Review

Cortical Hyperexcitability in the Driver's Seat in ALS

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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by the degeneration of cortical and spinal motor neurons. With no effective treatment available to date, patients face progressive paralysis and eventually succumb to the disease due to respiratory failure within only a few years. Recent research has revealed the multifaceted nature of the mechanisms and cell types involved in motor neuron degeneration, thereby opening up new therapeutic avenues. Intriguingly, two key features present in both ALS patients and rodent models of the disease are cortical hyperexcitability and hyperconnectivity, the mechanisms of which are still not fully understood. We here recapitulate current findings arguing for cell autonomous and non-cell autonomous mechanisms causing cortical excitation and inhibition imbalance, which is involved in the degeneration of motor neurons in ALS. Moreover, we will highlight recent evidence that strongly indicates a cardinal role for the motor cortex as a main driver and source of the disease, thus arguing for a corticofugal trajectory of the pathology.

Keywords: amyotrophic lateral sclerosis; excitability; upper motor neurons; neurodegeneration; motor cortex; microcircuit; interneurons; astrocytes

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a lethal disease that is primarily characterized by the loss of upper motor neurons (UMN) in the cortex and lower motor neurons (LMN) in the spinal cord [1,2]. Being one of the most common forms of adult motor neuron diseases, ALS occurs at an incidence of ~2/100,000 person per year and a prevalence of ~6/100,000 people [3]. An ever-growing list of genes and gene variants is linked to the development of ALS [4], but only a mere 5–10% of all cases are familial (fALS) due to inherited genetic mutations, while the large majority of cases (90–95%) are sporadic (sALS) [5–7]. Both forms of the disease progress rapidly. The initial muscle weakness, spasticity, and fasciculations soon worsen to cause severe paralysis and eventual fatal respiratory failure, typically within 3–5 years [8]. The mean age of onset of ALS is ~60 years, while in fALS the disease onset already occurs about 5 years earlier [9]. To date, only 40–60% of the familial cases could be linked to known mutations, such as the hexanucleotide repeat expansion in the *Chromosome 9 open reading frame 72* (C9orf72, 40% of fALS cases) or mutations in the *Superoxide dismutase 1* (SOD1, 20%), the *TAR DNA-binding protein* (TARDBP, 4%), or in the *Fused in sarcoma* (FUS, 3%) gene [7,10].

A common pathophysiological feature of ALS is the intracellular aggregation of proteinaceous deposits. The most frequently found (97% of all ALS cases) cytoplasmic aggre-

gates in fALS and sALS consist of the TAR DNA-binding protein of 43 kDa (TDP-43) [11,12]. *C9orf72* mutation carriers also display dipeptide repeat proteins [13]. Less frequent are aggregates of FUS, although the actual occurrence rate is a matter of debate [14,15], or SOD1 [16,17]. Genetic insight derived from fALS cases has enabled researchers to study the molecular mechanisms of the disease using animal and in vitro cell culture models. Although the complex molecular changes accompanying motor neuron loss remain to be fully elucidated, these studies have unearthed a plethora of compromised intracellular processes and pathways affecting motor neuron health, involving defective RNA processing [18], impaired oxidative stress regulation and mitochondrial function [19,20], irregularities in cytoskeletal structure affecting axonal transport [21,22], reduced intracellular Ca^{2+} handling capacity [23–25], or compromised energy metabolism [26]. None of which, however, has yet resulted in the development of an effective treatment strategy. ALS research in the past decades was strongly centered on the investigation of lower motor neurons in the spinal cord, while upper motor neuron pathology is understudied. A large body of evidence, however, emphasizes the cardinal role of the motor cortex in triggering UMN and LMN degeneration. As such, cortical hyperexcitability has been demonstrated in ALS patients and even asymptomatic ALS mutation carriers [27], but the molecular, cellular, and network mechanisms underlying cortical hyperexcitability are not completely understood. Both cell autonomous (e.g., altered intrinsic excitability of UMN) and non-cell autonomous mechanisms (e.g., alterations in the local microcircuitry caused by cells other than UMN, such as glia) are conceivable (for detailed review, see Gunes et al. [28]). In this review, we will summarize recent evidence for cortical hyperexcitability in both humans and rodent models of ALS and recapitulate the currently known underlying cellular and circuit mechanisms.

