We need ~~plenty of~~ ~~time~~ to solve ALS

Axol Bioscience, working with urgency to create better *in vitro* models of human disease.

AXOL

NEW! axoCells™ ALS Toolbox

Functional motor neurons from 6 iPSC lines to support ALS research

www.axolbio.com

Manufacturing better human disease models.

How iPSC technology is transforming drug discovery

In 2012, Professor Shinya Yamanaka and Sir John Gurdon were awarded the Nobel Prize "for the discovery that mature cells can be reprogrammed to become pluripotent." The ability to *reprogram* donated blood cells or fibroblast material from humans into stem cells and then the ability to *differentiate* these stem cells into end point cells is transforming the world of *in vitro* disease modelling.

With a consistent supply of functional end-point cells reflecting the phenotype of their donor and the ability to co-culture multiple cells, a new era of more human relevant and consistent *in vitro* models was born. With a strong tail wind of regulatory encouragement through the FDA Modernization Act, iPSC-derived models are now being used in the front-line of drug discovery for screening, mode-of-action and fundamental research.

Axol Bioscience, manufacturing functional cells, consistently and at scale

With over a decade of hard-earned experience, Axol Bioscience is recognised as a world leader in the quality manufacturing of functional iPSC-derived cells, at scale. Through this, we support the wider commercial life science industry with products, services, licensing, partnering and contract manufacturing.

We manufacture our **axoCells**™ at our ISO 9001-accredited production facility in Roslin, allowing us to reach over 1,300 customers in 57 countries, including all top pharma companies.

To support urgent work to find adequate treatments for ALS (amyotrophic lateral sclerosis), we continue to advance our range and characterization of iPSC-derived motor neurons and microglia, two key cells involved in the pathology of this condition.

In vitro models for ALS drug discovery

ALS (amyotrophic lateral sclerosis) is the most common form of motor neuron disease, where the progressive destruction of motor neurons leads to loss of muscular functions including walking, talking, swallowing and breathing. There is currently no cure. With treatment aimed at symptomatic relief and prolonging survival, most patients live only 3-5 years from the onset of symptoms[1].

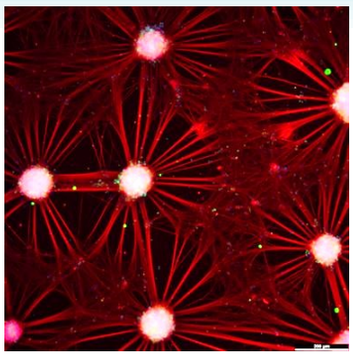
With limited treatment options and a predicted 69% increase in cases by 2040[2], attention has turned to *in vitro* ALS models that use human iPSCs from healthy or ALS patient donors.

The cells generated from these iPSCs retain the characteristics of their donors, enabling researchers to generate *in vitro* ALS models to improve knowledge of the disease or to screen potential therapies on a more human-relevant platform.

1 Xu L et al. doi: https://doi.org/10.1007/s00415-019-09652-y
2 Arthur, K et al. doi: https://doi.org/10.1038/ncomms12408

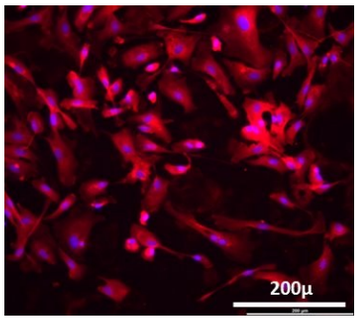
Key cell types involved in ALS:

- **Motor neurons:** Central to ALS pathophysiology is the progressive destruction of motor neurons, preventing communication between the brain/spinal cord and muscles.
    - axoCells Motor Neurons are functionally active in 10 days and demonstrate key marker expression (including HB9 and ChAT) and functional relevance in assays including electrophysiology and calcium imaging. Our ALS-derived motor neurons (ax0074, C9orf72 mutation) demonstrate phenotypic and functional differences compared to healthy control-derived motor neurons, including more fibrous neurites and higher firing frequency.



axoCells Motor Neurons stained for TUJ-1 (red) at day 21. X20 magnification

- **Microglia:** There is an overlap between ALS and frontotemporal dementia (FTD) with several genes implicated including C9orf72, the most common cause of familial ALS. As the main neuroinflammatory cell of the brain, microglia can be used to model the ALS-FTD overlap
    - Our axoCells Microglia are assay-ready in 7 days and express key markers (including Iba1, TMEM119, CX3CR1 and P2RY12), with functional relevance in assays including phagocytosis, chemotaxis and cytokine release. ALS-derived microglia (C9orf72) exhibit reduced phagocytosis of myelin basic protein compared to healthy control.



axoCells Microglia stained for CX3CR1 (red) and DAPI (blue) at day 7.

1 Badu-Mensah, A., Guo, X., McAleer, C.W. et al. Functional skeletal muscle model derived from SOD1-mutant ALS patient iPSCs recapitulates hallmarks of disease progression. Sci Rep 10, 14302 (2020). https://doi.org/10.1038/s41598-020-70510-3

Using axoCells™ **Motor Neurons** in ALS models

Motor neurons innervate muscle cells to control a range of voluntary and involuntary movements. The progressive destruction of motor neurons is central to neuromuscular conditions including **ALS**. axoCells Motor Neurons are in use in leading ALS research and drug discovery groups to fuel **advanced *in vitro* models of ALS**.

Assay ready from day 10

Express the **key markers** including HB9, MAP2, LIM3 and ChAT2

Demonstrated **functionality** in advanced *in vitro* models

Phenotypic characterization

We've **extensively characterized** our axoCells Motor Neurons for phenotypic relevance including **correct morphology (fig. 1)** and expression of **key cell markers via immunocytochemistry (fig. 2).**

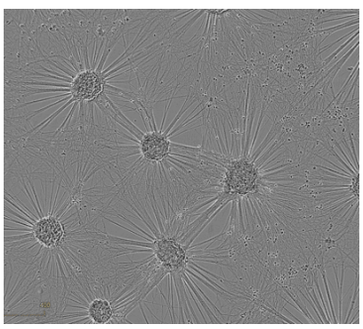


Figure 1. Phase contrast images of control axoCells Motor Neurons matured from progenitors over 21 days.

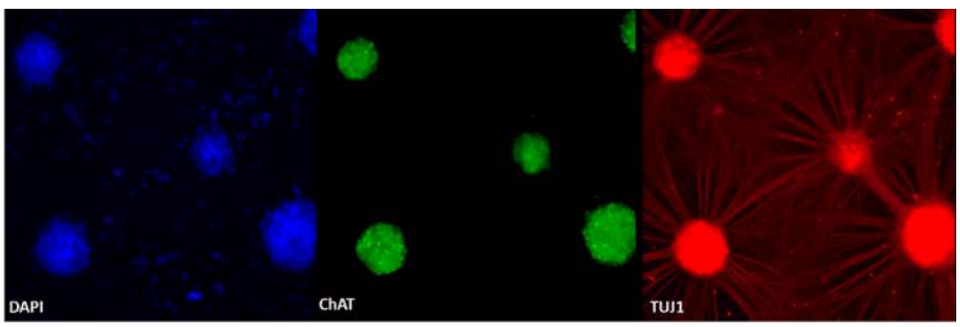
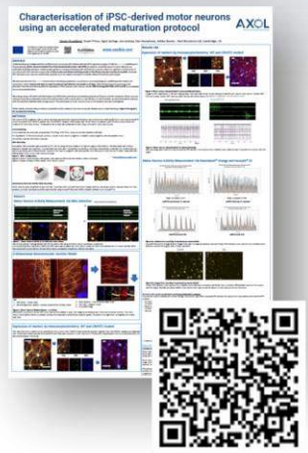


Figure 2. Immunocytochemistry of Day 21 mature axoCells Motor Neurons demonstrating presence of key markers (ChAT and TUJ1). Images captured on a Leica microscope x20 magnification.

How to accelerate your motor neuron research

1 Use Axol's Motor Neuron **Accelerator** to get your answers quicker

Enhanced maturation & short 10 day 'thaw to assay ready' time



Accelerator: We've developed an *in vivo* environment-mimicking supplement to reduce maturation times for our axoCells Motor Neurons from six weeks to just 10 days, with phenotypic and functional activity as assessed by morphology, immunocytochemistry and electrophysiology.

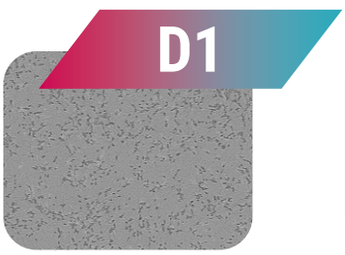
Scan the QR code to explore the data behind the development of our Accelerator Supplement.

2 Access Kits which include cells, media and supplements

Our kits come with cells, motor neuron maintenance media, GDNF, BDNF, CNTF and Accelerator. Additional third-party reagents are required to complete the protocol.

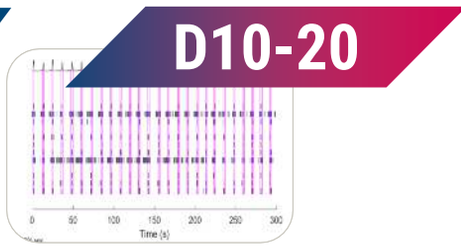
3 Use cells already validated on leading **MEA** platforms

Thawing and plating of cells in multi-well MEA plates.



Maturation of cells during cell maintenance in multi- well MEA plate. Feeding every second day.

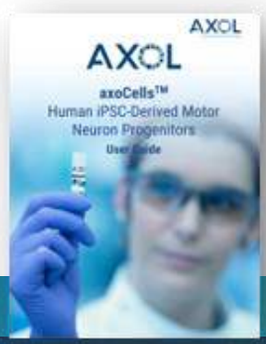
Motor neurons are assay ready at around day 10 with synchronised and regular burst firing soon after.

