Human iPSC-derived motor neurons for ALS modeling on the high-throughput Nanion SyncroPatch 384 platform

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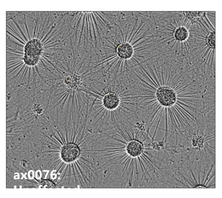
Introduction

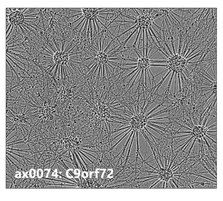
Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by motor neuron degeneration, leading to progressive muscle weakness and atrophy(1) Induced pluripotent stem cell (iPSC) technology enables the conversion of primary samples (blood or fibroblast) from a patient donor into a stem cell and from that stem cell, differentiated into functional cell types, including motor neurons. These motor neurons can be used in *in vitro* modeling when generated from high quality patient derived iPSC's and differentiated using a small molecule robust process to generate functionally qualified human iPSC-generated motor neurons. Axol Bioscience has generated motor neurons from a number of patient and unaffected donor samples. We have previously reported ALS relevant phenotypes using low-throughput electrophysiology. To investigate the utility of iPSC-derived motor neurons in a high-throughput setting, we collaborated with Nanion Technologies. We successfully demonstrated the application of these cells on the SyncroPatch 384 system and report ALS relevant differences between motor neurons from an ALS patient C9orf72 affected line and that of an unaffected donor.

axoCells™ human iPSC-derived motor neurons

Cell morphology



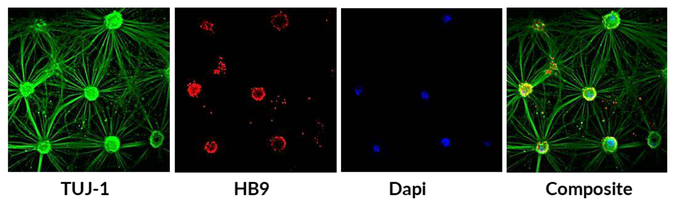
ax0076: Unaffected
From a male donor, 40-50 years old at time of donation



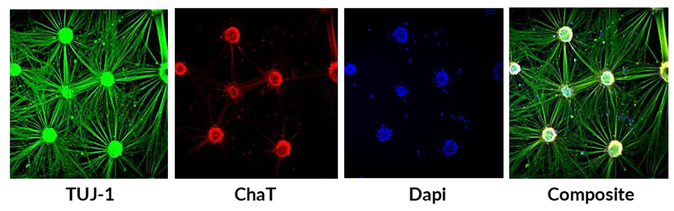
ax0074: C9orf72
From a female ALS donor with C9orf72 mutation, 64 years old at time of donation. Genotype C9orf72: >145 G4C2

Brightfield imaging at 10 days *in vitro* (DIV10)

Immunocytochemistry

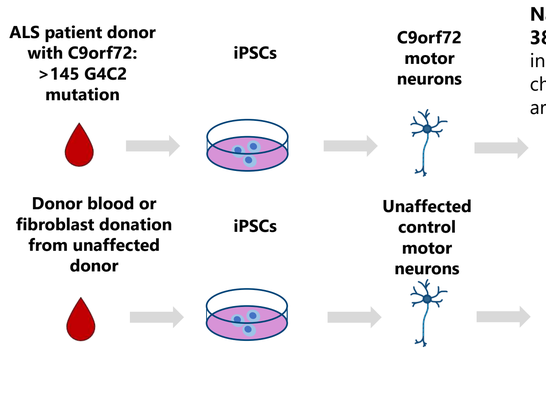


ax0076: Unaffected
Large uniform cell-body clusters. Thick cabling between cell bundles. Few fibrous thin neurites

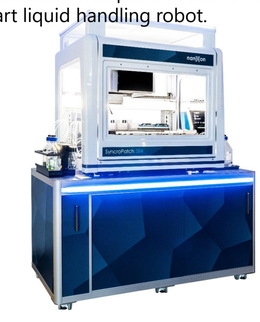


ax0074: C9orf72
Smaller irregular cell body clusters that are dispersed. Thinner cabling between cell bundles. More fibrous thin neurites

Display distinct functional and morphological phenotypes Tuj-1 = 100%, HB9 ≥ 90%, Chat ≥ 90%, Isl-1 ≥ 80%

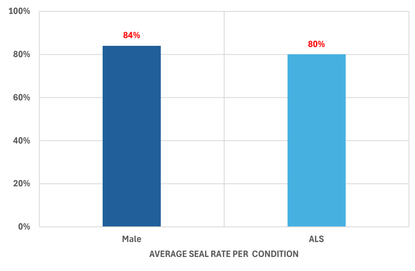


Nanion Technologies SyncroPatch 384 is an automated patch clamp instrument with 384 recording channels incorporated into a state-of-art liquid handling robot.



