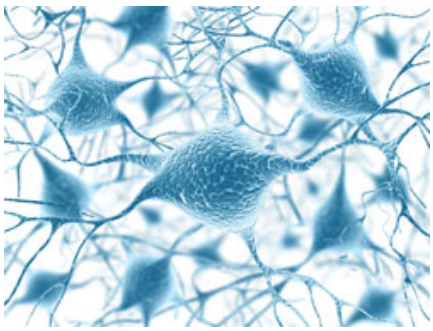
MOTOR NEURON DISEASES III

Pathophysiology, genetics, proteins, therapies

Bernard Schneider
October 2024



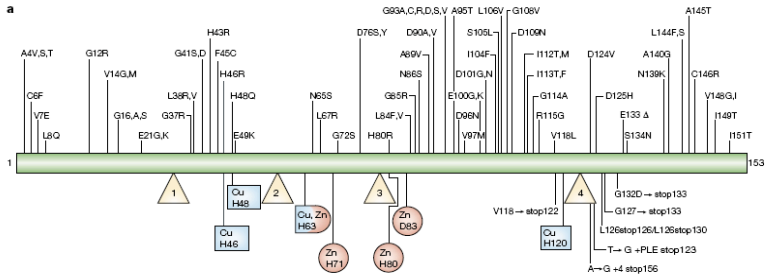
Lecture plan

1. **Motor system**
   - Overview
2. **Motor Neuron Diseases**
   - Clinical presentation
   - Molecular pathology
3. **Amyotrophic Lateral Sclerosis**
   - Clinical presentation, epidemiology, etiology
   - Molecular pathology: RNA metabolism
   - Non-cell autonomous mechanisms

ALS: genetic causes

SOD1 – Cu/Zn Superoxide dismutase

Cu/Zn SOD1 mutations
≈ 2% of all ALS cases
> 140 SOD1 mutations
Toxic gain of function



- ALS-causing mutations are located throughout the SOD1 polypeptide

- Cleveland & Rothstein, Nat Rev Neurosci 2001

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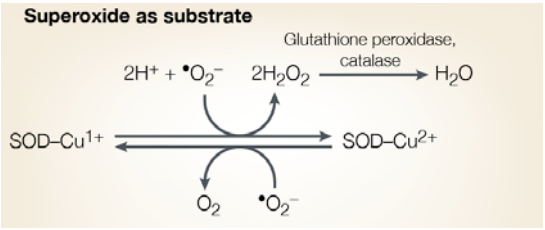
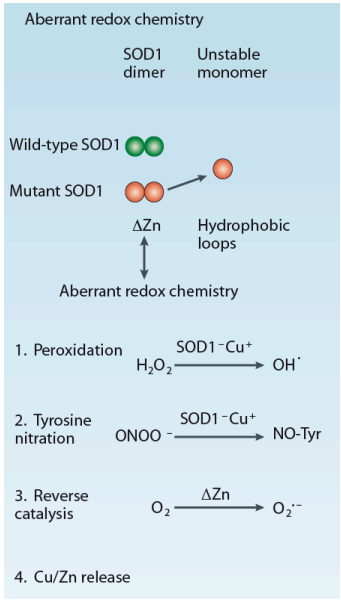
ALS: SOD1 pathology

SOD1: oxidative stress

Normal SOD1 dismutase activity → *Aberrant SOD1 activity in ALS ?*





SOD1 mutations lead to…

- aberrant chemistry of the active copper and zinc sites
- greater access of abnormal substrates to the active site (e.g. leading to peroxidation)
- clumsy handling of Cu and Zn ions (e.g. leading to reverse catalysis)

- Pasinelli P & Brown RH, Nat Rev in Neurosci 2006

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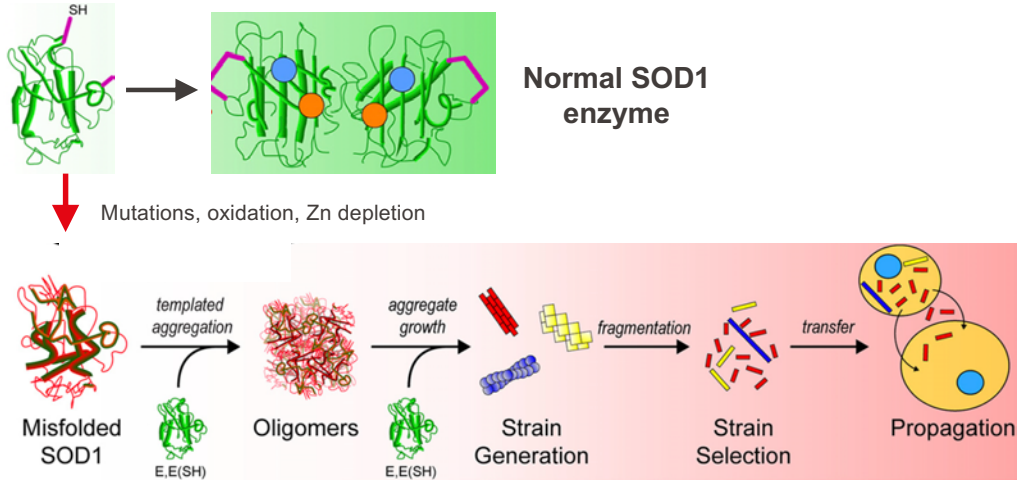
ALS: SOD1 pathology

