Neurotoxicity and ALS: Insights into Pathogenesis

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Contents

Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder of human motor neurons, characterized by a concomitant dysfunction of upper and lower motor neurons. The pathophysiological mechanisms underlying ALS are complex, involving multiple sequential steps (2 to 6), and mediated by an interaction between genetic, epigenetic, and environmental factors. Of relevance, the unique combination of upper and lower motor neuron dysfunction led to a dying forward hypothesis, which postulated that cortical hyperexcitability mediated motor neuron degeneration via a transsynaptic glutamatergic excitotoxic mechanism. Importantly, glutamate excitotoxicity has been consistently identified

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as a pathogenic mechanism in cell and transgenic animal models. Modulation of this neuronal hyperexcitability was shown to be neuroprotective in cell models. At a physiological level, cortical hyperexcitability has been identified as an early and specific feature in ALS patients, associated with motor neuron degeneration, clinical features, disease evolution, and adverse prognosis. Additionally, cellular protein accumulation (TDP-43), increased oxidative stress, mitochondrial dysfunction, defective axonal transport, and abnormalities of nonneuronal supporting cells further contribute to injury of critical target proteins and organelles within motor neuron, thereby resulting in neurotoxicity and degeneration in ALS. In this chapter, an updated overview of mechanisms contributing to neurotoxicity in ALS is provided, and potential therapeutic implications are discussed.

Keywords

ALS · Cortical hyperexcitability · Lower motor neuron · Oxidative stress · TDP-43 · Upper motor neuron

Abbreviations

ALS	Amyotrophic lateral sclerosis
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid
EAAT-2	Excitatory amino acid transporter-2
LMN	Lower motor neuron
NMDA	N-methyl-D-aspartate
TMS	Transcranial magnetic stimulation
SICI	Short interval intracortical inhibition
SICF	Short interval intracortical facilitation
SOD-1	Superoxide dismutase-1
TDP-43	TAR DNA binding protein-43
UMN	Upper motor neuron

1 Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and fatal neurodegenerative disorder of motor neurons and their connections, which is characterized by dysfunction of upper and lower motor neuronal compartments (Geevasinga et al., 2016; Kiernan et al., 2011). At a clinical level, the ALS phenotype is characterized by concurrent upper (UMN)* and lower motor neuron (LMN) signs (Kiernan et al., 2011, 2021), with fasciculations, muscle wasting, and weakness indicating LMN dysfunction, while slowness of movement, increased tone, hyperreflexia, and patterning of muscle weakness heralding UMN dysfunction. This unique combination of upper and lower motor neuron abnormalities led to the dying forward hypothesis, postulating that lower motor neuron degeneration was mediated by cortical hyperexcitability via a transsynaptic glutamatergic excitotoxic mechanism (Eisen et al., 1992). In contrast, primacy of lower motor neuronal dysfunction in ALS pathogenesis has been suggested

(Boillee et al., 2006), as has the notion that upper and lower motor neurons degenerate independently in a contiguous and random pattern (Ravits et al., 2007).

At a pathophysiological level, ALS appears to be a multifactorial process requiring between 2 and 6 sequential steps, mediated by a complex interaction of genetic, epigenetic, and environmental processes (Vucic et al., 2019, 2020). These complex pathways, including oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, and defective axonal transport systems (Fig. 1), along with abnormalities of nonneuronal supporting cells (astrocytes, microglia, and oligodendrocytes), may cause injury of critical target proteins and organelles within the motor neuron, thereby resulting in neurotoxicity and degeneration in ALS (Geevasinga et al., 2016). In this chapter, an overview for the role of neurotoxicity in ALS pathogenesis is provided, and potential therapeutic implications are discussed.

2 Cortical Hyperexcitability, Glutamate-Mediated Neurotoxicity, and ALS Pathogenesis

Cortical hyperexcitability was fist implicated in ALS pathogenesis by Charcot, with the identification of upper and lower motor neuron degeneration on pathological studies (Kiernan et al., 2011). Over a 100 years later, Eisen and colleagues proposed that cortical hyperexcitability mediated LMN degeneration via an anterograde glutamatergic mechanism, the *dying forward hypothesis* (Fig. 2) (Eisen et al., 1992). Loss of inhibitory and increased excitatory interneuronal activity within the primary motor cortex [M1] (Nihei et al., 1993) appear to mediate the development of cortical hyperexcitability in ALS. Profuse fasciculations, hyperreflexia, and spasticity represent the clinical manifestations of cortical hyperexcitability (Eisen & Weber, 2001).

Threshold tracking transcranial magnetic stimulation (TMS) has consistently identified cortical hyperexcitability as an important pathogenic feature of ALS, mediated by cortical disinhibition and an increase in cortical facilitation (Geevasinga et al., 2016). Reduction or absence of short interval intracortical inhibition (SICI), a biomarker of cortical inhibitory interneurons acting via $GABA_A$ receptor circuit function (Di Lazzaro et al., 2013), has been demonstrated to be an early and intrinsic feature of sporadic ALS and correlating with peripheral neurodegeneration (Vucic & Kiernan, 2006b). Cortical hyperexcitability was also reported to precede the development of lower motor neuron dysfunction, as measured by sensitive measures of LMN dysfunction such as needle electromyography (Menon et al., 2015b). Development of specific clinical features of ALS, such as the split hand phenomenon (Menon et al., 2014), and patterns of disease propagation (Menon et al., 2017) have also been associated with cortical hyperexcitability. Although it has been argued that cortical dysfunction may represent a compensatory mechanisms (Zanette et al., 2002), the findings of normal cortical excitability in ALS-mimicking disorders (Menon et al., 2015) and partial normalization of SICI with riluzole therapy (anti-glutamatergic agent utilised in ALS) (Vucic et al., 2013) argue for the pathogenic importance of cortical hyperexcitability ALS. This notion is further supported by

