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Impairments in Motor Neurons, Interneurons and Astrocytes Contribute to Hyperexcitability in ALS: Underlying Mechanisms and Paths to Therapy

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Abstract Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by the loss of motor neurons leading to progressive paralysis and death. Using transcranial magnetic stimulation (TMS) and nerve excitability tests, several clinical studies have identified that cortical and peripheral hyperexcitability are among the earliest pathologies observed in ALS patients. The changes in the electrophysiological properties of motor neurons have been identified in both sporadic and familial ALS patients, despite the diverse etiology of the disease. The mechanisms behind the change in neuronal signalling are not well understood, though current findings implicate intrinsic changes in motor neurons and dysfunction of cells critical in regulating motor neuronal excitability, such as astrocytes and interneurons. Alterations in ion channel expression and/or function in motor neurons has been associated with changes in cortical and peripheral nerve excitability. In addition to these intrinsic changes in motor neurons, inhibitory signalling through GABAergic interneurons is also impaired in ALS, likely contributing to increased neuronal excitability. Astrocytes have also recently been implicated in increasing neuronal excitability in ALS by failing to adequately regulate glutamate levels and extracellular K^+ concentration at the synaptic cleft. As hyperexcitability is a common and early feature of ALS, it offers a therapeutic and diagnostic target. Thus, understanding the underlying pathways and mechanisms leading to hyperexcitability in ALS offers crucial insight for future development of ALS treatments.

Keywords Hyperexcitability · Neuronal excitability · Amyotrophic lateral sclerosis · Excitotoxicity · Motor neurons

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Impact of Amyotrophic Lateral Sclerosis

Motor neurone disease (MND) describes a group of neurodegenerative diseases that cause the progressive loss of voluntary muscular control of the body [1]. The most severe form of MND is amyotrophic lateral sclerosis (ALS), which is characterised by the selective deterioration of the upper and lower motor neurons in the primary motor cortex and spinal cord [2]. All cases of ALS lead to progressive muscular weakness, often starting in the muscles of the limbs or face, with continued deterioration of motor neurons eventually leading to respiratory failure [1]. Based on a European epidemiology study conducted in 2010, the lifetime risk of ALS for women and men is 1:400 and 1:350, respectively, with an average age of onset between 50 and 60 years [3, 4]. In most cases, ALS progresses quickly, and patients succumb to the disease within 2–3 years after diagnosis. To date, the only available treatment for ALS is Riluzole, which prolongs life for approximately 2–3 months [5, 6]. Early diagnosis and intervention of ALS are central to slowing disease progression and improving the quality of life. However, current diagnostic tools lack definitive biomarkers and instead have to rely on clinical presentation of symptoms, as described by the El Escorial criteria [7]; thus, a diagnosis can potentially be delayed by up to 14 months [8, 9]. With the purpose of timely intervention in ALS, it is important to consider not only the biochemical changes in the

motor system during disease progression but also the changes prior to the onset of physical disease symptoms.

ALS: a Multifactorial Disease

The difficulty in diagnosing ALS and finding definitive biomarkers is due to differences in symptom presentation and speed of disease progression. The clinical heterogeneity present in ALS highlights the complexity of this neurodegenerative disease. For familial ALS (fALS), which accounts for 10% of ALS cases, more than 30 genes have already been implicated [10], with mutations in *C9ORF72* and *SOD1* accounting for a total of 52% of all fALS cases [11, 12]. However, it is estimated that 32% of genes associated with fALS are still unknown [12]. In the case of sporadic ALS (sALS), which accounts for the remaining 90% of ALS cases, a combination of environmental factors and genetic predisposition cause the onset of ALS. Presently, the genetic etiology of about 11% of sporadically occurring ALS cases is known [12]. As the list of implicated genes increases, it has become apparent that these mutations impact various cellular functions, such as the antioxidant response, protein degradation and RNA processing (reviewed by Robberecht and Philips [13]). It is important to study the individually affected pathways to comprehend ALS progression; however, better treatments may be developed through investigation of commonly shared pathologies.

Hyperexcitability Is a Common Feature of ALS

As ALS is a multifaceted disease, the biochemical pathways that cause motor neuron degeneration may differ somewhat between patients; however, there are common pathologies and symptoms that are shared amongst ALS patients. A well-documented pathophysiological feature observed in ALS patients is hyperexcitability [14]. In electrophysiological terms, hyperexcitability describes an increase or exaggerated response to a stimulus. The assessments of clinical and neurophysiological features in different fALS cohorts with *SOD1*, *FUS* or *C9ORF72* mutations showed that despite different genetic discrepancies, hyperexcitability was a common pathology [15–17]. Moreover, hyperexcitability has also been observed in large sALS cohorts [15, 17, 18]. The factors that instigate the changes in neural excitability over the course of disease onset and progression are not well understood as of yet.

Clinical Measurement of Hyperexcitability

ALS patients often present with fasciculations and cramps months prior to the clinical onset of ALS [14, 19]. These symptoms suggest an increase in neural activity and excitability and have been included into the diagnostic criteria as an early marker for ALS [20]. To further investigate these changes throughout disease progression in vivo, several clinical techniques are currently used, including transmagnetic stimulation (TMS) and nerve excitability test (NET). TMS provides a means to magnetically stimulate the primary motor cortex to produce electrical currents and measure the resulting evoked muscle responses using an electromyogram (EMG). Unlike TMS, NET directly applies an electrical stimulus to a desired nerve and measures the evoked response at the appropriate muscle; this method is generally used to assess peripheral nerve excitability.