Functional characterization of unaffected and ALS iPSC-derived motor neurons in HTS format with SyncroPatch 384

SyncroPatch 384 seal success rates



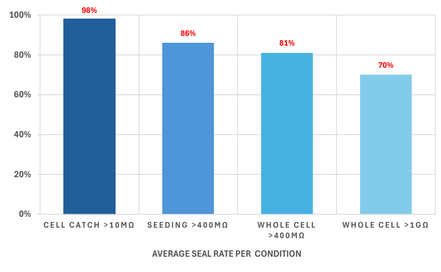
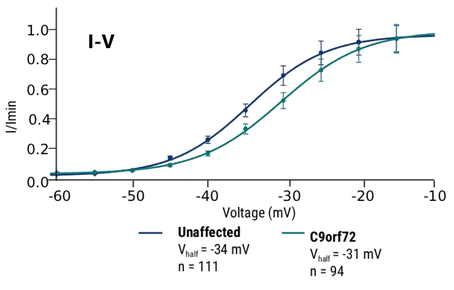


Figure 1. Seal success rates. Median seal resistance values were identical between unaffected and C9orf72 motor neurons ($p > 0.5$; Student's t test). After whole-cell configuration, between 76% and 88% of cells achieved a seal resistance > 400 MΩ (61% to 79% exceeded > 1 GΩ).

Sodium (Na^+) Channel I-V and Inactivation



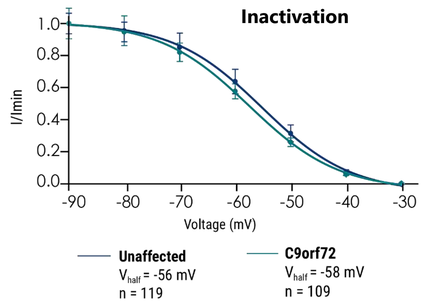


Figure 6. I-V & Inactivation (Normalized). The activation (Vhalf_act) and the inactivation (Vhalf_inact) were compared for unaffected and C9orf72 motor neurons. Sodium channels in unaffected and C9orf72 motor neurons showed comparable values for Vhalf_act and Vhalf_inact.

Potassium (K^+) Channels

ax0076: Unaffected

Fast activation and pronounced inactivation -> typical for transient outward K^+ current (I_A) (e.g. Kv4.x)

ax0074: C9orf72

Stable during depolarization and no or little inactivation -> typical for delayed rectifier K^+ currents (I_K) (e.g. Kv1.1, Kv1.2, Kv2.1)

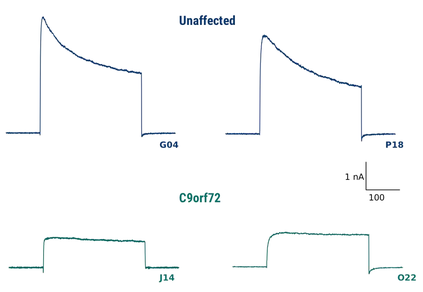


Figure 2. Potassium (K^+) currents of unaffected and C9orf72motor neurons following a voltage step protocol to 60 mV. Displaying very different wave form morphology.

Potassium (K^+) Channels parameter comparison

Cell type	SealR media n	SE	Cm median	SE	Current density median	SE
Unaffected	5.1GΩ	0.35	4.47pF	0.25	642pA/pF	32.50
C9orf72	4.0GΩ	0.39	3.18pF	0.18	359pA/pF	17.10

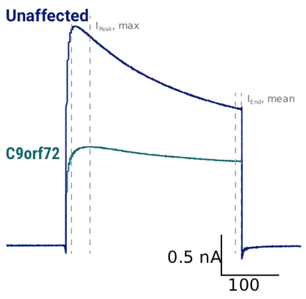


Figure 3. Electrophysiological parameter comparison. C9orf72 motor neurons showed lower values for amplitude which were highly significant ($p<0.001$) and slightly lower capacitance ($p<0.05$) compared to male (Student's t test). The current density was significantly reduced in ALS patients ($p<0.001$).

Potassium (K^+) Channels Inactivation properties

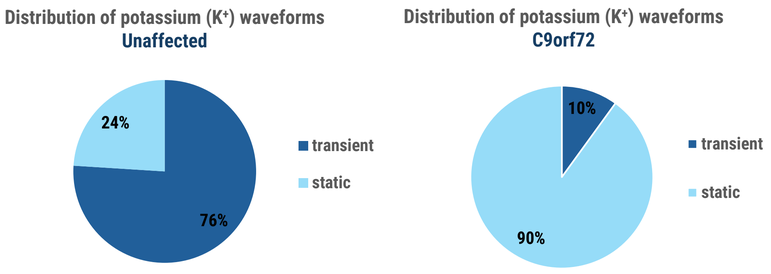


Figure 4. Inactivation significant difference in the distribution of K^+ currents with transient versus monotonic decay was observed between the unaffected and C9orf72 motor neurons.