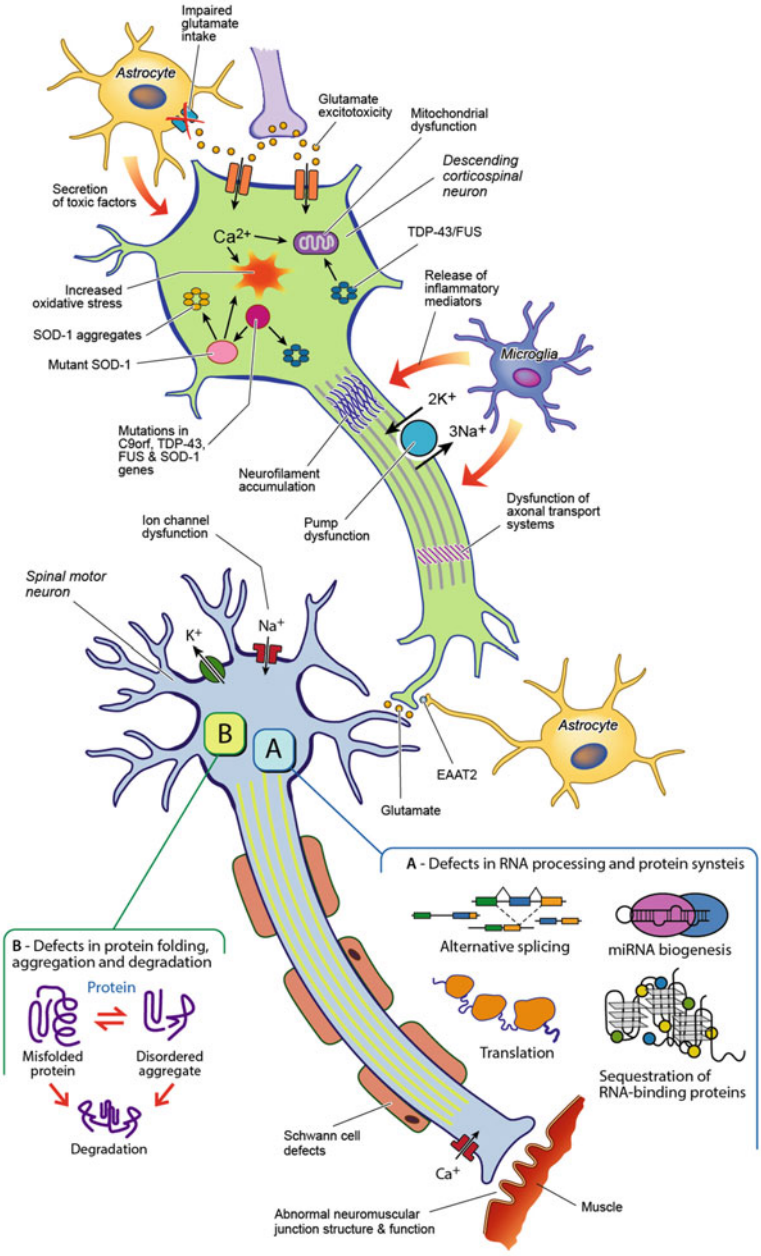

Fig. 1 Multiple interacting pathophysiological mechanisms appear to underlie the development of amyotrophic lateral sclerosis (ALS). Glutamate-mediated excitotoxicity is an important mechanism ALS progression seems to precede the clinical development of ALS. Dysfunction of the excitatory amino acid transporter type 2 (EAAT2), located on astrocytes, in part induces the development of glutamate excitotoxicity via excessive extracellular accumulation of glutamate.

findings that a greater reduction of SICI is associated with adverse prognosis in sporadic ALS (Shibuya et al., 2016).

Increased activity of cortical facilitatory circuits was also shown to be an important contributing mechanism for cortical hyperexcitability development in conjunction with reduction of SICI. Short interval intracortical facilitation, a biomarker of cortical excitatory circuit activity, was increased in sporadic ALS patients, and this increase was accompanied by reduction of SICI (Van den Bos et al., 2018). Index of excitation, a novel biomarker of cortical excitability, was increased in ALS suggesting that overactivity of the excitatory circuits appeared to contribute prominently to the development of hyperexcitability and was associated with greater functional disability in ALS.

Cortical hyperexcitability has also been reported in familial ALS cohorts, including phenotypes linked to mutations in the superoxide dismutase-1 (Vucic et al., 2008), fused in sarcoma (Williams et al., 2013) and c9orf72 genes (Geevasinga et al., 2015), and has been associated with peripheral neurodegeneration (Geevasinga et al., 2015; Vucic & Kiernan, 2010). Asymptomatic gene mutation carriers exhibited normal cortical function (Geevasinga et al., 2015; Vucic et al., 2008), with cortical hyperexcitability preceding the clinical development of familial ALS by ~4 months (Vucic et al., 2008). The findings from familial ALS cohorts have supported the notion that ALS is a multistep process (Chio et al., 2018), with cortical hyperexcitability potentially acting as an important pathogenic step.

3 Glutamate-Mediated Neurotoxicity

At a molecular level, cortical hyperexcitability appears to reflect glutamate excitotoxicity (Armada-Moreira et al., 2020). Glutamate is the major excitatory neurotransmitter in the central nervous system (Heath & Shaw, 2002), synthesized from reductive deamination of alpha-ketoglutarate or from the action of amino

Fig. 1 (continued) In addition, activation of nonneuronal cells (astrocytes and microglia) in ALS results in secretion of pro-inflammatory cytokines and other cytotoxic factors that ultimately results in further neurotoxicity and degeneration. In conjunction with glutamate-mediated excitotoxicity, other molecular processes induce neurotoxicity via multifactorial mechanisms. Within the neuron, mutations in a host of ALS-related genes, including C9orf72, superoxide dismutase-1 (SOD-1), TDP-43, and FUS, result in ALS via multiple mechanisms. Specifically, mutations in the SOD-1 gene result in toxic gain-of-function of the SOD-1 enzyme which affects a host of critical cellular organelles, such as DNA/RNA metabolism. In addition, mitochondrial dysfunction is a feature of ALS, linked to glutamate excitotoxicity and SOD-1 gene mutations, resulting in a reduced production of ATP and calcium sequestering ability, as well as an increase in free radical formation. Of further relevance, mitochondrial dysfunction may contribute to glutamate excitotoxicity. Ultimately, these multiple pathogenic processes result in critical cell dysfunction and motor neuron degeneration

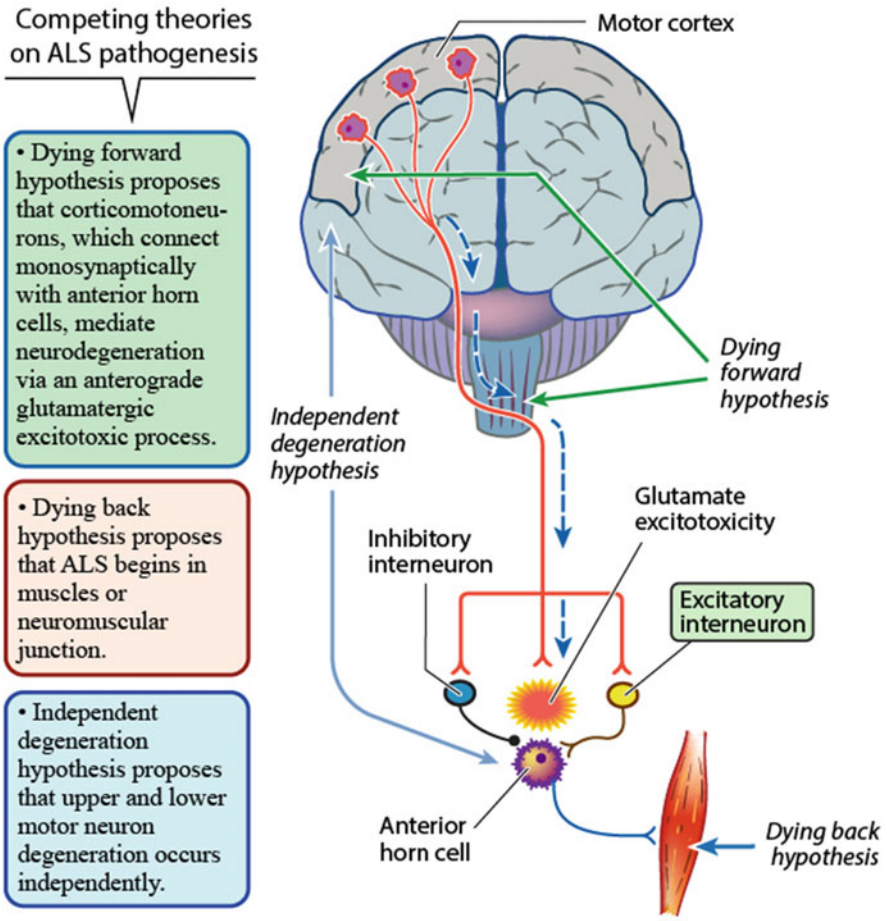

Fig. 2 The dying forward and dying back hypothesis of amyotrophic lateral sclerosis (ALS). The "dying forward" hypothesis proposed that ALS was primarily a disorder of the corticomotoneurons (highlighted in red), with anterior horn cell degeneration mediated via a transsynaptic anterograde glutamate-mediated excitotoxic process. In contrast, the dying back hypothesis proposed that ALS was primarily a disorder of the lower motor neurons with pathogens retrogradely transported from the neuromuscular junction to the cell body where these pathogens may exert their deleterious effects

acids of aminotransferases (Heath & Shaw, 2002). Approximately 20% of the total glutamate pool is stored in presynaptic nerve terminals, and during impulse transmission, glutamate is released from presynaptic neurons through the effects of depolarization, diffusing across the synaptic cleft to activate postsynaptic receptors. The excitatory signal is terminated by reuptake of glutamate from the synaptic cleft via specific transporters located on neurons and astrocytes, the main being excitatory amino acid transporter-2 [EAAT-2] (Dong et al., 1999). Within presynaptic astrocytes, glutamate is converted into glutamine by the enzyme

glutamine synthetase and recirculated to the neuron for resynthesis of glutamate (Heath & Shaw, 2002).