Cortical Hyperexcitability

Vucic and Kiernan [21] assessed neuronal conductivity on 23 MND patients with upper limb, lower limb or bulbar onset and 36 control subjects. For the TMS studies, motor-evoked potentials (MEP) were recorded from the abductor pollicis brevis. Initial TMS tests consisted of determining the unconditioned suprathreshold stimulus needed to produce and maintain a predefined MEP. This measurement is referred to as resting motor threshold (RMT). Two subgroups became apparent in this study: MND patients with severe upper motor neuron involvement showed an increase in RMT, whereas less-progressed cases showed a decrease in RMT compared to controls. This suggests that a smaller stimulus is needed to generate a response in less-progressed MND patients. In other words, patients in the early stages of MND showed a hyperexcitable motor cortex, whereas patients in the later stages of MND exhibited a hypoexcitable motor cortex, compared to the control group [21]. Concurrent with these findings, a separate TMS study in 24 ALS patients during the early stages of disease onset also found a reduction in patient RMT [22]. It was also found that ALS and MND patients early in disease progression present higher MEP amplitudes (i.e. bigger response) compared to controls (given an equivalent TMS stimulus), which is again suggestive of cortical hyperexcitability [21–23]. Assessing the inhibitory function of the cortex provided further insight into the establishment of cortical hyperexcitability in ALS patients. It was previously determined that a transcranial subthreshold stimulus could suppress the response of a following suprathreshold stimulus [24]. It is thought that the subthreshold stimulus activates low-threshold inhibitory circuits and thus increases the stimulus threshold to elicit an evoked response [25]. This phenomenon is referred to as short intracortical inhibition (SICI). Using TMS, ALS patients and control subjects were exposed to a subthreshold conditioning stimulus followed by a suprathreshold stimulus. It was found SICI was significantly lower in ALS patients compared to control subjects, i.e. the threshold to elicit an MEP was significantly reduced after

inhibitory stimuli [17, 21, 22, 26]. Consistent with MEP and RMT findings, SICI was significantly reduced in ALS patients with less severe symptoms. These findings indicate that cortical hyperexcitability occurs early in the disease and potentially dissipates with disease progression. Intriguingly, a 3-year longitudinal study of three pre-symptomatic *SOD1* mutation carriers, who developed clinical symptoms within 3 months of the study, found that SICI was completely absent in two patients and significantly reduced by 32% in the third subject at initial examination, suggesting there are changes in cortical excitability prior to the onset of symptoms [15]. Annual follow-ups showed a gradual reduction in SICI in the pre-symptomatic patients. In addition to a reduction in SICI, RMT was decreased in two pre-symptomatic patients; no increase in MEP amplitude was observed compared to the control group [15]. Unfortunately, due to the small sample size, it cannot be concluded whether cortical hyperexcitability is established prior to clinical onset. However, it is now well established that cortical hyperexcitability occurs early in disease progression and could thus offer a potential diagnostic and therapeutic target, although the mechanisms behind this phenomenon remain unknown.

Peripheral Hyperexcitability

Further exploration into neuronal excitability of ALS patients has revealed that in addition to cortical hyperexcitability, peripheral axons also demonstrate changes in their excitability [27]. Measurements of membrane excitability are generally recorded at the abductor pollicis brevis after stimulation of the median nerve. To describe the excitability properties in ALS patients, three main measurements will be discussed: firstly, the strength-duration constant (τ_{SD}) of an axon, which describes the rate at which the action potential current decreases [28]; secondly, the threshold electrotonus, which measures the change in threshold potential subsequent to a depolarizing or hyperpolarizing subthreshold current [29]; and thirdly, the refractory period of membrane excitability following a suprathreshold stimulus.

Peripheral nerve excitability studies have demonstrated that ALS patients exhibit a longer τ_{SD}, suggesting ALS patients exhibit a longer duration of excitability compared to healthy subjects for equal strength stimuli [27, 30, 31]. Correlating with this finding, a measurement of patient rheobase, which is defined as the minimal current needed to elicit an action potential (i.e. a measure of membrane excitability), was decreased, though not significantly [27, 32]. Both these findings indicate the axonal membranes of ALS patients are more excitable. Additionally, several threshold electrotonus studies demonstrated that the threshold potential following a conditioning subthreshold stimulus is significantly reduced in the majority of ALS patients [27, 29, 33]. The reduction of the threshold potential and thus increase in excitability are referred to as a type 1 response [30]. In some ALS cases, a subthreshold depolarizing pulse will lead to an increase in threshold potential, referred to as a type 2 response [30]. However, the type 2 response is a rare occurrence and has only been observed in rapidly deteriorating cases, whereas the type 1 response has been observed more frequently in both rapidly and slow-progressing cases of ALS [27, 30, 33]. Both type 1 and 2 responses are thought to underlie an imbalance in K^+ and Na^+ currents (the imbalance of K^+ and Na^+ is further discussed below). It is hypothesised that the type 1 response is due to a decrease in nodal K^+ conductance, whilst the type 2 response occurs due to regenerative depolarization [27, 30]. Further evidence suggesting the hyperexcitability of peripheral nerves in ALS was provided by studies focusing on the recovery cycle after a suprathreshold stimulus. The recovery cycle describes the axonal excitability after an action potential. Following an action potential in peripheral motor neurons, there is a period in which the axonal excitability increases. In ALS patients, the threshold potential during the superexcitability period is further reduced, indicative of increased excitability [27, 29, 33].

In addition to cortical hyperexcitability, there is convincing clinical evidence suggesting peripheral axon hyperexcitability is a common feature of ALS. It remains to be elucidated whether hyperexcitability is one of the causes of motor neuron degeneration or is a compensatory mechanism resulting from motor neuron degeneration. Nonetheless, studying the pathways and mechanisms of hyperexcitability will assist in the development of therapeutic agents to treat this disease.

Proposed Mechanisms of Hyperexcitability

As previously stated, ALS is a multifactorial disease. As such, it is likely that there are several contributing factors that could potentially cause cortical and peripheral hyperexcitability. The pathways that will be discussed in further detail are the following: the disruption of inhibitory circuits in the cortex, ionic channel dysfunction in ALS-derived motor neurons, dysregulation of K^+ homeostasis by astrocytes and glutamate-mediated excitotoxicity.

