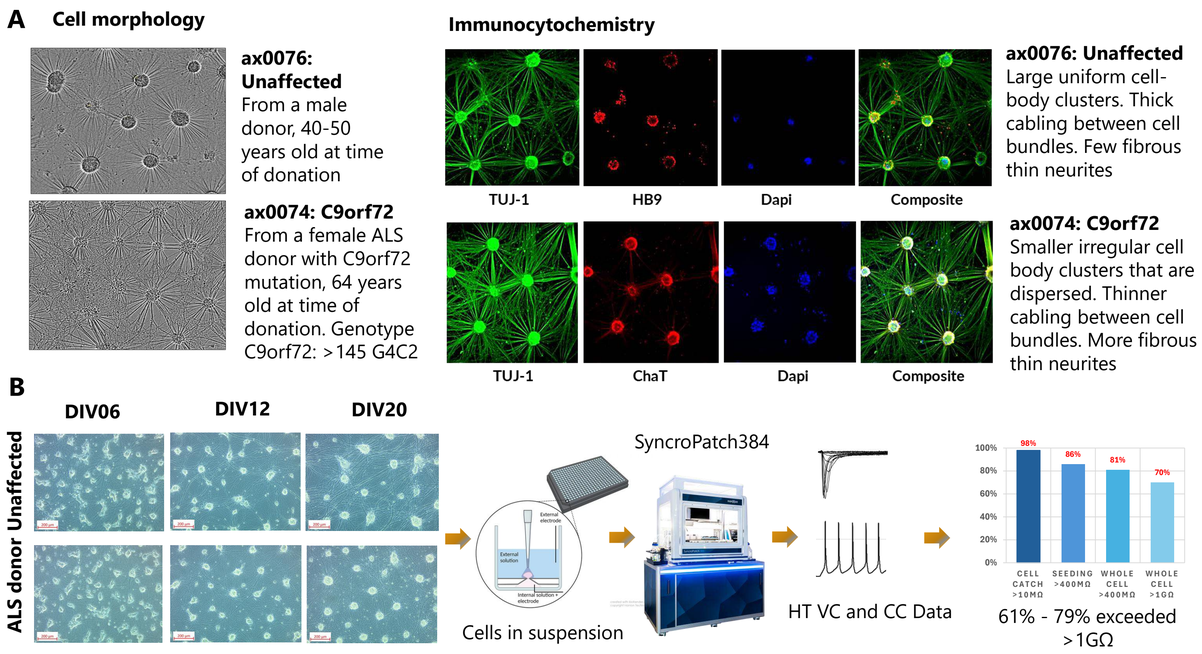
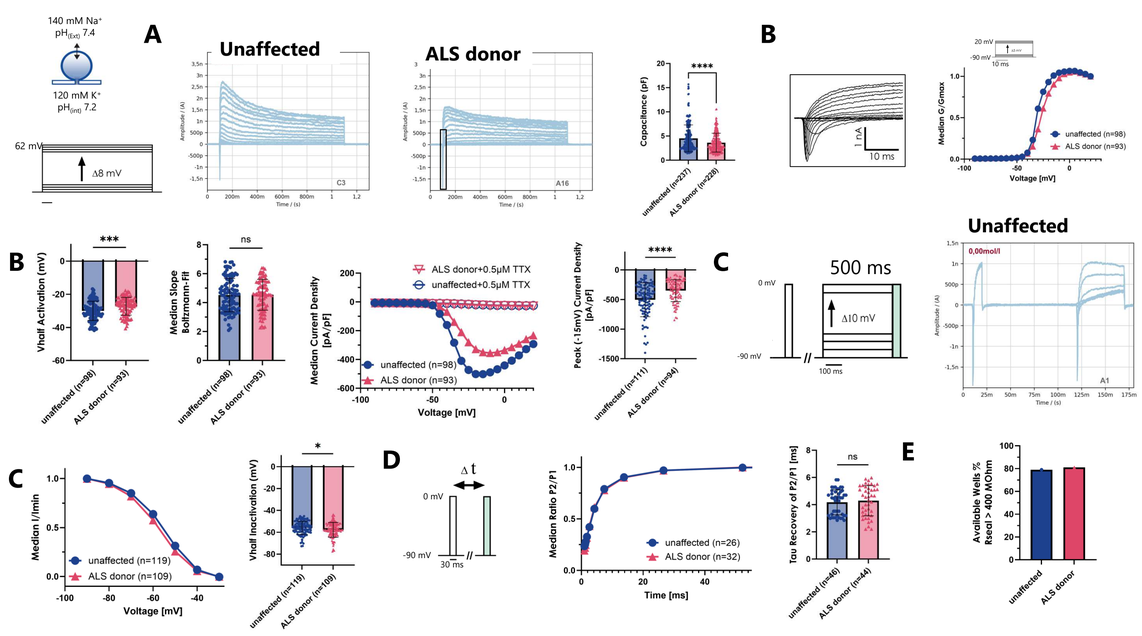
axoCells™ human iPSC-derived motor neurons