Protein misfolding and aggregation



"Aggregation - Gain of toxic function"
→ pathology develops in multiple CNS cell types

- *McAlary L et al, Front Mol Neurosci. 2019; 12, 262*

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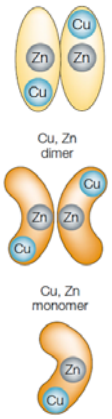
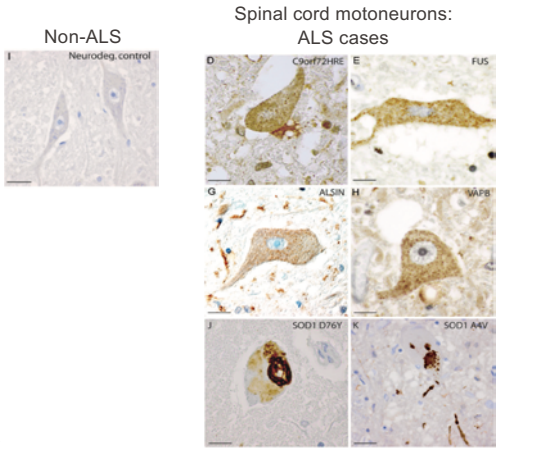
ALS: SOD1 pathology

Protein misfolding and aggregation



- SOD1 extremely stable (disulfide, metals)
- Mutations disrupt the structure
- Dimer dissociation, loss of metals
- Apomonomers are aggregation-prone
- Inclusions found in both FALS and SALS (humans and rodent models)
- Aggregates: toxic or (transiently) protective ?

Misfolded SOD1 deposition in ALS



Forsberg K et al, J Neurol Neurosurg Psychiatry. 2019;90(8):861-869

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ALS: SOD1 pathology

Mutated SOD1 fALS
Loss of function or gain of a toxic role ?

Gain of a toxic function

- Autosomal dominant inheritance
- SOD1 knock-out mice do not develop neurodegeneration
- Abnormal folding
- Experimental evidence for toxic activities

Loss-of-function

- Enzyme involved in reactive oxygen species detoxification

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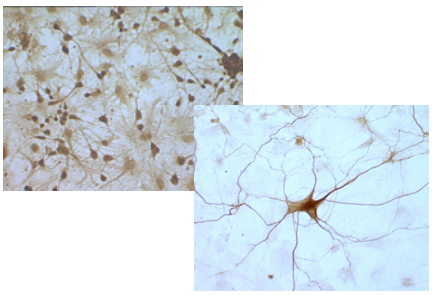
ALS: experimental models

Modeling ALS in the lab

In vitro



Primary motoneuron cultures

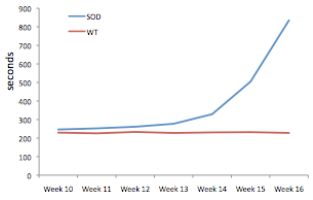
iPS-derived cell cultures

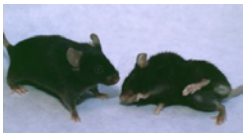
- Gurney ME, et al. Science 1994 264(5166)

In vivo

Mutated hSOD1 over-expression

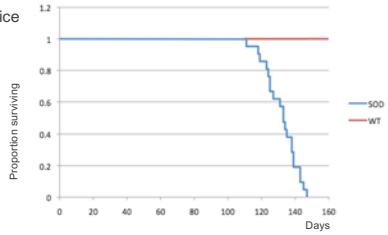
SOD1^{G93A} mice Swimming performance





Mutated hSOD1
(G93A, G37R, G85R)
(SOD1 promoter, ubiquitous expression)

SOD1^{G93A} mice Survival



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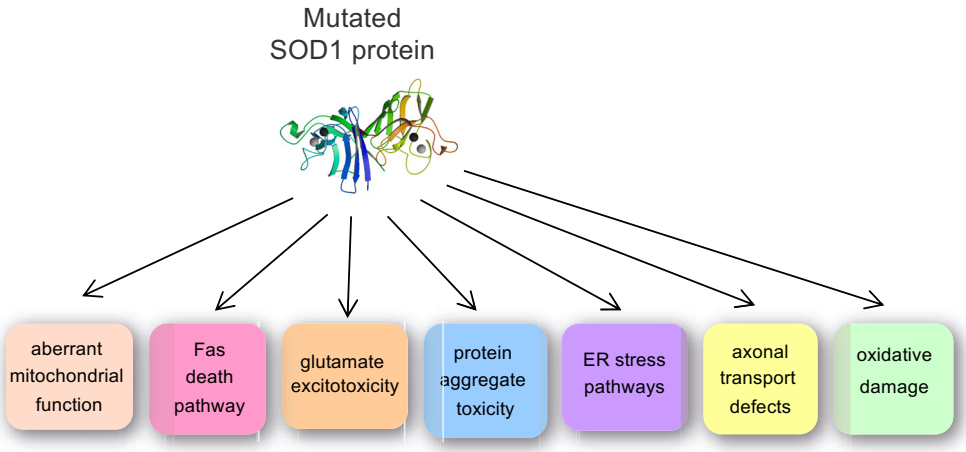
ALS: SOD1 pathology

