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# Amyotrophic lateral sclerosis (motor neuron disease): proposed mechanisms and pathways to treatment

**Emily F. Goodall and Karen E. Morrison**

**Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterised by loss of motor neurons. The cause of disease is unknown other than in the rare cases of familial disease arising from mutations in the superoxide dismutase 1 gene. Many theories for pathogenesis have been proposed – including oxidative stress, excitotoxicity, mitochondrial dysfunction and abnormal protein aggregation – based on studies of human post mortem tissue, research on animal models, and in vitro work. Here we review the evidence for the main pathogenic mechanisms and outline how they might interact to cause motor neuron death. Clinical trials have as yet failed to identify any truly effective therapies in ALS, with only riluzole providing a modest improvement in survival. Ongoing trials are exploring the value of antiglutamatergic agents, including the cephalosporin antibiotic ceftriaxone, as well as antioxidants, mitochondrial enhancers and anti-apoptotic drugs. It is likely that effective therapy will involve combinations of agents acting on different mechanisms. Gene therapy with neurotrophic factors will soon be in clinical trials, while work on stem cell therapy remains preclinical. In addition to finding effective therapies, research also needs to identify early disease markers because therapy is likely to be of most benefit when given early in the course of disease.**

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The most common adult-onset disorder of motor neurons, and the focus of this review, is amyotrophic lateral sclerosis (ALS) – better known in the UK as motor neuron disease (MND) and in the USA as Lou Gehrig's disease. Symptoms typically start in middle life and progress rapidly, leading to death from respiratory failure within 2–5 years. Clinical subtypes of ALS are defined according to the main site of weakness and whether upper or lower motor neurons are primarily involved (Fig. 1). Some features, such as onset at a young age (below 40 years) or presentation with purely upper or purely lower motor neuron features, are associated with longer survival. As many as 10% of patients with ALS survive for more than 10 years, suggesting that certain individuals harbour some form of protective factor. For details of clinical aspects of ALS and its management, the reader is referred to recent reviews (Refs 1, 2).

Despite the heterogeneous clinical findings, the pathological features in ALS are fairly homogeneous and usually restricted to the motor system (Table 1; Fig. 2). Reports of post mortem studies on patients who have received full respiratory support via invasive ventilation, and consequently survived with ALS for many years, have described typical ALS pathological features in areas of the brain other than the well-recognised motor system (Refs 3, 4). This suggests that the motor system is involved in ALS because it is particularly sensitive to the underlying pathological process rather than the disease being solely selective for motor neurons. The concept that ALS is a multisystem neurodegenerative disease is also supported by the observation that cognitive impairment is common in the disease, with nearly a third of ALS patients showing features compatible with frontotemporal lobar dementia (Ref. 5).

Many theories of ALS pathogenesis have been proposed, including oxidative stress, excitotoxicity, mitochondrial dysfunction, defective axonal transport and abnormal protein aggregation. The weight of evidence at present favours mitochondrial dysfunction acting with excitotoxicity to cause abnormal protein precipitation as key steps towards the final common path of neurodegeneration via an apoptotic mechanism. The aetiology is likely to be multifactoral, involving interplay of several mechanisms to initiate disease and propagate the spread of motor neuron cell death. In this review, we summarise the main theories, discuss some of the recent work aimed at identifying effective therapies and outline current areas of intense research activity.

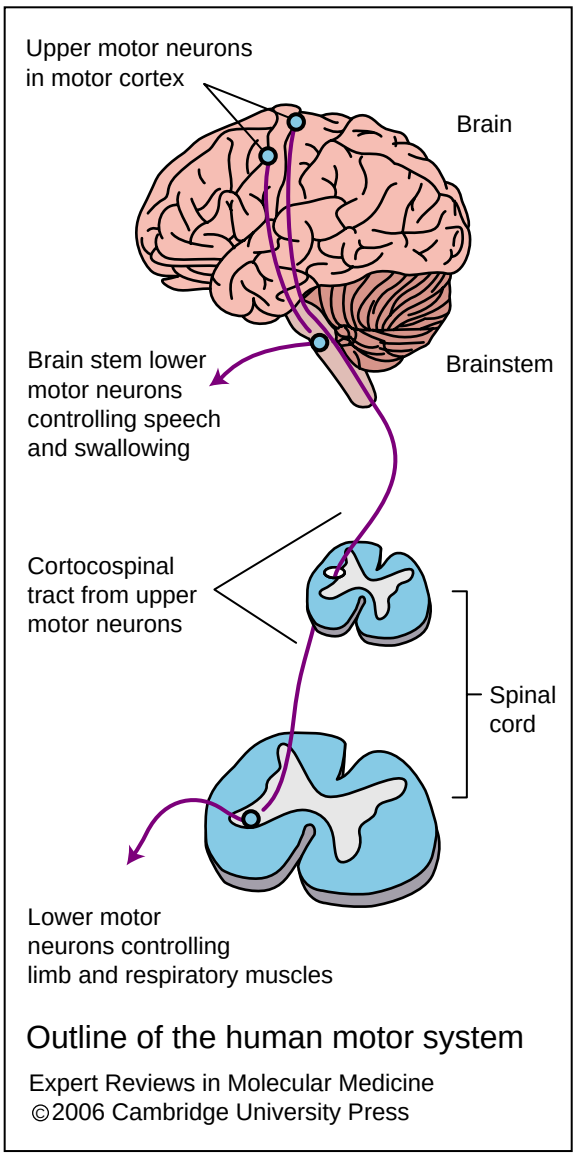


**Figure 1. Outline of the human motor system.** Upper motor neuron cell bodies are situated in the motor cortex and project axons via the corticospinal tracts to the spinal cord. There they synapse in the anterior horn with lower motor neurons, which project axons via peripheral nerves that then contact muscle fibres at the neuromuscular junction. Lower motor neurons originating in the brain stem that control speech and swallowing (bulbar motor neurons), and lower motor neurons that originate in the spinal cord that control limb and respiratory muscles, may both be affected. Damage to various combinations of upper and lower motor neurons occurs in human amyotrophic lateral sclerosis.



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**Table 1. Pathological features seen in amyotrophic lateral sclerosis**

| Feature | Description/location |
|---|---|
| Loss of motor neurons | Evident in cortex, brain stem and spinal cord |
| Axonal spheroids | Neurofilament aggregates in proximal axons of motor neurons |
| Bunina bodies | Eosinophilic bodies found in the soma of anterior horn cells; these are unique to amyotrophic lateral sclerosis |
| Ubiquitinated inclusions<br>'Skein-like' inclusions<br>'Lewy body-like' inclusions | Predominantly found in lower motor neurons<br>Threads/filamentous bodies<br>Compact, dense bodies |
| Hyaline inclusions | Large aggregates containing neurofilaments and other entrapped proteins in the soma of motor neurons |

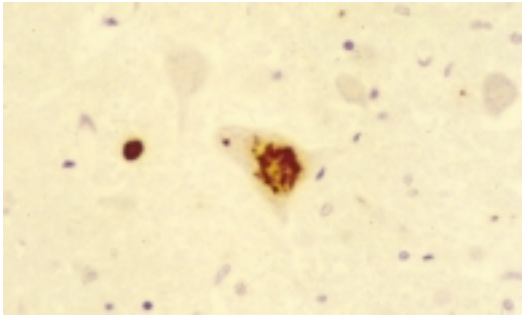
## Genetics

Between 5 and 10% of people with ALS have a history of the disease in first-degree relatives, most often compatible with autosomal dominant inheritance. The majority of familial cases are clinically and pathologically very similar to sporadic cases, leading to the hypothesis that they share common pathogenic mechanisms. Much current research involves studies of genetic factors in ALS, to seek loci and genes responsible for mendelian forms of the familial disease (FALS) and to identify genetic polymorphisms as risk factors in the more common sporadic ALS (SALS) (summarised in Table 2).