Disruption of Inhibitory Circuits

Interneurons in the CNS are one of the main regulators of neuronal signalling. The majority of interneurons in the cortex are inhibitory interneurons that use γ-aminobutyric acid (GABA) or glycine as a neurotransmitter. GABAergic interneurons play a major role in the regulation of neuronal excitability in the cortex. In healthy individuals, a subthreshold stimulus of the motor cortex generally can lead to the activation of inhibitory GABAergic interneurons, thus reducing subsequent excitability (SICI) [34]. However, in ALS patients, the reduction or complete

absence of SICI suggests either a dysfunction or loss of inhibitory interneurons (Fig. 1 (A)). A study of wobbler mice, an animal model of ALS, demonstrated a 72% reduction in GABA receptor-mediated inhibitory currents in layer 5 pyramidal neurons of the motor cortex [35]. Further studies on a mutant *Sod1* zebrafish model revealed that the first neuronal population displaying neuronal stress is interneurons and that the reduction of inhibitory currents or interneurons preceded any defects in motor neurons [36]. A recent study by Zhang and colleagues [37] used a *TDP-43^{A315T}* mouse model to demonstrate that impairments in GABAergic signaling contribute to cortical hyperexcitability. Moreover, the investigation of GABAergic signaling in this particular study revealed that somatostatin interneurons are hyperactive in *TDP-43^{A315T}* mice, compared to wild type. It remains to be demonstrated how hyperactive somatostatin interneurons are linked to cortical hyperexcitability. The authors proposed that hyperactive somatostatin interneurons disinhibit neurons in the motor cortex by inhibiting parvalbumin interneurons [37]. However, currently, there is no convincing evidence showing the direct interaction between somatostatin and parvalbumin interneurons in the motor cortex, thus questioning the validity of the proposed mechanism.

In regards to human findings that suggest interneuron involvement in ALS, post mortem histological studies for parvalbumin showed a reduction of parvalbumin positive cells [38, 39]. Besides the loss of GABAergic interneurons, messenger RNA (mRNA) expression analysis of post mortem primary motor cortex tissue of ALS patients also suggested a downregulation of expression of genes encoding the GABA receptor subunit α [40]. Positron emission tomography (PET) scans of ALS patients showed reduced binding of [^{11}C]flumazenil (a radiolabeled ligand that binds a subunit of the GABA receptor) in the motor cortex and several other cortical regions, compared to control subjects [41]. However, this study was not able to identify whether the loss of GABA receptor subunits was specific to a certain neuronal population.

The presented findings convincingly show an impairment of GABAergic signaling in ALS animal models and post mortem tissue from ALS patients. Whether the disruption of inhibitory circuits in ALS patients is due to a loss of GABAergic interneurons or a downregulation of GABA receptors on motor neurons, or a combination of both, is not known as of yet. However, several groups are currently in the process of studying the role of GABAergic interneurons in hyperexcitability and ALS disease progression.

Ionic Dysfunction Causes Hyperexcitability

In addition to the disruption of the inhibitory circuits, ion channel abnormalities, especially in the peripheral axons, have been implicated in causing hyperexcitability [30]. The increase in τ_{SD} seen in the aforementioned studies is attributed to an increase in persistent Na^+ currents [31, 42]. Persistent Na^+ channels have a low activation threshold and are generally associated with depolarizing the membrane to the threshold, which facilitates repetitive action potential firing. An increase in persistent Na^+ conductance in ALS patients could lead to the increase in membrane excitability observed in ALS patients (Fig. 1 (B)). An increase in persistent Na^+ currents was confirmed in cortical neurons of mutant *SOD1* mice [31]. Several studies have showed that the activation threshold of persistent Na^+ channels remained the same; however, there was a significant increase in the Na^+ current amplitude in cortical neurons of *SOD1* mice compared to healthy control mice [31, 43]. It is known that Riluzole slows the progression of ALS (albeit by only a few months), and one proposed mode of action of this drug is the inhibition of persistent Na^+ channels. Cortical neurons derived from the mutant *SOD1* mice treated with Riluzole showed a reduction in persistent Na^+ current [31]. In addition, the effects of Riluzole on cortical excitability have been shown to be transient in nature; sporadic ALS patients exhibiting cortical hyperexcitability that were treated with Riluzole demonstrated only temporarily increased SICI and longer refractory periods after 4 and 8 weeks of treatment before returning to baseline [44]. Riluzole treatment did not alter other biomarkers of hyperexcitability, such as MEP amplitude or τ_{SD} [44, 45]. The incomplete amelioration of hyperexcitability could explain the modest effect of Riluzole and further suggests that other mechanisms not targeted by Riluzole are contributing to hyperexcitability [44]. One factor that could contribute to this phenomenon is the reduction of K^+ conductance. It is hypothesized that the increase in membrane excitability observed subsequent to a subthreshold depolarizing pulse is due to an imbalance in K^+ and Na^+ conductance. The inward Na^+ currents which depolarize the membrane are not counterbalanced by the outward flow of K^+, thus increasing the membrane potential [33]. The hypothesis is further supported by an expression profile analysis of spinal cord motor neurons of sALS patients, which found a reduction in mRNA levels for several genes encoding paranodal fast K^+ channels *KCNA1*, *KCNA2* and M-current-associated K^+ channel *KCNQ2* [33, 46]. The reduction of K^+ channel expression was also confirmed in an immunohistological study of post mortem tissue of five ALS patients, one of whom was diagnosed with peripheral hyperexcitability. Densitometry analysis of spinal cord histology revealed approximately a two-fold reduction of Kv1.2 K^+ channels in the dorsal ventral roots of the spinal cord compared to matched controls; intriguingly, no reduction in Na^+ channels was found [47].

Whilst hyperexcitability could be a combination of both Na^+ and K^+ channel dysfunction, other cell types, such as astrocytes, may also contribute to this pathology. Current