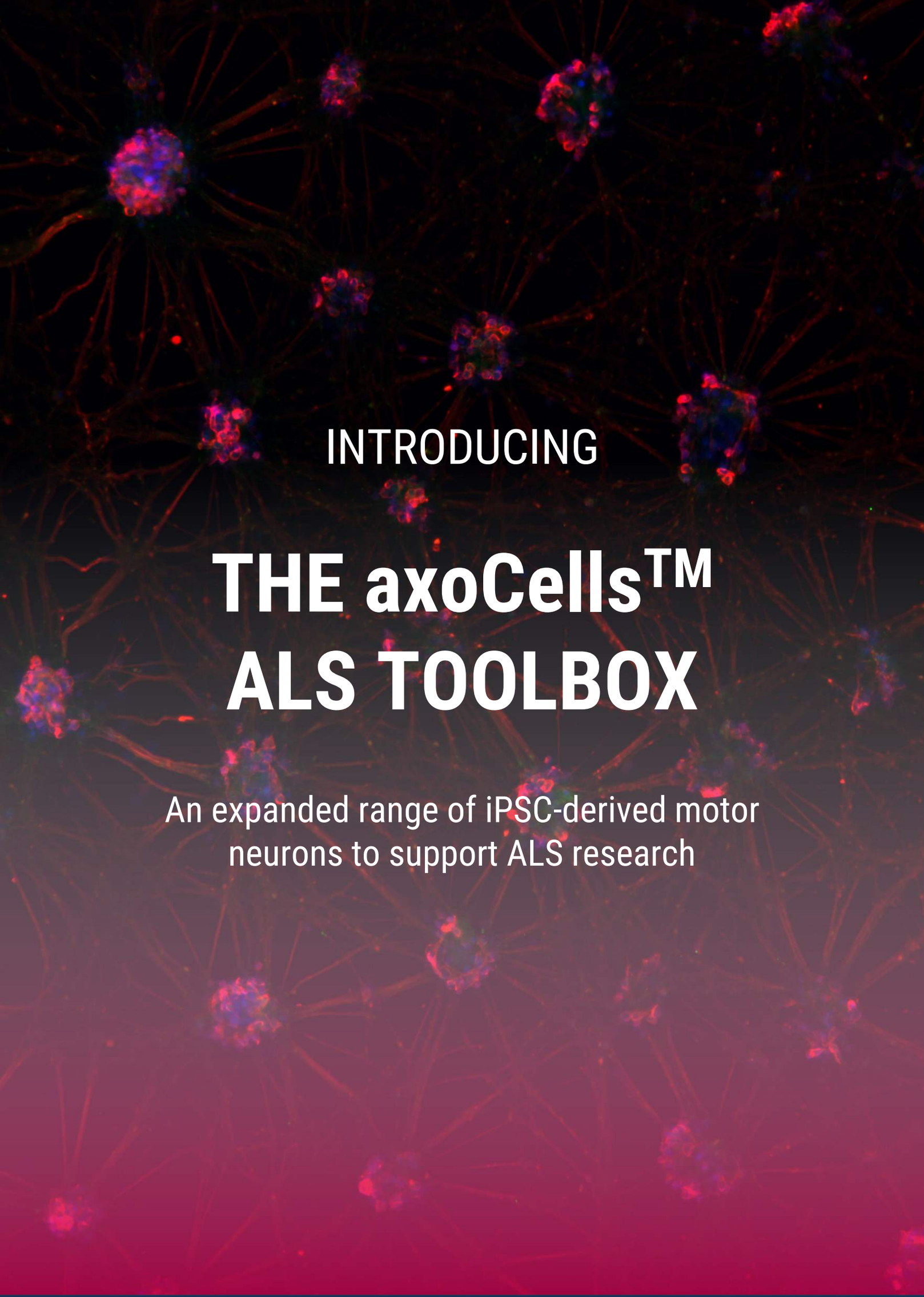


Day 10-20, acquisition of neural activity using the MEA. Non-invasive functional recording of neural networks and populations.

4 Get expert **help** to get you going faster

Download the protocol & contact support@axolbio.com

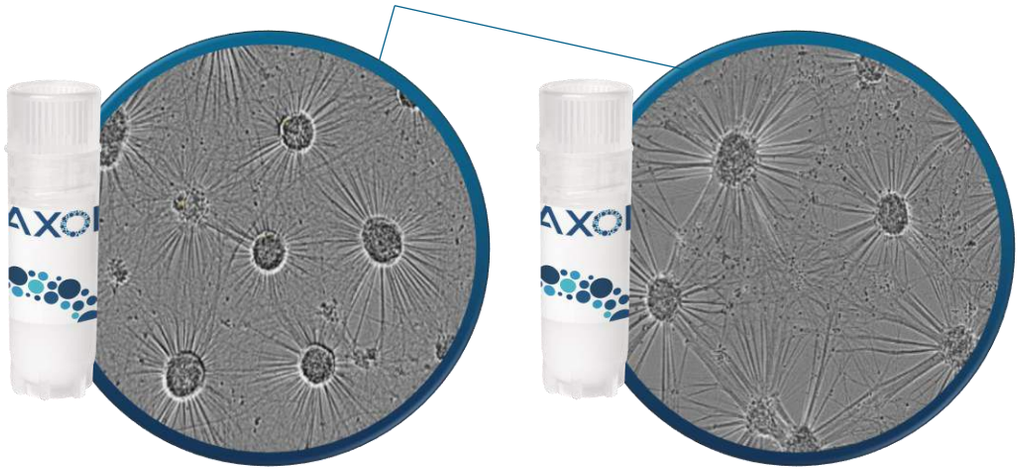



INTRODUCING
THE axoCells™
ALS TOOLBOX
An expanded range of iPSC-derived motor neurons to support ALS research

NEW! axoCells ALS Toolbox for Drug Discovery

Motor neurons derived from 6 donor lines

Unaffected donors
iPSC-derived motor neurons



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

ax0078: From a male donor, 74 years old at time of donation

C9orf72 carrying donor
iPSC-derived motor neurons

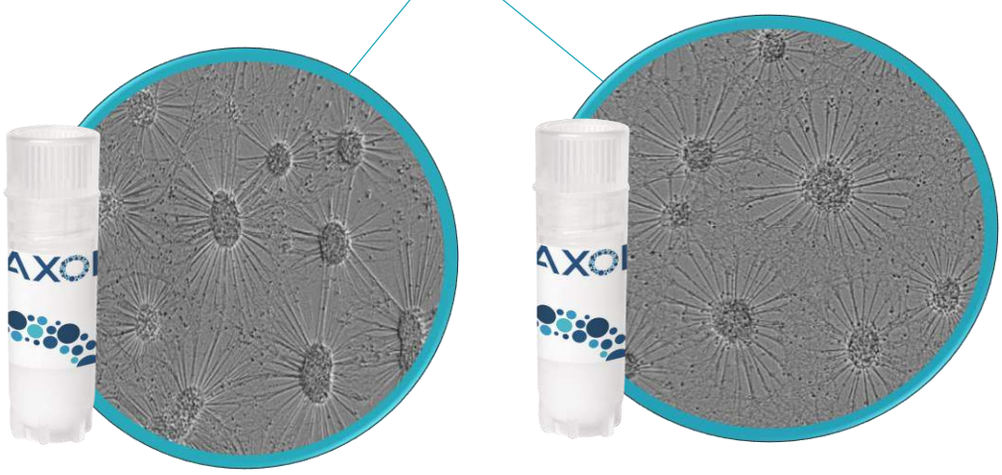
ax0073: From a **C9orf72** male who asymptomatic at time of sample. He is the sibling to ax0074 donor. 62 years old at time of donation.

[siblings]

ax0074:
From a female ALS donor with **C9orf72** mutation, 64 years old at time of donation

To support ALS research and drug discovery, Axol has developed an expanded set of 'ready to ship' motor neurons. With rapid 10 day to 'thaw to assay ready' protocols for axoCell motor neurons are backed with QC and functional QC, the new essential resource for ALS research.

ALS donors
iPSC-derived motor neurons



ax0735:
From a female ALS donor with **SOD1** mutation, 61 years old at time of donation

ax0079:
From a female ALS donor with **TDP43** mutation, 62 years old at time of donation

axoCells ALS Toolbox for Drug Discovery

Line and product information

Cells & Kits

≥2 million cells, supplied frozen.

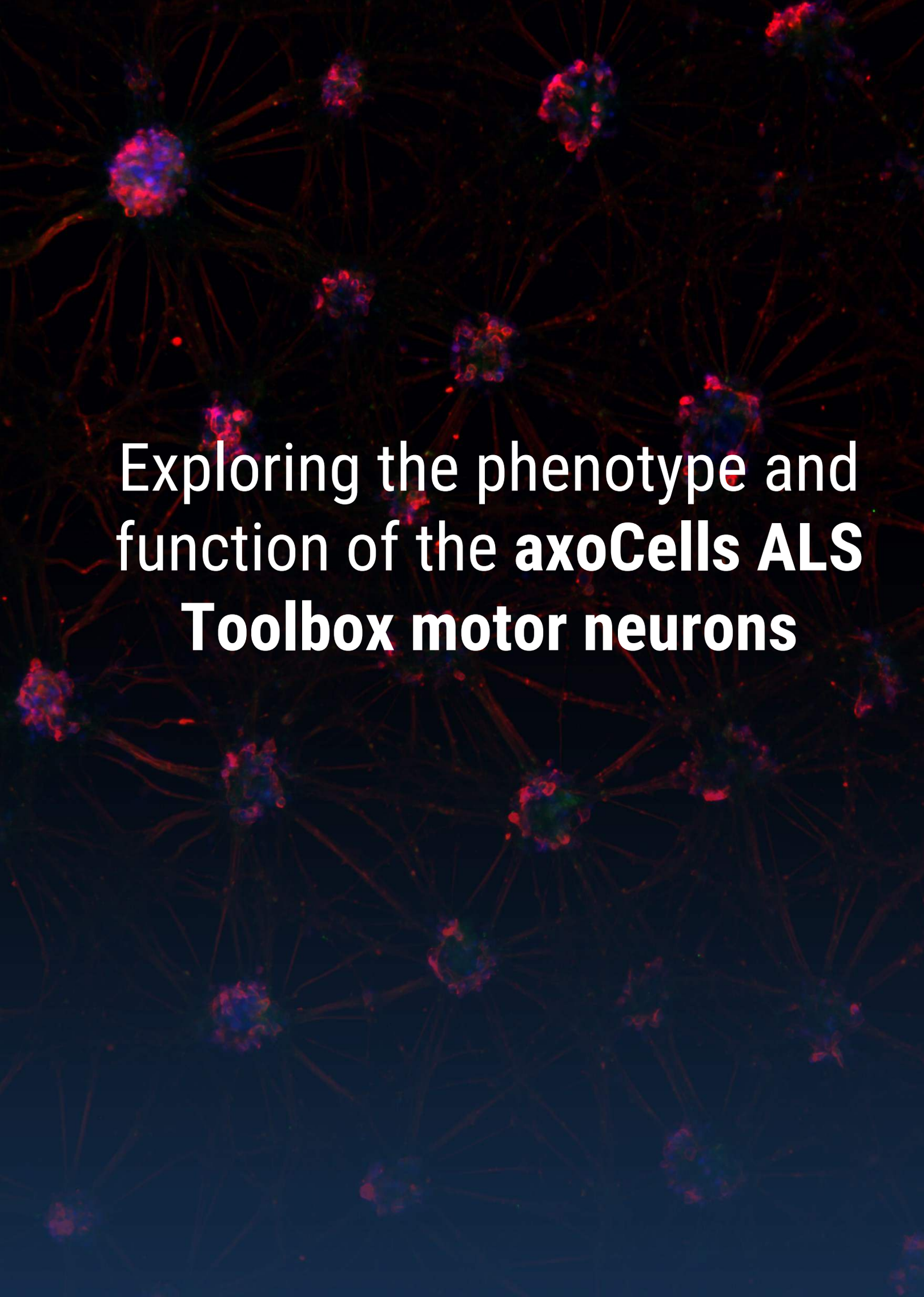
Kit contains cells, media and Accelerator, BDNF, GDNF, CNTF. Other third-party reagents may be required.