While two broad classes of glutamate receptors have been identified, ionotropic or metabotropic (Heath & Shaw, 2002), it's the activation of the inotropic receptors that appears to be pathogenic in ALS (Jaiswal, 2014), with excessive influx of Na^+ and Ca^{2+} ions (Heath & Shaw, 2002; Simeone et al., 2004). Based on pharmacological studies, three different groups of ionotropic receptors have been identified: (i) N-methyl-D-aspartate (NMDA), (ii) α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA), and (iii) kainite receptors. ***N-methyl-D-aspartate**** receptors are permeable to influx of Na^+ and Ca^{2+} and efflux of K^+ and mediate excitatory neurotransmission in complex physiological processes such as memory (Simeone et al., 2004). Separately, ***AMPA receptors*** mediate a rapid influx of monovalent ions (Na^+, K^+, and Cl^-) but unlike NMDA receptors are impermeable to Ca^{2+} ions (Heath & Shaw, 2002). Four AMPA receptor subtypes have been cloned (*Glu*R1-4), being composed of three transmembrane domains (M1, M3, M4) and a fourth cytoplasmic hairpin loop (M2), which contributes to the pore-lining region (Simeone et al., 2004). The AMPA receptor exists as a pentameric structure, which is formed by the arrangement of subunits to create receptor diversity (Heath & Shaw, 2002). The GluR2 subunit influences Ca^{2+} permeability, whereby AMPA receptors expressing immature GluR2 subunits are more permeable to Ca^{2+} ions*. Following activation of these AMPA receptors, excessive influx of Ca^{2+} results in neurodegeneration through activation of Ca^{2+}-dependent pathways (Heath & Shaw, 2002; Simeone et al., 2004).

In ALS, aberrant activation of NMDA and AMPA receptors leads to an excessive influx of Na^+ and Ca^{2+} ions resulting in neurodegeneration (Geevasinga et al., 2016). Evidence for glutamate excitotoxicity has been extensively demonstrated in molecular studies, with increased activity of NMDA receptors reported in spinal interneurons of transgenic SOD-1 mice (Jiang et al., 2009), as well as reduction in expression and function of EAAT2 transporter (Boillee et al., 2006). Downregulation of EAAT2 transporter may be a preclinical phenomenon in ALS (Gibb et al., 2007), with increased expression and activity of EAAT2 being neuroprotective in animal models (Rothstein et al., 2005). Accumulation of glutamate receptors has also been reported in ALS motor neurons and has been attributed to autophagosome dysfunction (Shi et al., 2019). Reversal of autophagosome dysfunction resulted in a slowing of neurodegeneration, providing a potential therapeutic target.

Separately, cell-specific molecular features render the motor neurons in ALS patients more vulnerable to glutamate toxicity. Increased expression of AMPA receptors with the GluR2 editing defect on spinal motor neurons has been well established in ALS (Van Damme et al., 2005), rendering them more permeable to Ca^{2+} influx and neurotoxicity (Cox et al., 2007; Heath & Shaw, 2002). Spinal motor neurons appear to lack the necessary Ca^{2+} ion buffering capacity due to reduced expression of proteins such as parvalbumin and calbindin D28k which are required to buffer intracellularly (Ince et al., 1993). At an anatomical level, ALS motor

neurons appear larger and exhibit greater distal dendritic branching rendering motor neurons more vulnerable to glutamate-induced electrical and metabolic stresses (Quinlan, 2011). The clinical effectiveness of the anti-glutamatergic agent riluzole in ALS (Bensimon et al., 1994) underscores the pathogenic importance of glutamate excitotoxicity in ALS.

Glutamate excitotoxicity has also been associated with increased axonal excitability, in part related to dysfunction of the Na^+/K^+ ATPase function (Naujock et al., 2016). Upregulation of persistent Na^+ currents has been reported in cortical neurons, suggesting a direct link between glutamate excitotoxicity and persistent Na^+ conductances (Pieri et al., 2009). At a peripheral level, upregulation of persistent Na^+ conductances and reduction in K^+ currents mediate hyperexcitability in ALS (Kanai et al., 2006; Vucic & Kiernan, 2010) and are associated with fasciculations and muscle cramps, adverse prognosis, and neurodegeneration (Vucic & Kiernan, 2006). Inhibition of persistent Na^+ conductances reduces muscle cramps (Park et al., 2015) and cortical and axonal hyperexcitability (Weiss et al., 2020) and appears to be neuroprotective (Pieri et al., 2009). Induced pluripotent cell models have demonstrated that neuronal hyperexcitability, mediated by reduction in K^+ currents, is pathogenic and inhibition of hyperexcitability appeared to be neuroprotective (Wainger Brian et al., 2014). A subsequent clinical trial demonstrated that inhibition of K^+ currents reduced cortical and axonal hyperexcitability (Wainger et al., 2020), although the study was not powered to detect a clinical effect.

The molecular mechanisms by which glutamate excitotoxicity exerts neurotoxicity remain to be fully elucidated, although an initial excessive influx of Na^+ and Cl^- ions along with water molecules leads to initial acute neuronal swelling (Shaw & Kuncl, 2002). Subsequently, an influx of Ca^{2+} ions results in increased intracellular Ca^{2+} ion concentration, activation of Ca^{2+}-dependent enzymatic pathways, and ultimately neuronal degeneration (Cox et al., 2007; Shaw & Kuncl, 2002). Glutamate excitotoxicity may also increase oxidative stress, further resulting in neurotoxicity (Maher & Davis, 1996).

It has also been argued that neuronal hyperexcitability may have neuroprotective benefits in ALS, a notion supported by some animal studies (Leroy et al., 2014; Saxena et al., 2013). Activation of metabotropic cholinergic receptors and mammalian target of rapamycin pathways (mTOR) was enhanced by neuronal hyperexcitability and appeared to exert neuroprotective effects (Saxena et al., 2013). In addition, early neuronal hyperexcitability failed to induce neurodegeneration in a separate SOD-1 mouse model (Leroy et al., 2014). These findings contrasted with contemporary transgenic mouse studies which disclosed that neuronal hyperexcitability induced pathology in ALS (Pieri et al., 2009; Wainger Brian et al., 2014). Hyperexcitability of cortical neuronal networks was also identified as an early feature in SOD-1 cell cultures, with evidence for upregulation of NMDA receptors and increased Na^+, Ca^{2+}, K^+, and Cl^- concentrations (Marcuzzo et al., 2019). Taken together, the cell line and animal study data provide overwhelming evidence for the importance of glutamate neurotoxicity in ALS pathogenesis.

4 Mitochondrial Dysfunction

Mitochondrial dysfunction is an important contributor to ALS pathogenesis and may act synergistically with glutamate excitotoxicity (Lederer et al., 2007). Mitochondria are intracellular organelles that are essential in bioenergetic homeostasis (ATP production), regulation of Ca^{2+} signaling, and cytosolic storage (Armada-Moreira et al., 2020). Glutamate excitotoxicity leads to excessive accumulation of Ca^{2+} within the mitochondria, resulting in membrane depolarization, impairment of energy generation, and oxidative stress (Lederer et al., 2007). Mitochondria are exquisitely sensitive to free radical damage, at both the protein and DNA level, which conversely exacerbates glutamate excitotoxicity through disruption of the normal voltage-dependent Mg^{2+}-mediated blockade of NMDA receptors (Heath & Shaw, 2002; Shaw & Kuncl, 2002).