2. Clinical Evidence for Cortical Hyperexcitability in ALS

The site of origin of the disease has long been a matter of debate. While some believe the initial pathology occurs at the level of lower motor neurons (LMN) in the spinal cord or even the neuro-muscular junction [29,30], with the cortex and UMN being affected in a retrograde process (termed ‘dying-back hypothesis’), others argue that the disease originates in the cortex and propagates in a corticofugal manner to subsequently affect LMN in the spinal cord [30,31]. The latter process was coined the ‘dying-forward’ hypothesis [31]. Several lines of evidence in fact strongly argue for a cortical origin of ALS [32,33]. These encompass the spreading pattern of the typical TDP-43 pathology, following anatomical connections through axonal projections and synaptic contacts [32]. Most importantly, electrophysiological measurements have revealed that cortical hyperexcitability is present in both sporadic and familial ALS patients and can occur even prior to disease onset in familial ALS cases [34–36]. Cortical hyperexcitability can be clinically assessed by means of threshold tracking paired-pulse transcranial magnetic stimulation (TMS) combined with motor-evoked potential (MEP) measurements (e.g., of the M. abductor pollicis brevis), as well as via electroencephalography (EEG) or functional MRI (fMRI) [37–42].

A number of parameters can be assessed to probe differences in cortical excitability when performing TMS, such as the resting motor threshold (RMT) and the MEP amplitude [43]. RMT represents the minimal stimulus intensity needed to excite the corresponding muscle [44] and was found to be decreased early in the disease, thereby reflecting UMN hyperexcitability [45,46]. In late-stage ALS patients, however, the RMT was increased along with a decrease in the MEP amplitude, indicative of gross denervation [47,48]. Cortical hyperexcitability could in principle result from either increased excitation or compromised intracortical inhibition. To disentangle both options, paired-pulse stimulation protocols can be applied, in which the interstimulus interval (ISI) determines the magnitude of the MEP. While very short ISIs (1–7 ms) depress the MEP, longer ISI (10–30 ms) can facilitate the MEP [36]. The degree of inhibition or facilitation can be assessed by altering the stimulus intensity of the 2nd pulse, the difference of which is represented in the short/long intracortical inhibition (SICI/LICI) and the intracortical facilitation (ICF) metric [36,38]

(Figure 1a–c). In ALS patients, the SICI was shown to be reduced [36,49–55] (Figure 1b), also in patients with subclinical UMN damage [56], indicating compromised GABA_B receptor mediated intracortical inhibition [57]. On the other hand, cortical facilitation was strongly increased, arguing for an elevated excitation [36,58–61] (Figure 1c). The phenomena of cortical hyperexcitability thus can be explained by a combined increase in excitation and impaired inhibition.

Figure 1. Assessment of motor cortex hyperexcitability and hyperconnectivity in ALS patients. (a) Schematic displaying paired-pulse transcranial magnetic stimulation (TMS) in patients. A magnetic coil is placed above the primary motor cortex (M1), and the resulting motor-evoked potential (MEP) is measured at the innervated muscle (typically the M. abductor pollicis brevis). (b) Example MEP depicting muscle potential changes (black—healthy controls, HC; white—ALS) triggered by a short interstimulus interval (ISI) between the conditioned stimulus (CS) and the test stimulus (TS) to facilitate short-interval intracortical inhibition (SICI). The TS needed to evoke a similar MEP is reduced in ALS, indicative of compromised intracortical inhibition. (c) To probe intracortical facilitation (ICF), the TS is applied after a longer ISI, which typically elicits a stronger MEP response compared to a single stimulus. The TS intensity needed to evoke a similar MEP observed after pair-pulse stimulation in healthy controls is reduced in ALS patients, arguing for elevated excitation within M1. (d) Functional magnetic resonance imaging (fMRI) studies demonstrate enhanced connectivity in somatosensory networks, as well as across (pre)frontal areas (FEF, frontal eye field) in ALS patients (data from Schulthess et al. [27], modified and used with permission of the authors and the publisher).

In addition to TMS, EEG is widely used to assess cortical excitability by measuring parameters such as event-related desynchronization/synchronization (ERD/ERS), enabling higher temporal resolution compared to TMS. ERD typically represents a correlate of an activated cortical network prior to the execution of a motor task [62], whereas ERS indicates an active inhibitory process of the cortical network after the motor task is performed [62]. In support of the notion of cortical hyperexcitability, ALS patients exhibit lower ERS, whereas task-dependent ERD is either decreased—likely reflecting UMN loss during later disease stages—or not significantly altered between ALS-patients and healthy controls [63,64]. These findings can be explained by both increased excitability of UMN and compromised intracortical inhibition during rest and motor task execution [46,61,65]. Corroborating EEG findings, a magnetoencephalography (MEG) study by Proudfoot et al. showed that

ALS patients have an enhanced cortical beta desynchronization during a visually cued lateralized motor task [66].