SOD1 neurotoxicity: the gain of a complex toxic function



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Motor neuron diseases: question 10

In SOD1^{G93A} mice (with 16 to 24 copies of the human SOD1 gene), blocking individual pathways leading to cell death only increases animal survival by 5-10%.
What is your interpretation (answers are ranked)?

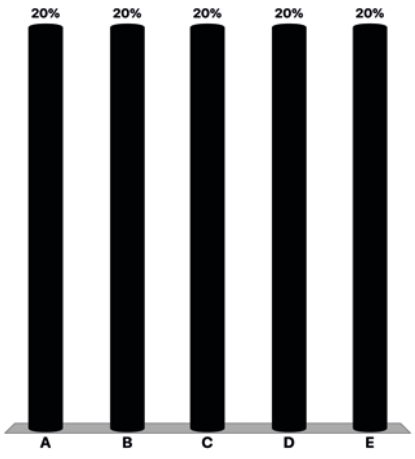
A. This animal model is not relevant for testing treatment efficacy

B. Multiple pathways act in parallel to cause neuronal degeneration

C. This indicates that it is poorly efficient to act downstream in the pathologic cascade

D. The exact cause of the pathology has not been identified yet

E. The level of expression of mutated SOD1 in transgenic mice is too high to reliably test therapeutic approaches



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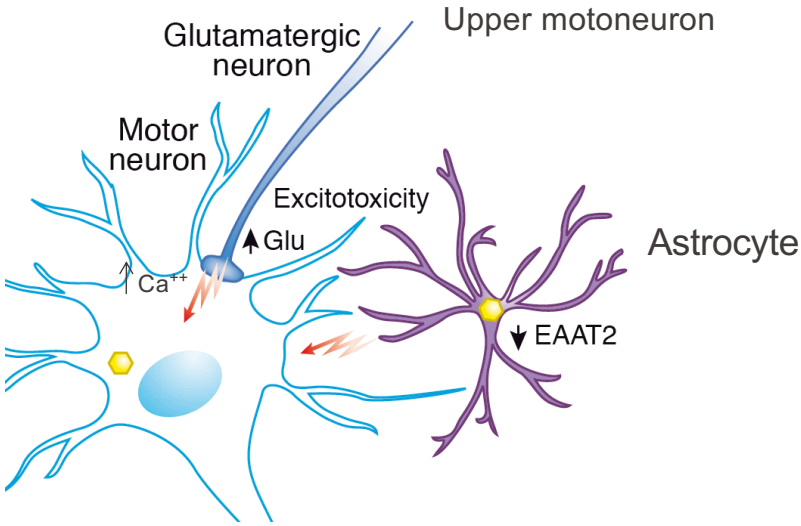
ALS: mechanisms

Excitotoxicity



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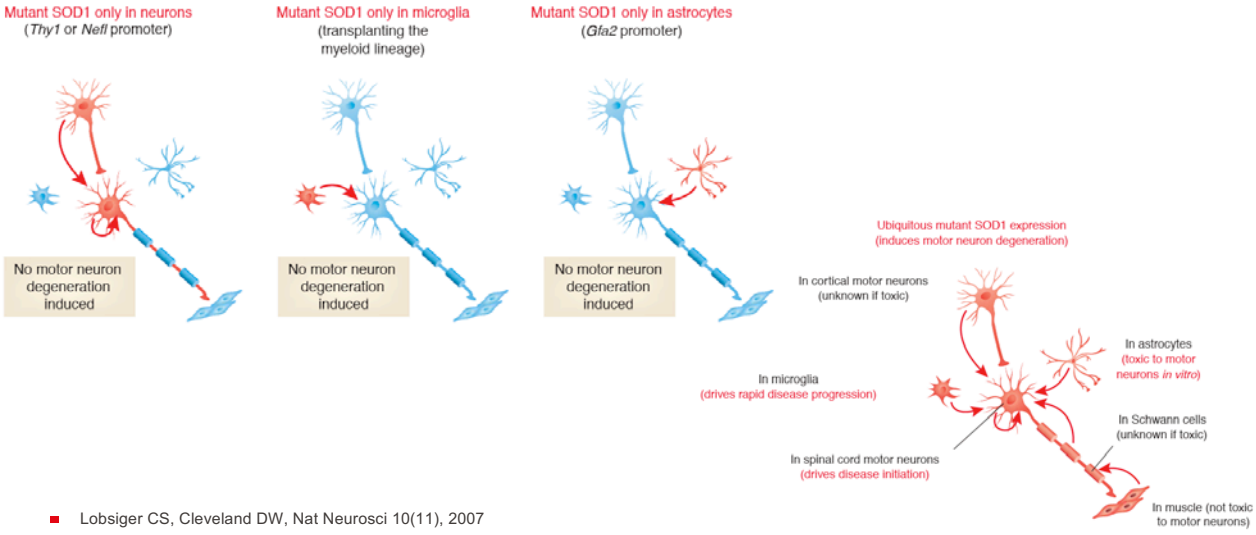
ALS: non-cell autonomous disease