Donor	Age at sampling	Details	Mutation	Reprogramming method	Cells	Kit
Unaffected donor	40-50	Male	None	Sendai	**ax0076**	ax0186
	74	Male	None	Episomal	**ax0078**	ax0178
C9orf72 carrying donor	62	Male, sibling of ax0074. Caucasian / Ashkenazi Familial history of ALS and dementia	C9orf72 repeat expansion	Sendai	**ax0073**	ax0183
ALS donor	61	Female	SOD1 Het D109Y (G>T)	Sendai	**ax0735**	ax01835
	62	Female	TARDBP A382T	Sendai	**ax0079**	ax0189
	64 Onset of ALS at 60 - death at 65.	Female, sibling of ax0074. Caucasian / Ashkenazi Familial history of ALS and dementia	C9orf72 repeat expansion	Sendai	**ax0074**	ax0184

Media and Supplements

Product Name	Product code	Quantity
axoCells™ Human CNTF Supplement, 20 µg	ax139888	20 µg
axoCells™ Human GDNF Supplement, 10µg	ax139855	10 µg
axoCells™ Human BDNF Supplement, 10 µg	ax139800	10 µg
axoCells™ Motor Neuron Maintenance Media, 200 ml	ax0072	200 ml
axoCells™ Motor Neuron Accelerator Supplement, 1 ml	ax0179	1 ml

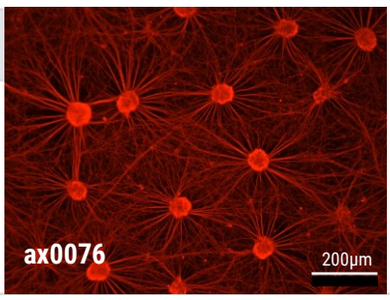
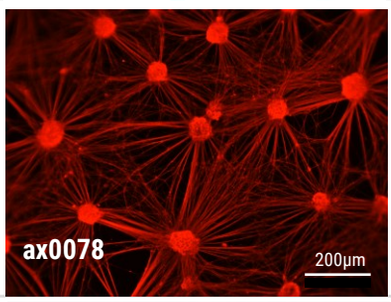
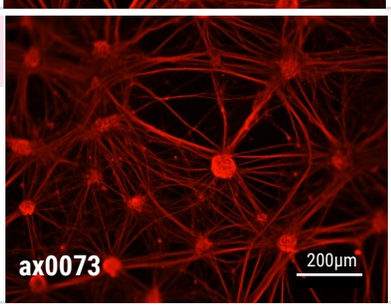
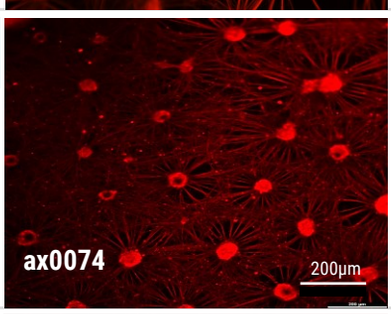
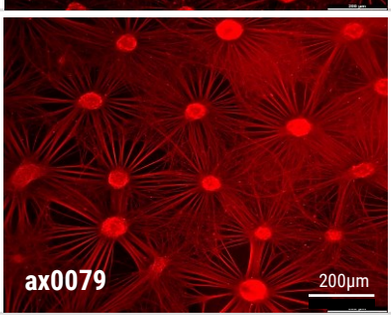
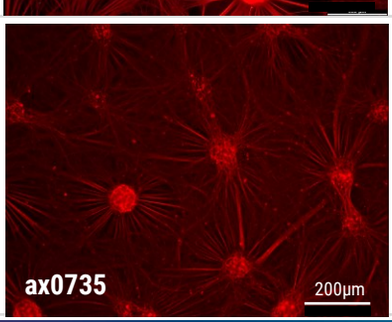
Additional third-party components may be required. Please refer to protocol for full list.



Exploring the phenotype and function of the **axoCells ALS Toolbox motor neurons**

Characterization of iPSC-derived motor neurons

Immunocytochemistry

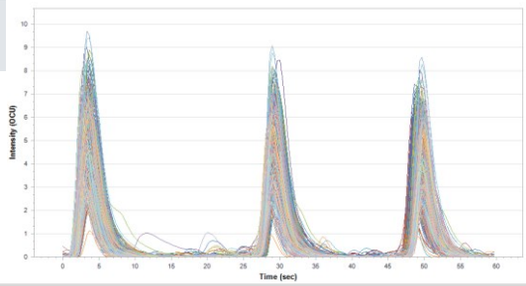
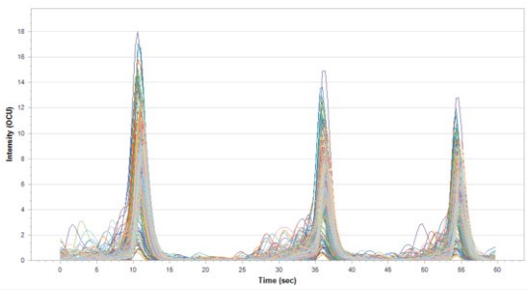
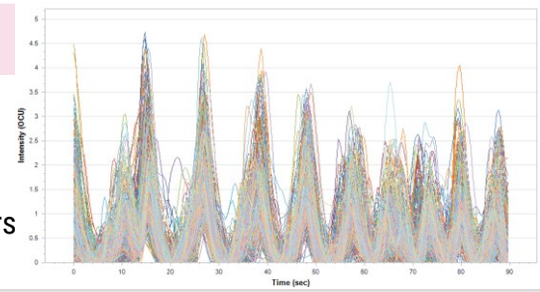
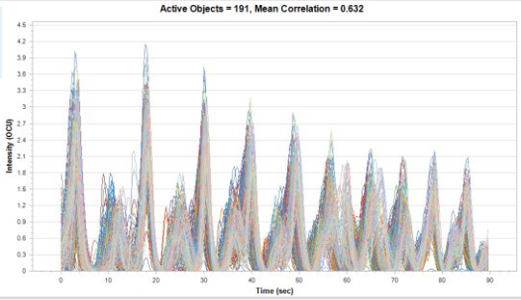
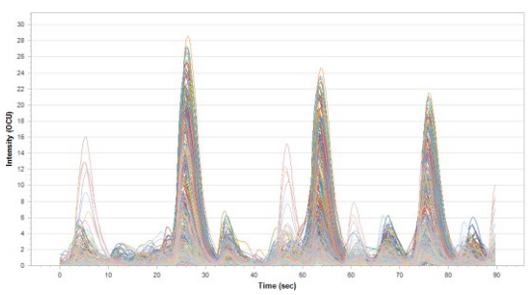
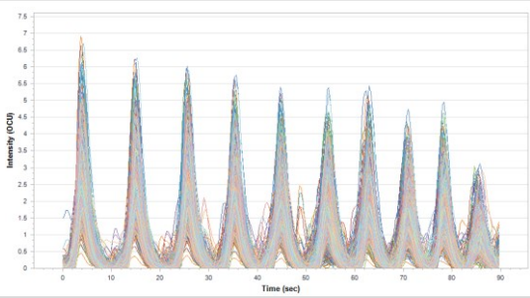
Donors		Key characteristics
Unaffected donors		
ax0076: Unaffected From a male donor, 40-50 years old at time of donation		1. Large uniform cell-body clusters 2. Thick cabling between cell bundles 3. Few fibrous thin neurites
ax0078: Unaffected From a male donor, 74 years old at time of donation		1. Large uniform cell-body clusters 2. Thick cabling between cell bundles 3. Few fibrous thin neurites
C9orf72 carrying donor		
ax0073: C9orf72 unaffected From a male unaffected donor, sibling to ax0074 donor, 62 years old at time of donation		1. Smaller irregular cell-body clusters that are dispersed 2. Thinner cabling between cell bundles 3. More fibrous thin neurites
ALS donors		
ax0074: C9orf72 From a female ALS donor with C9orf72 mutation, 64 years old at time of donation		1. Smaller irregular cell body clusters that are dispersed 2. Thinner cabling between cell bundles 3. More fibrous thin neurites
ax0079: TDP43 From a female ALS donor with TDP43 mutation, 62 years old at time of donation		1. Smaller irregular cell body clusters 2. Thinner cabling between cell bundles 3. More fibrous thin neurites
ax0735: SOD1 From a female ALS donor with SOD1 mutation, 61 years old at time of donation		1. Large, irregular cell body clusters 2. Thick cabling between cell bundles 3. A mix of thin and thick neurite cabling network

sibling

ICC images. Red: TUJ1 (Neuron-specific class III beta-tubulin)