### *SOD1* mutations in FALS

A key discovery in the field was the identification of mutations in the Cu/Zn superoxide dismutase 1 (*SOD1*) gene as the cause of approximately 20% of FALS and therefore 2% of all cases (Ref. 6). SOD1 is part of the cellular defence against oxidative stress; it catalyses the conversion of superoxide anions into hydrogen peroxide, which is then further metabolised. More than 100 mutations in *SOD1*, distributed throughout the gene, have been found in FALS patients (Ref. 7). An updated database of these can be found at http://www.alsod.org.

It is still not known why the mutant form of this abundant and ubiquitously expressed enzyme should be particularly toxic to motor neurons and cause ALS. Over a decade of extensive study has provided strong evidence for a toxic 'gain of function' rather than a loss of enzymatic function of the mutant enzyme. SOD1 enzymatic activity varies greatly depending on which mutation is present and does not correlate with disease severity, with some mutant forms maintaining full activity (Ref. 7). Mice with the *SOD1* gene knocked out do not develop overt ALS (Ref. 8), while transgenic mice overexpressing mutant forms of the SOD1 protein (mSOD1) develop an adult-onset progressive motor neuropathy phenotype (Ref. 9). They have become



Ubiquitinated inclusions in a motor neuron cell body from a patient with amyotrophic lateral sclerosis

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**Figure 2. Ubiquitinated inclusions in a motor neuron cell body from a patient with amyotrophic lateral sclerosis.** Part of a section through the ventral horn of the cervical spinal cord, obtained post mortem, is shown. The section is stained with anti-ubiquitin immunoperoxidase and counterstained with haematoxylin. Original magnification, 400×. (Photograph courtesy of Dr Martyn Carey, University Hospital Birmingham NHS Foundation Trust, Birmingham, UK.)



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**Table 2. Genetic factors in amyotrophic lateral sclerosis[a]**

| Disease | Locus | Gene | Inher. | Onset | Features | Refs |
|---|---|---|---|---|---|---|
| ALS1 | 21q22.21 | *SOD1* | AD/AR | Adult and juvenile | ALS phenotype; varies depending on mutation | 6 |
| ALS2 | 2q33 | Alsin | AR | Juvenile | ALS and PLS; slow progression | 15 |
| ALS3 | 18q21 | Unknown | AD | Adult | ALS phenotype | 145 |
| ALS4 | 9q34 | Senataxin | AD | Juvenile | Slow progression | 18 |
| ALS5 | 15q15.1-q21.1 | Unknown | AR | Juvenile | No pseudobulbar signs; slow progression | 146 |
| ALS6 | 16q12 | Unknown | AD | Adult | Short duration | 147, 148 |
| ALS7 | 20ptel | Unknown | AD | Adult | Short duration | 148 |
| ALS8 | 20q13.33 | VAPB | AD | Adult | Slow progression | 149 |
| ALS–FTD | 9q21-22 | Unknown | AD | Adult | ALS with fronto-temporal dementia | 150 |
| ALS with Parkinsonism and dementia | 17q21 | MAPT | AD | Adult | ALS disorder with Parkinsonism and dementia | 151 |
| Progressive lower MND | 2q13 | DCTN1 | AD | Adult | Lower motor neuron disorder | 78 |
| Sporadic ALS | 6q12 | VEGF | Risk factor | | ALS | 22 |
| | 22q12.1-q13.1 | Neurofilament heavy chain | Risk factor | | ALS | 19, 20, 21 |
| | MtDNA | Deletions | Risk factor | | ALS | 23, 24 |
| | 6q21.3 | HFE | Risk factor | | ALS | 25 |
| | 14q11.2 | Angiogenin | Risk factor | | ALS | 26, 27 |
| | 19q13.2 | ApoE (ε4) | Risk factor | | ALS | 152 |

[a] Loci for mendelian forms of ALS are listed followed by genes in which polymorphisms have been identified as risk factors in sporadic ALS.

Abbreviations: AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; ApoE; apolipoprotein E; AR, autosomal recessive; DCTN1, dynactin p150 subunit; FTD, fronto-temporal dementia; HFE, haemochromatosis gene (involved in iron metabolism); Inher., inheritance; MAPT, microtubule-associated protein Tau; MND, motor neuron disease; MtDNA, mitochondrial DNA; PLS, primary lateral sclerosis; SOD1, superoxide dismutase 1; VAPB, vesicle-associated membrane protein; VEGF, vascular endothelial growth factor.

the most popular and widely accepted animal model of ALS. Transgenic rats overexpressing various human *mSOD1* genes have also now been developed that likewise show features similar to the human disease (Refs 10, 11).

There are many theories as to why mSOD1 is toxic, including enhanced oxidative stress from aberrant free radical production, and protein misfolding leading to abnormal aggregation. There are also data to indicate that the toxicity of mSOD1 is non-cell-autonomous. Motor-neuron-specific expression of mSOD1 does not produce ALS in mice and neurodegeneration is delayed or eliminated when motor neurons expressing mSOD1 are surrounded by wild-type cells (Refs 12, 13). Recent work in spinal cord lysates from mSOD1 mice suggests that mSOD1, but not wild-type SOD1, interacts with chromogranins



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(components of neurosecretory granules) and is secreted extracellularly by motor neurons from where it triggers microgliosis and neuronal cell death (Ref. 14). Other proposed roles of mSOD1 in disease pathways and mechanisms are discussed in the relevant sections below.

### Other FALS disease loci and genes

Genetic linkage studies in pedigrees in which motor neuron disorder phenotypes are segregating have identified various other disease-causing loci and genes. For example, mutations in alsin cause autosomal recessive juvenile-onset forms of ALS and the upper motor neuron variant primary lateral sclerosis, and mutations in senataxin cause a slowly progressive autosomal dominant disorder. Alsin encodes a protein with three putative guanine-exchange factor domains that may activate small GTPases and have a role in signal transduction (Ref. 15). Several groups have now generated alsin knock-out mouse models, but only mild neurological changes have been reported in these animals to date (Refs 16, 17). Senataxin contains a DNA/RNA helicase domain, which may suggest mutations cause a defect in RNA processing (Ref. 18).

### Genetic association studies in ALS

Genetic association studies in ALS have provided important clues as to the principal pathways affected by disease. Examples of genetic polymorphisms that have been associated with ALS include deletions or insertions in the neurofilament heavy chain gene, deletions in the promoter of vascular endothelial growth factor (VEGF), mitochondrial DNA deletions, a polymorphism in the haemochromatosis gene *HFE* and, very recently, mutations in the angiogenin gene (see Table 2 and Refs 19, 20, 21, 22, 23, 24, 25, 26, 27). These are discussed in more detail below. As with all genetic association studies in complex disease, there is the potential for identifying spurious associations, for example because of small study sizes, population stratification and mis-matching of cases and controls (Ref. 28). The studies detailed above have generally been performed with large numbers of patients and appropriately matched controls, and have been reproduced in independent populations. In addition, there are plausible functional hypotheses, with some experimental evidence, to support the identified polymorphisms having roles in ALS pathogenesis.