Disruption of mitochondria transportation has also been reported in ALS and was also linked to glutamate excitotoxicity (MacAskill et al., 2009). The interruption of mitochondrial mobility may further compromise the energy supply to neuronal segments essential for maintenance of resting membrane potential and generation of action potentials. Structural mitochondrial abnormalities and aberrant aggregation have been reported as an early and presymptomatic feature in ALS (Jhanji et al., 2021), further contributing to pathogenesis. In addition, mitochondrial dysfunction may trigger activation of intrinsic apoptotic cell death pathways (via Bcl-2 family proteins), further contributing to ALS pathogenesis (Jhanji et al., 2021).

Mitochondrial dysfunction ultimately results in dysfunction of the cellular metabolic pathways and bioenergetic defects in ALS motor neurons and non-neuronal supporting cells [astrocytes and oligodendrocytes] (Vandoorne et al., 2018). Consequently, the metabolic demands of motor neurons are not satisfied leading to apoptosis and neurodegeneration (Vandoorne et al., 2018). Improving cellular bioenergy by increased intracellular NAD^+ and ATP levels with nanocrystalline gold compound CNM-Au8 was shown to be neuroprotective in transgenic ALS disease models (Zhou et al., 2020). Specifically, SOD-1 transgenic mice treated with CNM-Au8 exhibited improved clinical scores and survival. A phase II trial investigating the safety and efficacy (reflected by motor unit counts) of CNM-Au8 in sporadic ALS patients is being undertaken [RESCUE-ALS, NCT04098406] (Vucic et al., 2021), as is a parallel platform trial (Healey Platform trial) investigating multiple agents using a single set of inclusion/exclusion criteria, centralized randomization and assessment of participant data, and a shared placebo group. Separately, numerous other drug candidates aimed at improving mitochondrial function have been investigated, although the results have been disappointing (Kiernan et al., 2021). The discrepancy between preclinical and therapeutic studies could relate to limitations of animal and cell models or that mitochondrial dysfunction may not be a critical pathological event in ALS.

5 Oxidative Stress

Oxidative stress has also been implicated in ALS pathogenesis and linked to glutamate-mediated excitotoxicity and mitochondrial dysfunction (Kiernan et al., 2011, 2021). The role for oxidative stress was first raised with the uncovering of mutations in the SOD-1 gene [21 q22.1], which was postulated to exert pathogenic effect's aberrant cytotoxic activity of the SOD-1 enzyme (Siddique et al., 1991). Increased production of reactive oxygen species, such as hydroxyl and free radicals (Liu et al., 1998), along with nitration of protein tyrosine residues (Crow et al., 1997), has been reported in ALS implying a pathogenic effect of oxidative stress. In addition, upregulation of pro-inflammatory cytokines (Hensley et al., 2006), including nitric oxide and interleukin (IL)-1, IL-6, and IL-12, has been reported in ALS and attributed to oxidative stress. Separately, impairment of stress granule formation has been reported in ALS and associated with TAR DNA binding protein-43 (TDP-43) accumulation and neurodegeneration (Romano et al., 2020). While functional effectiveness of the antioxidant agent edaravone was reported (Writing Group EM-ASG, 2017), thereby implying a pathogenic relevance for oxidative stress in ALS, the benefits were minor, not accompanied by changes in respiratory function or muscle strength and were evident in a highly selected cohort (Writing Group EM-ASG, 2017). A recent real-life study failed to establish an effect of edaravone on disease progression, respiratory function, and survival (Lunetta et al., 2020). As for mitochondrial dysfunction, oxidative stress may represent a downstream pathogenic effect, contributing to pathogenesis but not being an initiating mechanism.

6 Protein Aggregation and Neurotoxicity

Aggregation of hyperphosphorylated TDP-43 in cortical neurons is a neuropathological hallmark of ALS evident in ~97% of cases (Al-Sarraj et al., 2011; Neumann et al., 2006). The predominating specific patterns of TDP-43 pathology include (i) glial [22% of cases], (ii) mixed neuronal and glial [59% of cases], and (iii) neuronal [7% of cases] (Williams et al., 2017). TDP-43 inclusions are evident in both demented and non-demented ALS patients, with inclusion density increasing with disease progression and being associated with development of cognitive impairment (Wilson et al., 2001). Importantly, accumulation of TDP-43 in extramotor regions has been associated with cognitive impairment (Gregory et al., 2020), and the degree of TDP-43 pathology differentiates ALS from the ALS-frontotemporal dementia syndrome (Prudlo et al., 2016). Additionally, regional cortical aggregation of TDP-43 is associated with specific cognitive abnormalities, such that TDP-43 pathology in the orbitofrontal, dorsolateral prefrontal, medial prefrontal, and ventral anterior cingulate cortices manifests as executive dysfunction, while pathology within the inferior frontal gyrus, transverse temporal area, middle, inferior temporal gyri, and angular gyri is associated with language dysfunction (Gregory et al., 2020). As a consequence of the clinical-

pathological correlation, ALS has been reclassified as a primary neurodegenerative cortical disorder (Eisen et al., 2017).

The precise pathophysiological mechanisms by which TDP-43 causes neurotoxicity remains to be elucidated, although multiple processes have been advanced (Prasad et al., 2019). At a physiological level, the wild-type TDP-43 protein appears to be critical for multiple cellular processes, including regulation of RNA metabolism, mRNA transport, microRNA maturation, and stress granule formation (Prasad et al., 2019). TDP-43 is predominantly located within the nucleus, although it shuttles between the nuclear and cytoplasmic compartments depending on physiological requirements, and this movement is regulated by specific proteins (de Boer et al., 2020). Of relevance to brain function, TDP-43 appears critical for normal development of central neuronal cells in early stages of embryogenesis (Sephton et al., 2010). In ALS, the pathological hallmark is cytosolic mislocalization and increased nuclear clearance of TDP-43 (de Boer et al., 2020), thereby suggesting two non-mutually exclusive disease mechanisms, loss of nuclear function and cytoplasmic gain-of-function. Underscoring this notion are transgenic mouse studies disclosing that overexpression of TDP-43 is associated with neurotoxicity, while "knocking-out" of the TARDBP gene, which encodes the TDP-43 protein, is embryonically lethal (Xu et al., 2010). Pathogenic missense mutations in the TARDBP gene have been reported in sporadic and familial forms of ALS (Chiò et al., 2020), resulting in toxic gain-of-function (including protein aggregation propensity and cytotoxicity), as well as formation of neurotoxic amyloid-like fibrils and larger stress granules with an impaired ability to maintain RNA homeostasis (Neumann et al., 2006). In addition, nuclear depletion of TDP-43 leads to transcriptional dysregulation and splicing defects, with ensuing neurotoxicity (Melamed et al., 2019).

Posttranslational TDP-43 protein modifications, including hyperphosphorylation, ubiquitination, acetylation, poly ADP-ribosylation, and cysteine oxidation, may also induce aberrant TDP-43 aggregation and result in neurotoxicity by impairing the ability of TDP-43 to regulate DNA/RNA and protein-protein interactions (Prasad et al., 2019). Pathogenic TDP-43 oligomers have also been reported in ALS and exert pathogenic effects by increasing the protein's propensity to cytoplasmic aggregation, cross-seeding, and prion-like behavior (Fang et al., 2014). The notion of a centrifugal propagation of TDP-43 pathology, whereby pathogenic TDP-43 aggregation begins in the prefrontal cortex and propagates along axonal fibers (Brettschneider et al., 2013), may be explained by this prion-like spread in ALS. Additionally, mutated TDP-43 may exert pathogenic effects by increasing the propensity for liquid-liquid phase separation (LLPS)*, resulting in dysregulation of nucleocytoplasmic transport, increased clearance of nuclear TDP-43, and protein aggregation (McGurk et al., 2018). Mitochondrial dysfunction, impairment of endocytosis, dysregulation of metal ion homeostasis, and interference with chromatin remodeling are additional neurotoxic mechanisms implicated in TDP-43 pathogenesis (Prasad et al., 2019).