Transgenic mouse models:
mutated SOD1 has to be ubiquitously expressed to generate ALS pathology



- Lobsiger CS, Cleveland DW, Nat Neurosci 10(11), 2007

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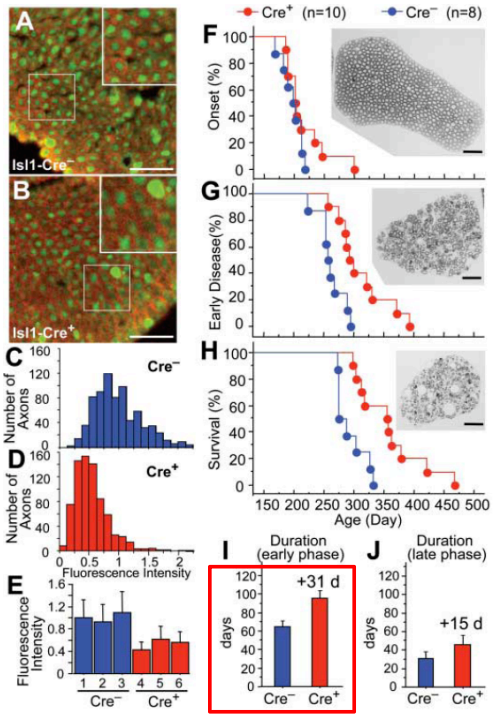
ALS: non-cell autonomous disease

The role of mutated SOD1 in motor neurons: ALS onset

- Cre-lox system to inactivate mutated SOD1 overexpression in specific cell types.
- Reduction of mutated SOD1 in motor neurons only (Islet1-Cre) retards disease progression in the early phase of the disease.



- S. Boillée et al., Science 312, 1389 (2006)

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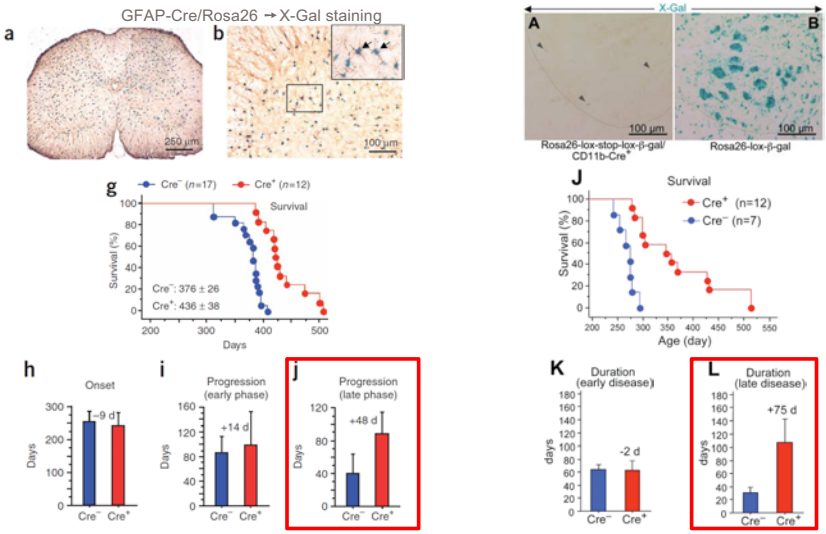
ALS: non-cell autonomous disease

Expression of mutated SOD1 in glial cells determines late disease progression

Astrocytes | **Microglial cells**



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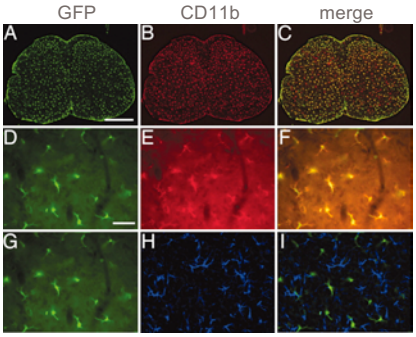
ALS: non-cell autonomous disease

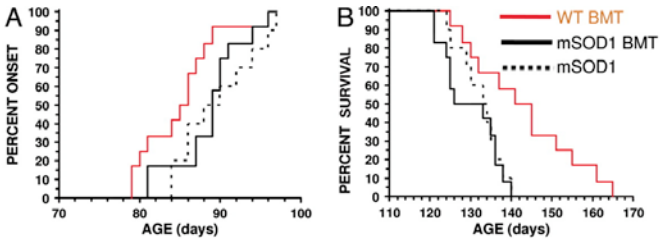
Wild-type microglial cells from bone marrow slow down disease progression in fALS mice