## Exogenous factors in ALS

Figure 3 summarises some of the mechanisms considered to be important in ALS pathogenesis. As with other complex diseases, the aetiology of ALS is likely to involve both genetic and environmental factors. The only risk factors for ALS that have been consistently identified in epidemiology studies are increasing age, male sex and smoking (Ref. 29). Other agents implicated in some studies include agricultural work, exposure to lead or mercury, athletic activity, milk ingestion, work in the textile or plastic industries, mechanical trauma and exposure to welding or soldering (Ref. 30). Recently, military service in the Gulf and playing top-level Italian professional football have been investigated as risk factors but the evidence for either of these having a role remains controversial (Refs 31, 32).

Rare geographical foci of disease have previously been cited as providing supporting evidence for external factors in ALS pathogenesis, such as the neurotoxin beta-methylaminoalanine (BMAA), derived from cycad nuts, in the ALS Parkinsonism dementia complex (ALS–PDC) of Guam (Ref. 30). However, this ALS–PDC disorder shows pathological features consistent with a tauopathy rather than those typical of ALS, and the relevance of extrapolating environmental aetiologies based on this disorder to standard ALS has been questioned (Ref. 33).

Another exogenous factor for ALS that has received attention in the past is viral infection, given the selective vulnerability of motor neurons to certain viruses such as the poliovirus (Ref. 34). Recently, the existence of ALS-like syndromes in a small number of human immunodeficiency virus (HIV)-infected patients, and the discovery of enteroviral and retroviral sequences in ALS patients, have reopened the debate (Refs 35, 36, 37). The evidence for viral involvement remains contradictory, but more-sensitive technologies may yet find a place for it in ALS pathogenesis.

## Proposed pathogenic mechanisms in ALS

### Oxidative stress

Motor neuron damage as a result of oxidative stress is a key hypothesis in ALS. Oxidative damage increases with age so fits in with the middle-life onset of the disease. Several studies have confirmed the presence of elevated oxidative metabolism in ALS, such as the detection of increased biochemical markers of oxidative injury in post mortem samples from patients (Ref. 38).



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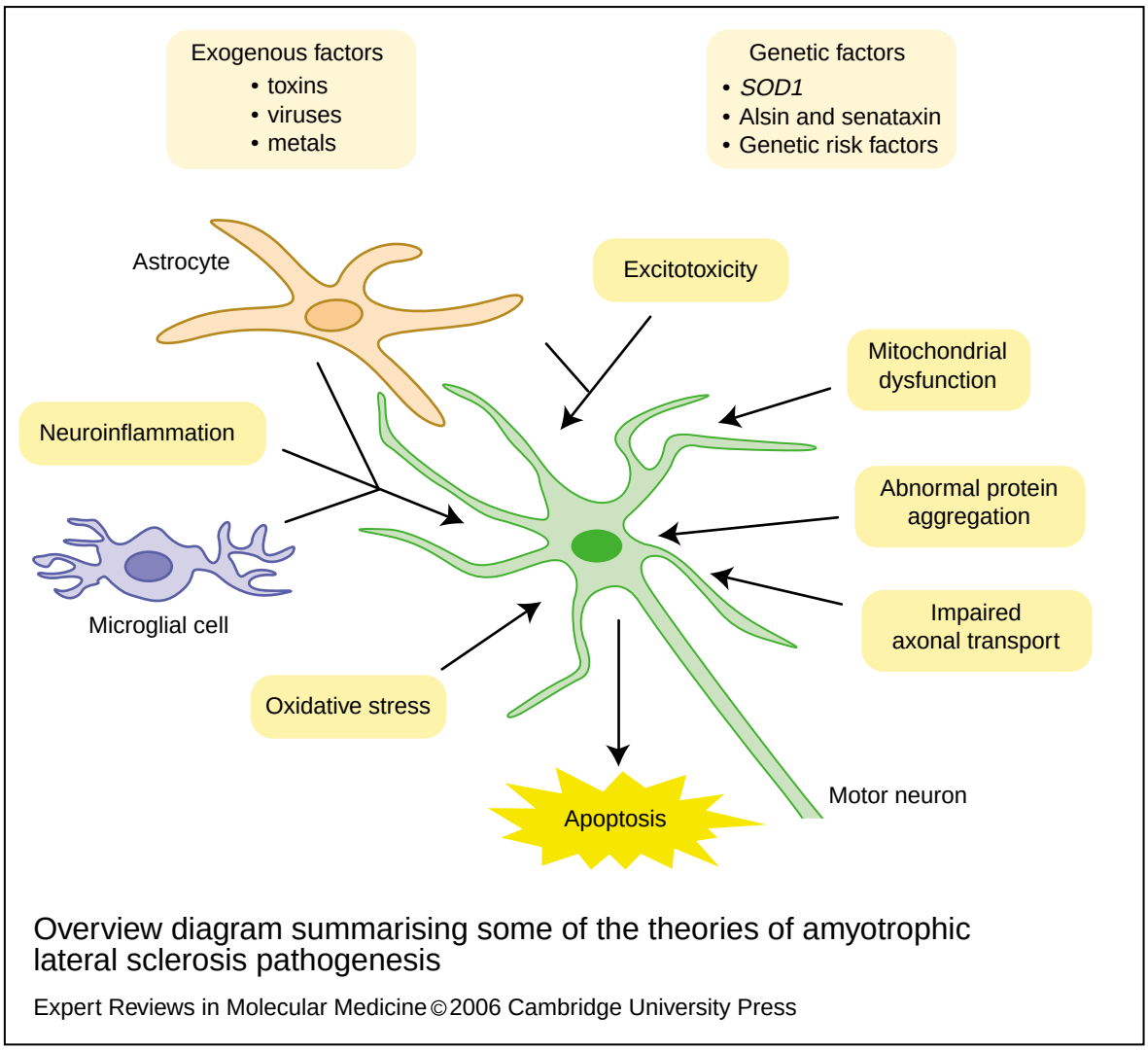


Overview diagram summarising some of the theories of amyotrophic lateral sclerosis pathogenesis



**Figure 3. Overview diagram summarising some of the theories of amyotrophic lateral sclerosis pathogenesis.** It is likely that different mechanisms act in different patients, with both genetic and exogenous factors being important. The final mechanism of motor neuron death in amyotrophic lateral sclerosis is likely to be via a process resembling apopotosis.

mSOD1 has the capacity to catalyse the production of reactive oxygen species (ROS) such as superoxide anions, peroxynitrite and hydroxyl radicals. mSOD1 transgenic mice show elevated levels of protein and lipid oxidation at both pre- and post-symptomatic stages (Ref. 39). Oxidative stress might also link with other proposed disease mechanisms such as excitotoxicity and axonal transport defects. Excitotoxicity leads to increased intracellular calcium, which in turn leads to increased nitric oxide formation. Peroxynitrite, generated by the reaction of superoxide anions and nitric oxide, can subsequently lead to oxidative damage (Ref. 40). Nitration may target neurofilament proteins, disrupting their phosphorylation and affecting axonal transport (Ref. 41).

## Excitotoxicity

Glutamate excitotoxicity is another mechanism implicated in ALS pathogenesis, via mechanisms that include disruption of intracellular calcium homeostasis and free radical production (Ref. 42). Sodium-dependent glutamate transporters tightly control glutamate levels in the synapse, particularly the glial cell excitatory amino acid transporter 2 (EAAT2) (Fig. 4a).