Hexanucleotide gene expansion [9p21 (G_4C_2)] in the dominantly inherited c9orf72 gene* has been identified as a major cause of familial (~40%) and sporadic

(4.1–8.3%) ALS (DeJesus-Hernandez et al., 2011; Renton Alan et al., 2011). The pathophysiological mechanisms by which c9orf72 gene causes neurodegeneration remains to be fully elucidated, although three potential mechanisms have been proposed: (i) haploinsufficiency of the normal c9orf72 gene (Cooper-Knock et al., 2015), (ii) RNA-based toxicity of the transcribed repeat or protein-based toxicity via translation of the hexanucleotide containing RNA to form dipeptide protein repeats [DPR] (DeJesus-Hernandez et al., 2011; Donnelly Christopher et al., 2013), and (iii) non-ATG (RAN) translation leading to formation of DPRs and dysregulation of ubiquitin-proteasome system and nucleocytoplasmic transport (Zhang et al., 2016). Importantly, accumulation of TDP-43 and p62 positive inclusions is a pathological hallmark in c9orf72-associated ALS (Al-Sarraj et al., 2011). At a molecular level, c9orf72 expansions induce the formation of aberrant stress granules (Todd et al., 2020), as well neuronal hyperexcitability (Wainger Brian et al., 2014) and aberrant activation of cellular protective mechanisms in response to glutamate neurotransmission (Seminary et al., 2020). From a therapeutic perspective, the utility of antisense oligonucleotides (ASOs) to reduce toxic gain-of-function has shown promise in preclinical studies (Ly & Miller, 2018). Specifically, binding of ASOs upstream of the intrinsic expansion reduced RNA foci and dipeptide aggregates, increased survival from glutamate excitotoxicity, and abrogated aberrant gene expression patterns (Donnelly Christopher et al., 2013). Additionally, ASOs targeted against het c9orf72 gene reduce TDP-43 pathology and neurodegeneration (Cook et al., 2020). Interestingly, behavioral and cognitive deficits were improved in the transgenic c9orf72 mouse models (Jiang et al., 2016), suggesting potential therapeutic effects in ALS patients. Consequently, phase I multicenter study is currently underway to determine the safety, pharmacokinetic profile, and efficacy of the ASO, BIIB078, in c9orf72 ALS (***NCT03626012***).

Antisense oligonucleotide treatment strategies have also been developed for SOD-1-associated familial ALS. Of relevance, mutations in the SOD-1 gene lead to conformational instability and misfolding of the SOD-1 peptide, resulting in formation of toxic intracellular aggregates in motor neurons and glial cells (Zetterstrom et al., 2007). Importantly, SOD-1 knockout mice fail to develop motor neuron degeneration (Picher-Martel et al., 2016), suggesting that lowering SOD-1 protein levels could be a viable therapeutic strategy (Ly & Miller, 2018). Studies of ASOs targeting the SOD-1 gene in mouse and nonhuman primates demonstrated widespread distribution of ASOs within the central nervous system, reduction of SOD-1 mRNA and protein levels, and extension of survival (McCampbell et al., 2018). The clinical feasibility of this therapeutic approach was established in a first-in-man phase I study that intrathecally delivered ASO 333611 (ISIS-SOD1RX) in SOD-1-familial ALS patients and was shown to be well tolerated (Miller et al., 2013). More recently, intrathecal administration of the more potent ASO (Tofersen) was shown to be safe and to exhibit biological effectiveness in a dose escalating phase I/II study [NCT02623699] (Miller et al., 2020). These promising findings have led to the phase III VALOR study to assess the efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of Tofersen in SOD-1 familial ALS.

Although ASOs have shown some therapeutic promise in monogenic gain-of-function mutations, such as SOD-1- and C9orf72-related ALS, a challenge remains in developing therapies for the broader ALS patient community especially considering disease heterogeneity. Given that TDP-43 appears to be a pathophysiological hallmark of ALS (Ly & Miller, 2018), therapies aimed at reducing TDP-43 expression and aggregation may be of utility in a larger population of ALS patients. While direct manipulation of the TARDBP gene is lethal (Sephton et al., 2010), ASOs targeting ataxin-2 in TDP-43 mouse models were shown to reduce formation of TDP-43 aggregates, slow disease progression, and prolong survival (Becker et al., 2017). Additionally, inhibition of stress granule formation by ataxin-2 ASOs suppressed nucleocytoplasmic transport defects and neurodegeneration in C9orf72 disease models (Zhang et al., 2018). A phase I study is currently underway investigating the safety, tolerability, and pharmacokinetics of the ASO BIIB105 in ALS patients harboring the ataxin-2 gene expansion (NCT04494256). Given the preclinical finings and pending the successful completion of the phase I study, targeting the ataxin-2 gene may be of potential utility in a broader ALS cohort.

7 Conclusion

Amyotrophic lateral sclerosis appears to be a multistep process mediated by a complex interaction between genetic, epigenetic, molecular, and environmental processes. Glutamate neurotoxicity appears to be an early feature of ALS, associated with neurodegeneration, clinical features, and disease progression, suggesting that ALS is a primary brain disorder. Underscoring this notion are pathological findings of widespread TDP-43 aggregation in cortical neurons and supporting cells (astrocytes and microglia). In addition, TDP-43 pathology is associated with common ALS genetic mutations (c9orf72), evolves with disease progression, and correlates with emergence of cognitive dysfunction. Other molecular processes including mitochondrial dysfunction, oxidative stress, abnormalities of axonal transport, and toxic gain-of-function induced by mutant SOD-1 enzymes contribute to ALS pathogenesis. Emerging therapeutic strategies utilizing repurposed compounds that target specific dysfunctional molecular pathways, along with antisense oligonucleotide (genetic) treatment strategies, will undoubtedly result in development of much needed therapeutic strategies.

8 Cross-References

- ▶ Biomarkers of Neurotoxicity Inform Mechanisms of Vulnerability and Resilience in Dopaminergic Neurons
- ▶ Excitotoxicity and Amyotrophic Lateral Sclerosis
- ▶ Glutamate and Neurodegeneration in the Retina
- ▶ Glutamate as a Neurotoxin
- ▶ Glutamate Neurotoxicity Related to Energy Failure

► Glutamate Neurotoxicity, Transport and Alternate Splicing of Transporters
► Microglial Cell Dysregulation in the Aged Brain and Neurodegeneration
► The NMDA Receptor System and Developmental Neurotoxicity

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References

Al-Sarraj, S., King, A., Troakes, C., Smith, B., Maekawa, S., Bodi, I., et al. (2011). P62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. *Acta Neuropathologica, 122*, 691–702.

Armada-Moreira, A., Gomes, J. I., Pina, C. C., Savchak, O. K., Gonçalves-Ribeiro, J., Rei, N., et al. (2020). Going the extra (synaptic) mile: Excitotoxicity as the road toward neurodegenerative diseases. *Frontiers in Cellular Neuroscience, 14*, 90.

Becker, L. A., Huang, B., Bieri, G., Ma, R., Knowles, D. A., Jafar-Nejad, P., et al. (2017). Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. *Nature, 544*, 367–371.

Bensimon, G., Lacomblez, L., & Meininger, V. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *The New England Journal of Medicine, 330*, 585–591.