Tx of GFP-expressing bone marrow cells



- PU.1-/- mice: lack mΦ, neutrophils, T and B cells, **CNS microglia**
- Need bone marrow Tx to survive
- Crossed with fALS $SOD1^{G93A}$ mice

- Tx of wild-type bone marrow cells
  Tx of $SOD1^{G93A}$ bone marrow cells



- Beers D.R. et al., PNAS 103(43), 2006

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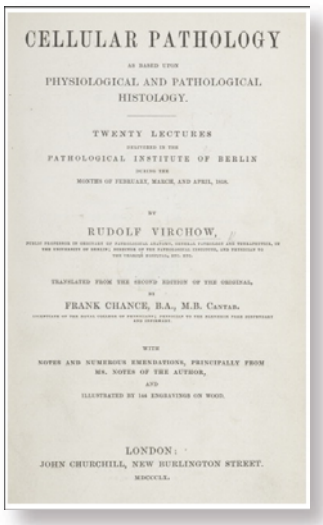
ALS: non-cell autonomous disease

SOD1 fALS: challenging the notion of ***cell autonomous*** disease

« theory of the cell state »

Metazoans = complex colonies of protozoan-like cells with a highly evolved division of labor

Rudolf Virchow (German physician, 1821-1902)
Claude Bernard (French physiologist, 1813-1878)


CELLULAR PATHOLOGY

AS DERIVED UPON PHYSIOLOGICAL AND PATHOLOGICAL HISTOLOGY.

TWENTY LECTURES DELIVERED IN THE PATHOLOGICAL INSTITUTE OF BERLIN DURING THE MONTHS OF FEBRUARY, MARCH, AND APRIL, 1858.

BY

RUDOLF VIRCHOW,

TRANSLATED FROM THE SECOND EDITION OF THE ORIGINAL, BY

FRANK CHANCE, B.A., M.B. Cantab.

WITH NOTES AND NUMEROUS EMENDATIONS, PRINCIPALLY FROM MS. NOTES OF THE AUTHOR, AND ILLUSTRATED BY 186 ENGRAVINGS ON WOOD.

LONDON: JOHN CHURCHILL, NEW BURLINGTON STREET. MDCCCLVI.

Neurodegenerative disorders are often considered « cell autonomous », mechanistically resulting from damage within individual cell types

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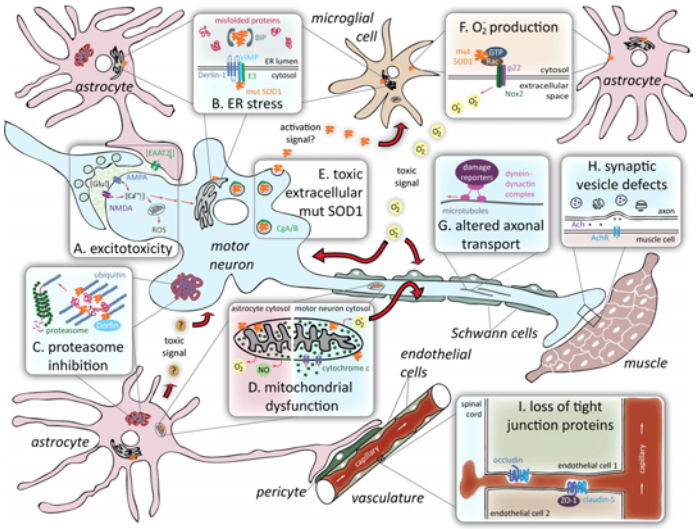
ALS: non-cell autonomous disease

Proposed mechanisms of toxicity in SOD1-mediated ALS



- Ilieva H et al. J Cell Biol 2009;187:761-772

JCB

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Motor neuron diseases: question 11

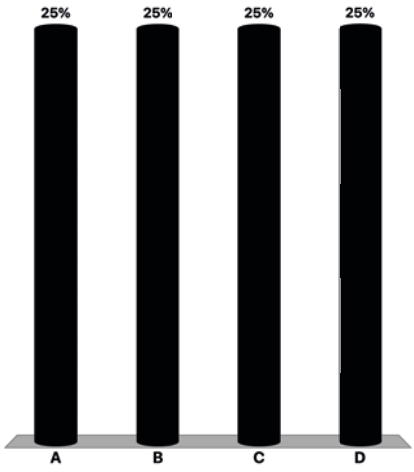
From the previous experiments on the role of mutated SOD1 in various cell types, which one(s) are the correct conclusion(s)?

A. It will be impossible to achieve therapeutic efficacy by suppressing SOD1 toxicity only in motoneurons

B. Strategies that prevent astroglial and microglial cell activation are more effective than the ones targeting motoneurons

C. At the time the disease is diagnosed, mainly strategies targeting glial/microglial pathology should be applied

D. Mutated SOD1 leads to a pathogenic crosstalk between neuronal and glial cells



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ALS: SOD1 pathology

What did we learn from the ALS models based on mutated SOD1?