A) Brightfield imaging at 10 days *in vitro* (DIV10) and β-III-Tubulin (a neuronal microtubules marker; TUJ-1) immunostaining revealed clear morphological differences from ALS – and unaffected donor lines. The ALS donor carries an pathogenic hexanucleotide repeat expansion in chromosome 9 open-reading frame 72 (C9ORF72), the most common genetic cause of Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (1,2).

B) Cell culture maturation of unaffected - and ALS donor iPSCs and workflow for high throughput voltage – (VC) and current clamp recordings (CC) using Nanions' Syncropatch384.

Altered voltage-gated Nav channel properties in ALS neurons



A) Depolarizing steps evoked voltage-dependent currents in both groups. Cell capacitance was significantly reduced in ALS-derived neurons compared with unaffected controls.

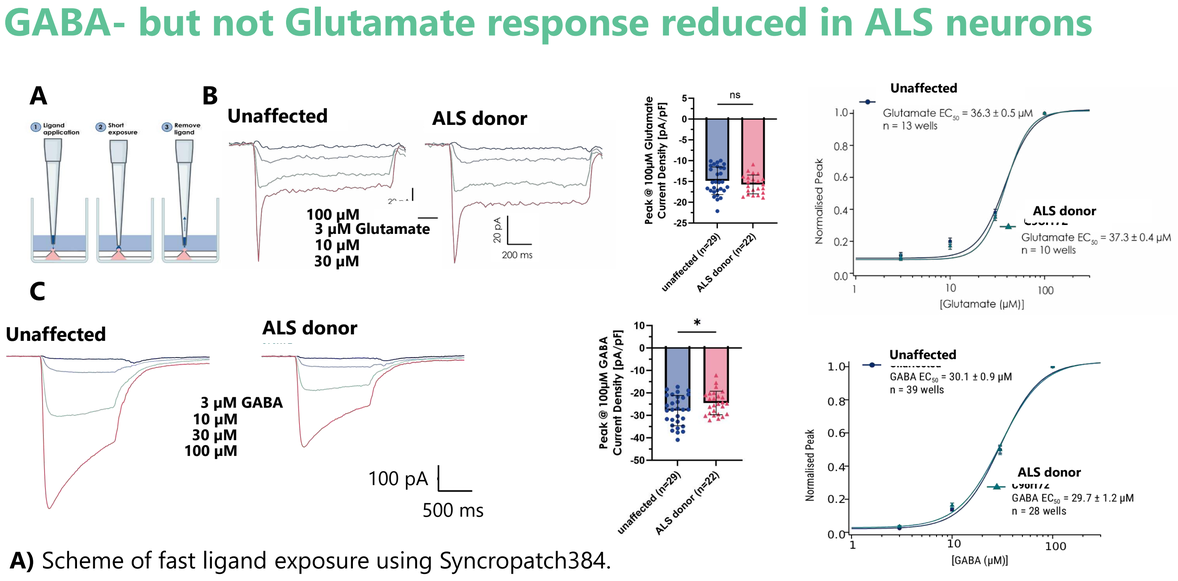
B) Nav currents were normalized to cell capacitance and plotted as conductance-voltage(G/Gmax) relationships. Boltzmann fits revealed a small but significant depolarizing shift in activation Vhalf in ALS neurons with unchanged slope. Peak current densities (PCD) were TTX-sensitive (0.5 µM) and significant lower in ALS neurons.

C) Steady-state inactivation was measured using a 500 ms pre-pulse describing macroscopic "intermediate" inactivation (reflecting mix of channels undergoing fast /slow inactivation) and showed a modest but significant hyperpolarizing shift in ALS neurons.

D) To further assess inactivation properties, we measured recovery from inactivation after a depolarizing pre-pulse of 30 ms (P1; indicative of fast inactivated). The normalized recovery following the pre-pulse yielded no difference in Tau for ALS – vs unaffected neurons.

E) Examples comparison of available wells for whole cell recording was similar for both cell types indicating no major difference in sealing properties of the isolated cells.