Boillee, S., Vande Velde, C., & Cleveland, D. W. (2006). ALS: A disease of motor neurons and their nonneuronal neighbors. *Neuron, 52*, 39–59.

Brettschneider, J., Del Tredici, K., Toledo, J. B., Robinson, J. L., Irwin, D. J., Grossman, M., et al. (2013). Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Annals of Neurology, 74*, 20–38.

Chio, A., Mazzini, L., D'Alfonso, S., Corrado, L., Canosa, A., Moglia, C., et al. (2018). The multistep hypothesis of ALS revisited: The role of genetic mutations. *Neurology, 91*, e635–ee42.

Chiò, A., Mazzini, L., & Mora, G. (2020). Disease-modifying therapies in amyotrophic lateral sclerosis. *Neuropharmacology, 167*, 107986.

Cook, C. N., Wu, Y., Odeh, H. M., Gendron, T. F., Jansen-West, K., Del Rosso, G., et al. (2020). C9orf72 poly(GR) aggregation induces TDP-43 proteinopathy. *Science Translational Medicine, 12*, 559.

Cooper-Knock, J., Kirby, J., Highley, R., & Shaw, P. J. (2015). The spectrum of C9orf72-mediated neurodegeneration and amyotrophic lateral sclerosis. *Neurotherapeutics, 12*, 326–339.

Cox, L., Kirby, J., & Shaw, P. (2007). Pathogenesis of motor neurone disease. In M. Kiernan (Ed.), *The motor neurone disease handbook* (pp. 26–55). Australasian Medical Publishing Company Limited.

Crow, J. P., Sampson, J. B., Zhuang, Y., Thompson, J. A., & Beckman, J. S. (1997). Decreased zinc affinity of amyotrophic lateral sclerosis-associated superoxide dismutase mutants leads to enhanced catalysis of tyrosine nitration by peroxynitrite. *Journal of Neurochemistry, 69*, 1936–1944.

de Boer, E. M. J., Orie, V. K., Williams, T., Baker, M. R., De Oliveira, H. M., Polvikoski, T., et al. (2020). TDP-43 proteinopathies: A new wave of neurodegenerative diseases. *Journal of Neurology, Neurosurgery, and Psychiatry, 92*, 86–95.

DeJesus-Hernandez, M., Mackenzie Ian, R., Boeve Bradley, F., Boxer Adam, L., Baker, M., Rutherford Nicola, J., et al. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron, 72*, 245–256.

Di Lazzaro, V., Ranieri, F., Profice, P., Pilato, F., Mazzone, P., Capone, F., et al. (2013). Transcranial direct current stimulation effects on the excitability of corticospinal axons of the human cerebral cortex. *Brain Stimulation, 6*, 641–643.

Dong, H., Zhang, P., Song, I., Petralia, R. S., Liao, D., & Huganir, R. L. (1999). Characterization of the glutamate receptor-interacting proteins GRIP1 and GRIP2. *The Journal of Neuroscience, 19*, 6930–6941.

Donnelly Christopher, J., Zhang, P.-W., Pham Jacqueline, T., Haeusler Aaron, R., Mistry Nipun, A., Vidensky, S., et al. (2013). RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron, 80*, 415–428.

Eisen, A., & Weber, M. (2001). The motor cortex and amyotrophic lateral sclerosis. *Muscle & Nerve, 24*, 564–573.

Eisen, A., Kim, S., & Pant, B. (1992). Amyotrophic lateral sclerosis (ALS): A phylogenetic disease of the corticomotoneuron? *Muscle & Nerve, 15*, 219–224.

Eisen, A., Braak, H., Del Tredici, K., Lemon, R., Ludolph, A. C., & Kiernan, M. C. (2017). Cortical influences drive amyotrophic lateral sclerosis. *Journal of Neurology, Neurosurgery, and Psychiatry, 88*, 917–924.

Fang, Y. S., Tsai, K. J., Chang, Y. J., Kao, P., Woods, R., Kuo, P. H., et al. (2014). Full-length TDP-43 forms toxic amyloid oligomers that are present in frontotemporal lobar dementia-TDP patients. *Nature Communications, 5*, 4824.

Geevasinga, N., Menon, P., Nicholson, G. A., Ng, K., Howells, J., Kril, J. J., et al. (2015). Cortical function in asymptomatic carriers and patients with C9orf72 amyotrophic lateral sclerosis. *JAMA Neurology, 72*, 1268–1274.

Geevasinga, N., Menon, P., Özdinler, P. H., Kiernan, M. C., & Vucic, S. (2016). Pathophysiological and diagnostic implications of cortical dysfunction in ALS. *Nature Reviews. Neurology, 12*, 651–661.

Gibb, S. L., Boston-Howes, W., Lavina, Z. S., Gustincich, S., Brown, R. H., Jr., Pasinelli, P., et al. (2007). A caspase-3-cleaved fragment of the glial glutamate transporter EAAT2 is sumoylated and targeted to promyelocytic leukemia nuclear bodies in mutant SOD1-linked amyotrophic lateral sclerosis. *The Journal of Biological Chemistry, 282*, 32480–32490.

Gregory, J. M., McDade, K., Bak, T. H., Pal, S., Chandran, S., Smith, C., et al. (2020). Executive, language and fluency dysfunction are markers of localised TDP-43 cerebral pathology in non-demented ALS. *Journal of Neurology, Neurosurgery, and Psychiatry, 91*, 149–157.

Heath, P. R., & Shaw, P. J. (2002). Update on the glutamatergic neurotransmitter system and the role of excitotoxicity in amyotrophic lateral sclerosis. *Muscle & Nerve, 26*, 438–458.

Hensley, K., Mhatre, M., Mou, S., Pye, Q. N., Stewart, C., West, M., et al. (2006). On the relation of oxidative stress to neuroinflammation: Lessons learned from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *Antioxidants & Redox Signaling, 8*, 2075–2087.

Ince, P., Stout, N., Shaw, P., Slade, J., Hunziker, W., Heizmann, C. W., et al. (1993). Parvalbumin and calbindin D-28k in the human motor system and in motor neuron disease. *Neuropathology and Applied Neurobiology, 19*, 291–299.

Jaiswal, M. K. (2014). Selective vulnerability of motoneuron and perturbed mitochondrial calcium homeostasis in amyotrophic lateral sclerosis: Implications for motoneurons specific calcium dysregulation. *Molecular and Cellular Therapies, 2*, 26.

Jhanji, R., Behl, T., Sehgal, A., & Bungau, S. (2021). Mitochondrial dysfunction and traffic jams in amyotrophic lateral sclerosis. *Mitochondrion, 58*,102–110.

Jiang, M., Schuster, J. E., Fu, R., Siddique, T., & Heckman, C. J. (2009). Progressive changes in synaptic inputs to motoneurons in adult sacral spinal cord of a mouse model of amyotrophic lateral sclerosis. *The Journal of Neuroscience, 29*, 15031–15038.

Jiang, J., Zhu, Q., Gendron, T. F., Saberi, S., McAlonis-Downes, M., Seelman, A., et al. (2016). Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. *Neuron, 90*, 535–550.

Kanai, K., Kuwabara, S., Misawa, S., Tamura, N., Ogawara, K., Nakata, M., et al. (2006). Altered axonal excitability properties in amyotrophic lateral sclerosis: Impaired potassium channel function related to disease stage. *Brain, 129*, 953–962.

Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., et al. (2011). Amyotrophic lateral sclerosis. *Lancet, 377*, 942–955.

Kiernan, M. C., Vucic, S., Talbot, K., McDermott, C. J., Hardiman, O., Shefner, J. M., et al. (2021). Improving clinical trial outcomes in amyotrophic lateral sclerosis. *Nature Reviews. Neurology, 17*, 104–118.