- Mechanisms of cellular toxicity induced by mutated SOD1
- Non-cell autonomous disease

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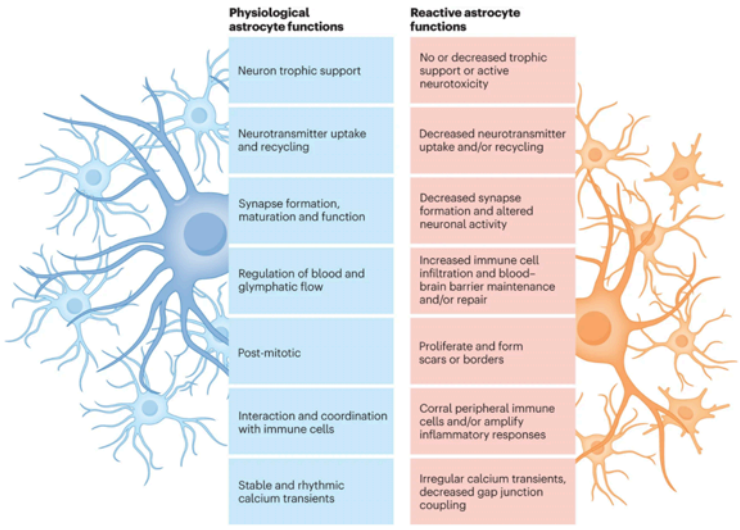
ALS: non-cell autonomous disease

Reactive astrocytes



- *Nature Reviews Neurology volume 19, pages 395–409 (2023)*

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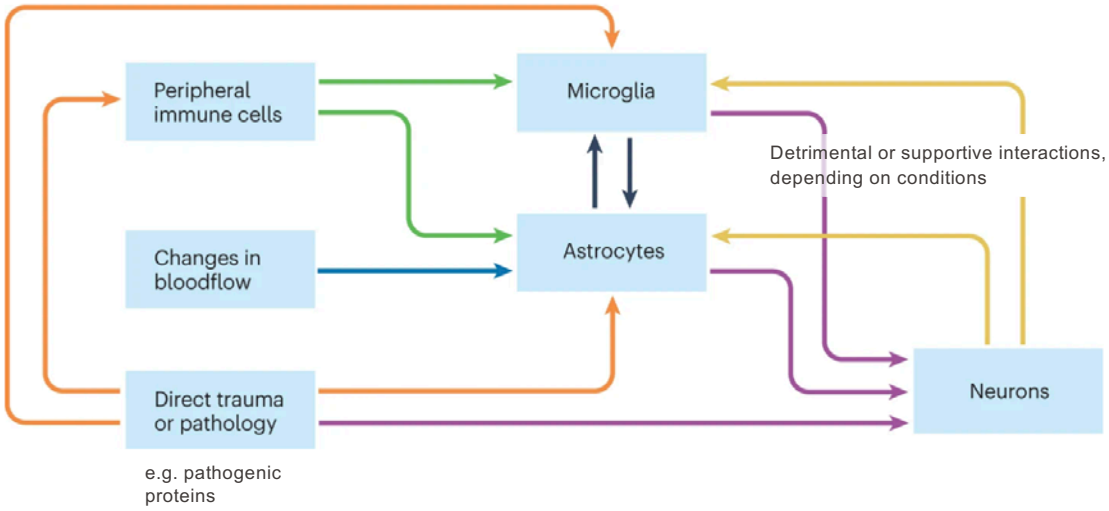
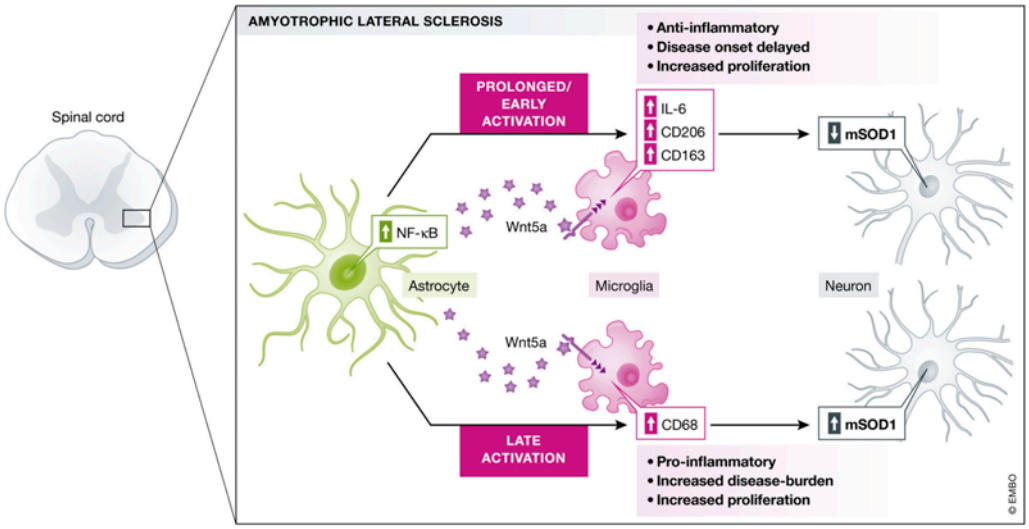
The role of astrocytes within the inflammatory cascade