Characterization of iPSC-derived motor neurons

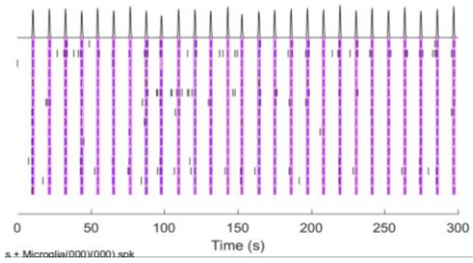
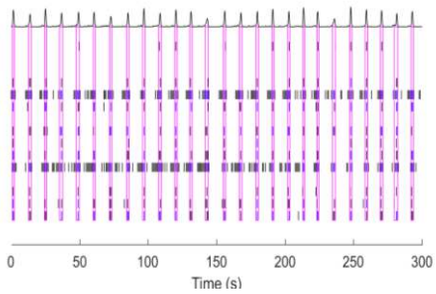
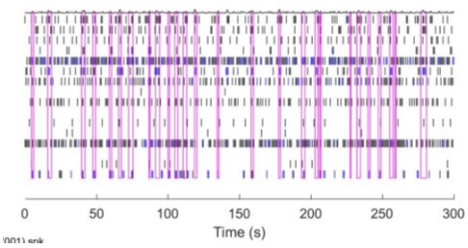
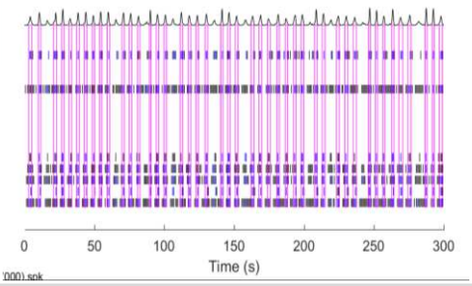
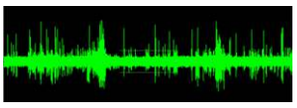
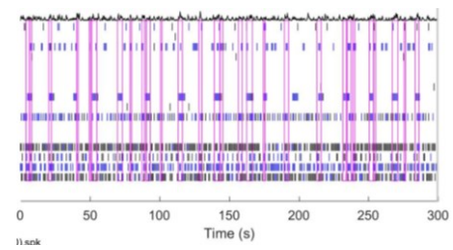
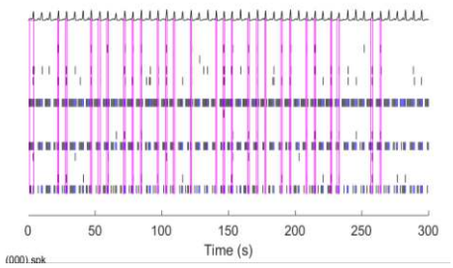
Spontaneous Neuronal Activity

Donors		Key characteristics
Unaffected donors		
ax0076: Unaffected From a male donor, 40-50 years old at time of donation		1. Highly synchronized (mean correlation >0.9) 2. Higher mean burst duration 3. Lower burst rate
ax0078: Unaffected From a male donor, 74 years old at time of donation		1. Highly synchronized (mean correlation >0.9) 2. Higher mean burst duration 3. Lower burst rate
C9orf72 carrying donor		
ax0073: C9orf72 unaffected From a male unaffected donor, sibling to ax0074 donor, 62 years old at time of donation		1. Hyperexcitable phenotype 2. Less synchronized 3. Lower mean burst duration 4. Higher burst rate
ALS donors		
ax0074: C9orf72 From a female ALS donor with C9orf72 mutation, 64 years old at time of donation		1. Hyperexcitable phenotype 2. Less synchronized (mean correlation ~0.7) 3. Lower mean burst duration 4. Higher burst rate
ax0079: TDP43 From a female ALS donor with TDP43 mutation, 62 years old at time of donation		1. Less synchronized 2. Higher burst rate
ax0735: SOD1 From a female ALS donor with SOD1 mutation, 61 years old at time of donation		1. Highly synchronized 2. Higher burst rate

sibling

Characterization of iPSC-derived motor neurons

Multi-Electrode Array

Donors		Key characteristics
Unaffected donors		
ax0076: Unaffected From a male donor, 40-50 years old at time of donation		 1. Synchronized burst firing 2. Regular burst events with low IBI SD 3. Short burst durations 4. Constant spike amplitude
ax0078: Unaffected From a male donor, 74 years old at time of donation		 1. Synchronized burst firing 2. Regular burst events with low IBI SD 3. Short burst durations 4. Constant spike amplitude
C9orf72 carrying donor		
ax0073: C9orf72 unaffected From a male unaffected donor, sibling to ax0074 donor, 62 years old at time of donation		 1. Hyperexcitable phenotype 2. Less synchronized burst firing 3. Irregular burst events with higher IBI SD 4. Short burst durations 5. Variable spike amplitude
ALS donors		
ax0074: C9orf72 From a female ALS donor with C9orf72 mutation, 64 years old at time of donation		 1. Hyperexcitable phenotype 2. Less synchronized burst firing 3. Irregular burst events with higher IBI SD 4. Short burst durations 5. Variable spike amplitude
ax0079: TDP43 From a female ALS donor with TDP43 mutation, 62 years old at time of donation		 1. Less synchronized burst firing 2. Irregular burst events with higher IBI SD 3. Extremely long burst durations 4. Variable spike amplitude
ax0735: SOD1 From a female ALS donor with SOD1 mutation, 61 years old at time of donation		 1. Highly synchronized burst firing 2. Short & medium duration burst trains 3. Constant spike amplitude

sibling

EDITORIAL:

Observing hyperexcitability, a classic trait of ALS, in iPSC-derived motor neurons.

A critical aspect of ALS pathophysiology is the hyperexcitability of motor neurons, a phenomenon that has garnered significant attention in the study of this disease. Hyperexcitability refers to an increased responsiveness of motor neurons to synaptic inputs, leading to an abnormal increase in firing rate. In the long term this increased electrical activity can lead to excitotoxicity which is believed to contribute to the degeneration of motor neurons and the progression of ALS.

Mechanisms of Hyperexcitability in ALS Motor Neurons

The hyperexcitability of motor neurons in ALS can be attributed to several underlying mechanisms. One key factor is the altered function of ion channels that regulate neuronal excitability. Motor neurons express a variety of ion channels, including voltage-gated sodium (Na+), potassium (K+), and calcium (Ca^{2+}) channel, all of which play critical roles in the generation and propagation of action potentials. Another factor is that ALS enhances motor neuron vulnerability to glutamate-mediated excitotoxicity via AMPA receptors leading to calcium overload. In ALS, there is evidence that the function of these ion channels is dysregulated.

How to read hyperexcitability in MEA electrode traces:

1. Higher frequency - more bursts (synchronised events) happening in the same time scale
2. More activity ('noise') in between the bursts
3. Lack of synchronization of bursts

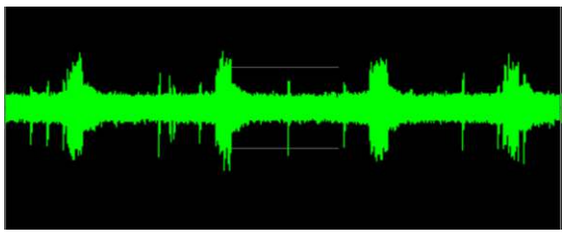
How hyperexcitability is observed in *in vitro studies using multielectrode array (MEA).*

MEA enables non-invasive measurement of electrically-active cells within a familiar multi-well cell culture format. MEA measurements are based on the detection of extracellular field potentials, which are the electrical signals generated by the activity of neurons or other excitable cells. These potentials arise due to the flow of ions (such as sodium, potassium, and calcium) across the cell membrane during action potentials or other electrical events.

'Raw' data is seen in real-time as an electrode trace. A simple way to visualize this neural network behavior is with a raster plot. Each tick mark represents the time, on the x axis, that a spike occurred. Each row of tick marks represents the spikes from a single electrode. Several consecutive spikes make a burst (shown in blue) and when multiple electrodes burst at the same time, this is represented as network event (pink box).

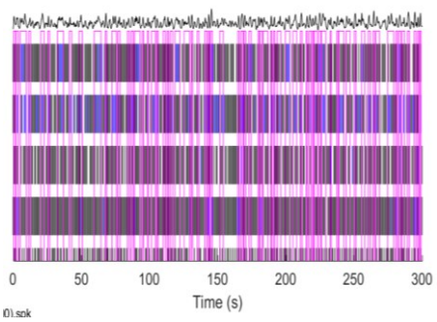
Differences between cells can be observed in connection to the overall firing rate, the synchronization of firing, the length of bursts and the amplitude of bursts.

1a. Electrode trace from ax0078

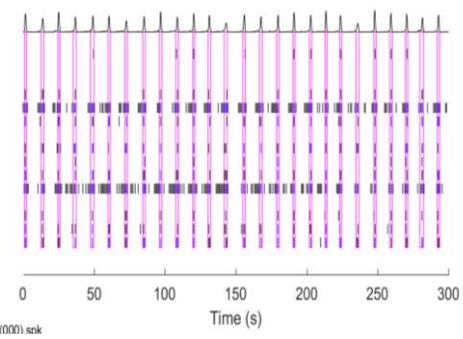


Overview data presented in form of raster plot captures the difference between the synchronised burst firing of ax0078 unaffected donor motor neurons (1b, left) and the increase in firing rate and loss of synchronisation in ALS-affected ax0079 TDP43-mutant motor neurons (1c, right.)

1b. **ax0078**



1c. **ax0079 TDP43**



Intrinsic Properties of ALS Motor Neurons

ALS motor neurons exhibit intrinsic properties that contribute to their hyperexcitability. These include changes in the expression and function of ion channels, as previously mentioned, but also involve alterations in other cellular components, such as the cytoskeleton, which may affect the structural and functional integrity of the neurons.

For example, ALS motor neurons may show a reduction in the expression of small conductance calcium-activated potassium (SK) channels, which normally act to hyperpolarize the membrane following an action potential and prevent excessive firing. The downregulation of SK channels can therefore lead to prolonged depolarization and increased excitability. Additionally, mutations in ALS-linked genes, such as SOD1, TDP-43, and FUS, can directly or indirectly affect the expression and function of these channels, further contributing to the hyperexcitability phenotype.

