ALS and Hyperexcitability

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Overview

- History
- What we know
- What supports our theories
- What we don't know
- Future directions
ALS History

- **1869**: First identified as a disease by French neurologist Jean-Martin Charcot
  - Trained as a pathologist
  - Detailed work in the pathophysiology of ALS
  - Called Charcot’s disease in many countries

- **1939**: Brought to national and international attention when Lou Gehrig was diagnosed with the disease.

[Image of Jean-Martin Charcot](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3064755/)

[Image of Lou Gehrig](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3064755/)
ALS Research Accelerates

As the decades have seen discoveries in ALS—genes such as SOD1, FDA approval of riluzole—the number of publications about ALS has exploded.

Source: Merit E. Cudkowicz, MD

What we know

Clinical Presentation

- Weakness and muscle atrophy
  - Limbs
  - Bulbar
  - Respiratory
- Difficulty swallowing, speaking, breathing
- Muscle cramps
- Fasciculations: tongue, limbs
- UMN signs
- Cognitive changes ~ 20% of ALS patients develop FTD
What we know

Clinical Presentation

- Neuropathy Pattern 5 - Asymmetric distal weakness without sensory symptoms + UMN signs\(^1\)
- Neuropathy Pattern 8 - Focal midline proximal symmetric weakness (neck/trunk extensor + bulbar + diaphragm) + UMN signs\(^1\)
- Myopathy Pattern 7 - Bulbar (tongue, pharyngeal, diaphragm), symmetric. \(^2\)

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Criteria for ALS diagnosis

Clinical Presentation

When diagnosing ALS, the Awaji-shima consensus recommendations look for either "clinical" or "electrophysiological" evidence of ...

| Clinically definite + | • UMN + LMN signs in bulbar region + ≥ 2 spinal regions; or
|                       | • UMN + LMN signs in 3 spinal regions*
| Clinically probable   | • UMN + LMN signs in ≥ 2 spinal regions and “with some UMN signs necessarily rostral to (above) the LMN signs”
| Clinically possible   | • UMN + LMN signs in 1 spinal region; or
|                       | • UMN signs in ≥ 2 spinal regions; or
|                       | • LMN signs are found rostral to UMN signs,
|                       | • ONLY AFTER the appropriate neuroimaging and laboratory test are performed to exclude other possible differential diagnosis that may mimic ALS

*Spinal regions: upper limbs/ lower limbs/ thoracic/ bulbar
Studies conducted between 1865 to 1869

- Lesions within the lateral column in the spinal cord resulted in chronic progressive paralysis and contractures (no atrophy of muscles).
- Lesions of the anterior horn of the spinal cord resulted in paralysis without contractures (with atrophy of muscles).

These findings supported his hypothesis at the time that the motor component of the spinal cord consisted of a two-part system, and that the location of the lesion results in a varying clinical presentation.
What we know
Genetics

- SOD 1 (superoxide dismutase 1) mutation
- C9orf72 mutation
- Fused-in sarcoma (FUS) mutation
- TARDBP

Key to the normal functioning of motor neurons and other cells
What we know

C9orf72: 30 - 40%
SOD1: 15 - 20%
TARDBP: 5%
FUS: 5%

Turner et al. Lancet Neurol 2013
What we know
Pathophysiology - cellular level

Facts supporting theories

1. Dying-back hypothesis of motoneuron degeneration - oxidation and inflammation -
2. Excitotoxic hypothesis of neurodegeneration - glutamate -
3. Hyperexcitability hypothesis - Na⁺ and K⁺ channels -
Hyperexcitability and Hyperexcitation

Intrinsic electrical cell properties

↑ Calcium entrance

Synaptic input

Ligand-gated: AMPA, NMDA

Voltage-activated: N/P-, L-, T-type

Second-messenger-activated: IP3, G-protein receptors

Ca\(^{2+}\)-dependent enzymatic pathways

Neuronal Degeneration

Ca$^{+2}$ toxicity

What triggers motoneuron degeneration?

Mouse models
- Motoneurons – not equally affected.
- Motoneurons exhibit differential vulnerability and follow an orderly degeneration.

