# Altered Electrophysiology and Calcium Dynamics in SOD1-Associated Amyotrophic Lateral Sclerosis

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## Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective degeneration of motor neurons in the spinal cord, brain stem, and motor cortex[8]. The mechanism of neurodegeneration in ALS is not fully understood, but experimental observations in animal and human models of ALS point to the activation and dysfunction of various pathways that may ultimately lead to motor neuron death.

ALS can be broadly classified into two groups based on etiology: familial and sporadic. Familial cases of ALS (fALS) are linked to genetic mutations and account for approximately 10% of total ALS cases[2]. Over 30 genes have been implicated in fALS, one of the most well-studied being *superoxide dismutase 1* (*SOD1*)[5]. Mutations in the *SOD1* gene have been linked to approximately 20% of total fALS cases and confer a toxic gain of function in the protein that results in abnormal aggregation, particularly in late stages of ALS. In heterogeneous neuron cultures, all cells incorporate SOD1 aggregates, but motor neurons die at much higher rates than other neuron types, showing that SOD1 aggregates are selectively toxic to motor neurons. Additionally, though aggregates are not detectable during early stages of ALS, mutated SOD1 affects motor neurons in a variety of ways and has been linked to a variety of negative effects including mitochondrial swelling and increased endoplasmic reticulum stress[1].

Experiments in mouse and human pluripotent stem cell (hPSC) models of SOD1-linked fALS have shown that motor neurons undergo distinct electrophysiological changes compared to control and wild type motor neurons throughout disease progression[4]. These changes correspond to distinct phases of excitability that can be observed and modulated using whole-cell patch clamp analysis. Mouse models have also shown changes in calcium and sodium currents, highlighting the role of individual ion channels and ions in ALS pathology and neurodegeneration[2, 3, 5]. Previous work in our labs has shown that motor neurons treated with SOD1 aggregates have significantly depolarized resting membrane potentials and higher action potential thresholds compared to healthy controls, linking the toxic effects of SOD1 aggregates to electrophysiological changes.

## Background

- Cultured motor neurons become electrically active after about 3 weeks in culture and electrical properties can be examined using patch clamp analysis
- Motor neurons treated with SOD1 aggregates have a significantly higher resting membrane potential and action potential threshold than healthy motor neurons
- Motor neurons lose their ability to fire repetitive action potentials following current injection[9]

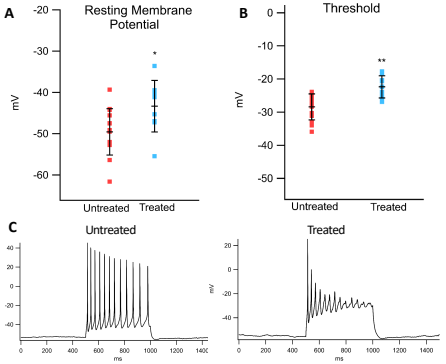


**Figure 1.** Treating hESC-derived motor neurons with SOD1 aggregates significantly changes electrophysiological properties. Statistical analyses performed using Wilcoxon ranked sum test, *p<0.05, **p<0.01. A) Comparing motor neurons treated with SOD1 aggregates (n = 15) to untreated control cells (n = 8) shows that treated motor neurons have a significantly higher resting membrane potential. B) Treated motor neurons have a significantly higher action potential threshold than untreated motor neurons. C) Representative recordings from untreated and treated motor neurons stimulated at 500 pA for 500 ms show loss of repetitive firing pattern.

## Theories of Neurodegeneration

Glutamate-Mediated Excitotoxicity and Calcium-Related Apoptosis

Mouse and human models of ALS have shown that motor neurons are hyperexcitable before neuron dysfunction, degeneration, and symptom onset[12]. Hyperexcitable motor neurons fire more often than healthy controls, leading to an increase in calcium influx which can have detrimental effects on the cell. Motor neurons are particularly vulnerable to calcium overload because they have a lower endogenous ability to buffer calcium than other neuron types and tend to express many calcium-permeable channels[7]. Calcium ions are important secondary messengers within neurons, but in large amounts can trigger endoplasmic reticulum stress, proteasome impairment, and the unfolded protein response, all of which can lead to neuron death[8].