- *Nature Reviews Neurology volume 19, pages 395–409 (2023)*

ALS: non-cell autonomous disease

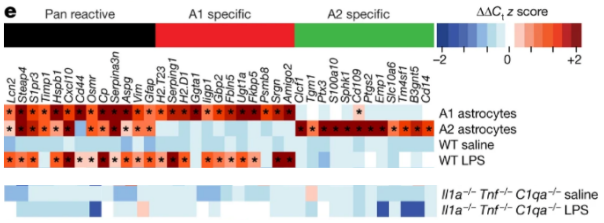
Example of crosstalk between glial cells



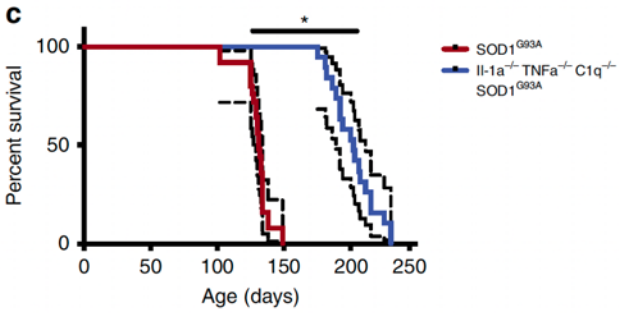
- *The EMBO Journal (2018)37:e100130https://doi.org/10.15252/embj.2018100130*

Example of crosstalk between glial cells

Different subtypes of reactive astrocytes



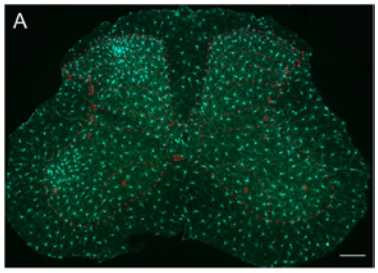
KO of factors to suppress 'A1' astrocyte prolongs the survival of ALS mice



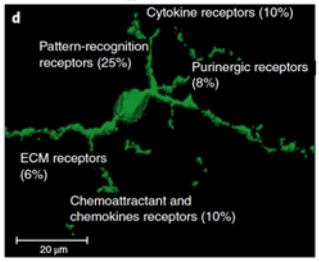
- *Nat Commun. 2020 Jul 27;11(1):3753. doi: 10.1038/s41467-020-17514-9*
- *Nature, 2017;541(7638):481-487. doi: 10.1038/nature21029.*