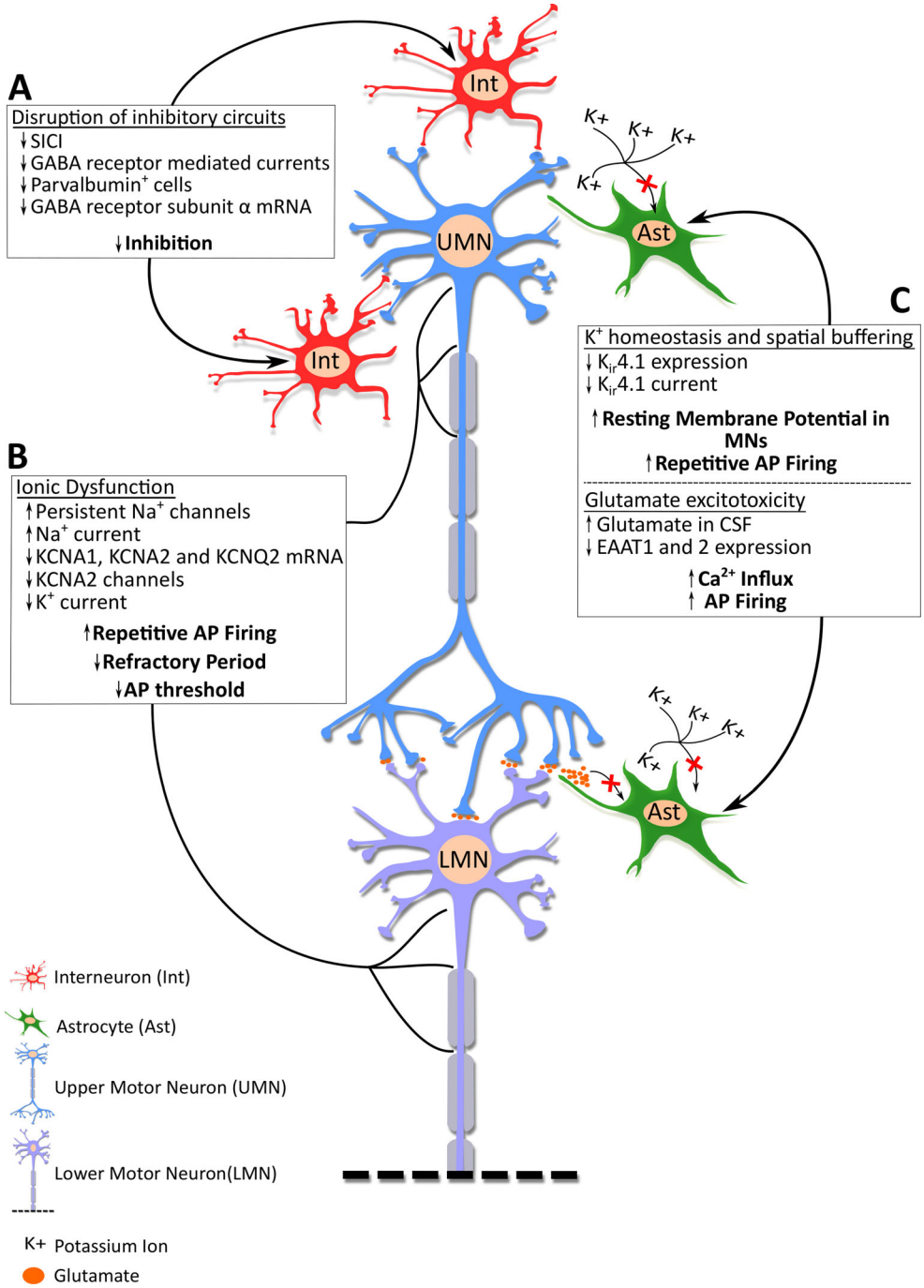

Fig. 1 An overview of mechanisms contributing to hyperexcitability in ALS. This schematic summarises the pathological changes in interneurons, motor neurons and astrocytes that are likely contributing to hyperexcitability in ALS. (*A*) Functional impairments of interneurons are likely to increase neuronal excitability. A reduction of short intracortical inhibition (SICI), GABA receptor-mediated currents, parvalbumin$^+$ cells and GABA receptor subunit α expression suggests an impairment of inhibitory circuits leading to a reduction of upper motor neuron inhibition. (*B*) Intrinsic changes to Na^+ and K^+ channels in upper and lower motor neurons lead to ionic imbalance. Increases in persistent Na^+ channels and Na^+ currents will cause a decrease in the refractory period and increase the repetitive firing. Moreover, reduction in potassium channel expression (KCNA1 and 2, KCNQ2) and K^+ currents will lead to a decrease in action potential (AP) threshold. (*C*) Impaired astrocytic function contributes to hyperexcitability in ALS. Astrocytes show reduced expression of inward-rectifying K^+ 4.1 (K_{ir}4.1) channel and its associated current. These findings are suggestive of a reduction in potassium buffering and thus an increase of extracellular K^+ concentration. The implication of increased extracellular K^+ is an increase in the resting membrane potential and repetitive firing. Moreover, evidence suggests that astrocytes are also impaired in their ability to take up glutamate causing glutamate excitotoxicity. Increases in excitatory neurotransmitter glutamate in patient cerebrospinal fluid (CSF) and a reduction in the excitatory amino acid transporters (EAAT) 1 and 2 in astrocytes support this theory. The increase in extracellular glutamate will lead to overstimulation of lower motor neurons and cause increased Ca^{2+} influx and increased AP firing

resources to study the affected currents are limited; TMS studies do not provide the possibility to isolate specific currents. Whole-cell patch clamping using brain slices of ALS animal models affords some insight into the channel function; however, channel distribution and characteristics of human motor neurons potentially differ (as do the causes of ALS) and thus, findings in ALS animal models do not correlate well with human pathophysiology.

Glutamate Excitotoxicity

Neuronal transmission and excitability are regulated by both intrinsic pathways of motor neurons and extrinsic mechanisms of other cells, for example interneurons. An important cell type that governs neuronal transmission is the astrocyte. This type of glial cell is responsible for the regulation of neurotransmitter clearance at the synaptic cleft. The uptake of glutamate, the main excitatory neurotransmitter, by astrocytes is especially important in protecting neurons from overstimulation and the associated damage. It has been proposed that hyperexcitability in ALS patients results from overstimulation of motor neurons by excessive glutamate. Glutamate-mediated excitotoxicity can contribute to neuronal death by increasing influx of Ca^{2+}. As motor neurons possess low Ca^{2+}-buffering capacities, the increase in Ca^{2+} leads to activation of various enzymes, as well as interference of mitochondrial function, eventually causing cell death [48]. Several studies investigating the mode of action of Riluzole suggest that excessive glutamate is a contributor to motor neuron degeneration in ALS [49]. In addition to inhibiting persistent Na^+ currents, it is thought that Riluzole also inhibits glutamate-mediated excitotoxicity by inhibiting the release of glutamate and blocking the post-synaptic response of glutamate [50]. Deregulation of glutamate metabolism and clearance is indicated by an increase of glutamate in cerebrospinal fluid (CSF) derived from ALS patients [51]. In accordance with this, analysis of glutamate transporter expression in post mortem ALS tissue found a reduction of excitatory amino acid transporters (EAAT1 and EAAT2), which are both glutamate transporters that are specifically expressed in astrocytes [52]. Expression of the glutamate transporter (GLT-1) in mutant SOD1 mice was also found to be less pronounced in astrocytes, thereby making neurons more vulnerable to excitotoxicity [53]. There have been contradicting studies in regard to the mechanism of glutamate transporter downregulation in astrocytes. One study suggested that errors in mRNA processing and aberrant splicing of *EAAT2* mRNA lead to the reduction of EAAT2 receptors [54]. Although an independent study also confirmed alternative splicing forms of *EAAT2* in post mortem ALS tissue, this study was not able to confirm a significant difference in *EAAT2* splicing in control tissue [55].