Innervating fast-contracting fatigable motor units (FF motoneurons)

Innervating fast-contracting fatigue-resistant motor units (FR motoneurons)

Slow motor units (S motoneurons)

More vulnerable

Lower amounts of Ca buffering proteins

Resistant to the disease

Hyperexcitability

Hyperexcitation

Ca$^{+2}$ toxicity theory

Ca is normally kept low in all cells (Ca buffering proteins)

The role of oxidative stress in degeneration of the neuromuscular junction in amyotrophic lateral sclerosis

Model of motor nerve terminal dysregulation in ALS

Decline in synaptic function from the presynaptic terminals:
- Oxidative stress
- Compromised mitochondria
- Increased intracellular Ca\(^{2+}\)

Inflammatory agents

Loss of trophic support

Neurodegeneration

Progressive loss of motoneurons and degradation of the NMJ

Complex nature of ALS + failure of monotherapies suggest:
- Antioxidant + anti-inflammatory therapy
  (enhance the restoration of the redox balance)
Normal glutamate regulation

Rapidly taken up by astrocytic glutamate transporters (EAAT2)

- Vesicular glutamate
  Released and binds to ionotropic receptors (postsynapse)

- Astrocytes and microglia release non vesicular glutamate into the extrasynaptic extracellular space → activate extrasynaptic NMDA receptors.

Spillover from the synaptic cleft can activate perisynaptic mGluR5
Excitotoxic Hypothesis of Neurodegeneration

Chronic Glutamate Toxicity in Neurodegenerative Diseases—What is the Evidence?

Jan Lewerenz* and Pamela Maher**
Front. Neurosci., 16 December 2015

Glutamate transporters in astrocytes

System $\text{X}_c^+$

L-Glut$^+$

Releases glutamate from astrocytes and microglia

Loss of post-synaptic structures including dendrites and cell bodies

Excessive activation of iGluRs

Normal glutamate regulation

Glutamate Regulation in SOD1-linked ALS

Mutant SOD1

Free radicals

inactivate

Glutamate transporters (EAAT2)
The pharmacology and mechanism of action of riluzole

A. Doble, PhD

*Neurology* December 1996 vol. 47 no. 6 Suppl 4 233S-241S

Excessive glutaminergic synaptic activity

↓

Calcium overload

↓

Cell death

Riluzole

↓

Blocks NMDA receptors (noncompetitive)

↓ some of the postsynaptic effects of glutamate

Inhibits the release of glutamate from cultured neurons

http://www.neurology.org/content/47/6_Suppl_4/233S.abstract
Hyperexcitability Hypotheses

Na$^+$ and K$^+$ channels
Normal Physiology

- Depolarization and Repolarization
Hyperexcitability Hypothesis of Neurodegeneration

Induced pluripotent stem cells (iPSC) - in vitro

[SOD1]
Motor neuron from ALS patient

↓ voltage-gated K+ currents (compared to controls)

Hyperexcitability

Motor neuron Death

↑ survival of SOD1 motor neurons

Retigabine (activator of K+ channels)

Correction of SOD1 mutation

Supports the hypothesis that motor neuron hyperexcitability may contribute to motor neuron degeneration in ALS

*unknown pathways
Threshold tracking studies = indicative of widespread dysfunction in axonal ion channel conduction

Protocol for testing measures of axonal excitability
by stimulation of median nerve at the wrist

↑ persistent Na⁺ channel conduction
Abnormalities of the K⁺ channel function

Predispose axons to generation of fasciculation and cramps

Tests for axonal excitability: SR, TSD, C/T relationship, TES (evaluate axonal MB)
Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons.

Axonal excitability properties in amyotrophic lateral sclerosis.
Vucic S, Kiernan MC.

↑ persistent Na\(^+\) channel conduction
↓ in K\(^+\) currents
What is hyperexcitability?

Increased persistent Na\(^+\) channel conduction + Reduction in K\(^+\) currents

These abnormalities predispose axons to generate fasciculation and cramps.
Where does it occur?

The Puzzling Case of Hyperexcitability in Amyotrophic Lateral Sclerosis
Jong Seok Bae, Neil G. Simon, Parvathi Menon, Steve Vucic, Matthew C. Kiernan

Where does it occur?