Although limited in number, functional magnetic resonance imaging (fMRI) investigations were also employed in clinical ALS studies. fMRI is a widely used imaging technique that detects changes in blood oxygenation in the brain as a proxy for neuronal activity in the respective region [67,68]. Multiple studies reported a stronger response to a motor task in ALS patients compared to healthy subjects [69–71]. Resting state fMRI also revealed an increased functional connectivity of the motor cortex not only within the sensorimotor network [72,73] and the prefrontal association cortex [74], but also within the brainstem, ventral attention, and default mode network in sALS patients [27,75] (Figure 1d). Interestingly, changes in functional connectivity between the cerebellum and a network comprised of the precuneus, the cingulate, and the middle frontal lobe were already observed in presymptomatic mutation carriers [39].

The electrophysiological findings of compromised intracortical inhibition were further substantiated by proton magnetic resonance spectroscopy (^1H -MRS) measurements, revealing reduced GABA levels in ALS patients [76] and demonstrated a reduction of glutamate-glutamine levels upon riluzole treatment [77]. Additional histological and transcriptomic analyses demonstrated decreased GABA_A receptor densities and a downregulation of the $\alpha 1$ -subunit and upregulation of $\beta 1$ -subunit mRNA levels in the post-mortem motor cortex of ALS patients [78]. Furthermore, a downregulation of N-methyl-D-aspartate receptors (NMDAR) and alterations in the expression of particular α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) subunits in ALS were revealed by immunohistochemistry and RT-PCR [79]. These investigations, however, were conducted in post-mortem tissue, and thus reflect end-stage alterations.

Taken together, electrophysiological and imaging studies have revealed an early increase in cortical excitability in ALS, which is likely based on a combined increase in excitation and a decrease in inhibition.

3. Human-Derived Induced Pluripotent Stem Cells to Study Altered Motor Neuron Excitability in ALS

Patient-derived induced pluripotent stem cells (iPSCs) have emerged as a powerful tool to study the molecular and cellular mechanisms of neurological diseases. The method has also been widely used in ALS research. However, the majority of studies thus far have again focused on the investigation of iPSC-derived spinal motor neurons, while only limited information on cortical neurons, or more specifically upper motor neurons, is currently available [80]. Electrophysiological recordings conducted on iPSC-derived LMN cultures revealed somewhat conflicting results. While early (2–4 weeks old) cultures harboring *SOD1*, *C9orf72*, or *TARDBP* mutations displayed hyperexcitability and increased spontaneous activity levels [81,82], prolonged culture renders these ALS LMN hypoactive, such that after 7–10 weeks reduced spontaneous activity and decreased F-I gain was detected in conjunction with fewer synaptic inputs [82]. Indeed, hypoexcitability and/or hypoactivity of LMNs has been reported by others in iPSC-derived LMNs harboring a *C9orf72* repeat expansion measured at 9–10 weeks in culture [83], in four-week-old *SOD1* mutation LMN cultures [84]; and in seven-week-old LMN cultures from *FUS* and *SOD1* mutation carriers [85]. A caveat of these studies, however, is the incomplete functional characterization and motor neuron subtype classification of the investigated LMNs. It is well established that LMN subtypes differ in their vulnerability in ALS [86], an effect that is insufficiently modelled in iPSC-derived LMN cultures to date and thus further complicates the interpretation of the data. In contrast to the many studies conducted on iPSC-derived spinal motor neurons, very few reports have so far addressed the excitability alterations of cortical neurons. Recently, Perkins et al. found that iPSC-derived cortical neurons harboring *C9orf72* repeat expansions display enhanced network burst activity and increased synaptic inputs when cultured for 4–6 weeks, corroborating the cortical hyperexcitability seen in patients (Figure 1d, Perkins et al. [87]). Again, cell type specificity

was not further characterized in this study. Though informative, cell culture models come with inherent limitations, such as the absence of a tightly regulated network consisting of intricately connected diverse types of neurons and glia cells, as well as the rather short lifetime of those cells studied. Recent advances in the field have led to the development of more complex systems, such as brain organoid slice cultures [88] or even human 3D cortico-motor assembloids that recapitulate the complete cortico-spinal-muscle circuit [89]. These latest advancements are promising and will be instrumental for future research into the circuit mechanisms involved in ALS as well as other neurodegenerative diseases.