GABA- but not Glutamate response reduced in ALS neurons

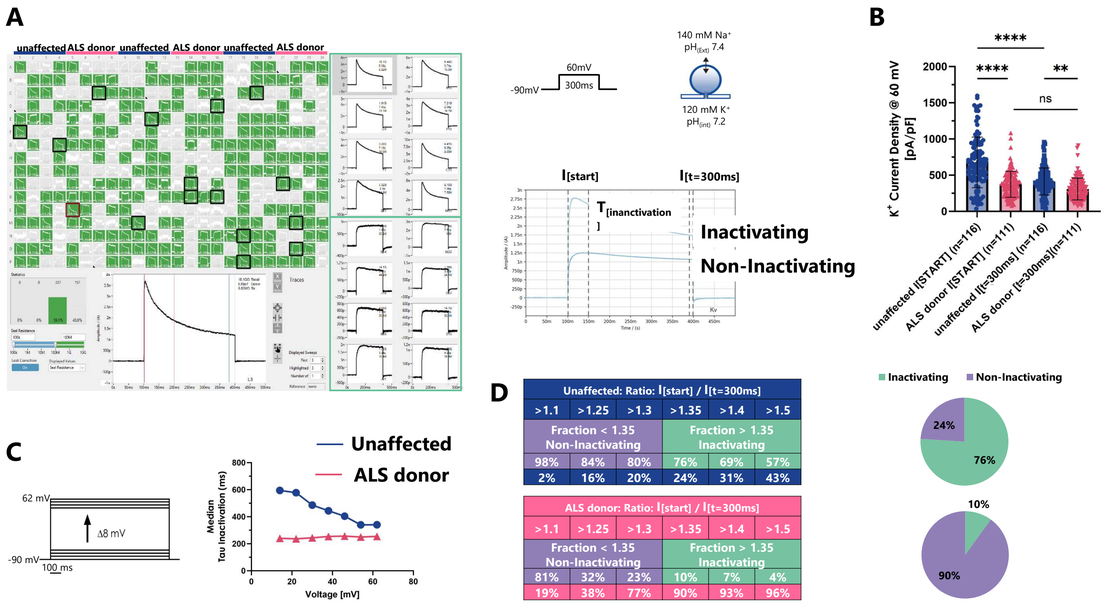


A) Scheme of fast ligand exposure using Syncropatch384.

B) Application of increasing Glutamate concentrations revealed dose-dependent increase of inward currents with no significant difference in PCD for ALS – vs unaffected neurons. The data fitted to Hill-equation yielded EC_{50} of 37.3 ± 0.4 µM for ALS – vs 36.3 ± 0.5 µM for unaffected neurons.

C) Similar experiments were conducted for responses to GABA with a small but significant reduction in PCD for ALS – vs unaffected neurons with EC_{50} similar with 27.3 ± 1.2 µM for ALS – vs 30.1 ± 0.9 µM for unaffected neurons.