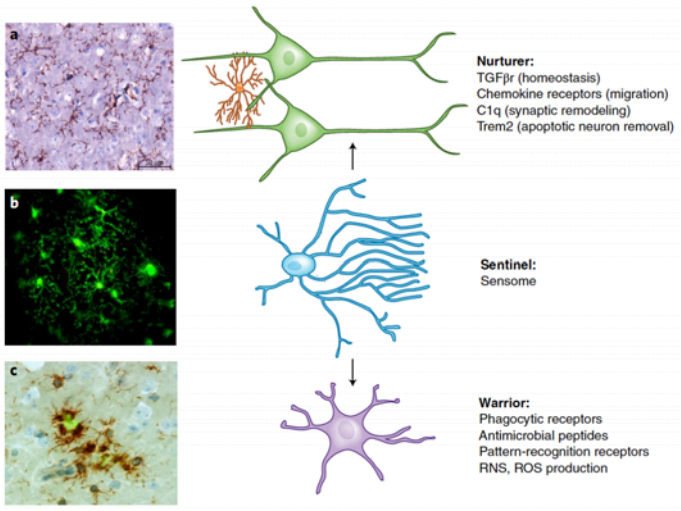
Microglia: a versatile cell type

Mouse spinal cord



Ontology of the sensome

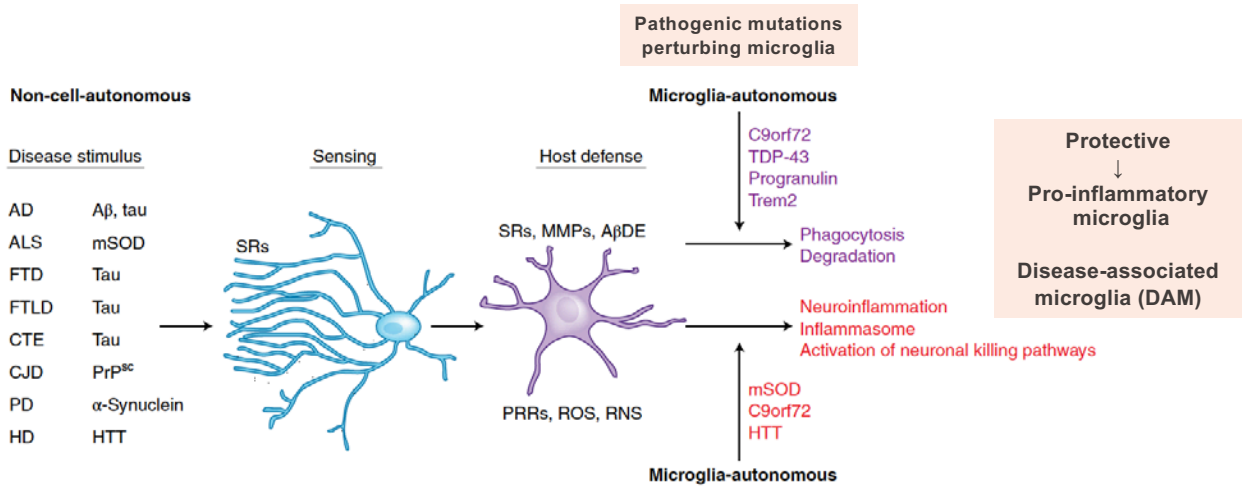




- Hickmann S et al, Nature Neuroscience 21, 1359–1369, 2018

ALS: non-cell autonomous disease

Microglia: a versatile cell type



- Hickmann S et al, Nature Neuroscience 21, 1359–1369, 2018

- SR: 'sensing' receptors
- PRR: pattern recognition receptors

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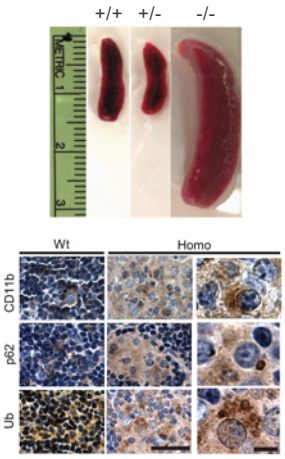
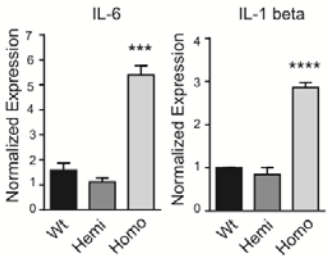
ALS: non-cell autonomous disease

Decreased C9orf72 expression in **C9orf72−/− mice** and C9-ALS patients leads to altered microglial function and neuroinflammation

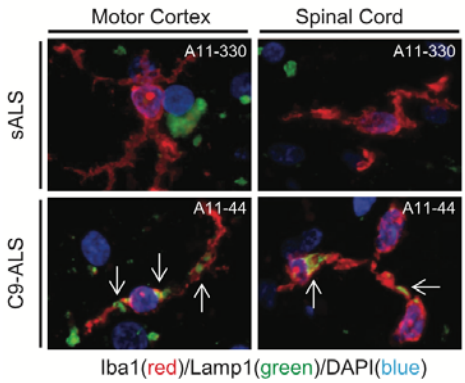
Splenomegaly: accumulation of engorged macrophage-like cells



mRNA expression in microglia isolated from C9orf72−/− mice



Accumulation of phagocytic material (Lamp1) in C9-ALS microglia



- O'Rourke et al, Science 351 (6279), 2016

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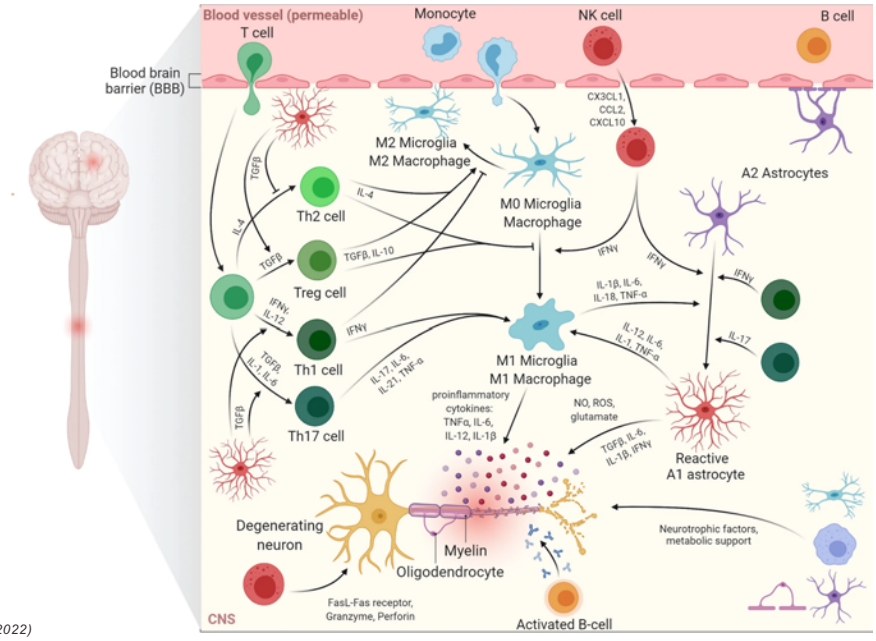
ALS: non-cell autonomous disease

The role of the immune system in ALS



- *Molecular Neurodegeneration vol 17: 22 (2022)*