Hyperexcitability seems to develop in the cortical origins of ALS

Related to disrupted integrity (in the neocortex) between the projection neurons (excitatory - glutamate) and interneurons (inhibitory - GABA)

Excitotoxicity

Neuroprotective in cell culture models

↓ in GABAergic inhibition: degradation of cortical neurons and old animals
Transcranial Magnetic Stimulation (humans)

Cortical threshold: lowest in early ALS (Vucic, 2008)

Cortical Hyperexcitability

Threshold tracking protocols (ionic mechanisms of axonal dysfunction)

Na/K involvement: axolemma depolarization (Bae, 2013)

Peripheral Axonal Hyperexcitability
Hyperexcitability

Drugs

Mexiletine: Reported to alleviate cramping in spinocerebellar ataxia patients (2003)


**The effects of mexiletine on excitability properties of human median motor axons.**
Kuwabara S¹, Misawa S, Tamura N, Kanai K, Hiraga A, Ogawara K, Nakata M, Hattori T.

- Originally approved for treatment of cardiac arrhythmia
- Na⁺ channel blocker ➔ ↓ peripheral nerve excitability
- May slow progression of the disease
- ↓ cramps frequency and severity in ALS patients
- Prevented cell death induced by astrocytes expressing mutant human SOD1
- Protects against motor neuron death
- Observed ↓ in Na⁺ conductances
- Safety concerns: increased mortality and cardiac arrest
Hyperexcitability

Drugs

*Ranolazine*

- **Na\(^+\) channel blocker** → ↓ peripheral nerve excitability
- May slow progression of the disease
- May ↓ cramps in ALS patients

Originally approved for treatment of chronic angina

↓ **Na\(^+\) current and intracellular Ca\(^{2+}\) accumulation**

Protects against motor neuron death
What we don’t know
Are vulnerable motoneurons hyperexcitable?

mSOD1 embryos: far from disease onset

- Unchanged excitability
- More vulnerable (Fast fatigable)
- Hypoexcitability

Resistant to the disease (Slow)

- Unlike to be responsible for later degeneration

Hyperexcitable
Recruited at lower current

SOD1 adult
G93A
G85R

What we don’t know

Are vulnerable motoneurons hyperexcitable?

Induced pluripotent stem cells from patients with ALS

Contradictory results:

- SOD1 patient-derived motoneuron – **hyper**excitable (decrease in K current)
- C9ORF72 patient-derived motoneuron – **hypo**excitable (increased KCNQ3 expression)
- TARDBP and C9ORF72 ALS patients: **hyper**excitability (transient) –> **hypo**excitability (decrease in the Na and K current generating the action potential)

Adult motoneurons loose ability to fire repetitively

Why?

Does it lead to degeneration?

What we don’t know
Pathophysiology - cellular level

- Motoneurons:
  - ↓glycinergic current
  - ↓glycine synapses
  - ↓ventral root response (after dorsal root stimulation)
  - Enlarged cholinergic C-button synapses (impinging on motoneurons)
- Astrocytes
  - Impaired glutamate transporter (↓glutamate clearance from synaptic cleft)
- Chromaffin cells
  - ↓nicotine currents = hypoexcitable

Do they contribute to disease?
Are they linked to altered excitability and motoneuron degeneration?

How is hyperexcitability manifested in ALS patients

- LMN hyperexcitability: Fasciculation and cramps
  - Fasciculations may present early in the disease: early identification in hyperexcitable motor system —> may help predict ALS development and allow early neuroprotective intervention
- UMN hyperexcitability: increased DTRs/clonus
Future Directions

- Pilot studies to test drugs that act on nerve axonal hyperexcitability
  - Determine safety and tolerability
  - Some insight on efficacy
- Multi-center studies
  - Effect on disease progression
Difficulties

- Pathophysiologic evidences in ALS are heavily based in mice models (genetic mutations for ALS), however, familial ALS represents only 5-10% of ALS patients.

- Complexity of ALS: acting in one pathophysiologic mechanism may not be sufficient - maybe a more successful approach should target more than one mechanism.

- ALS is considered a rare disease: 5,000 people diagnosed in the United States each year.
Summary

- **We know:**
  - Important discoveries have accelerate the research in ALS: SOD1, C9orf72, riluzole, mouse models
  - Tendency to continue to grow: stem cells, genetic testing, drug trials
  - Clinical patterns (including UMN and LMN signs)

- **We don’t know yet:**
  - Pathophysiology may be a combination of mechanisms involving NMJ, axons, synapse cleft with changes noticed in the cortex and peripheral nerves.

- **We hope**
  - Drug repurposing trials being conducted may be promising for the treatment of ALS symptoms, disease progression and may provide additional information in pathophysiology
“It is a capital mistake to theorize before one has data. Insens-ibly one begins to twist facts to suit theories, instead of theo-ries to suit facts.”

- Arthur Conan Doyle