4. Evidence for Cortical Hyperexcitability in ALS Rodent Models

Mouse models offer the great advantage of direct experimental access to individual, characterized elements of the motor cortex circuitry, which enables an in-depth molecular and cellular investigation across disease-stages. Numerous rodent models have been thus generated in the past, based on the genetic mutations identified in fALS patients. However, the investigation of UMN or cortical dysfunction is clearly underrepresented in mouse studies, largely due to the fact that UMN seem less affected in mice vs. humans, and importantly, the direct monosynaptic connection of UMN and LMN is lacking in rodents. Recent studies now demonstrate that UMN dysfunction is also present in rodent models and shares features of the human pathology. As such, there is also evidence for cortical hyperexcitability in mouse models of ALS, akin to the human disease (for review, see Gunes et al. [28]). Most critically, a recent study now demonstrates that cortical hyperactivity is sufficient to drive LMN degeneration, even in the absence of a genetic mutation [90]. In their study Haidar et al. used a chemogenetic approach to chronically drive neurons in motor cortex, which caused UMN loss, corticospinal tract degeneration, and gliosis, and importantly also caused the degeneration of LMN and the neuromuscular junction resulting in impaired motor function [90]. This seminal work therefore not only strongly supports the corticofugal origin of ALS, but also for the first time demonstrates that neuronal hyperactivity alone is sufficient to cause downstream degeneration.

What are the mechanisms leading to early cortical hyperexcitability? Alterations in the net motor cortex excitability can be driven by intrinsic (cell-autonomous) mechanisms, affecting the main motor cortex output population—the UMN (e.g., synaptic deficits caused by altered neurotransmitter receptor expression levels and alterations in active/passive membrane properties caused by deficits in voltage gated channels, etc.)—and/or by extrinsic (non-cell autonomous) mechanisms, inflicted by cells other than UMN, such as altered glutamatergic or GABAergic neurons or glia cells. It has already become obvious from TMS and electrophysiological studies that cortical hyperexcitability in ALS patients is based on a combination of events and likely involves the dysfunction of multiple cell types within and beyond the primary motor cortex (M1).

5. Circuit Mechanisms Involved in Cortical Hyperexcitability

The M1 microcircuit consists of excitatory pyramidal neurons (PN) and local inhibitory interneurons (Figure 2a). Depending on their projection target, PN can be further categorized into intratelencephalic (IT) and pyramidal tract neurons (PT, aka UMN, also known as corticospinal motor neurons (CSMN) or corticospinal tract (CST) neurons and Betz cells in humans). IT neurons are found in layer 2/3, 5, and 6, with corticocortical (CC) neurons projecting transcallosally and corticostriatal (CStr) neurons projecting to the striatum. UMN are embedded in layer 5B of M1 (Figure 2a). They are multi-projectional, and downstream target regions include both the ipsi- and contra-lateral brainstem and the spinal cord, with branches also innervating the ipsilateral cortex and some subcortical regions [91]. The strongest feedforward excitatory drive to layer 5 neurons is provided by layer 2/3 PN (Figure 2a), followed by intralaminar projections within layer 5. Activity levels and information processing in M1 is regulated by a diverse set of local and remote inhibitory neurons, which constitute ~20% of the cortical neuronal population [92,93]. Based on the expression of typical markers, three major non-overlapping subtypes of interneurons are

known: parvalbumin (PV), somatostatin (SST), and 5-HT3a (with the majority of them being vasoactive intestinal peptide (VIP)) expressing interneurons [94–96] (Figure 2a). While PV and SST target PN directly at the level of their somata (PV) and the dendritic compartment (SST), respectively, VIP regulate the circuit via disinhibition, i.e., by inhibiting PV and SST. Notably, SST are also inhibiting PV to allow for further fine-tuning of information processing within neural circuits [97,98]. Glutamatergic (excitatory) and GABAergic (inhibitory) neurons in the M1 circuitry are further regulated by neuromodulatory inputs [91,99–101]. Several circuit elements were shown to be structurally and functionally altered in various models of the disease (Figure 2b, Gunes et al. [28]), causing a disruption of the tightly controlled excitation/inhibition balance in M1:

Figure 2. Alterations of primary motor cortex (M1) microcircuit elements in ALS. **(a)** In the healthy M1 microcircuitry upper motor neurons (UMNs), aka, pyramidal tract neurons (PT, red), located within cortical layer 5b receive feedforward excitatory synaptic input primarily from intratelencephalic (IT, light gray) neurons in layer 2–3 and, to a lesser degree, intralaminar from layer 5 IT. Long-range feedforward input is provided by the (pre)frontal and somatosensory cortex, the contralateral primary motor cortex (M1), and the thalamus (TH). Excitation is controlled by a number of inhibitory GABAergic interneurons, which express either parvalbumin (PV), somatostatin (SST), or vasoactive intestine peptide (VIP) (dark gray). Neuromodulatory input further shapes information processing in M1 and is provided by projections from the locus coeruleus (LC; releasing norepinephrine (NE)), the ventral tegmental area (VTA; releasing dopamine (DA)), the dorsal raphe (DR; releasing serotonin (5-HT)), or the tuberomammillary nucleus (TN; releasing histamine (HA)). **(b)** In ALS, hypoactive PV and hyperactive SST interneurons were reported, as well as increased long-range feedforward excitatory synaptic inputs and elevated functional connectivity, while neuromodulatory inputs were found to be compromised. Line thickness reflects connectivity strength.