Moreover, motor neurons in ALS may also exhibit changes in the expression of synaptic proteins, which can alter synaptic strength and plasticity. These alterations can lead to increased synaptic input onto motor neurons, further enhancing their excitability. The loss of inhibitory inputs, such as those mediated by GABAergic or glycinergic interneurons, can also contribute to the hyperexcitability phenotype, as the balance between excitation and inhibition in the motor neuron circuitry becomes disrupted.

Genetic and Environmental Contributions

The hyperexcitability of motor neurons in ALS is influenced by both genetic and environmental factors. Mutations in several genes associated with familial forms of ALS, such as SOD1, C9orf72, TARDBP (encoding TDP-43), and FUS, have been linked to hyperexcitability in motor neurons. For example, SOD1 mutations are known to cause oxidative stress and mitochondrial dysfunction, which can lead to the dysregulation of ion channels and calcium homeostasis, contributing to hyperexcitability.

The expansion of GGGGCC repeats in the C9orf72 gene, the most common genetic cause of ALS, has been associated with the formation of toxic RNA foci and dipeptide repeat proteins, which can disrupt normal cellular function and contribute to neuronal hyperexcitability. Similarly, mutations in TARDBP and FUS, which encode RNA-binding proteins involved in RNA processing and transport, can lead to the accumulation of toxic protein aggregates and dysregulation of gene expression, further exacerbating the hyperexcitability of motor neurons.

Environmental factors, such as exposure to toxins, oxidative stress, and inflammation, can also contribute to the development of hyperexcitability in ALS motor neurons. For instance, chronic exposure to environmental toxins like pesticides and heavy metals has been linked to an increased risk of ALS, potentially through their effects on neuronal excitability and synaptic function. Oxidative stress, which is a common feature in ALS, can lead to the modification of ion channels and other proteins involved in maintaining neuronal excitability, further promoting hyperexcitability.

Observations from the ALS iPSC-derived motor neuron toolbox

In the characterization of this expanded set of iPSC-derived motor neurons, we used MEA, SNA and imaging to observable key traits. By building in this characterization into the manufacturing QC and functional QC processes, we recorded multiple lines and multiple production runs (differentiations) to ensure the traits were reproducible and were not just a consequence of a single differentiation run.

In both the ‘unaffected control’ lines we saw synchronization, short burst events and constant signal amplitude. In comparison, motor neurons from the ALS lines and the unaffected sibling control motor neuron harbouring the C9orf72 mutation, demonstrated a reproducible loss of regular synchronous firing and different degrees of a hyperexcitability phenotype. This is in accordance with the understood ALS clinical pathology and supports the case for the use of these cells in experimental *in vitro* models.

Read more:

- *Xie, M., Pallegar, P.N., Parusel, S. et al. Regulation of cortical hyperexcitability in amyotrophic lateral sclerosis: focusing on glial mechanisms. Mol Neurodegeneration **18**, 75 (2023). https://doi.org/10.1186/s13024-023-00665-w*
- *G Lorenzo Odierna, Steve Vucic, Marcus Dyer, Tracey Dickson, Adele Woodhouse, Catherine Blizzard, How do we get from hyperexcitability to excitotoxicity in amyotrophic lateral sclerosis?, Brain, Volume 147, Issue 5, May 2024, Pages 1610–1621, https://doi.org/10.1093/brain/awae039*
- *Nathan Pavey, Andrew Hannaford, Mehdi van den Bos, Matthew C Kiernan, Parvathi Menon, Steve Vucic, Distinct neuronal circuits mediate cortical hyperexcitability in amyotrophic lateral sclerosis, Brain, Volume 147, Issue 7, July 2024, Pages 2344–2356, https://doi.org/10.1093/brain/awae049*
- *Wainger BJ, Kiskinis E, Mellin C, Wiskow O, Han SS, Sandoe J, Perez NP, Williams LA, Lee S, Boulting G, Berry JD, Brown RH Jr, Cudkowicz ME, Bean BP, Eggan K, Woolf CJ. Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. Cell Rep. 2014 Apr 10;7(1):1-11. doi: 10.1016/j.celrep.2014.03.019. Epub 2014 Apr 3. PMID: 24703839; PMCID: PMC4023477. https://pubmed.ncbi.nlm.nih.gov/24703839/*

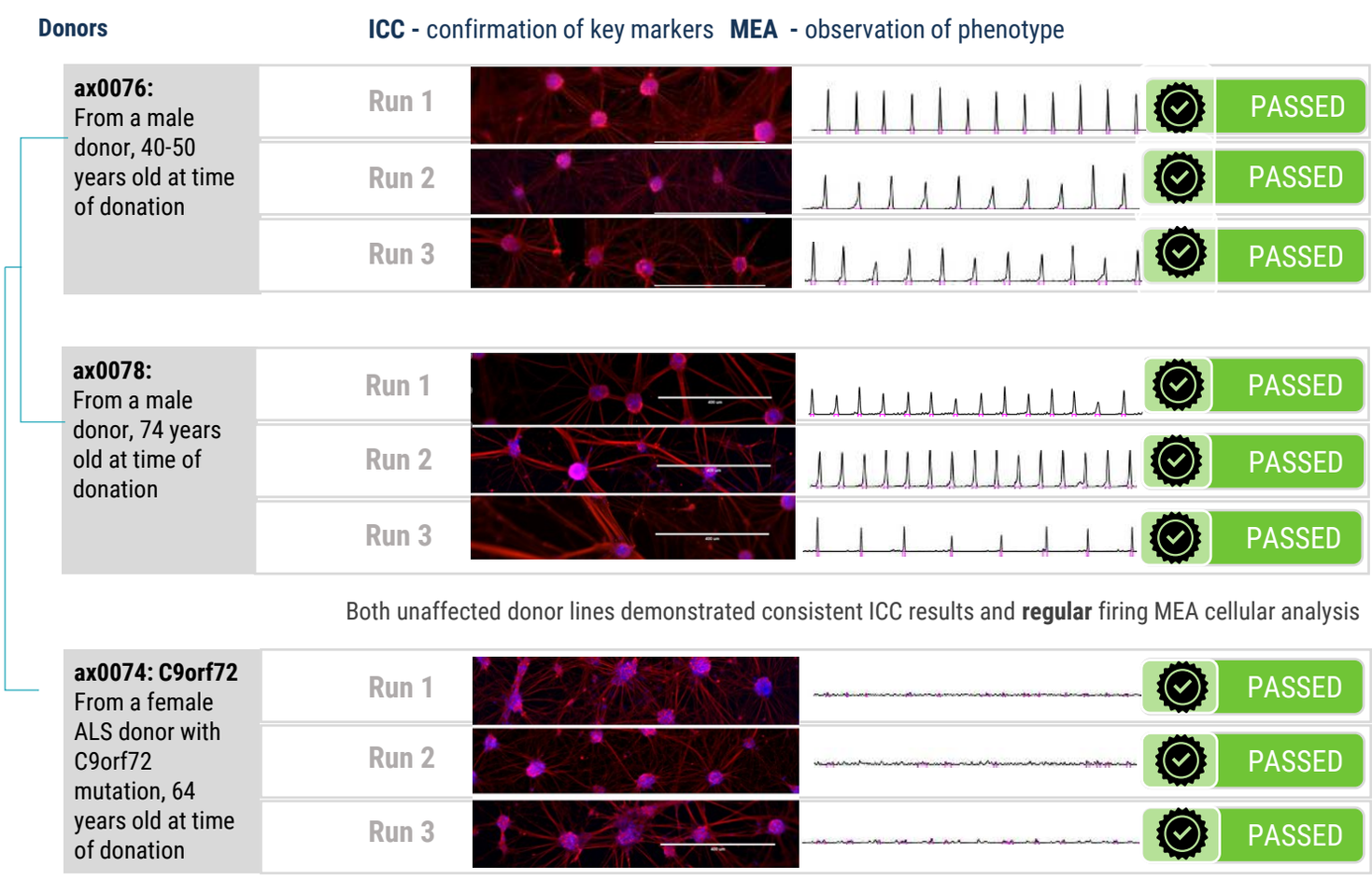
Consistently different.

Unaffected vs ALS on multiple manufacturing runs showing functional consistency, batch to batch.

The ability to manufacture cells of consistent functionality and performance, and cells from different lines that capture their inherent phenotypes, are the hallmarks of a quality iPSC manufacturing process. At Axol we have spearheaded quality manufacturing systems in the iPSC arena to support the development of better models for human disease.