Glutamate levels in the cerebrospinal fluid (CSF) are elevated in at least a subset of ALS



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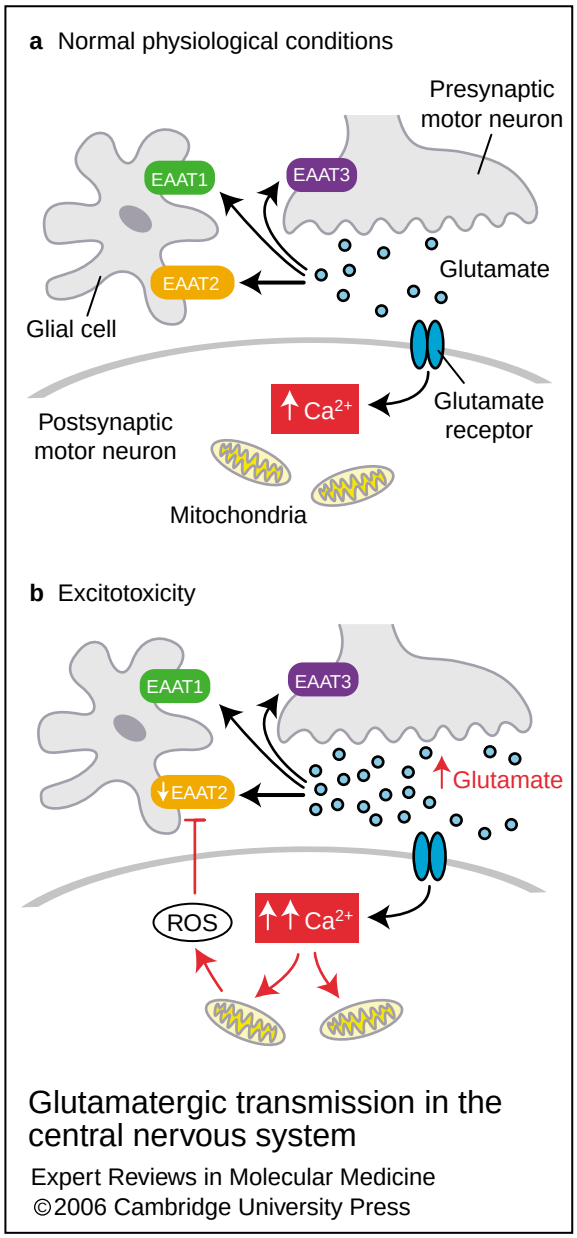


**Figure 4. Glutamatergic transmission in the central nervous system.** *(See next column for legend.)*

**Figure 4. Glutamatergic transmission in the central nervous system.** (a) A glutamatergic synapse under normal physiological conditions. Glutamate is released from the presynaptic nerve terminal and diffuses into the synaptic cleft. It acts on several glutamate receptors on the postsynaptic neuron. The action of glutamate in the cleft is terminated by its rapid reuptake via glutamate transporter proteins. EAAT1 and EAAT2 are expressed on glial cells; EAAT3 is mainly on presynaptic motor neurons. EAAT2 is responsible for most glutamate reuptake in the human brain. Under normal physiological conditions postsynaptic activation of glutamate receptors results in a small rise in intracellular calcium that can be buffered in the cell. (b) A glutamatergic synapse under conditions of excitotoxicity. When excess glutamate is present, there is a greater elevation in intracellular calcium postsynaptically. This triggers mitochondrial production of reactive oxygen species (ROS), which then inhibit glial EAAT2 function. This leads to further increases in glutamate concentrations in the synapse and further rises in postsynaptic calcium levels.

patients (Refs 43, 44). This elevation may be attributed to loss of EAAT2 function, as decreased levels of the transporter have been found in some post mortem ALS brains (Ref. 45). It is not clear how EAAT2 disruption occurs in ALS. mRNA levels of the transporter are normal and there are no genomic DNA sequence changes in EAAT2 in ALS patients (Refs 46, 47, 48). Reports of aberrant RNA processing of EAAT2 specifically in ALS patients have not been confirmed (Refs 49, 50).

The oxidative stress evident in ALS might also promote increased excitotoxicity, as glutamate transporters are particularly susceptible to disruption by oxidants, and oxidative modifications to the transporter have been reported in ALS and the mSOD1 mouse model (Refs 40, 51) (Fig. 4b).

## Abnormal protein precipitation/aggregation

Abnormal protein aggregates, including Bunina bodies, ubiquitinated inclusions and neurofilament-rich hyaline inclusions are pathological hallmarks of ALS (Ref. 52) (Table 1). There is ongoing debate as to whether these aggregates play a key role in pathogenesis, are harmless by-products of the degeneration process, are beneficial (via the sequestration of toxic proteins), or are harmful (via the sequestration of proteins essential to normal cellular function) (Ref. 53).

mSOD1 misfolds and forms aggregates, evident in FALS cases and in transgenic mSOD1 mice (Ref. 54). Other proteins, such as the copper chaperone for SOD1 (CCS), neuronal glutamate transporters, and heat shock proteins (HSPs) 70 and 40, co-immunoprecipitate with mSOD1 in these aggregates, lending weight to a harmful sequestering effect (Refs 55, 56). Partial inhibition of the proteasome in mSOD1 animals triggers large-aggregate formation, and the overexpression of chaperone proteins enhances survival (Refs 57, 58).



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## Mitochondrial abnormalities

In addition to providing energy through oxidative phosphorylation, mitochondria have roles in calcium homeostasis, free radical production and cell death pathways. These features, as well as an age-related decline in their function, have led to the hypothesis that mitochondrial dysfunction is important in ALS (Ref. 59). Mitochondria from ALS patients show abnormal morphology, elevated calcium levels and decreased activity of respiratory chain complexes I and IV (Ref. 60). One patient with an ALS-type disorder was found to have a mutation in cytochrome c oxidase, a complex IV subunit gene, encoded on mitochondrial DNA (mtDNA) (Ref. 61). There are also reports of multiple mtDNA deletions, diminished total mtDNA levels and a high frequency of mtDNA mutations in ALS (Refs 23, 24).

Cell culture studies seeking to determine the functional significance of mtDNA changes in ALS have been conducted using cytoplasm hybrid (cybrid) cell lines. Patient and control platelets (containing no nucleus) were fused with neuroblastoma cells (with depleted mitochondria) to create cybrid cells differing only in their mtDNA. The ALS cybrid cells had several defects including altered mitochondrial ultrastructure, decreased complex I activity and decreased calcium storage by the mitochondria (Ref. 62).

Vacuolated mitochondria are a striking and early feature of disease in some strains of mSOD1 mice, including the most commonly used G93A model. The degree of vacuolation correlates with the decline in muscle strength (Ref. 63). Studies using in vitro cellular models have also shown that mSOD1 disrupts mitochondrial morphology and function (Refs 64, 65).

## Cytoskeletal defects

As mentioned above, abnormal protein aggregation is a pathological hallmark in ALS. Neurofilament proteins (neuron-specific intermediate filaments) are the most abundant structural protein in mature motor neurons, and aggregates of neurofilament proteins in the cell body and proximal axons of motor neurons are commonly seen in ALS.

Experiments in transgenic mice have revealed a key role for neurofilaments in normal motor neuron function. Mice overexpressing human wild-type or mutant neurofilament protein develop motor neuropathies and undergo massive motor neuron cell death (Refs 66, 67, 68). Further experiments using the mSOD1 mouse model have produced some intriguing results. Increased expression of the neurofilament heavy chain delays disease onset and increases the survival of G37R SOD1 mice by as much as six months (Ref. 69). The reason for this remains unclear but two theories have been proposed. First, the accumulated neurofilament protein might provide a buffer for other deleterious processes such as increases in intracellular calcium from excitotoxicity or aberrant protein modification caused by oxidative stress (Refs 69, 70). Second, trapping neurofilament protein in the cell body might reduce the burden on axonal transport (Ref. 71).