Lederer, C. W., Torrisi, A., Pantelidou, M., Santama, N., & Cavallaro, S. (2007). Pathways and genes differentially expressed in the motor cortex of patients with sporadic amyotrophic lateral sclerosis. *BMC Genomics, 8*, 26.

Leroy, F., Lamotte d'Incamps, B., Imhoff-Manuel, R. D., & Zytnicki, D. (2014). Early intrinsic hyperexcitability does not contribute to motoneuron degeneration in amyotrophic lateral sclerosis. *eLife, 3,* e04046.

Liu, R., Althaus, J. S., Ellerbrock, B. R., Becker, D. A., & Gurney, M. E. (1998). Enhanced oxygen radical production in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Annals of Neurology, 44*, 763–770.

Lunetta, C., Moglia, C., Lizio, A., Caponnetto, C., Dubbioso, R., Giannini, F., et al. (2020). The Italian multicenter experience with edaravone in amyotrophic lateral sclerosis. *Journal of Neurology, 267*, 3258–3267.

Ly, C. V., & Miller, T. M. (2018). Emerging antisense oligonucleotide and viral therapies for amyotrophic lateral sclerosis. *Current Opinion in Neurology, 31*, 648–654.

MacAskill, A. F., Rinholm, J. E., Twelvetrees, A. E., Arancibia-Carcamo, I. L., Muir, J., Fransson, A., et al. (2009). Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. *Neuron, 61*, 541–555.

Maher, P., & Davis, J. B. (1996). The role of monoamine metabolism in oxidative glutamate toxicity. *The Journal of Neuroscience, 16*, 6394–6401.

Marcuzzo, S., Terragni, B., Bonanno, S., Isaia, D., Cavalcante, P., Cappelletti, C., et al. (2019). Hyperexcitability in cultured cortical neuron networks from the G93A-SOD1 amyotrophic lateral sclerosis model mouse and its molecular correlates. *Neuroscience, 416*, 88–99.

McCampbell, A., Cole, T., Wegener, A. J., Tomassy, G. S., Setnicka, A., Farley, B. J., et al. (2018). Antisense oligonucleotides extend survival and reverse decrement in muscle response in ALS models. *The Journal of Clinical Investigation, 128*, 3558–3567.

McGurk, L., Gomes, E., Guo, L., Mojsilovic-Petrovic, J., Tran, V., Kalb, R. G., et al. (2018). Poly (ADP-Ribose) prevents pathological phase separation of TDP-43 by promoting liquid demixing and stress granule localization. *Molecular Cell, 71*, 703–17.e9.

Melamed, Z., López-Erauskin, J., Baughn, M. W., Zhang, O., Drenner, K., Sun, Y., et al. (2019). Premature polyadenylation-mediated loss of stathmin-2 is a hallmark of TDP-43-dependent neurodegeneration. *Nature Neuroscience, 22*, 180–190.

Menon, P., Kiernan, M. C., & Vucic, S. (2014). Cortical dysfunction underlies the development of the split-hand in amyotrophic lateral sclerosis. *PLoS One, 9*, e87124.

Menon, P., Geevasinga, N., Yiannikas, C., Howells, J., Kiernan, M., & Vucic, S. (2015a). The sensitivity and specificity of threshold-tracking transcranial magnetic stimulation for the diagnosis of amyotrophic lateral sclerosis: A prospective study. *Lancet Neurology, 14*, 478–484.

Menon, P., Kiernan, M. C., & Vucic, S. (2015b). Cortical hyperexcitability precedes lower motor neuron dysfunction in ALS. *Clinical Neurophysiology, 126*, 803–809.

Menon, P., Geevasinga, N., van den Bos, M., Yiannikas, C., Kiernan, M. C., & Vucic, S. (2017). Cortical hyperexcitability and disease spread in amyotrophic lateral sclerosis. *European Journal of Neurology, 24*, 816–824.

Miller, T. M., Pestronk, A., David, W., Rothstein, J., Simpson, E., Appel, S. H., et al. (2013). An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: A phase 1, randomised, first-in-man study. *Lancet Neurology, 12*, 435–442.

Miller, T., Cudkowicz, M., Shaw, P. J., Andersen, P. M., Atassi, N., Bucelli, R. C., et al. (2020). Phase 1-2 trial of antisense oligonucleotide tofersen for SOD1 ALS. *The New England Journal of Medicine, 383*, 109–119.

Naujock, M., Stanslowsky, N., Bufler, S., Naumann, M., Reinhardt, P., Sterneckert, J., et al. (2016). 4-Aminopyridine induced activity rescues hypoexcitable motor neurons from amyotrophic lateral sclerosis patient-derived induced pluripotent stem cells. *Stem Cells, 34*, 1563–1575.

Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., et al. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science, 314*, 130–133.

Nihei, K., McKee, A. C., & Kowall, N. W. (1993). Patterns of neuronal degeneration in the motor cortex of amyotrophic lateral sclerosis patients. *Acta Neuropathologica, 86*, 55–64.

Park, S. B., Vucic, S., Cheah, B. C., Lin, C. S., Kirby, A., Mann, K. P., et al. (2015). Flecainide in amyotrophic lateral sclerosis as a neuroprotective strategy (FANS): A randomized placebo-controlled trial. *eBioMedicine, 2*, 1916–1922.

Picher-Martel, V., Valdmanis, P. N., Gould, P. V., Julien, J. P., & Dupré, N. (2016). From animal models to human disease: A genetic approach for personalized medicine in ALS. *Acta Neuropathologica Communications, 4*, 70.

Pieri, M., Carunchio, I., Curcio, L., Mercuri, N. B., & Zona, C. (2009). Increased persistent sodium current determines cortical hyperexcitability in a genetic model of amyotrophic lateral sclerosis. *Experimental Neurology, 215*, 368–379.

Prasad, A., Bharathi, V., Sivalingam, V., Girdhar, A., & Patel, B. K. (2019). Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis. *Frontiers in Molecular Neuroscience, 12*, 25.

Prudlo, J., König, J., Schuster, C., Kasper, E., Büttner, A., Teipel, S., et al. (2016). TDP-43 pathology and cognition in ALS: A prospective clinicopathologic correlation study. *Neurology, 87*, 1019–1023.

Quinlan, K. A. (2011). Links between electrophysiological and molecular pathology of amyotrophic lateral sclerosis. *Integrative and Comparative Biology, 51*, 913–925.

Ravits, J., Paul, P., & Jorg, C. (2007). Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. *Neurology, 68*, 1571–1575.

Renton Alan, E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J. R., et al. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron, 72*, 257–268.

Romano, N., Catalani, A., Lattante, S., Belardo, A., Proietti, S., Bertini, L., et al. (2020). ALS skin fibroblasts reveal oxidative stress and ERK1/2-mediated cytoplasmic localization of TDP-43. *Cellular Signalling, 70*, 109591.

Rothstein, J. D., Patel, S., Regan, M. R., Haenggeli, C., Huang, Y. H., Bergles, D. E., et al. (2005). Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature, 433*, 73–77.

Saxena, S., Roselli, F., Singh, K., Leptien, K., Julien, J.-P., Gros-Louis, F., et al. (2013). Neuroprotection through excitability and mTOR required in ALS motoneurons to delay disease and extend survival. *Neuron, 80*, 80–96.

Seminary, E. R., Santarriaga, S., Wheeler, L., Mejaki, M., Abrudan, J., Demos, W., et al. (2020). Motor neuron generation from iPSCs from identical twins discordant for amyotrophic lateral sclerosis. *Cell, 9, 571*.

Sephton, C. F., Good, S. K., Atkin, S., Dewey, C. M., Mayer, P., 3rd, Herz, J., et al. (2010). TDP-43 is a developmentally regulated protein essential for early embryonic development. *The Journal of Biological Chemistry, 285*, 6826–6834.