Sodium (Na^+) Channels parameter comparison

Cell type	SealR median	SE	Cm median	SE	Current density median	SE
Unaffected	4.8GΩ	0.51	3.5pF	0.67	-338pA/pF	24.36
C9orf72	4.5GΩ	1.21	3.0pF	0.57	-223pA/pF	14.2

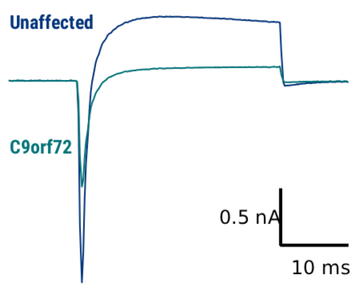
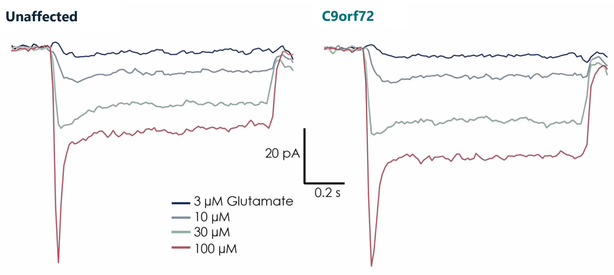


Figure 5. Sodium Na^+ current. Amplitudes and success rates were compared over one experiment day. NaV currents from C9orf72 motor neurons were significantly smaller in amplitude compared to unaffected motor neurons ($p<0.001$).

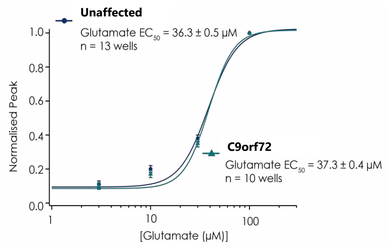
Neurotransmitter response

Glutamate

Current Amplitude



EC50

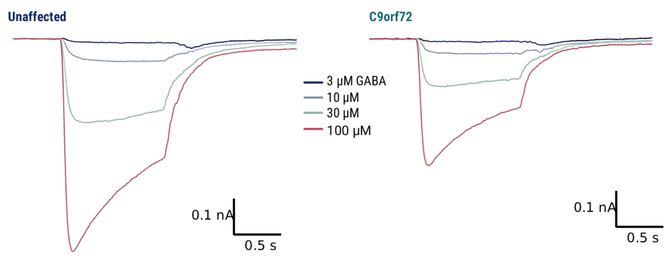


Cell type	EC_{50} Integral	# data points	# wells	Ipeak @ 100mM average	SE
Unaffected	36µM	100	13	-95pA	4pA
C9orf72	37µM	52	10	-110pA	12pA

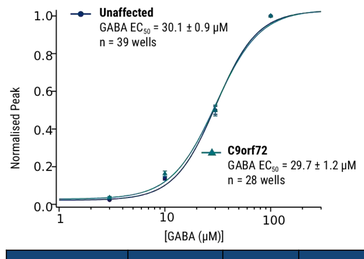
Figure 7a. Neurotransmitter response-Glutamate. No differences were observed between unaffected and C9orf72 motor neurons when comparing current amplitude or EC50 values for the Glutamate or Glycine responses, glycine not shown here.

Gaba

Current Amplitude



EC50



Cell type	EC_{50} Integral	# data points	# wells	Ipeak @ 100mM average	SE
Unaffected	31µM	156	39	-603pA	56pA
C9orf72	31µM	112	28	-361pA	37pA

Figure 7b. Neurotransmitter response-Gaba. Differences in current amplitudes are found between the unaffected and C9orf72 exposure groups, but no differences are observed for the EC50 values for Gaba responses.

Conclusion

axoCells™ motor neurons were matured over 10–20 days and integrated into high-throughput workflows using Nanion Technologies' SyncroPatch 384 platform and proprietary harvesting protocol. The protocol delivers a near-complete catch rate, with around 80% of cells forming high-quality seals in whole-cell configuration, enabling consistent and scalable electrophysiological recordings.

Syncropatch data showed clear functional differences between the unaffected and C9orf72 motor neurons. C9orf72 motor neurons exhibited significant differences for Potassium current shape, density and inactivation waveform distribution when compared to the unaffected neurons.

A distinct difference was also observed for Sodium current density, however activation and inactivation profiles ($V_1/_2$ values) remained similar, suggesting comparable sodium channel types across both populations. These channels were also identified to be TTX-sensitive.

Responses to Glutamate and GABA revealed similar EC_{50} values in both groups. However, C9orf72 motor neurons consistently showed reduced GABAergic responses, showing potential shifts in inhibitory signaling, a feature relevant to ALS pathology.

These results highlight the adaptability and value of axoCells™ motor neurons which combined with automated patch-clamp platforms generates scalable, phenotypically relevant ALS modeling, with high success capture rates and data quality for HTS drug screening.

References

• Gregory, J.M., Fagegaltier, D., Phatnani, H. et al. (2020) Genetics of Amyotrophic Lateral Sclerosis. Curr Genet Med Rep 8, 121–131. https://doi.org/10.1007/s40142-020-00194-8