Neurofilament heavy chain gene deletions are found in 1% of SALS cases (Refs 19, 20, 21). Mutations in the neurofilament light chain have been associated with forms of hereditary motor neuropathy (Refs 72, 73).

Peripherin and α-internexin are two other intermediate-filament proteins that co-localise with neurofilaments and form part of the axonal inclusion bodies in ALS. Overexpression of peripherin or α-internexin in transgenic mice causes motor neuron degeneration (Refs 74, 75). Peripherin is encoded by a single gene and has splice variants of 56, 58 and 61 kDa. Peripherin 61 is toxic to primary motor neuron cultures, even at low levels, and has been detected in the spinal cord of SALS patients (Ref. 76).

## Axonal transport

Axonal transport, both retrograde and anterograde, is especially important in motor neurons because of their large size. The mSOD1 mouse shows axonal transport defects as one of the earliest pathological features, and recent reports suggest this may even occur in embryonic development (Ref. 77).

The dynein–dynactin complex is involved in fast retrograde transport. Mutations in the p150 subunit of dynactin have been reported in a family with an unusual lower motor neuron disorder that begins with vocal cord paralysis (Ref. 78). Neuronal expression of this p150 'glued' dynactin mutant in transgenic mice causes a progressive motor neuron disorder with up to 60% of motor neurons lost by the end stage of disease (Ref. 79). Furthermore, mice overexpressing the p50 subunit of dynactin have reduced axonal transport and develop progressive motor neuron degeneration (Ref. 80). The 'legs at odd angles' (LOA) mouse model also shows defects in axonal transport and motor neuron degeneration, caused by mutations in the dynein heavy chain (Ref. 81). Interestingly,



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crossing this mouse with the mSOD1 mouse results in amelioration of the disease, later onset and increased survival (Ref. 77), an effect similar to that seen when the neurofilament heavy chain gene is overexpressed in mSOD1 mice, as mentioned above (Ref. 69).

The kinesin proteins are important components of anterograde axonal transport. Mutations in the genes encoding kinesin proteins have been found in human cases of hereditary spastic paraplegia (SPG10) and in forms of hereditary motor and sensory neuropathy but have not yet been described in any ALS cases (Refs 82, 83).

## Neuroinflammation

Microglia are the immune cells of the central nervous system (CNS) and have a complex relationship with neurons as they can be both protective and cytotoxic. In the healthy brain microglia exist in a resting state, defined by small cell bodies and minimal expression of surface antigens. Upon injury they are rapidly activated, releasing a range of factors such as glutamate, nitric oxide, ROS, proinflammatory cytokines and prostaglandins (Ref. 53). Studies of post mortem ALS brain and spinal cord tissue suggests potent activation and proliferation of microglia in the areas of the CNS affected by disease (Ref. 84). The same is true for the mSOD1 mouse model, with prolonged and sustained microglial activation before symptom onset and throughout the course of disease (Ref. 85). Mediators released by activated microglia also have effects on astrocytes, causing them to down-regulate expression of neurotrophic factors and release additional neuroinflammatory mediators that further activate microglia, thus creating a potentially lethal cycle (Ref. 86).

Cyclooxygenase 2 (COX-2) is a crucial enzyme in the production of prostaglandins and thus has an important role in neuroinflammation. Elevated levels of COX-2 and one of its key downstream products, prostaglandin $E_2$ ($PGE_2$), have been measured in ALS post mortem tissue and mSOD1 mice (Refs 87, 88). Several studies have shown a link between glutamate and increases in neuronal COX-2 levels. Glutamate receptor antagonists inhibit COX-2 activation in neurons and COX-2 inhibitors can attenuate glutamate-stimulated prostaglandin production (Ref. 89). Transgenic mice overexpressing COX-2 in neuronal cells show increased sensitivity to excitotoxicity (Ref. 90). In addition, COX-2 can produce ROS as a by-product of prostaglandin production, contributing to oxidative stress (Ref. 91).

In summary, although there is increasing evidence for a role of neuroinflammation in ALS, it is unclear whether this is a primary cause or a consequence of neurodegeneration. A function of such inflammation in the propagation of disease via the release of various mediators is an attractive hypothesis.

## Abnormalities in hypoxia-regulated genes

Recent research has highlighted the potential involvement of hypoxia-regulated genes in ALS pathogenesis. This stems from an unexpected discovery in transgenic mice with a targeted deletion of the hypoxia-response element of the VEGF gene. Researchers studying the relevance of hypoxic regulation of VEGF-dependent angiogenesis found that these mice developed a late-onset, progressive neurological disorder with ALS-like neuropathology (Ref. 92). A large genetic study subsequently revealed associations between polymorphisms in the promoter and leader sequences of the VEGF gene and increased risk of developing ALS in Belgian, Swedish and UK (Birmingham) populations (Ref. 22). Other studies have not replicated these findings, due in part to differences in the frequency of the 'at risk' haplotype in the controls from the different populations (Refs 22, 93, 94). VEGF is thought to influence motor neurons via direct neurotrophic effects and via its action to maintain blood flow to highly metabolically active motor neurons (Ref. 22). The reduced vascular perfusion of neurons that occurs with age, the increased frequency of ALS in males (about 1.6:1), who are more likely to develop vascular disease compared with premenopausal females, and the increased risk of ALS developing in smokers compared with non-smokers provide circumstantial support for this mechanism (Refs 29, 95).

Angiogenin is another hypoxia-induced angiogenic factor with similar functions to VEGF. Very recent studies have revealed that mutations in this gene are risk factors in SALS, particularly in patients of Scottish and Irish descent, and are causative in some FALS cases (Refs 26, 27).

## Apoptosis

Most evidence points to the final mechanism of cell death in ALS being by a programmed cell-death pathway with features resembling apoptosis (Ref. 96). Apoptosis is an energy-dependent



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process characterised by cell shrinkage, condensation of the cytoplasm and nucleus, membrane blebbing, DNA fragmentation and the maintenance of organelle integrity. While morphological studies in post mortem spinal cords and motor cortex from ALS patients have shown many of these features, not all are found in all studies (Ref. 96). This may be because, although motor neurons are dysfunctional for long periods in the disease, cell death occurs very rapidly once apoptosis is activated, leaving only a short time frame for detection of apoptotic markers (Ref. 97).

Apoptosis can be triggered by three mechanisms: activation of cell-surface receptors of the tumour necrosis family such as Fas; cytochrome c release from the mitochondria; and stress to the endoplasmic reticulum. These are all tightly controlled via a wide range of proteins including the caspase family of cysteine proteases, the Bcl-2 family of pro- and anti-apoptotic proteins, and inhibitors of apoptosis proteins (IAPs). Members of these key protein families have been intensely studied in the mSOD1 models and in human ALS.