5.1. Alterations of Upper Motor Neurons

Naturally, UMN have been the center of attention when it comes to studying M1 pathology in ALS. These studies revealed structural abnormalities, which, however, seemingly differ between mouse models: While, in SOD1^{G93A} transgenic (tg) mice, a reduction of dendritic spine density (the structural correlates of excitatory synapses) along with a reduced cell complexity (fewer dendrites) were observed [102,103], an increase in apical and basal dendritic spine densities in TDP43^{Q331K} tg mice was found [104]. Functionally, UMNs were shown to be hyperexcitable across various ALS mouse models. In the SOD1^{G93A} mouse model layer 5 PNs (which largely comprise UMN) display age- and disease-stage specific alterations in their intrinsic properties. Already in neonatal tg mice (P5–6), UMNs are hyperexcitable, followed by a normalization during pre-symptomatic stages (P14–P70),

but there is a reoccurrence of hyperexcitability during symptomatic stages ($\geq P90$) [105,106]. However, in contrast others also reported UMN hypoexcitability during the early symptomatic phase [107]. Similar findings were made in the TDP-43^{A315T} mouse model, in which layer 5 PN displayed a higher firing frequency during the pre-symptomatic stage [2,104]. In agreement with these studies, 30 days of induced expression of TDP-43^{ΔNLS} in adult mice also lead to intrinsic hyperexcitability in M1 layer 5 PN, highlighting the role of TDP-43 cytoplasmic mislocalization in driving cortical hyperexcitability [108]. Furthermore, also in the wobbler mouse model of ALS, which is based on a spontaneous mutation in the *VPS54* gene in the C57BL/Fa strain [109], layer 5 PN are hyperexcitable, as shown by an increased input resistance and strongly reduced current threshold (the minimal injected current needed to elicit an action potential) [110].

5.2. Increased Excitatory Inputs to UMNs

In addition to intrinsic alterations of UMNs, there is also evidence for an augmented synaptic input onto them, witnessed as increased miniature excitatory synaptic current (mEPSC) frequencies in UMNs in presymptomatic SOD1^{G93A} [103] and in TDP-43^{Q331K} tg mice [104]. The source of these excitatory synaptic inputs remains elusive. Based on the microcircuit connectivity, the main input source for layer 5 PN (UMNs) are layer 2/3 PN. Notably, layer 2/3 PN are also hyperexcitable in the SOD1^{G93A} model [105] and more active in the FUS^{ΔNLS} mouse model, as witnessed by increased spontaneous activity [111], further supporting the notion of excess excitatory drive onto UMNs. Additional excess synaptic inputs might also result from long-range projections. Indeed, connectivity changes were reported in SOD1^{G93A} mice [74], with increased synaptic inputs from S1 and contralateral M2 already during early-symptomatic stages, which further expanded to include the thalamus, contralateral M1, auditory cortex, and caudoputamen at later stages of the disease (Figure 2b, Commisso et al. [74]).