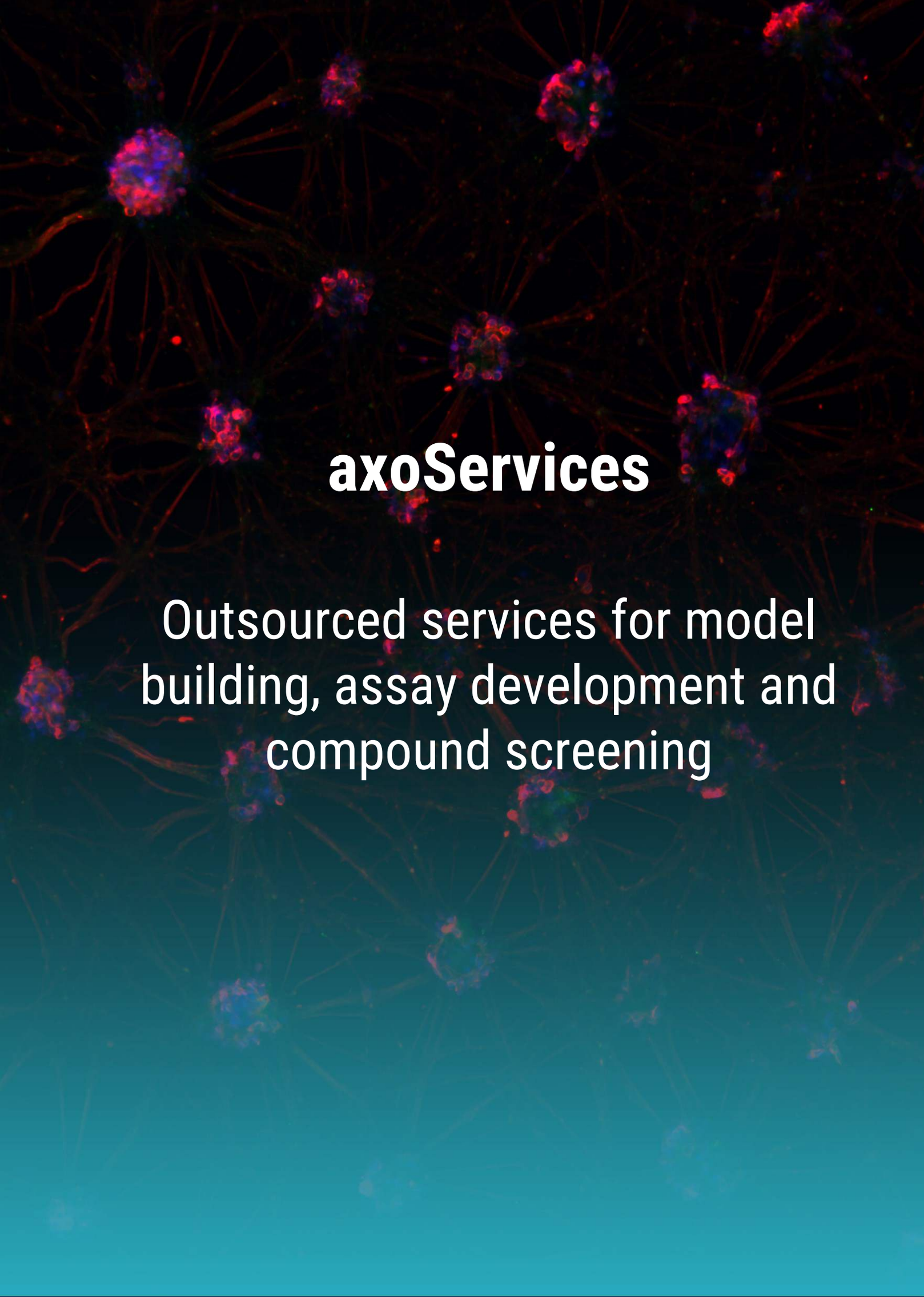
In line with our approach to ISO 9001 accreditation, we compared the phenotype and MEA read out from 3 runs from 3 healthy donors to examine run to run control and line to line difference. All runs passed QC criteria for commercial release.

QC data from 3 manufacturing ('differentiation') runs



Using these fQC readouts, we are able to consistently see clear phenotypic differences between healthy and ALS motor neurons. Healthy lines (ax0076 and ax0078) show very regular burst firing which is synchronised. Motor neurons derived from an ALS patient (ax0074) show much more irregular burst firing with more activity between bursts.

ICC images. Red: TUJ1 (Neuron-specific class III beta-tubulin)



axoServices

Outsourced services for model building, assay development and compound screening

axoServices™

Outsourced services to support iPSC drug discovery and research

SOURCING & iPSC QUALITY MANAGEMENT

Services include patient recruitment, line **sourcing**, ethics and consent checks, primary material **management**, iPSC quality control, **bank creation** and expansion.

iPSC TECHNICAL SERVICES

Services include large scale **reprogramming** projects, iPSC generation from primary material, manufacturing scale **differentiation** to a range of cells including , cortical excitatory neurons, interneurons, sensory neurons, striatal neurons, motor neurons, microglia, astrocytes, ventricular & atrial cardiomyocytes, QC and fQC**, gene-editing** for creation of genetically engineered iPSCs.

MODEL BUILDING & ASSAY DEVELOPMENT

Services include model **building** in monoculture and co-culture, format development including MPS, custom **protocol** design and optimization, model **characterization**, exploring phenotypes, treatment and endpoints, **integration** with MPS and other platforms and **scaling-up** of models for CROs, higher throughput platforms.

COMPOUND TESTING ON iPSC MODELS

Services include compound toxicity and effect screening using a range of in-house assays including multi-electrode array, flow cytometry, cellular imaging, real-time imaging, plate-based assays and 'omics.

iPSCs? What can we do to help?

Through axoServices, we partner with drug discovery groups, from the very largest to the very earliest in assay development, model creation and compound testing. Each project is unique but built on the shoulders of giants. Our project managers look to maximize efficiency and success always through the best spirit of collaboration and partnership.

Discussion
NDA
Design
Agreement

Modelling
Which lines and cells
Mono- or Co-
Characterization

Experiment
Compound modalities
Treatment regimes
Endpoint, midpoint assays

Data management
Quality control
Data analysis
Data mining

Communication
Regular updates
Final reports & data

axoServices™ Differentiation

We offer a QC-rich full-service differentiation process from human iPSCs to endpoint cells. Potential endpoint cells include **cortical excitatory neurons, cortical inhibitory interneurons, striatal neurons, astrocytes, microglia, sensory neurons, motor neurons, myotubes (as made-to-order), atrial cardiomyocytes and ventricular cardiomyocytes.**

What does an axoServices Differentiation project look like?

Step 1: Kick-off meeting with the Axol iPSC experts

- Decades of experience in the room
- Open, two-way communication
- Rapid follow-up with competitive quote

Step 2: Extensive iPSC characterization

- Flow cytometry to confirm pluripotency-associated marker expression
- Ensure high-quality starting material to maximize project success

Step 3: Directed differentiation and optimization

- Our proprietary differentiation process uses small molecules to generate footprint-free endpoint cells
- Built-in optimization provides safety net for project challenges
- Robust target cell populations for QC, custom assays and shipping

Step 4: QC and custom assays

- Rigorous checks for contaminants and optimal post-thaw viability
- ICC to confirm expression lineage-specific markers
- Custom assays capabilities to match project requirements
- Desired end-point cells are shipped with media and instructions

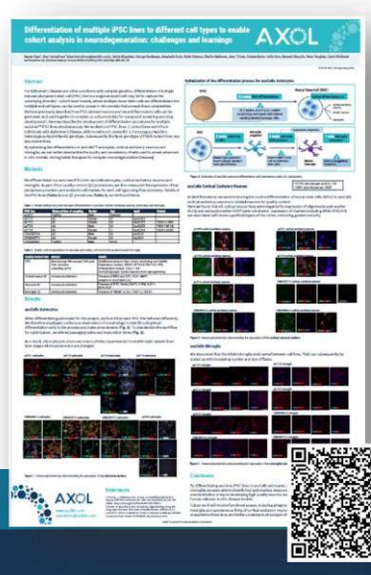
Step 5: Ongoing support

- Continued communication and advice from iPSC experts
- Ease the adaptation of cells into your experimental system
- An open channel for future projects, collaborations and partnerships

Timelines are usually >4 weeks but differ based on specific endpoint cell.

Differentiation of multiple iPSC lines to different cell types to enable cohort analysis in neurodegeneration: challenges and learnings

Scan the QR code to explore this poster where we demonstrate our differentiation expertise. Here we describe the development of differentiation procedures for multiple axoLines™ iPSC lines simultaneously, including 2 control lines and 6 from individuals with Alzheimer's Disease, with mutations in presenilin 1, homozygous ApoE4 or heterozygous ApoE4/ApoE3 genotype. By optimizing the differentiation of astrocytes, cortical excitatory neurons and microglia, we can better standardize the quality and consistency of cells used to power advanced *in vitro* models, driving better therapies for complex neurodegenerative diseases.



axoServices™ Compound Screening

We combine our **iPSC expertise** with a **comprehensive suite of custom assays** to deliver efficient compound/phenotypic screening, with a specific emphasis on the quality and communication of assay data. We can help you to generate **robust pre-clinical data** to validate compounds, or narrow down a large compound library for further **in-depth experimental analysis**.

What does an axoServices Compound Screening project look like?

Step 1: Kick-off meeting with the Axol iPSC experts

- Decades of experience in the room
- Open, two-way communication
- Rapid follow-up with competitive quote

Step 2: Assay design and optimization

- In-house expertise to guide design and maximize project success
- Small-scale pilot experiments to optimize assay selection
- Progression to higher throughput screening to enhance reproducibility

Step 3: Data analysis and reporting

- Across the service project, all raw data is stored and lab notebooks are maintained in keeping with data management requirements
- Full written report produced upon project completion with data-sharing meeting for full transparency

Step 4: Ongoing support

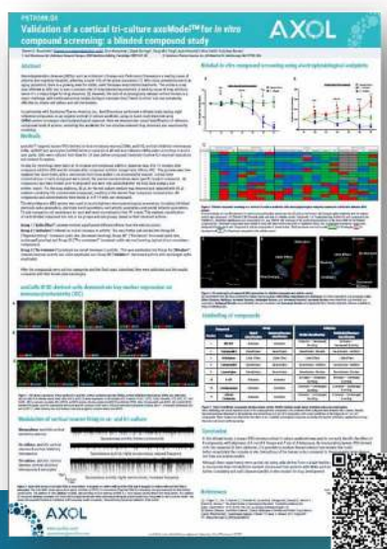
- Continued communication and advice from iPSC experts
- Ease the adaptation of cells into your experimental system
- An open channel for future projects, collaborations and partnerships

Validation of a cortical tri-culture axoModel™ for in vitro compound screening a blinded compound study

Scan the QRD code to read this poster demonstrating our expertise in compound screening.

In partnership with Sumitomo Pharma America, Inc., we performed a blinded study testing eight reference compounds on an isogenic cortical tri-culture axoModel, using an Axion multi-electrode array (MEA) system to measure electrophysiological response.

Here we demonstrate robust identification of reference compound mode of actions, validating this axoModel for use in human-relevant drug discovery and neurotoxicity screening.



Resources

Characterisation of iPSC-derived motor neurons using an accelerated maturation protocol

Steven Broadbent, Stuart Prime, Signe Springe, Joe Lyndsay, Sian Humphreys, Ashley Barnes - Axol Bioscience Ltd, Cambridge, UK

The Platforma Project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 951890

www.axolbio.com

Abstract

Understanding neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) requires a supply of cells for *in vitro* modelling and drug screening. **Motor neurons derived from induced pluripotent stem cells (iPSC)** provide an unlimited source of such cells and can potentially facilitate precision medicine approaches to compound testing when derived from somatic cells from patients. Furthermore, in co-culture with iPSC-derived muscle cells, **a fully human preclinical screening model of the Neuromuscular Junction is possible**. However, iPSC-derived motor neurons traditionally required up to six weeks maturation to exhibit relevant functional phenotypes.