In mSOD1 mice there is sequential activation of at least two caspases, and broad-spectrum caspase inhibitors extend survival (Refs 98, 99). Motor neurons from the mice show increased susceptibility to Fas signalling (Ref. 100). There are increases in the pro-apoptotic Bax and Bad proteins and a decrease in the protective Bcl-2 protein (Ref. 101). Increased expression of Bcl-2 in the model slows disease progression and extends survival (Ref. 102). Furthermore, mSOD1 protein, but not wild type, binds and aggregates Bcl-2 in the spinal cord mitochondria of transgenic mice, providing a direct link between mitochondrial apoptotic pathways and mSOD1 toxicity (Ref. 103). IAPs suppress caspase activity and protect cells from apoptosis. Levels of mRNA for X-linked inhibitor of apoptosis protein (XIAP), one of the most potent apoptosis inhibitors, are reduced in symptomatic mSOD1 mice and the protein is inactivated in end-stage disease (Ref. 104). When expressed in the spinal motor neurons of mSOD1 mice, XIAP delayed the onset of disease and slowed progression (Ref. 105).

In human ALS spinal cord tissue, increases in caspase-1 and -9 activation have been detected (Refs 98, 105). Alterations in the balance and subcellular compartmentalisation of pro- and anti-apoptotic members of the Bcl-2 family in a direction favouring apoptosis have also been detailed (Refs 106, 107).

## Selective vulnerability

Understanding why motor neurons are particularly vulnerable in ALS may be important for deciphering pathogenic mechanisms and finding treatments. Significant features are likely to include the extreme size of motor neurons, their high metabolic activity, their sensitivity to mitochondrial dysfunction, their elevated neurofilamnet content and their reduced capacity to buffer calcium (Ref. 108).

Motor neurons contain a large number of calcium-permeable AMPA glutamate receptors and lack the calcium-binding proteins pavalbumin and calbindin V28K, making them susceptible to excitotoxic damage (Ref. 108). AMPA channels are composed of several different subunits. The GluR2 subunit, which blocks calcium permeability, is not present in most motor neurons. This means that calcium can rapidly enter the cell through these channels, increasing the intracellular concentration and inducing ROS production by the mitochondria (Ref. 109). As discussed above, glutamate transport proteins may be damaged by oxidative mechanisms and this can lead to a feed-forward hypothesis of disease propagation. Excess glutamate causes increased ROS production and oxidative stress that in turn damages the glutamate transport proteins, resulting in even more extracellular glutamate and oxidative stress (Ref. 40) (Fig. 4b).

Recent work has also suggested a specific role for mitochondria in calcium buffering in motor neurons. Mitochondrial preparations from motor neurons take up large amounts of calcium in response to high intracellular calcium concentrations. This reaction is not seen in mitochondrial preparations from other cell types such as forebrain GABAergic neurons that are well-calcium-buffered, and are unaffected in ALS (Ref. 110).

## Clinical implications and trials

Clinical trials have been performed in ALS for many years, with over 20 trials reported in the last decade (Ref. 111). As yet, the only agent that has been shown to confer improved survival in ALS is riluzole (Rilutek®; Aventis Pharma SA, France) (Refs 112, 113). The benefit is modest, prolonging life for an average of about 3 months if the drug is taken for 18 months. Riluzole does



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not represent a cure, nor even a very effective treatment, and the search for better therapeutic agents continues.

Clinical trials in ALS are difficult and expensive to perform. Trials must involve many sites to allow enrolment of sufficient numbers for statistical validity. Various modifications to trial design have been proposed to try to maximise the power of information obtained with the minimum number of patients, including the recently suggested use of futility or non-superiority tests for Phase II studies (Ref. 114). The disease is fortunately fairly rare, but this means the incentive for large pharmaceutical companies to direct resources to ALS is not great. Advances in patient care, such as assisted ventilation via nasal intermittent positive-pressure ventilation, care in multidisciplinary teams and tube feeding have improved survival such that historical controls are not valid (Refs 1, 2). Survival or the requirement for assisted ventilation are now generally agreed as the most robust end-points in trials, rather than functional measures of muscle strength, which can be difficult to standardise. There is still no reliable surrogate marker for early disease in ALS. Most researchers believe that therapeutic agents will be of most benefit if given early in the disease. Various neuraxis imaging modalities [magnetic resonance imaging (MRI), diffusion MRI, functional MRI, and single photon emission computed tomography (SPECT)] have been examined, but no specific and sensitive imaging features to allow early diagnosis have been identified (Ref. 115). Likewise, no serum or CSF markers of early disease have yet been found (Ref. 116).

## Use of data from mouse and cell models of ALS

Many of the agents undergoing clinical trials in ALS have shown good effects in the mSOD1 mouse model, both in reducing the rate of disease progression and in prolonging survival. However, the benefits in the mouse have translated into clinically efficacy only in the case of riluzole. Possible reasons for this have recently been reviewed (Ref. 117). These include (1) difficulties in extrapolating equivalent doses from mouse to man, (2) species differences in the anatomy of the motor system, the permeability of the blood–brain barrier, the ratio of neurons to glial cells and in the immune response, and (3) the fact that the mSOD1 rodent models involve huge overexpression of the human mSOD1 gene [17-fold overexpression in the G85R mSOD1 mouse and 40-fold overexpression in the G93R mouse according to a recent paper (Ref. 118)] compared with the single gene dose effect in human mSOD1-mediated FALS. There is clearly scope for developing further animal models that more closely resemble the human disease. Many of the drug trials in the mouse model report results on giving the agents early, before the mice develop clinically apparent weakness, which is of questionable relevance in human ALS in the absence of an early identified surrogate marker for disease. We recommend that human trials should be performed only when agents have shown benefit in animal models when given after disease onset and when such benefit has been replicated in different laboratories.

The Neurodegeneration and Drug Screening Consortium was recently established in the USA by the National Institute of Neurological Disorders and Stroke (NINDS) to screen compounds of potential benefit in various neurological diseases, including ALS (Ref. 119). Twenty-six laboratories tested 1040 compounds in 29 different in vitro and in vivo assays, some of which assessed pathogenic pathways of potential relevance in ALS such as oxidative stress and excitotoxicity (Ref. 119). One promising result from this screen was the identification of beta-lactam antibiotics, including penicillin and certain cephalosporins, to stimulate expression of rat glutamate transporter GLT-1 (equivalent to human EAAT2) mRNA in neuronal tissue (Ref. 120). Clinical trials of ceftriaxone, the longest-acting beta-lactam antibiotic, are now under way in ALS (see http://www.alsa.org).

## Previous and ongoing trials

Some of the compounds assessed in completed and ongoing clinical trials are listed in Box 1. Trials of cocktails of therapies – combining agents that act on different proposed mechanisms, such as minocycline, riluzole and nimodipine – have given excellent results in the mouse model (Ref. 121). It seems likely that successful therapy in ALS will similarly involve a mix of agents, acting synergistically on various mechanistic pathways to interrupt the final common pathway of motor neuron degeneration.

## Antioxidants and mitochondrial agents

Many antioxidants, such as vitamin E, *N*-acetyl-L-cyteine (NAC) and catalase, have shown benefit



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in the mSOD1 mouse model, but not unfortunately in human ALS clinical trials (Ref. 122) (Box 1). Creatine, an agent that promotes glutamate uptake into synaptic vesicles and stabilises the mitochondrial energy transfer complex through inhibiting the opening of the mitochondrial permeability transition pore, showed great promise when administered orally to mSOD1 mice, with even greater improvement in survival to that seen with riluzole (Ref. 123). Unfortunately, three large, placebo-controlled trials in human ALS have recently reported no benefit of the agent (Refs 124, 125, 126).

Coenzyme Q10 is a co-factor of the electron-transport chain and functions as a free radical scavenger in mitochondrial membranes. It also prolongs survival in mSOD1 mice, and a double-blind controlled trial in ALS patients is now under way (see http://www.alsa.org).