5.3. Reduced Cortical Inhibition

Excitation/inhibition (E/I) imbalance can be caused by increased excitation as well as compromised inhibition. In addition to enhanced intrinsic excitability and excitatory inputs onto UMNs, several lines of evidence highlight a parallel reduction in synaptic inhibition in M1 in various mouse models of the disease. Impaired inhibition was evident by reduced inhibitory postsynaptic currents (IPSCs) in UMNs in SOD1^{G93A} [112], TDP-43^{A315T} mice [2] and in the wobbler mouse model [110], which could be based on a loss of interneurons or particularly inhibitory synapses and/or functional interneuronal deficits. Indeed, our recent work in the FUS^{ΔNLS} model suggests that primarily inhibitory synapses are affected and less abundant, while overall PV interneuron number was not significantly altered [111]. There are contradictory findings regarding the density of subtype-specific interneurons in SOD1^{G93A} mice. While some have reported no change in the density of PV-, SST-, or VIP-expressing interneurons [113,114], others observed a decrease [112]. In the wobbler mouse model, the density of PV and SST interneurons was decreased [110]. Functionally, mouse-line and disease-stage specific alterations of interneuron subtypes were also identified. In SOD1^{G93A} mice, whole-cell recordings of PV interneurons revealed variable changes throughout the course of the disease [105]. While the excitability of PV interneurons was not altered presymptomatically, they turned hyperexcitable during the symptomatic stage (P90–101) [105]. Others, however, have reported hypoexcitability of PV interneurons in presymptomatic SOD1^{G93A} mice [112] (Figure 2b). Notably, in TDP-43^{A315T} mice Zhang et al. observed hyperactive SST, which suppressed PV activity, thus causing layer 5 PN disinhibition [2] (Figure 2b). Importantly, means to restore PV activity levels in the motor cortex either by ablating hyperactive SST in TDP-43^{A315T} mice [2] or through direct chemogenetic stimulation of PV in SOD1^{G93A} mice [112] restored layer 5 PN excitability and firing rates [2,112], further emphasizing the role of interneurons in cortical ALS pathophysiology.

5.4. Altered Neuromodulation

In addition to glutamatergic and GABAergic inputs, neuronal activity levels are furthermore regulated by a number of neuromodulators, which also seem to be altered in ALS patients and mouse models of the disease [115]. These alterations affect the dopaminergic system, as evidenced by compromised dopaminergic signaling in the striatum in ALS patients [116,117], a finding that is corroborated by mouse studies, in which SOD1^{G1H} mice display a reduction of dopamine levels and the loss of dopaminergic neurons (Figure 2b, Kostic et al. [118]). Moreover, serotonin levels were also found to be affected. In ALS patients, a loss of serotonergic neurons in the brainstem was reported, and also in SOD1^{G86R} mice reduced levels of serotonin in the brainstem and spinal cord were observed [119]. For a more detailed description of neuromodulatory disturbances, please see this excellent review [115].

6. A Potential Role of Astrocytes in Cortical Hyperexcitability

Another key player in cortical and spinal motor circuits, regulating synaptic efficacy and information processing, are astrocytes. Astrocytes serve numerous physiological functions such as the regulation of neurotransmitter concentration in the synaptic cleft, the maintenance of ion homeostasis, and the supply of energy substrates to neurons, through which they modulate energy metabolism [120,121]. Several molecular alterations of astrocytes were identified in ALS patients, presumably compromising the maintenance of neurotransmitter and ion homeostasis in the parenchyma [122,123]. A large part of the existing literature on the causal involvement of astrocytes in ALS is based on studies conducted in spinal cord preparations or cultured spinal motor neurons [124–127]. Whether these observations are also applicable to cortical astrocytes in the disease remains to be clarified. The most extensively studied molecular alteration is a reduced expression of the astrocytic excitatory amino acid transporter 2 (EAAT2, Figure 3). Its downregulation was identified in both the spinal cord of SOD1^{G85R} and SOD1^{G93A} and in the amyotrophic lateral sclerosis–parkinsonism–dementia complex (ALS-PDC) mouse model [124–127], as well as in the motor cortex and spinal cord of late-stage ALS patients [122,126,128], potentially exposing neurons to glutamate-mediated excitotoxicity. This mechanism is also shared by multiple neurodegenerative diseases [129–131]. Furthermore, impaired extracellular potassium clearance, due to a reduced expression of the astrocytic inward-rectifying (Kir4.1) channel and a dysregulation of Na⁺/K⁺ ATPases were reported in both SOD1^{G93A} tg rats (cortex) [132] and SOD1^{G93A} tg mice (spinal cord) [133], a finding, which was also observed in iPSC-derived astrocytes from ALS patients (SOD1 mutation carriers) [123,133,134] (Figure 3). In addition to maintaining ion homeostasis in the brain, astrocytes also provide neuronal energy substrates, such as lactate [135,136]. Lactate is largely produced in astrocytes through glycolysis and glycogenolysis and is exported to the extracellular space through monocarboxylate transporters (MCTs), where it is taken up by neurons through MCTs (Figure 3). Interestingly, lactate was also shown to modulate neuronal activity levels and plasticity [135], rendering it an important molecule potentially involved in alterations of the motor neuron circuitry in ALS. Notably, reduced lactate levels were detected in the motor cortex of SOD1^{G93A} tg mice by *in vivo* ¹H magnetic resonance spectroscopy (¹H-MRS) already during the early symptomatic phase [135,137,138], as well as in a SOD1^{G93A} tg astrocyte-MN (wt) co-culture system (Figure 3, Madji Hounoum et al. [139]).