As discussed previously, hyperexcitability is one of the earliest changes observed in ALS pathology. The mechanisms leading to cortical and peripheral hyperexcitability in motor neurons likely involve the dysregulation of inhibitory circuits and dysfunction of intrinsic ion channels. However, the role of glial cells (such as astrocytes), which support neuronal transmission and are essential in maintaining neuronal integrity, should not be underestimated. Not only do astrocytes play an essential role in regulating glutamate levels at the synaptic cleft but they also play an essential role in regulating extracellular ion concentration.

Potassium Homeostasis and Spatial Buffering

Extracellular K^+ concentration directly impacts on neuronal depolarization and excitability. Excess K^+ concentration can also lead to synchronized neuronal activity, which might underlie symptoms, such as fasciculations and cramps that are evident in the early stages of ALS. Rapid increases of extracellular K^+, which are mainly due to neuronal discharges, can be decreased by neuronal reuptake through Na^+/K^+ pumps, through simple passive diffusion or through astrocytic clearance. Through this process, astrocytes play a key role in mediating neuronal hyperexcitability (Fig. 1 (C)). There are two mechanisms by which astrocytes carry out K^+ clearance (reviewed in [56]). The first is "potassium uptake", which involves astrocytes sequestering K^+ ions by K^+ cotransporters ($Na^+/K^+/2Cl^-$) and Na^+-K^+ pumps (Na^+/K^+ ATPase). This mechanism also involves water influx into the cell and causes overall swelling of the astrocyte. The second mechanism referred to as "spatial buffering" involves astrocytes mediating inward flow of K^+ current (through K^+ inward-rectifying channels, (K^+_{ir})) from high to low K^+ concentrations by employing membrane voltage differences between the local K^+ reversal potential and the astrocytic network membrane potential. As K^+ ions are flowing from one astrocyte to other astrocytes through intracellular connections (gap junctions), this mechanism does not involve the accumulation of K^+, water influx or swelling.

Although K^+ clearance is mediated through several channels, $K_{ir}4.1$ channels that are selectively expressed in astrocytes, are considered to be the prime candidate for mediating K^+ buffering [57]. Consistent with the importance of K^+ spatial buffering to normal functioning, $K_{ir}4.1$ knockout mice exhibit epileptic seizures, growth retardation and premature lethality at the age of 2 weeks [57, 58]. A recent study by Bataveljic and colleagues showed that the expression level of $K_{ir}4.1$ was significantly reduced in a transgenic $SOD1^{G93A}$ rat model [59]. Furthermore, the K^+_{ir} current density decreased compared to wild-type astrocytes, indicating a reduction of the K^+-buffering capabilities by astrocytes [59], which will consequently affect K^+ homeostasis. An increase in extracellular K^+ can lead to depolarization of the resting membrane potential and an increase in overall excitability. Further evidence for the contribution of K^+ dysregulation

was identified by a clear correlation between downregulation of $K^+_{ir}4.1$ channels from astrocytes and disease progression in an ALS animal model [60]. Additionally, motor neurons were highly sensitive to increased K^+ levels, as even a two-fold increase in K^+ concentration (10 mM) led to neuronal cell death.

Increasing evidence suggests that astrocytes actively contribute to the deterioration of motor neurons in ALS [61–65]. Several in vitro studies have reported that astrocytes from ALS patients cause toxicity and selective death to spinal cord- and stem cell-derived non-ALS motor neurons [61, 62]. In recent years, the role of astrocytes and other glial cells in neuroinflammation has been a major focus of research. However, astrocytes also play a crucial role in supporting neuronal signalling; thus, it is crucial to study whether astrocytic function in maintaining extracellular homeostasis is compromised in ALS leading to hyperexcitable motor neurons.

Conclusion and Future Directions

Hyperexcitability is a common pathology in ALS patients despite differences in genetic or environmental factors. Histological studies on post mortem tissue, and in animal models and clinical studies have provided some insight into the development of hyperexcitability in ALS. It has become evident that many intrinsic and extrinsic factors are able to contribute to the mechanism of hyperexcitability. Besides intrinsic impairments in ion channels of motor neurons, diminished functions of other cell types, such as interneurons and astrocytes, are also likely to contribute to the pathology. A large body of evidence regarding hyperexcitability in the onset and progression of ALS has been gathered using these studies. Although it is widely accepted that hyperexcitability occurs in ALS, it is not yet well understood how hyperexcitability contributes to motor neuron degeneration [66]. Leroy et al. [67] and Delestrée et al. [68] have argued that hyperexcitability only occurs in a small subset of motor neurons and is likely not to contribute to the actual motor neuron degeneration in ALS. The mechanisms and consequences of hyperexcitability are therefore not yet understood in enough detail to make a valid conclusion. Future studies need to focus on determining the full extent of changes in excitability in populations of motor neurons and other neurons.