## Antiglutamatergic agents

The beneficial effect of riluzole in ALS is thought to be due to its antiglutamatergic effects – by inhibiting glutamic acid release, by non-competitive blocking of NMDA (*N*-methyl-D-aspartate) receptor-mediated responses, and via

### Box 1. Trialled therapies in amyotrophic lateral sclerosis[a]

#### Therapeutic agents previously trialled in amyotrophic lateral sclerosis

**Antioxidants**
Vitamin E
*N*-Acetyl-L-cysteine
Selegiline
D-Penicillamine

**Antiglutamatergic agents**
Riluzole[b]
L-Threonine
Branched chain amino acids
Dextromethorphan
Lamotrigine
Gabapentin
Topiramate

**Calcium regulators**
Verapamil
Nimodipine

**Mitochondrial enhancers**
Creatine

**Immunomodulatory and anti-inflammatory agents**
Cyclophosphamide
Cyclosporine
Celecoxib
Azathioprine
Total lymphoid irradiation
Intravenous immunoglobulin

**Neurotrophic agents**
Ciliary neurotrophic factor
Glial derived neurotrophic factor
Brain derived neurotrophic factor
Insulin-like growth factor
Thyrotrophin-releasing hormone
Xaliproden

**Antiviral therapies**
Guanidine
Guanidine and amantidine
Interferon-α
Isoprinosine

#### Agents currently undergoing clinical trials in amyotrophic lateral sclerosis

**Antiglutamatergic/anti-excitotoxic agents**
Ceftriaxone
Talampanel
Tamoxifen

**Antioxidants**
AEOL 10150

**Mitochondrial enhancers**
Coenzyme Q10

**Anti-apoptotic agents**
Minocycline
Sodium phenylbutyrate
Arimoclomol

**Immunomodulatory/anti-inflammatory agents**
Glatiramer acetate
Thalidomide

**Proteasome inhibitors**
Ritonavir

[a] Data from Refs 111, 122, 126 and 128, and websites http://www.alsa.org and http://www.alscenter.org.
[b] Riluzole is the only agent that has been shown to prolong survival in amyotrophic lateral sclerosis in randomised, placebo-controlled clinical trials.



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a direct action on voltage-dependent sodium channels (Ref. 127). Several other trials of agents targeted against glutamate excitotoxicity have sadly been disappointing. Such agents have included gabapentin, topiramate, verapamil, lamotrigine and dextromethorphan (Ref. 128). The identification of beta-lactam antibiotics in increasing EAAT2 expression is intriguing and the results of the recently begun clinical trial of ceftriaxone are keenly awaited (see above).

### Anti-inflammatory and anti-apoptotic agents

Much current interest is focused on the tetracycline antibiotic minocycline, as this has been the most effective agent in prolonging life in the rodent mSOD1 model (Ref. 129). The exact mechanism is unclear but thought to be via inhibiting microglial activation and modulating apoptosis (Ref. 130). The antibiotic has good CNS penetration when taken orally, and clinical trials in various neurodegenerative disorders, including ALS, are now under way (see http://www.alsa.org).

A trial of Copaxone (glatiramer acetate), widely used in the treatment of multiple sclerosis, has recently begun. In the mSOD1 model administration of the drug improved survival by almost 25% via the proposed mechanisms of increased T-cell derived interferon γ and enhanced glutamate transporter expression (Ref. 131).

Arimoclomol is one of a novel family of 'smart drugs', co-inducing the expression of HSPs only under times of cellular stress. Treatment with arimoclomol after symptom onset in mSOD1 mice delayed disease progression and increased survival, suggesting that chaperone induction may be a good target for effective therapy in humans (Ref. 132). Human trials are currently under way in the USA (see http://hedwig.mgh.harvard.edu/alsconsortium/trials.html).

### Growth factors

As the striking pathological feature in ALS is motor neuron loss, another key therapeutic strategy has been treatment with various nerve growth factors. Ciliary neurotrophic factor (CNTF), glial derived neurotrophic factor (GDNF) and insulin-like growth factor (IGF-1) all showed good effects in mSOD1 models, but trials in human ALS have been disappointing. IGF-1 showed a modest effect to slow progression of functional impairment and decline in health-related quality of life in an American trial, but this result was not confirmed in a subsequent European study (Refs 133, 134). A third large study is currently under way (see http://www.alsa.org). The disappointing results with neurotrophic factors may be due to their low bioavailability and poor delivery to motor neurons, factors that will potentially be overcome with new gene therapy approaches.

### Gene therapy

Gene therapy holds promise for ALS, as it has the potential to deliver treatments to damaged motor neurons and overcome the difficulty of crossing the blood–brain barrier. Many strategies are under investigation, including the delivery of genes encoding neurotrophic factors, anti-apoptotic drugs and antioxidants using a range of viral vectors administered by direct injection into the affected areas of the CNS, or remotely via intramuscular injection and retrograde transport to motor neuron cell bodies, or by ex vivo gene transfer (Ref. 135). At present, gene therapy approaches are directed to preclinical studies in the mSOD1 model. Retrograde adeno-associated virus (AAV) delivery systems for IGF-1 and GDNF have shown benefits in the mSOD1 mice (Refs 136, 137). Intramuscular injections of lentiviral-tagged VEGF have also proved successful in this rodent model (Ref. 138), and proof of principle studies to establish delivery of VEGF via this route in primates are under way. Intramuscular injection of an AAV vector containing the anti-apoptotic Bcl-2 gene has also been effective in the mSOD1 mouse (Ref. 139).

An alternative approach with gene therapy is to 'knock down' genes that might be causing degeneration, such as mSOD1. There are now successful reports, again in mSOD1 mice, of amelioration of disease using small interfering RNAs (RNAi) targeted to SOD1 (Refs 140, 141). While this method may prove valuable in treating mSOD1-mediated FALS, other targets will have to be found to treat the much more common, non-SOD1-mediated, sporadic disease.

### Stem cell therapy

Stem cell therapy in ALS has recently received a lot of publicity. There have been media reports of rapid beneficial effects in ALS patients of controversial treatments using fetal olfactory ensheathing cells in China, but none of these 'successes' has been confirmed (Ref. 142). A UK