Voltage-Gated K^+ Current properties differ in ALS derived neurons

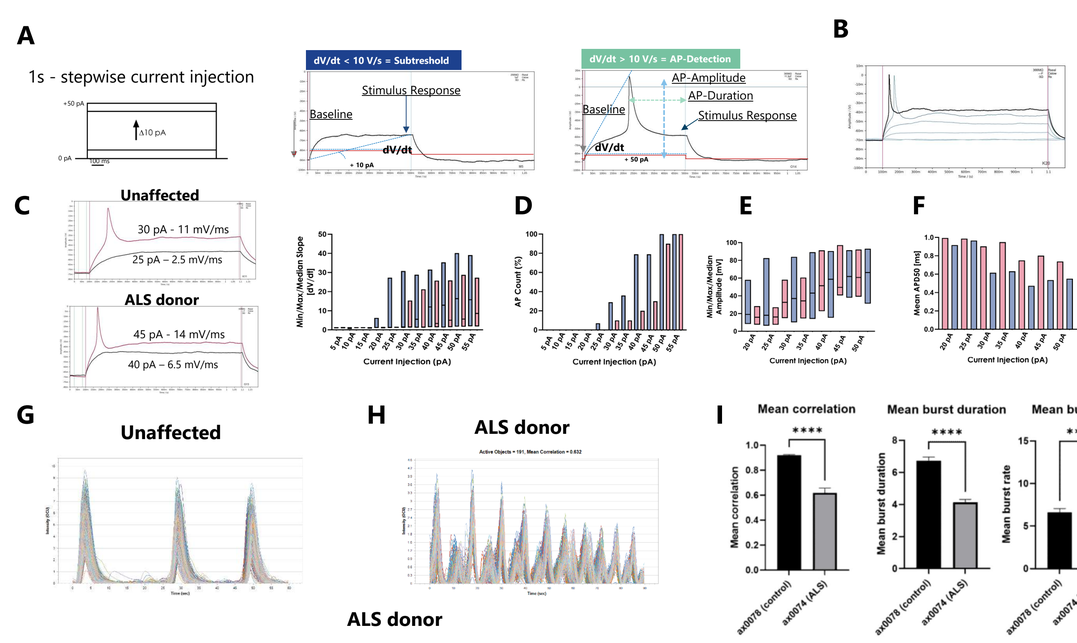


A) Depolarizing voltage steps in physiological solutions evoked outward currents consistent with voltage-gated K^+ channels highlighted with the Syncropatch384 acquisition software. Macroscopic appearance displayed an inactivating as well as a non-inactivating current phenotype indicative for the heterogeneity within a cell population, but also between unaffected – and ALS donor derived neurons.

B) Whereas unaffected - showed a higher PCD over ALS neurons, the current after 300 ms is significantly reduced in unaffected – whereas not different in ALS neurons. This indicates a higher prevalence of an inactivating phenotype in unaffected neurons that is further supported by the finding that the inactivation time constant (τ), obtained from mono-exponential fits, displayed voltage dependence in unaffected but not ALS neurons **(C)**.

D) The ratio of initial to sustained current ($I_{[t=300\ ms]}$ at +60 mV) confirmed a higher prevalence of inactivating currents in unaffected cells and a more non-inactivating phenotype in ALS donor–derived cells, consistent with differences in K_v channel composition, modulation/ signaling, and/or expression.

Impaired neuronal excitability in ALS neurons



A) Current-clamp recordings were used to assess neuronal excitability; action potentials (APs) were detected using a dV/dt threshold >10 mV/ms, enabling analysis of AP amplitude and duration.

B) Representative AP-positive recording from a cell held at V_{target} of -70 mV where stepwise current injections were used.

C-D) Unaffected neurons showed higher mean dV/dt and generated more APs per current step.

E-F) AP amplitude and duration were not different between groups, indicating preserved AP waveform properties.

G, H, I) This is further support by recording of sspontaneous neuronal activity (SNA) after 21 DIV on the IncuCyte S3 (Sartorious). The system is using optical readout (genetically-encoded fluorescent calcium indicator (GECI) to investigate activity and connectivity of neuronal tissue. Unaffected neurons exhibited highly synchronized firing, characterized by a higher mean burst duration and a lower burst rate compared to ALS neurons indicating an impaired excitability phenotype that could be likely reflecting abnormalities in ion channel function, membrane excitability/ action potential generation and cellular Ca^{2+} handling.

Conclusion

The SyncroPatch 384 can be used to biophysically characterize iPSC derived motor neurons to record voltage – and ligand gated ion channels as well as APs in the current clamp mode with the possibility to record ionic conductance and cellular excitability within the same cell and/or at body temperature (3,4).

Comparison Nav, Kv and GABA channels reveal differences in current densities and activation/inactivation properties suggesting ion channel remodeling within the ALS donor derived phenotype vs healthy donor cells.

Possible dysfunctional membrane excitability was shown in a reduced propensity of AP generation in patch clamp for ALS donor derived cells that was further confirmed by irregular, high-frequent Ca^{2+} transients.

A loss of action potential output in patient derived iPSC cells carrying the C9ORF72 ALS mutation has been suggested to contribute to downstream degenerative pathways that ultimately lead to loss in ALS (5).

References

1). Renton, A.E. et al. *Neuron* 72, 257-268 (2011). 2) DeJesus-Hernandez, M. et al. *Neuron* 72, 245-256 (2011). 3) Seibertz, F. et al, Commun Biol. 2022 Sep 15;5(1):969. 4) Kriegeskorte, S. et al. J Gen Physiol (2023) 155 (9): e202213312. 5). Devlin, A.C. et al. Nat Commun 6, 5999 (2015).

QC-Critera: Rseal > 400 Mohm start/end; Capacitance > 1.5pF; Rseries < 15 MOhm; INav > -200 pA; IKv > 200 pA, Iglu/GABA > -100 pA; Fit criteria: R^2 > 0.95; for statistical analysis either TTest or Anova was used to test for statistical significance in GraphPad Prism.

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