Animal models have provided some mechanistic details; however, to fully understand the contribution of hyperexcitability to disease onset and progression, it is essential to translate and confirm findings into a humanised disease model to allow ongoing assessment of the pathology. The development of induced pluripotent stem cell (iPSC) technology has provided a new perspective to study neurodegenerative disease mechanisms. Patient-derived cells can be reprogrammed and differentiated into specific cell types, which can recapitulate disease-associated phenotypes. ALS patient iPSC-derived models offer the opportunity to study the mechanisms of hyperexcitability and develop and test potential drug therapies in a living human cell model. Ion channels offer promising targets to delay the onset of hyperexcitability and potentially ALS. An example of a promising ion channel modulator currently in phase II trials for ALS is Mexiletine, a Na^+ channel blocker, which reduced fasciculations and cramps [69, 70]. Using an iPSC-derived ALS model, Wainger and colleagues have established that the anticonvulsant drug Retigabine (which is currently in phase II clinical trials) has a neuroprotective effect on iPSC-derived motor neurons from ALS patients [71]. Retigabine works by activating Kv7 (KCNQ) channels [71], which are thought to be downregulated in ALS [33, 46]. Treatment with Retigabine has been shown to attenuate the hyperexcitability and increased cell death associated with ALS in iPSC-derived motor neurons carrying mutations in *C9ORF72*, *SOD1* or *FUS* [71].

The generation of a patient-derived co-culture system consisting of the affected cell types including (but not limited to) motor neurons, interneurons and astrocytes will provide an essential tool to investigate the interactions within the neuronal and glial networks contributing to hyperexcitability and motor neuron degeneration in ALS. Such studies will provide an ability to study the mechanisms of hyperexcitability in human patient neurons but are dependent on the following: (i) efficient differentiation of appropriate cell types, (ii) neural cell types developing physiologically relevant networks in vitro and (iii) demonstration of a functional disease phenotype [72]. Overcoming these experimental challenges will allow the identification of mechanisms and permit preclinical testing of potential drugs. Initial studies do suggest that patient-derived motor neurons, at least from some patients, exhibit hyperexcitability in vitro [71, 73]. Identifying the mechanisms of hyperexcitability will thus provide a broader platform for the development of diagnostic and therapeutic strategies.

References

1. Cleveland DW, Rothstein JD (2001) From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat Rev Neurosci 2:806–819. doi:10.1038/35097565
2. Kiernan JA, Hudson AJ (1991) Changes in sizes of cortical and lower motor neurons in amyotrophic lateral sclerosis. Brain 114(Pt 2):843–853
3. Logroscino G, Traynor BJ, Hardiman O, Chiò A, Mitchell D, Swingler RJ, Millul A, Benn E et al (2010) Incidence of amyotrophic lateral sclerosis in Europe. J Neurol Neurosurg Psychiatry 81: 385–390. doi:10.1136/jnnp.2009.183525
4. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, Burrell JR, Zoing MC (2011) Amyotrophic lateral sclerosis. Lancet 377:942–955. doi:10.1016/S0140-6736(10)61156-7