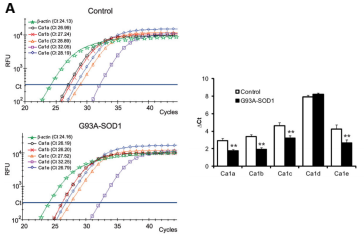


**Figure 2.** Chang and Martin (2016) show increased high voltage-activated calcium currents in G93A-SOD1 mice. A) RT-qPCR shows that G93A-SOD1 MNs have increased expression of calcium channel subunits compared to controls. B) Representative voltage clamp recordings from control and G93A-SOD1 motor neurons. C) *I-V* plots show peak calcium current at different potentials; calcium current from G93A-SOD1 MNs was significantly larger at -10, 0, and 10 mV.

Mitochondrial Dysfunction

In addition to triggering various apoptotic cellular pathways, calcium overload can also damage mitochondria. Mitochondria have the endogenous ability to buffer calcium, but like neurons, too much calcium can have negative effects on both the mitochondria and the cell[7]. When mitochondria experience calcium overload, they release pro-apoptotic factors that activate various pathways that lead to cell death. Additionally, with increased calcium influx, mitochondria increase production of reactive oxygen species (ROS) in the electron transport chain. In healthy individuals, ROS are captured by SOD1 proteins produced by both the mitochondria and the cell, so they do not oxidize and damage lipid membranes, other proteins and enzymes, and genetic material. In individuals with SOD1-associated ALS, not only is there increased ROS production following mitochondrial calcium overload, they also lack fully functional SOD1 proteins, resulting in increased oxidative stress and damage[10]. Beyond inducing mitochondrial calcium overload, mutated SOD1 has been found in aggregates inside the mitochondrial matrix and on the outer membrane. These aggregates may negatively impact mitochondrial function, resulting in decreased ATP production[11].

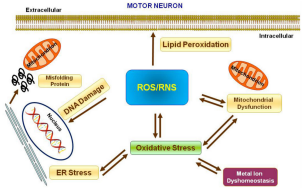


**Figure 3.** Schematic from Sirabella et al (2018) shows various negative cellular effects of increased ROS production and oxidative stress.

Protein Aggregation

SOD1 aggregates have been found post mortem in the spinal cords of ALS patients, leading to the hypothesis that aggregates play a role in disease pathology and neurodegeneration. Experiments using a heterogeneous culture of human iPSC-derived motor neurons show that SOD1 aggregates are incorporated by all cell types but are selectively toxic to motor neurons. In motor neurons that have incorporated SOD1 aggregates, proteasome activity is significantly decreased compared to controls, leading to a further buildup of proteins and a loss of homeostasis. Protein aggregation can also lead to increased ER stress and activation of the unfolded protein response (UPR). While neuroprotective when activated for short periods of time, chronic activation of the UPR leads to cellular dysfunction and apoptosis[7]. Additionally, mutated SOD1 has been proposed to act in a prion-like manner, causing WT SOD1 to misfold and causing increased aggregation[1].

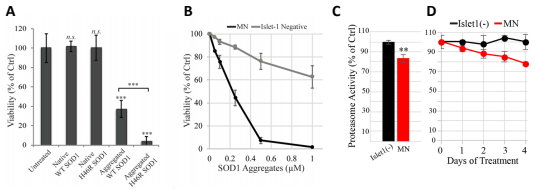


**Figure 4.** Benkler et al (2018) show that H46R-SOD1 aggregates are selectively toxic to motor neurons. A) WT and H46R-SOD1 aggregates were significantly more toxic to MNs than native forms. B) SOD1 aggregate toxicity was dose dependent and impacted MNs more than other neuron types (Islet-1 Negative). C) Proteasome activity was significantly decreased in MNs compared to Islet-1 negative cells. D) After treated cells were normalized to untreated controls, MNs showed decreased proteasome activity as a percentage of total.