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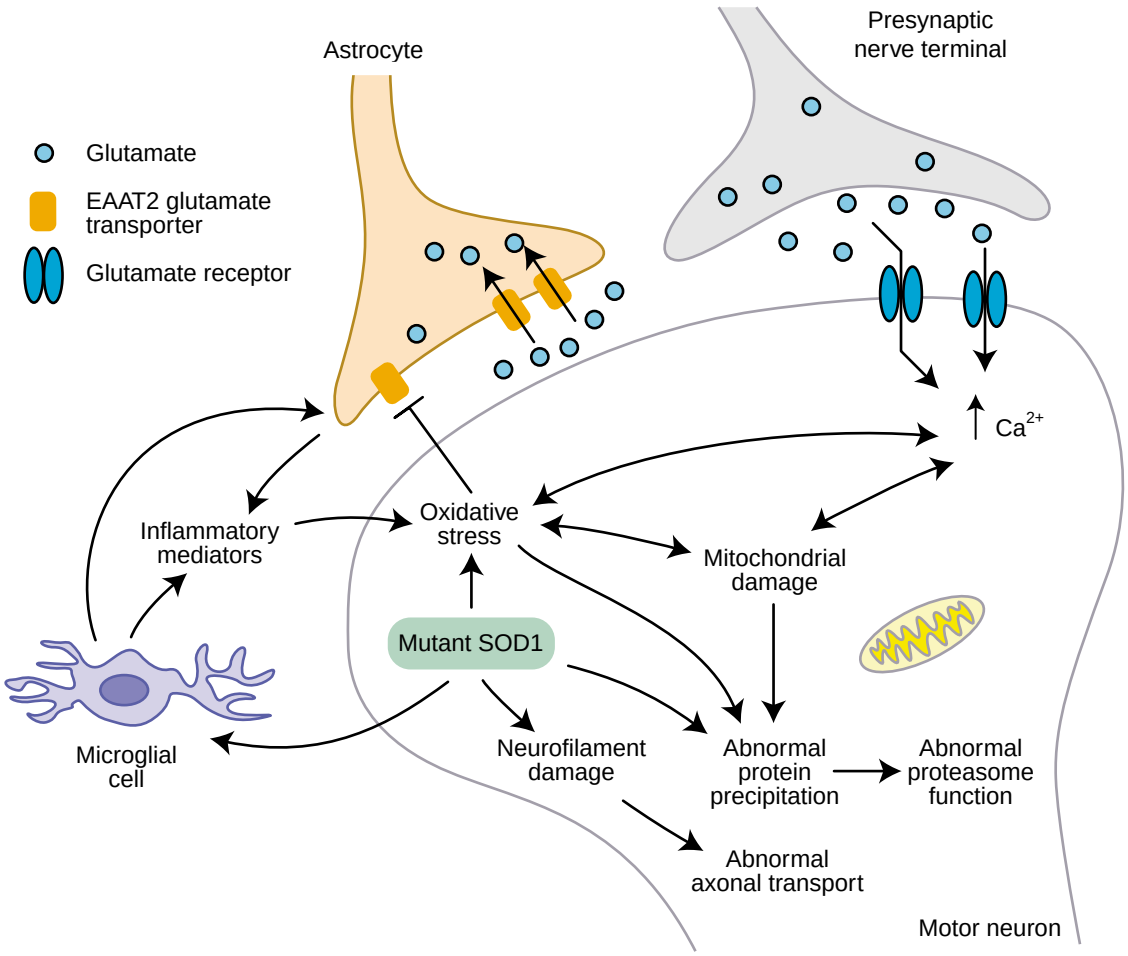


Model to show how some of the proposed mechanisms in amyotrophic lateral sclerosis could interact to cause motor neuron degeneration



**Figure 5. Model to show how some of the proposed mechanisms in amyotrophic lateral sclerosis could interact to cause motor neuron degeneration.** Normal superoxide dismutase 1 (SOD1) functions as a detoxification enzyme, catalysing the conversion of superoxide ions to hydrogen peroxide, which is then converted to water by catalase and glutathione. Mutant SOD1, as seen in some familial amyotrophic lateral sclerosis (FALS) cases, may be toxic because it is unstable, forming aggregates in the motor neuron cytoplasm, axoplasm and mitochondria. These aggregates may interfere with normal proteasome function, and also with other cellular processes, such as neurofilament function, leading to impaired axonal transport and further toxic aggregation. Mutant SOD1 can also trigger oxidative reactions by various means including by increasing levels of peroxynitrite, which can then cause damage through the formation of hydroxyl radicals or via nitration of tyrosine residues on proteins. Mutant, but not normal, SOD1 has also been recently reported to be secreted extracellularly, from where it can trigger microglial activation. Oxidative stress impairs mitochondrial function and inhibits the function of EAAT2, the main glial glutamate transporter protein, responsible for most of the reuptake of synaptically released glutamate. Glutamate excess causes neurotoxicity by increasing intracellular calcium, which enhances oxidative stress and mitochondrial damage. Mitochondrial damage in turn leads to further oxidative stress, enhancing the pathogenic cascade. Microglial activation is seen in both familial and sporadic ALS. Inflammatory mediators released by activated microglia cause additional release of neuroinflammatory proteins from astocytes causing further inflammation and oxidative stress. Ultimately, neuronal cell death in ALS is thought to occur via a programmed cell-death pathway with features resembling apoptosis.



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licence for therapeutic human cloning in ALS research has been granted, but this is for work in cell culture to identify mechanistic pathways rather than for direct therapy using stem cells [see R0158 at http://www.hfea.gov.uk/Research/HFEAresearchlicences/]. Human embryonic stem cells can be coaxed to express key motor neuron genes in vitro but this is still a long way from showing that such stem cells can integrate into the central and peripheral nervous system to reconstitute damaged motor neuron pathways (Ref. 143). A more realistic prospect for stem cell therapy in human ALS may be to focus on glial stem cells, using them to deliver neurotrophic or other factors to modulate disease progression. Stem cell research, while exciting, is firmly in the realm of preclinical studies at present.

## Outstanding research questions

This review has highlighted various possible pathogenic mechanisms in ALS. Figure 5 summarises some of the main proposed mechanisms and outlines how they might interact. Their relative importance in initiating and then propagating the disease is not clear. Potentially effective therapeutic compounds are being identified from studies of pathogenic pathways and from high-throughput cell-culture and other model-system screens. While ALS is pathologically fairly homogeneous, different mechanisms are likely to be involved in individual patients. It is anticipated that successful therapy will involve combinations of agents acting on these different mechanisms, hopefully synergistically. The success of translating promising results from the best available animal model to patients with ALS has been limited to date, and more research towards better animal and cell models of the disease is encouraged. Therapy is likely to be most successful if started early in the disease and identification of early surrogate markers of ALS should be a research priority. Stem cell therapy is still a way off, and should remain so until appropriate preclinical studies have been performed.

In conclusion, as judged by the increasing numbers attending conferences such as the 16th International ALS Symposium, scientists and clinicians interested in ALS are working together to further research into this dreadful disease (Ref. 144). We predict that therapies that make a difference will be available within the next 10 years. Our patients deserve better than the few months of improved survival that riluzole offers.

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## Further reading, resources and contacts

**Publications**

Good reviews concerning pathogenic mechanisms in amyotrophic lateral sclerosis:

Rowland, L.P. and Shneider, N.A. (2001) Amyotrophic lateral sclerosis. N Engl J Med 344, 1688-1700, PubMed: 11386269

Cleveland, D.W. and Rothstein, J.D. (2001) From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat Rev Neurosci 2, 806-819, PubMed: 11715057

Two fairly recent books that detail clinical features, epidemiology, mechanisms and potential therapies in amyotrophic lateral sclerosis:

Leigh, P.N. and Swash, M. (1995) Motor neuron disease, Springer-Verlag, London, UK

Brown, R.H.J., Meininger, V. and Swash, M., eds (2000) Amyotrophic lateral sclerosis, Martin Dunitz, London, UK

**Websites**

The website of ALSA, the largest US national health organisation dedicated solely to amyotrophic lateral sclerosis, provides clinical information on the disease and updates of current research funded by the association and other organisations. It is particularly useful for up-to-date information on current US clinical trials:

http://www.alsa.org

The website of the MND Association, the main national UK organisation dedicated to the support of people with motor neuron disease and their carers, provides clinical and care information, summarises current and previous clinical trials, and provides updates of current research:

http://www.mndassociation.org

The Washington University website provides a frequently updated resource on clinical and research aspects of many neuromuscular diseases, including motor neuron diseases:

http://www.neuro.wustl.edu/neuromuscular/index.html

## Features associated with this article

### Figures



### Tables



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