Figure 3. Altered molecular profile of astrocytes in ALS. The downregulation of astrocytic excitatory amino acid transporter 2 (EAAT2) could cause increased synaptic glutamate and postsynaptic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and N-methyl-D-aspartate receptor (NMDAR) activation and increased Ca^{2+} levels. Reduced expression of the inward rectifier potassium channel (Kir4.1) disrupts K^+ homeostasis, resulting in excess K^+ in the synaptic cleft. Reduced extracellular lactate levels might affect neuronal energy supply and alter activity levels and plasticity. Reactive astrocytes are induced by microglia secreting inflammatory cytokines interleukin 1 alpha ($\text{IL-1}\alpha$), tumor necrosis factor ($\text{TNF-}\alpha$), and the complement component 1, subcomponent q (C1q). Saturated lipids in apolipoprotein E (APOE), and apolipoprotein J (APOJ) lipoparticles were identified as the toxic factors secreted by reactive astrocytes, triggering lipoapoptosis.

In addition to the altered physiological function of astrocytes in ALS, there is intriguing evidence suggesting that astrocytes secrete neurotoxic factors, causing motor neuron (MN) degeneration and death [140,141]. Most studies are based on in vitro experiments, in which cultured primary LMN are exposed to media obtained from cultured ALS transgenic astrocytes or co-culture systems of astrocyte-MN [140–144]. MN bathed in astrocytic conditioned media obtained from $\text{SOD1}^{\text{G93A}}$ mice or co-cultured with iPSC-derived astrocytes from patients positive for the C9orf72 repeat expansion display acute hyperexcitability with increased voltage-gated Na^+ currents and repetitive firing prior to MN death [143,145]. Earlier work had already identified a highly proliferative subtype of cultured astrocytes obtained from $\text{SOD1}^{\text{G93A}}$ tg rats, which seemingly secreted toxic factors [146]. In line with this, more recent work also demonstrates that reactive astrocytes can secrete toxic factors [147,148]. In an attempt to prevent the formation of reactive astrocytes, Guttenplan et al. crossed $\text{SOD1}^{\text{G93A}}$ tg mice with a triple knockout mouse, lacking $\text{IL-1}\alpha$, $\text{TNF}\alpha$, and C1qa [148]. Those three factors are released by microglia and induce the formation of reactive astrocytes [147]. The lack of those three factors strongly slowed down disease progression, thereby yielding the longest lifespan prolongation

hitherto observed in the SOD1^{G93A} tg model [148]. In their new study, Guttenplan et al. succeeded in narrowing down the involved toxic factors released by reactive astrocytes and surprisingly identified saturated lipids found in apolipoprotein E (APOE) and apolipoprotein J (APOJ) lipoparticles [144]. These lipoparticles might be taken up by neurons through lipid transporters or ApoE particles to cause lipoapoptosis (Figure 3, Smolič et al. [149]). Whether these pathways are also causally involved in the degeneration of UMN and LMN in ALS remains to be determined. It also remains open whether astrocyte reactivity and the corresponding secretome represent a unifying process shared by different forms of the disease, because recent evidence suggests a mutation specific molecular heterogeneity of reactive astrocytes in ALS [150]. These exciting recent data will surely spur future studies, shedding more light onto the molecular mechanisms by which astrocytes compromise cortical and spinal circuits in ALS.