We demonstrate use of an *in vivo* environment-mimicking supplement, to produce a more physiological, modified growth medium for maturation, which **reduces the time to reach functional maturity from six weeks to 10 days**. Cells showed typical morphology with abundant neurites and were positive for expression of the mature motor neuron marker **SMI-32 along with HB9, ChAT and Isl-1**, as assessed by immunocytochemistry.

Recordings using a 48-well multi-electrode array (MEA) demonstrate synchronised spontaneous firing at a similar timepoint. Motor neurons matured using the accelerated protocol formed junctions with muscle cells in a microfluidic co-culture system, as demonstrated by staining with fluorescently labelled alpha-bungarotoxin. The phenotype of motor neurons from an ALS patient was also investigated.

These rapidly matured cells provide an invaluable tool for research into neuromuscular disease and a model allowing a **higher throughput for compound screening**.

METHODS

Cell culture of iPSC progenitor cells to mature cell types was performed using Axol Bioscience user protocols and media (ax0072) for motor neurons (ax0078 control and ax0074 ALS C9ORF72, greater than 145 GGGGCC repeats). Initial studies used Motor Neuron Accelerator (ax0179) with a poly-D-lysine and SureBond XF matrix (day 18 plus) . Subsequently the process was accelerated further using a vitronectin matrix (day 10 plus).

Immunostaining:

Immunostaining was conducted using standard PFA fixing, 0.3% Triton, using commercially available antibodies.

For visualisation of the neuromuscular junction, cultured motor neuron progenitor or skeletal muscle progenitor cells were seeded into a microfluidic chamber with 450um channels

MEA Recording:

Extracellular field potentials were acquired at 37°C, 5% CO_2 using the Axion Maestro Pro high-throughput MEA platform. 48-wells plates with a 350µm separation between each well-floor mounted electrode were used. This enabled simultaneous recording of extracellular potentials from 16 electrodes per well at a sampling rate of 20kHz/channel. Cultured motor neuron progenitor cells were spotted onto the array in a 10µl volume of media. These were then differentiated in-situ to form their mature cell types.

Figure 1. MEA Configuration.
Figures A, B and C show the axion MEA system, well plate and MEA array that resides in wells of the plate.
Figure D. (phase contrast) of MEA coated motor neuron culture.

* *www.platforma-project.com*

Spontaneous Neuronal Activity (SNA) Recording:

Motor neurons were transfected at day 2 and day 7 post-thaw with Incucyte® Neuroburst Orange Lentivirus, encoding a calcium reporter driven from the synapsin promoter. Spontaneous activity was recorded using Incucyte® Neuronal Activity Analysis Software on an Incucyte® S3.

RESULTS

Motor Neuron Activity Measurement Via MEA Detection

Synchronized clustered firing

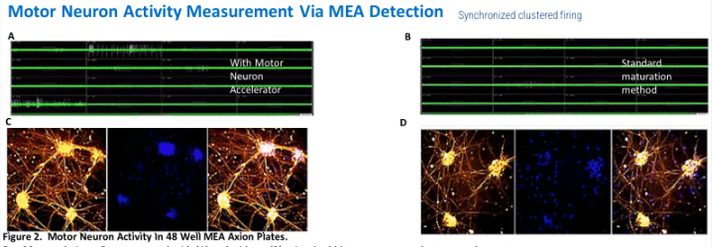
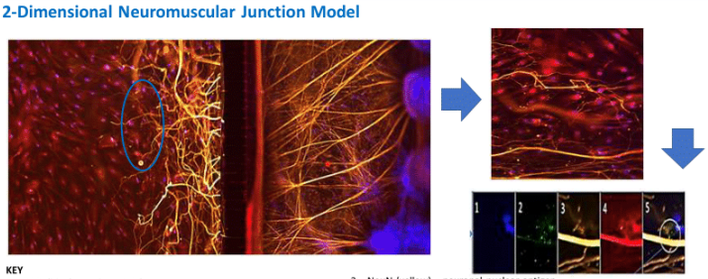


Figure 2. Motor Neuron Activity In 48 Well MEA Axion Plates.
Day 20 post plating – Data generated with (A) and without (B) using Axol Motor neuron accelerator supplement.
Immunocytochemistry staining for SMI32 and DAPI were used to assess the motor neuron cluster formation and development of mature neurites within the culture with (C) and without (D) Axol Motor neuron accelerator supplement, without vitronectin.

2-Dimensional Neuromuscular Junction Model



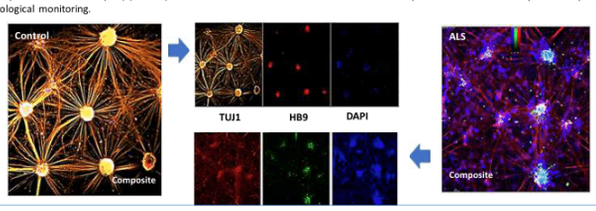
KEY

1. DAPI (blue) - nuclear stain)
2. Alpha-Bungarotoxin (green) – synaptic acetylcholine binding marker
3. NeuN (yellow) – neuronal nuclear antigen
4. Titin (red) – skeletal muscle
5. Composite image

Figure 3. Motor Neuron/ Skeletal Muscle – co-Culture.
Microfluidic co-culture of human iPSC motor neurons and skeletal muscle. The images show development of the neuromuscular junction. The motor neuron nerve (yellow, NeuN) completely overlaps the postsynaptic acetylcholine receptors (green, fluorescent α-bungarotoxin conjugates) and muscle (red, titin)

Expression of markers by immunocytochemistry: WT and C9Orf72 mutant

iPSC used were from a healthy donor (ax0078) and from a donor with C9ORF72 hexanucleotide expansion (greater than 145 GGGGCC repeats) and diagnosed with amyotrophic lateral sclerosis (ALS) (ax0074). Both lines were matured to motor neurons for 10 days with characterization by immunocytochemistry and electrophysiological monitoring.



Results ctd.

Expression of markers by immunocytochemistry: WT and C9Orf72 mutant

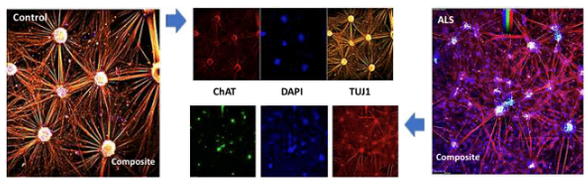


Figure 4. Motor neuron characterization by immunohistochemistry.
Imaged at 20X magnification in 96 well imaging plates. Cells were fixed at day 10 post plating and stained with mature motor neuron markers HB9, ChAT and nuclear marker DAPI. Neurons were matured with Motor Neuron Accelerator supplement and vitronectin matrix.

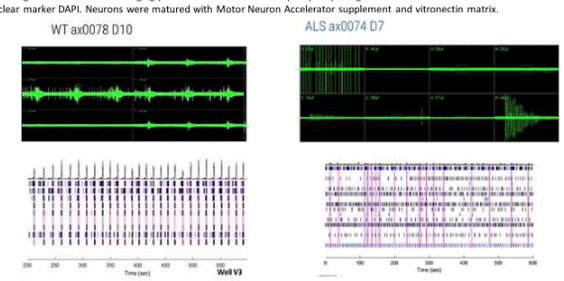


Figure 5. Motor neuron characterization by electrophysiology
MEA recordings were made from day 10-matured iPSC-derived motor neurons from a healthy donor (left) and day 7-matured from a donor with ALS (right)

Motor Neuron Activity Measurement Via Neuroburst® Orange and Incucyte® S3

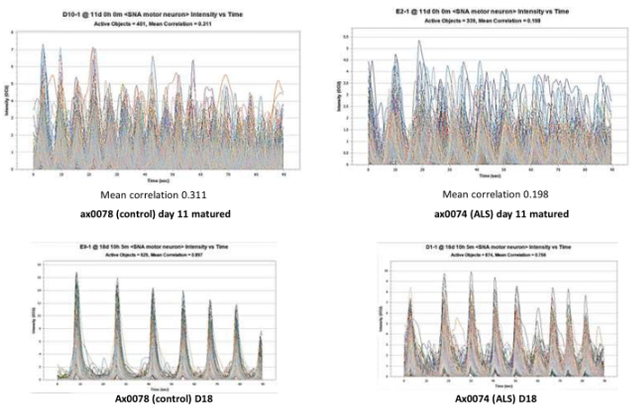


Figure 6a. Analysis from recording of spontaneous neural activity.
Incucyte® Neuroburst Orange lentiviral reagent was used to image spontaneous neuronal firing in iPSC-derived motor neurons from a healthy donor (left) and one with ALS (right), after 11 days maturation

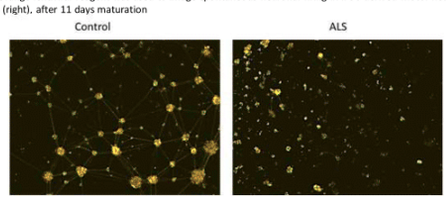
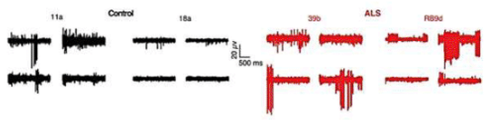


Figure 6b. Images from recording of spontaneous neural activity[1].
Firing in iPSC-derived motor neurons from a healthy donor (left) are more evenly distributed than in similarly differentiated neurons from a donor with ALS (right). Neurites are clearly visible in the control motor neurons and far less so in motor neurons from an ALS donor.

The ALS *in vitro* model recapitulates results from SOD1A4V/+ mutation

To prove the model correlated with clinical findings, the authors[2] used MEA to evaluate iPSC-derived ALS neurons from two patients with the $SOD1^{A4V/+}$ mutation.

- ALS neurons showed increased neural firing compared to control cells. Maestro recordings confirmed clinical findings of neural hyperexcitability compared to controls.
- Below are representative recordings from four out of 64 MEA electrodes in control (11a, 18a) and $SOD1^{A4V/+}$ mutant (39b, RB9d)-derived motor neurons cultured for 28 days on the arrays. MEA recordings revealed increased spontaneous firing in the mutant-derived neurons compared to control-derived neurons, confirming previous clinical findings and further validating the in vitro model.