## Acknowledgements

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## Electrophysiology

Hyperexcitability

Mouse and human models of SOD1-associated fALS have shown that motor neurons are hyperexcitable early in ALS progression, often even before symptom onset[8]. Motor neurons fire both spontaneous and evoked action potentials at significantly higher frequencies than wild type cells[12]. Hyperexcitability may be caused by an increase in the influx of sodium and calcium ions. Experiments in mice have shown that hyperexcitable motor neurons have significantly larger sodium currents compared to control motor neurons and that firing rates can be reduced with Riluzole treatment, a compound that blocks voltage-gated sodium channels[6]. RT-qPCR also shows that calcium channels are upregulated in motor neurons with SOD1 mutations, resulting in larger calcium currents that further depolarize the cell and contribute to the hyperexcitable phenotype[2].

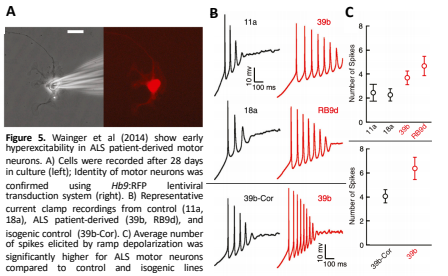


**Figure 5.** Wainger et al (2014) show early hyperexcitability in ALS patient-derived motor neurons. A) Cells were recorded after 28 days in culture (left); Identity of motor neurons was confirmed using *Hb9*-RFP lentiviral transduction system (right). B) Representative current clamp recordings from control (11a, 18a), ALS patient-derived (39b, RB9d), and isogenic control (39b-Cor). C) Average number of spikes elicited by ramp depolarization was significantly higher for ALS motor neurons compared to control and isogenic lines ($p<0.05$, Mann-Whitney U Test).

Hypoexcitability

Motor neuron hypoexcitability is another distinct electrophysiological phase that occurs during ALS progression. Human models of SOD1 ALS show that hypoexcitability is a late phenotype that is usually followed by cell death[4]. In this phase, motor neurons fire spontaneous action potentials at significantly lower frequencies than healthy cells and lose their ability to fire action potentials repetitively in response to an injected current. Sodium channels are thought to also play a role in hypoexcitability because both mouse and human models of fALS show that in late stages of disease, the size of sodium currents decreases and sodium channels are downregulated[4, 9]. The role of potassium channels in hypoexcitability remains unclear, but both potassium and sodium channels are essential in action potential generation. As a result, decreases in the activity or expression of either channel type will impact motor neuron excitability.

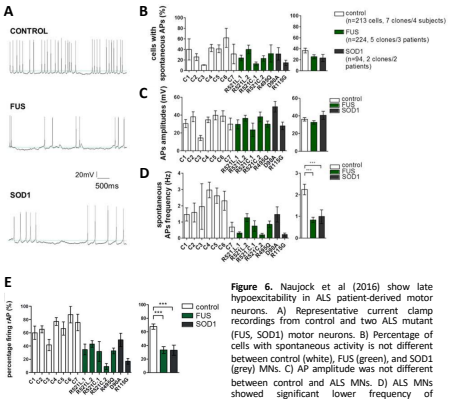


**Figure 6.** Naujock et al (2016) show late hypoexcitability in ALS patient-derived motor neurons. A) Representative current clamp recordings from control and two ALS mutant (FUS, SOD1) motor neurons. B) Percentage of cells with spontaneous activity is not different between control (white), FUS (green), and SOD1 (grey) MNs. C) AP amplitude was not different between control and ALS MNs. D) ALS MNs showed significant lower frequency of spontaneous APs. E) Smaller percentage of ALS MNs were capable of firing repetitive APs than controls.