5. Bensimon G, Lacomblez L, Meininger V (1994) A controlled trial of riluzole in amyotrophic lateral sclerosis. N Engl J Med 330:585–591. doi:10.1056/NEJM199403033300901
6. Miller RG, Mitchell JD, Moore DH (2012) Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane Database of Systematic Reviews
7. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases (2000) El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 1:293–299
8. Chiò A (1999) ISIS survey: an international study on the diagnostic process and its implications in amyotrophic lateral sclerosis. J Neurol 246(Suppl 3):III1–III5
9. Bowser R, Turner MR, Shefner J (2011) Biomarkers in amyotrophic lateral sclerosis: opportunities and limitations. Nat Rev Neurol 7:631–638. doi:10.1038/nrneurol.2011.151
10. Chen S, Sayana P, Zhang X, Le W (2013) Genetics of amyotrophic lateral sclerosis: an update. Mol Neurodegener 8:28. doi:10.1186/1750-1326-8-28
11. Ling S-C, Polymenidou M, Cleveland DW (2013) Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. Neuron 79:416–438. doi:10.1016/j.neuron.2013.07.033
12. Renton AE, Chiò A, Traynor BJ (2014) State of play in amyotrophic lateral sclerosis genetics. Nat Neurosci 17:17–23. doi:10.1038/nn.3584
13. Robberecht W, Philips T (2013) The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci 14:248–264. doi:10.1038/nrn3430
14. Bae JS, Simon NG, Menon P, Vucic S, Kiernan MC (2013) The puzzling case of hyperexcitability in amyotrophic lateral sclerosis. J Clin Neurol 9:65–74. doi:10.3988/jcn.2013.9.2.65
15. Vucic S, Nicholson GA, Kiernan MC (2008) Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. Brain 131:1540–1550. doi:10.1093/brain/awn071
16. Blair IP, Williams KL, Warraich ST, Durnall JC, Thoeng AD, Manavis J, Blumbergs PC, Vucic S et al (2010) FUS mutations in amyotrophic lateral sclerosis: clinical, pathological, neurophysiological and genetic analysis. J Neurol Neurosurg Psychiatry 81:639–645. doi:10.1136/jnnp.2009.194399
17. Williams KL, Fifita JA, Vucic S, Durnall JC, Kiernan MC, Blair IP, Nicholson GA (2013) Pathophysiological insights into ALS with C9ORF72 expansions. J Neurol Neurosurg Psychiatry 84:931–935. doi:10.1136/jnnp-2012-304529
18. Mills KR, Nithi KA (1997) Corticomotor threshold is reduced in early sporadic amyotrophic lateral sclerosis. Muscle Nerve 20:1137–1141. doi:10.1002/(SICI)1097-4598(199709)20:9<1137::AID-MUS7>3.0.CO;2-9
19. de Carvalho M, Swash M (2013) Fasciculation potentials and earliest changes in motor unit physiology in ALS. J Neurol Neurosurg Psychiatry 84:963–968. doi:10.1136/jnnp-2012-304545
20. de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, Mills K, Mitsumoto H et al (2008) Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol 119:497–503. doi:10.1016/j.clinph.2007.09.143
21. Vucic S, Kiernan MC (2006) Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. Brain 129:2436–2446. doi:10.1093/brain/awl172
22. Menon P, Kiernan MC, Vucic S (2015) Cortical hyperexcitability precedes lower motor neuron dysfunction in ALS. Clin Neurophysiol 126:803–809. doi:10.1016/j.clinph.2014.04.023
23. Weber M, Eisen A, Stewart H, Hirota N (2000) The split hand in ALS has a cortical basis. J Neurol Sci 180:66–70. doi:10.1016/S0022-510X(00)00430-5
24. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P et al (1993) Corticocortical inhibition in human motor cortex. J Physiol Lond 471:501–519
25. McClintock SM, Freitas C, Oberman L, Lisanby SH, Pascual-Leone A (2011) Transcranial magnetic stimulation: a neuroscientific probe of cortical function in schizophrenia. Biol Psychiatry 70:19–27. doi:10.1016/j.biopsych.2011.02.031
26. Ziemann U, Winter M, Reimers CD, Reimers K, Tergau F, Paulus W (1997) Impaired motor cortex inhibition in patients with amyotrophic lateral sclerosis evidence from paired transcranial magnetic stimulation. Neurology 49:1292–1298. doi:10.1212/WNL.49.5.1292
27. Vucic S, Kiernan MC (2006) Axonal excitability properties in amyotrophic lateral sclerosis. Clin Neurophysiol 117:1458–1466. doi:10.1016/j.clinph.2006.04.016
28. Bostock H, Rothwell JC (1997) Latent addition in motor and sensory fibres of human peripheral nerve. J Physiol 498:277–294
29. Bostock H, Cikurel K, Burke D (1998) Threshold tracking techniques in the study of human peripheral nerve. Muscle Nerve 21:137–158. doi:10.1002/(SICI)1097-4598(199802)21:2<137::AID-MUS1>3.0.CO;2-C
30. Bostock H, Sharief MK, Reid G, Murray NMF (1995) Axonal ion channel dysfunction in amyotrophic lateral sclerosis. Brain 118:217–225. doi:10.1093/brain/118.1.217
31. Pieri M, Carunchio I, Curcio L, Mercuri NB, Zona C (2009) Increased persistent sodium current determines cortical hyperexcitability in a genetic model of amyotrophic lateral sclerosis. Exp Neurol 215:368–379. doi:10.1016/j.expneurol.2008.11.002
32. Mogyoros I, Kiernan MC, Burke D, Bostock H (1998) Strength-duration properties of sensory and motor axons in amyotrophic lateral sclerosis. Brain 121:851–859. doi:10.1093/brain/121.5.851
33. Kanai K, Kuwabara S, Misawa S, Tamura N, Ogawara K, Nakata M, Sawai S, Hattori T et al (2006) Altered axonal excitability properties in amyotrophic lateral sclerosis: impaired potassium channel function related to disease stage. Brain 129:953–962. doi:10.1093/brain/awl024
34. Wagle-Shukla A, Ni Z, Gunraj CA, Bahl N, Chen R (2009) Effects of short interval intracortical inhibition and intracortical facilitation on short interval intracortical facilitation in human primary motor cortex. J Physiol 587:5665–5678. doi:10.1113/jphysiol.2009.181446
35. Moser JM, Bigini P, Schmitt-John T (2013) The wobbler mouse, an ALS animal model. Mol Gen Genomics 288:207–229. doi:10.1007/s00438-013-0741-0
36. McGown A, McDearmid JR, Panagiotaki N, Tong H, Al Mashhadi S, Redhead N, Lyon AN, Beattie CE et al (2013) Early interneuron dysfunction in ALS: insights from a mutant sod1 zebrafish model. Ann Neurol 73:246–258. doi:10.1002/ana.23780
37. Zhang W, Zhang L, Liang B, Schroeder D, Zhang Z, Cox GA, Li Y, Lin D-T (2016) Hyperactive somatostatin interneurons contribute to excitotoxicity in neurodegenerative disorders. Nat Neurosci 19:557–559. doi:10.1038/nn.4257
38. Nieto-Gonzalez JL, Moser J, Lauritzen M, Schmitt-John T, Jensen K (2011) Reduced GABAergic inhibition explains cortical hyperexcitability in the wobbler mouse model of ALS. Cereb Cortex 21:625–635. doi:10.1093/cercor/bhq134
39. Nihei K, McKee AC, Kowall NW (1993) Patterns of neuronal degeneration in the motor cortex of amyotrophic lateral sclerosis patients. Acta Neuropathol 86:55–64
40. Petri S, Krampfl K, Hashemi F, Grothe C, Hori A, Dengler R, Bufler J (2003) Distribution of GABAA receptor mRNA in the motor cortex of ALS patients. J Neuropathol Exp Neurol 62:1041–1051. doi:10.1093/jnen/62.10.1041
41. Lloyd CM, Richardson MP, Brooks DJ, Al-Chalabi A, Leigh PN (2000) Extramotor involvement in ALS: PET studies with the