1. Video available to view at Axol's booth no. 617

2. Wainger BJ. *et al*. Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. Cell Rep. 2014 Apr 10;7(1):1-11.

CONCLUSION

Motor neurons matured for 10 days with a more physiological medium with Motor Neuron Accelerator (ax0179) show typical expression of HB9 and ChAT and a high level of neuraxis. Synchronized spontaneous firing is also evident after only 10 days of maturation, recorded using a multiple electrode array, or using the Incucyte® Neuroburst Orange Lentivirus.

Motor neurons derived from iPSC carrying a C9ORF72 hexanucleotide expansion (greater than 145 GGGGCC repeats) exhibit earlier (day 7) activity, but with longer burst trains and decreased synchronization. The distribution of firing across the well, as visualized on the Incucyte®, is uniform in the wildtype motor neurons, but heterogenous in the ALS motor neurons.

iPSC-derived motor neurons have been shown to exhibit a clinically relevant phenotype by electrophysiology or using the Incucyte activity module. Using a medium supplement whose components mimic the physiological cellular environment, motor neurons ca progenitors in only 10 days, providing a disease-relevant model for medium throughput compound screening or mechanistic studie

Case study: iPSC-Derived Motor Neurons and Microglia From ALS Background Display Disease Phenotype

The overlap between **ALS and FTD** (frontotemporal dementia) has led researchers to investigate the role of neuroinflammation in ALS development. This has led to increasing interest in the role of microglia, the main neuroinflammatory cell, in the development of ALS. In collaboration with **Sartorius**, we investigated the **morphology and functional performance** of ALS-derived motor neurons and microglia compared to healthy control cells. Here we present key highlights from the project.

Spontaneous neuronal activity analysis on day 18 axoCells Motor Neurons

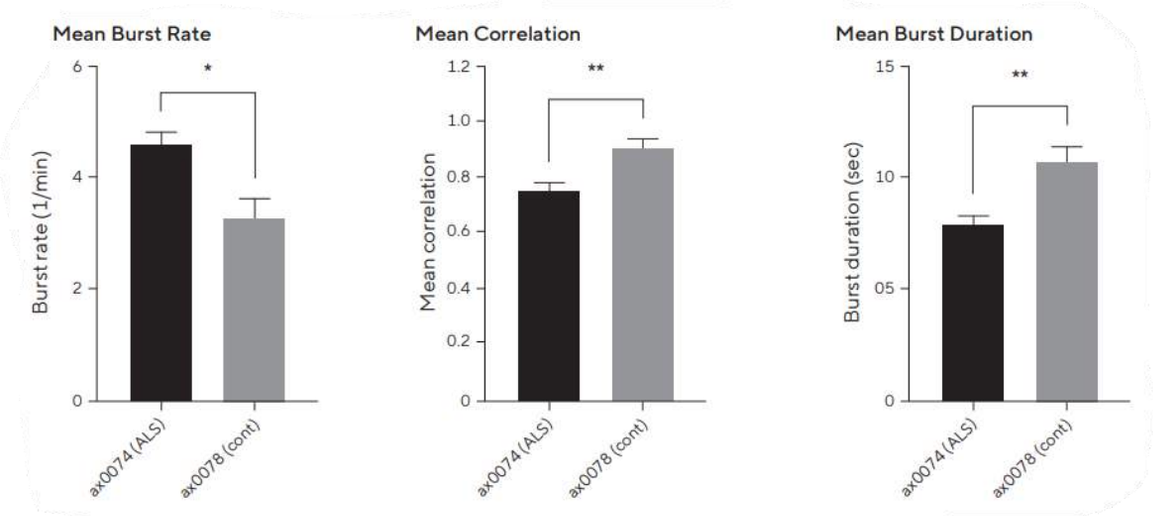


Figure 5. The IncuCyte® Neuronal Activity Software Module was used to monitor and analyze calcium signaling. Statistical significance was assessed using a non-parametric T-test, *p<0.05, **p<0.01. ALS motor neurons exhibited significantly higher burst rates compared to the control line, along with lower burst duration and lower mean correlation (hence less synchronized burst firing).

Phagocytosis of myelin basic protein (MBP) by axoCells Microglia.

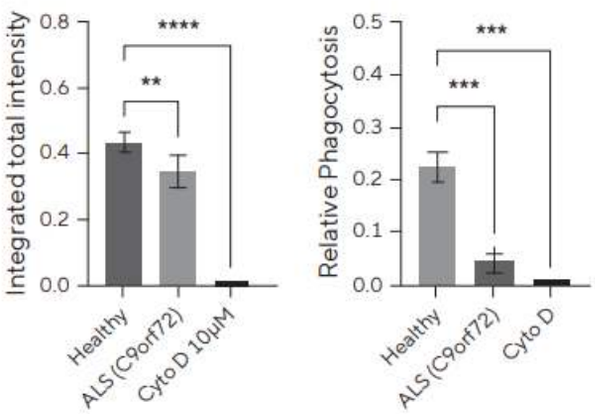


Figure 6. Phagocytosis of pHrodo® for IncuCyte® labeled MBP was assessed on an Incucyte® Live-Cell Analysis System up to 24h using fresh and cryopreserved microglia. Cytochalasin D (10 µM) was used as a control. Statistical significance was assessed using a one-way ANOVA, **p<0.01, ***p<0.001, ****p<0.001

This indicates that ALS microglia are affected by the C9orf72 expansion in ALS with decreased phagocytosis, demonstrating a useful assay to identify an ALS-like phenotype.

In this project, we demonstrated distinct **ALS-like phenotypes** in axoCells Motor Neurons and microglia derived from ALS iPSCs, which can be quantified and used to inform future drug discovery purposes. As part of this project, we also looked into **morphology**, **immunocytochemistry** and **electrophysiology** (data available in the full whitepaper), demonstrating the wide range of useful assays for investigating ALS-like phenotypes in motor neurons and microglia.

Scan the QR code to access the full whitepaper

axoCells ALS Toolbox for Drug Discovery
Product information

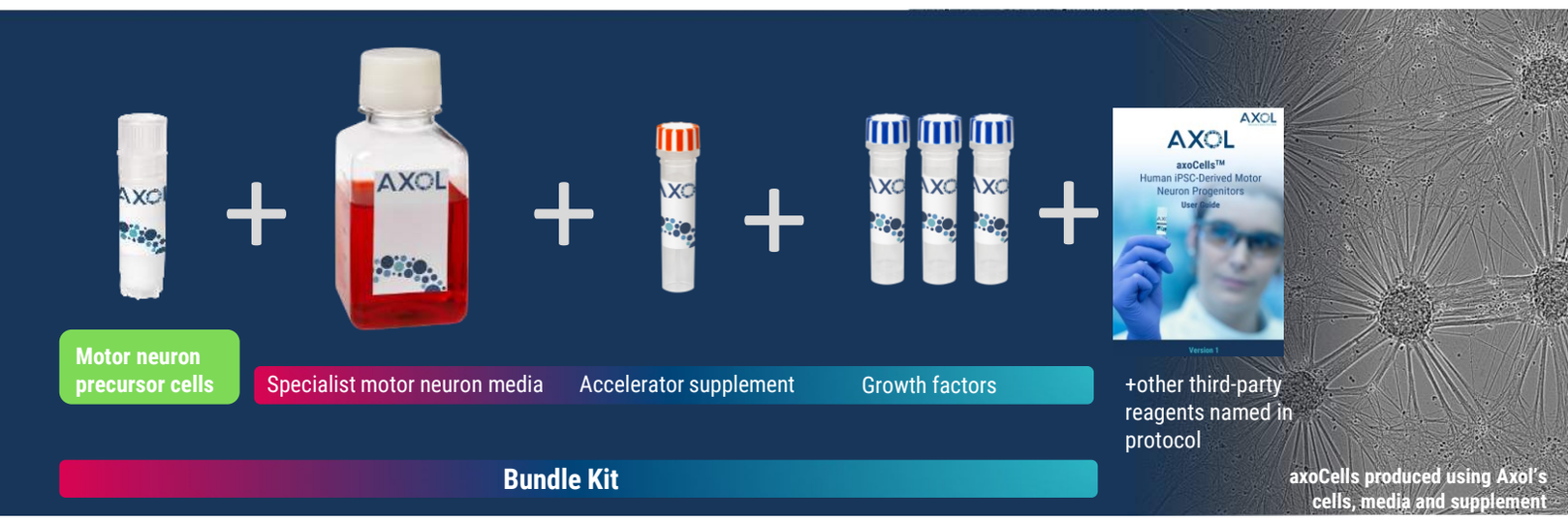
Axol Bioscience manufacturers specialist precursor cells, media and supplements to achieve high performance *in vitro* models.

Use our motor neuron precursor cells + specialist iPSC media + Accelerator supplement + growth factors to achieve functional motor neurons.

How to make high performing axoCells motor neurons

Cells and Kits

Product Name	Quantity	Cells only	Bundle Kit (Cells + media + supplements)
Human iPSC-Derived Motor Neurons (unaffected male 1)	>2M cells	ax0076	ax0186
Human iPSC-Derived Motor Neurons (unaffected male 2)	>2M cells	ax0078	ax0178
Human iPSC-Derived Motor Neurons (male, C9orf72 carrying)	>2M cells	ax0073	ax0183
Human iPSC-Derived Motor Neurons (female, ALS, SOD1)	>2M cells	ax0735	ax01835
Human iPSC-Derived Motor Neurons (female, ALS, TDP43)	>2M cells	ax0079	ax0189
Human iPSC-Derived Motor Neurons (female, ALS, C9orf72)	>2M cells	ax0074	ax0184



Specialist Media and Supplements – available separately or included in kit

Product Name	Product code	Quantity
axoCells™ Human CNTF Supplement, 20 µg	ax139888	20 µg
axoCells™ Human GDNF Supplement, 10µg	ax139855	10 µg
axoCells™ Human BDNF Supplement, 10 µg	ax139800	10 µg
axoCells™ Motor Neuron Maintenance Media, 200 ml	ax0072	200 ml
axoCells™ Motor Neuron Accelerator Supplement, 1 ml	ax0179	1 ml

For a quotation, email **operations@axolbio.com** or contact your local distributor.

iPSCs? How can we help?

operations@axolbio.com

support@axolbio.com

www.axolbio.com