7. Implications for Potential Circuit-Level Based Therapeutic Strategies

Unfortunately, despite the profound insight into the molecular mechanisms causing motor neuron degeneration in ALS an efficient treatment or cure for this devastating disease is still lacking. To date, riluzole, which blocks glutamate release and neurotransmission [151], is (in addition to edaravone) the only FDA-approved drug for the treatment of ALS. However, the beneficial effect on the survival of ALS patients is unfortunately minimal and varies between patients [152–154]. The effects in mouse models are also variable, ranging from no effect on disease onset and survival to a modest prolongation of life expectancy (for review see [155–157]). Notably, little is known about the effect of riluzole at the circuit level in the cortex and spinal cord or whether potential adaptive (homeostatic) mechanisms upon chronic treatment might exist. Since riluzole indiscriminately affects glutamatergic neurotransmission, it might also compromise activity levels of inhibitory interneurons in vivo. The net effect on the circuitry in vivo thus still remains elusive. In light of previous reports emphasizing the involvement of interneurons in the development of cortical hyperexcitability in ALS, a more targeted approach to interfere with dedicated circuit elements is thus strongly desirable. Owing to the recent impressive advancements in chemogenetic approaches and adeno-associated virus (AAV)-mediated gene therapy, circuit-level therapy in humans has now come within reach. To target specific cell types in the motor circuitry, chemogenetic tools such as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) and Pharmacologically Selective Effector Molecules (PSEMs)/Pharmacologically Selective Actuator Modules (PSAMs) systems have been successfully used in mice [112]. PV-expressing interneurons were chronically activated in SOD1^{G93A} tg mice during the pre-symptomatic and symptomatic stages via DREADDs and the application of its ligand clozapine-n-oxide (CNO), which delayed the development of motor deficits [112]. In humans, AAV-mediated gene therapy has already been approved by the FDA for the treatment of diseases such as lipoprotein lipase deficiency or spinal muscular atrophy, and many more clinical trials to combat various diseases are under way [158–160]. While we still largely lack long-term data to fully evaluate the efficacy and potential side-effects, the first clinical trials are overall extremely promising. However, to date, genetic means to manipulate the excitability of neurons in humans have never been tested. Thus, in addition to the already acknowledged risks of mutagenesis, haemato-, hepato- and neurotoxicity, one further needs to establish the application of chemogenetic tools, including the optimal stimulation paradigm and the development of highly selective agonists. Cell type specificity could be achieved by a combination of AAV serotypes and/or promoters and enhancers, such as, for inhibitory neurons, the *mDlx* [161] or a novel *GAD65* promoter [162]. The newest generations of AAVs now also enable brain-wide transgene expression in non-human primates, while causing less liver toxicity [163], thereby further enlarging the toolbox. Based on previous mouse studies, one can picture future therapeutic approaches based on the selective modulation of interneurons (PV, SST), UMNs themselves (potentially also targeted retrogradely, Genç et al. [164]), and astrocytes.

A modified CRISPR-based gene editing method involving an intein-mediated trans-splicing system that enables single-base editing of *SOD1* has overcome the transient therapeutic effect of antisense oligonucleotides (ASOs) and RNA interference (RNAi)-based gene therapy and achieved a permanent knock down of mutant *SOD1* expression in vivo [165]. The group employed the intrathecal delivery of dual AAV9 particles into the spinal cord to interfere with the expression of SOD, which resulted in a predominant targeting of astrocytes and led to a 40% reduction in intracellular *SOD1* inclusions, preserved neuromuscular function, and a prolonged life expectancy [165]. Alternatively, transplantation studies are also conceivable with the aim of replenishing the circuitry with healthy astrocytes. Indeed, several astrocyte transplantation therapies have entered phase I/IIa clinical studies (ClinicalTrials.gov: ID-NCT03482050; ID-NCT02943850; ID-NCT02478450), in which healthy astrocytes derived from human embryonic stem cell or human neural progenitor cells expressing glial cell-derived neurotrophic factor (GDNF) are transplanted. Multiple studies in ALS rodent models have demonstrated the therapeutic potential of this astrocyte-based cell transplantation/replacement approach in delaying disease onset and slowing down disease progression [166–169]. However, as we still lack long-term data, one needs to be cautious and carefully monitor possible side-effects, such as a potential gain of toxic neuroinflammatory properties of transplanted astrocytes [170]. Overall, these recent approaches hold great promise for the development of targeted, cell type specific interventions, which will hopefully lead to a much-needed clinical breakthrough.

8. Concluding Remarks

We here have summarized electrophysiological findings from both ALS patients and mouse models of the disease, demonstrating strong evidence for cortical hyperexcitability in ALS. We disentangle the underlying cellular and circuit mechanisms and discuss recent data showing, for the first time, a clear link between cortical hyperactivation and subsequent downstream MN degeneration and loss in the spinal cord. Together these data provide compelling evidence for a cortical origin and a consecutive corticofugal trajectory of the disease. We also review reports that suggest cortical hyperexcitability is a consequence of a combination of cell-autonomously elevated UMN excitability and increased excitatory inputs, paralleled by compromised inhibition. The resulting imbalance in excitation and inhibition is notably a feature shared by various other neurodegenerative diseases such as Alzheimer's disease [171–174], Huntington's disease [175], multiple sclerosis [176], Parkinson's disease [177,178], and spinal muscular atrophy [179], as well as psychiatric diseases such as schizophrenia [180] and even glioblastoma [181]. The underlying circuit mechanisms causing E/I imbalance in dedicated areas of the brain likely differ between these disorders, as does its role for the neurodegenerative process typical of those diseases. However, these recent findings open up new avenues for promising therapeutic strategies based on precise circuit-level interventions, which could, for instance, be achieved through adeno-associated viral vectors. To this end, however, more detailed insight into the cell-type specific alterations in the human cortex is still needed.

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