Shaw, P., & Kuncl, R. (2002). Current concepts in the pathogenesis of ALS. In R. W. Kuncl (Ed.), *Motor neuron disease* (pp. 37–73). WB Saunders.

Shi, Y., Hung, S. T., Rocha, G., Lin, S., Linares, G. R., Staats, K. A., et al. (2019). Identification and therapeutic rescue of autophagosome and glutamate receptor defects in C9ORF72 and sporadic ALS neurons. *JCI Insight, 5*, e127736.

Shibuya, K., Park, S. B., Geevasinga, N., Menon, P., Howells, J., Simon, N. G., et al. (2016). Motor cortical function determines prognosis in sporadic ALS. *Neurology, 87*, 513–520.

Siddique, T., Figlewicz, D. A., Pericak-Vance, M. A., Haines, J. L., Rouleau, G., Jeffers, A. J., et al. (1991). Linkage of a gene causing familial amyotrophic lateral sclerosis to chromosome 21 and evidence of genetic-locus heterogeneity. *The New England Journal of Medicine, 324*, 1381–1384.

Simeone, T. A., Sanchez, R. M., & Rho, J. M. (2004). Molecular biology and ontogeny of glutamate receptors in the mammalian central nervous system. *Journal of Child Neurology, 19*, 343–360.

Todd, T. W., McEachin, Z. T., Chew, J., Burch, A. R., Jansen-West, K., Tong, J., et al. (2020). Hexanucleotide repeat expansions in c9FTD/ALS and SCA36 confer selective patterns of neurodegeneration in vivo. *Cell Reports, 31*, 107616.

Van Damme, P., Braeken, D., Callewaert, G., Robberecht, W., & Van Den Bosch, L. (2005). GluR2 deficiency accelerates motor neuron degeneration in a mouse model of amyotrophic lateral sclerosis. *Journal of Neuropathology and Experimental Neurology, 64*, 605–612.

Van den Bos, M. A. J., Higashihara, M., Geevasinga, N., Menon, P., Kiernan, M. C., & Vucic, S. (2018). Imbalance of cortical facilitatory and inhibitory circuits underlies hyperexcitability in ALS. *Neurology, 91*, e1669–e1e76.

Vandoorne, T., De Bock, K., & Van Den Bosch, L. (2018). Energy metabolism in ALS: An underappreciated opportunity? *Acta Neuropathologica, 135*, 489–509.

Vucic, S., & Kiernan, M. C. (2006a). Axonal excitability properties in amyotrophic lateral sclerosis. *Clinical Neurophysiology, 117*, 1458–1466.

Vucic, S., & Kiernan, M. C. (2006b). Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain, 129*, 2436–2446.

Vucic, S., & Kiernan, M. C. (2010). Upregulation of persistent sodium conductances in familial ALS. *Journal of Neurology, Neurosurgery, and Psychiatry, 81*, 222–227.

Vucic, S., Nicholson, G. A., & Kiernan, M. C. (2008). Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. *Brain, 131*, 1540–1550.

Vucic, S., Lin, C. S.-Y., Cheah, B. C., Murray, J., Menon, P., Krishnan, A. V., et al. (2013). Riluzole exerts central and peripheral modulating effects in amyotrophic lateral sclerosis. *Brain, 136*, 1361–1370.

Vucic, S., Westeneng, H. J., Al-Chalabi, A., Van Den Berg, L. H., Talman, P., & Kiernan, M. C. (2019). Amyotrophic lateral sclerosis as a multi-step process: An Australia population study. *Amyotroph Lateral Scler Frontotemporal Degener, 20*, 532–537.

Vucic, S., Higashihara, M., Sobue, G., Atsuta, N., Doi, Y., Kuwabara, S., et al. (2020). ALS is a multistep process in South Korean, Japanese, and Australian patients. *Neurology, 94*, e1657–e1e63.

Vucic, S., Kiernan, M. C., Menon, P., Huynh, W., Rynders, A., Ho, K. S., et al. (2021). Study protocol of RESCUE-ALS: A Phase 2, randomised, double-blind, placebo-controlled study in early symptomatic amyotrophic lateral sclerosis patients to assess bioenergetic catalysis with CNM-Au8 as a mechanism to slow disease progression. *BMJ Open, 11*, e041479.

Wainger Brian, J., Kiskinis, E., Mellin, C., Wiskow, O., Han Steve, S. W., Sandoe, J., et al. (2014). Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. *Cell Reports, 7*, 1–11.

Wainger, B. J., Macklin, E. A., Vucic, S., McIlduff, C. E., Paganoni, S., Maragakis, N. J., et al. (2020). Effect of ezogabine on cortical and spinal motor neuron excitability in amyotrophic lateral sclerosis: A randomized clinical trial. *JAMA Neurology, 78*, 186–196.

Weiss, M. D., Macklin, E. A., McIlduff, C. E., Vucic, S., Wainger, B. J., Kiernan, M. C., et al. (2020). Effects of mexiletine on hyperexcitability in sporadic amyotrophic lateral sclerosis: Preliminary findings from a small phase II randomized controlled trial. *Muscle Nerve, 63*, 371–383.

Williams, K. L., Fifita, J. A., Vucic, S., Durnall, J. C., Kiernan, M. C., Blair, I. P., et al. (2013). Pathophysiological insights into ALS with C9ORF72 expansions. *Journal of Neurology, Neurosurgery, and Psychiatry, 84*, 931–935.

Williams, S. M., Khan, G., Harris, B. T., Ravits, J., & Sierks, M. R. (2017). TDP-43 protein variants as biomarkers in amyotrophic lateral sclerosis. *BMC Neuroscience, 18*, 20.

Wilson, C. M., Grace, G. M., Munoz, D. G., He, B. P., & Strong, M. J. (2001). Cognitive impairment in sporadic ALS: A pathologic continuum underlying a multisystem disorder. *Neurology, 57*, 651–657.

Writing Group EM-ASG. (2017). Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: A randomised, double-blind, placebo-controlled trial. *Lancet Neurology, 16*, 505–512.

Xu, Y. F., Gendron, T. F., Zhang, Y. J., Lin, W. L., D'Alton, S., Sheng, H., et al. (2010). Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. *The Journal of Neuroscience, 30*, 10851–10859.

Zanette, G., Tamburin, S., Manganotti, P., Refatti, N., Forgione, A., & Rizzuto, N. (2002). Different mechanisms contribute to motor cortex hyperexcitability in amyotrophic lateral sclerosis. *Clinical Neurophysiology, 113*, 1688–1697.

Zetterstrom, P., Stewart, H. G., Bergemalm, D., Jonsson, P. A., Graffmo, K. S., Andersen, P. M., et al. (2007). Soluble misfolded subfractions of mutant superoxide dismutase-1s are enriched in spinal cords throughout life in murine ALS models. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 14157–14162.

Zhang, Y. J., Gendron, T. F., Grima, J. C., Sasaguri, H., Jansen-West, K., Xu, Y. F., et al. (2016). C9ORF72 poly(GA) aggregates sequester and impair HR23 and nucleocytoplasmic transport proteins. *Nature Neuroscience, 19*, 668–677.

Zhang, K., Daigle, J. G., Cunningham, K. M., Coyne, A. N., Ruan, K., Grima, J. C., et al. (2018). Stress granule assembly disrupts nucleocytoplasmic transport. *Cell, 173*, 958–71.e17.

Zhou, Q., Zhu, L., Qiu, W., Liu, Y., Yang, F., Chen, W., et al. (2020). Nicotinamide riboside enhances mitochondrial proteostasis and adult neurogenesis through activation of mitochondrial unfolded protein response signaling in the brain of ALS SOD1(G93A) mice. *International Journal of Biological Sciences, 16*, 284–297.