GABAA ligand [11C]flumazenil. Brain 123:2289–2296. doi:10.1093/brain/123.11.2289
42. Vucic S, Kiernan MC (2010) Upregulation of persistent sodium conductances in familial ALS. J Neurol Neurosurg Psychiatry 81: 222–227. doi:10.1136/jnnp.2009.183079
43. Kuo JJ, Siddique T, Fu R, Heckman CJ (2005) Increased persistent Na+ current and its effect on excitability in motoneurones cultured from mutant SOD1 mice. J Physiol 563:843–854. doi:10.1113/jphysiol.2004.074138
44. Geevasinga N, Menon P, Ng K, Bos MVD, Byth K, Kiernan MC, Vucic S (2016) Riluzole exerts transient modulating effects on cortical and axonal hyperexcitability in ALS. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 0:1–9. doi:10.1080/21678421.2016.1188961
45. Vucic S, Lin CS-Y, Cheah BC, Murray J, Menon P, Krishnan AV, Kiernan MC (2013) Riluzole exerts central and peripheral modulating effects in amyotrophic lateral sclerosis. Brain 136:1361–1370. doi:10.1093/brain/awt085
46. Jiang Y-M, Yamamoto M, Kobayashi Y, Yoshihara T, Liang Y, Terao S, Takeuchi H, Ishigaki S et al (2005) Gene expression profile of spinal motor neurons in sporadic amyotrophic lateral sclerosis. Ann Neurol 57:236–251. doi:10.1002/ana.20379
47. Shibuya K, Misawa S, Arai K, Nakata M, Kanai K, Yoshiyama Y, Ito K, Isose S et al (2011) Markedly reduced axonal potassium channel expression in human sporadic amyotrophic lateral sclerosis: an immunohistochemical study. Exp Neurol 232:149–153. doi:10.1016/j.expneurol.2011.08.015
48. Van Den Bosch L, Van Damme P, Bogaert E, Robberecht W (2006) The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. Biochim Biophys Acta (BBA) - Mol Basis Dis 1762: 1068–1082. doi:10.1016/j.bbadis.2006.05.002
49. Rothstein J (1994) Excitotoxic mechanisms in the pathogenesis of amyotrophic lateral sclerosis. Adv Neurol 68:7–20–7
50. Doble A (1996) The pharmacology and mechanism of action of riluzole. Neurology 47:S233–S241
51. Rothstein JD, Tsai G, Kuncl RW, Clawson L, Cornblath DR, Drachman DB, Pestronk A, Stauch BL et al (1990) Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. Ann Neurol 28:18–25. doi:10.1002/ana.410280106
52. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW (1995) Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol 38:73–84
53. Pardo AC, Wong V, Benson LM, Dykes M, Tanaka K, Rothstein JD, Maragakis NJ (2006) Loss of the astrocyte glutamate transporter GLT1 modifies disease in SOD1G93A mice. Exp Neurol 201: 120–130. doi:10.1016/j.expneurol.2006.03.028
54. Lin C-LG, Bristol LA, Jin L, Dykes-Hoberg M, Crawford T, Clawson L, Rothstein JD (1998) Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. Neuron 20:589–602. doi:10.1016/S0896-6273(00)80997-6
55. Nagai M, Abe K, Okamoto K, Itoyama Y (1998) Identification of alternative splicing forms of GLT-1 mRNA in the spinal cord of amyotrophic lateral sclerosis patients. Neurosci Lett 244:165–168. doi:10.1016/S0304-3940(98)00158-X
56. Kofuji P, Newman E (2004) Potassium buffering in the central nervous system. Neuroscience 129:1045–1056
57. Haj-Yasein NN, Jensen V, Vindedal GF, Gundersen GA, Klungland A, Ottersen OP, Hvalby O, Nagelhus EA (2011) Evidence that compromised K+ spatial buffering contributes to the epileptogenic effect of mutations in the human Kir4.1 gene (KCNJ10). Glia 59: 1635–1642. doi:10.1002/glia.21205
58. Djukic B, Casper KB, Philpot BD, Chin L-S, McCarthy KD (2007) Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J Neurosci 27:11354–11365. doi:10.1523/JNEUROSCI.0723-07.2007
59. Bataveljić D, Nikolić L, Milosević M, Todorović N, Andjus PR (2012) Changes in the astrocytic aquaporin-4 and inwardly rectifying potassium channel expression in the brain of the amyotrophic lateral sclerosis SOD1G93A rat model. Glia 60:1991–2003. doi:10.1002/glia.22414
60. Kaiser M, Maletzki I, Hülsmann S, Holtmann B, Schulz-Schaeffer W, Kirchhoff F, Bähr M, Neusch C (2006) Progressive loss of a glial potassium channel (KCNJ10) in the spinal cord of the SOD1 (G93A) transgenic mouse model of amyotrophic lateral sclerosis. J Neurochem 99:900–912. doi:10.1111/j.1471-4159.2006.04131.x
61. Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S (2007) Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat Neurosci 10:615–622. doi:10.1038/nn1876
62. Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, Song S, Likhite S et al (2011) Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. Nat Biotechnol 29:824–828. doi:10.1038/nbt.1957
63. Philips T, Robberecht W (2011) Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. The Lancet Neurology 10:253–263. doi:10.1016/S1474-4422(11)70015-1
64. Das MM, Svendsen CN (2015) Astrocytes show reduced support of motor neurons with aging that is accelerated in a rodent model of ALS. Neurobiol Aging 36:1130–1139. doi:10.1016/j.neurobiolaging.2014.09.020
65. Almad AA, Doreswamy A, Gross SK, Richard J-P, Huo Y, Haughey N, Maragakis NJ (2016) Connexin 43 in astrocytes contributes to motor neuron toxicity in amyotrophic lateral sclerosis. Glia 64:1154–1169. doi:10.1002/glia.22989
66. Leroy F, Zytnicki D (2015) Is hyperexcitability really guilty in amyotrophic lateral sclerosis? Neural Regen Res 10:1413–1415. doi:10.4103/1673-5374.165308
67. Leroy F, d'Incamps BL, Imhoff-Manuel RD, Zytnicki D (2014) Early intrinsic hyperexcitability does not contribute to motoneuron degeneration in amyotrophic lateral sclerosis. eLife 3:e04046. doi:10.7554/eLife.04046
68. Delestrée N, Manuel M, Iglesias C, Elbasiouny SM, Heckman CJ, Zytnicki D (2014) Adult spinal motoneurones are not hyperexcitable in a mouse model of inherited amyotrophic lateral sclerosis. J Physiol 592:1687–1703. doi:10.1113/jphysiol.2013.265843
69. Shibuya K, Misawa S, Kimura H, Noto Y-I, Sato Y, Sekiguchi Y, Iwai Y, Mitsuma S et al (2015) A single blind randomized controlled clinical trial of mexiletine in amyotrophic lateral sclerosis: efficacy and safety of sodium channel blocker phase II trial. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 16:353–358. doi:10.3109/21678421.2015.1038277
70. Weiss MD, Macklin EA, Simmons Z et al (2016) A randomized trial of mexiletine in ALS safety and effects on muscle cramps and progression. Neurology 86:1474–1481. doi:10.1212/WNL.0000000000002507
71. Wainger BJ, Kiskinis E, Mellin C, Wiskow O, Han SSW, Sandoe J, Perez NP, Williams LA et al (2014) Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. Cell Rep 7:1–11. doi:10.1016/j.celrep.2014.03.019
72. Engel M, Do-Ha D, Muñoz SS, Ooi L (2016) Common pitfalls of stem cell differentiation: a guide to improving protocols for neurodegenerative disease models and research. Cell Mol Life Sci 73: 3693–3709. doi:10.1007/s00018-016-2265-3
73. Devlin A-C, Burr K, Borooah S, Foster JD, Cleary EM, Geti I, Vallier L, Shaw CE et al (2015) Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability. Nat Commun 6:5999. doi:10.1038/ncomms6999