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Abstract: Amyotrophic lateral sclerosis is a fatal adult-onset neurodegenerative disease characterized by progressive muscular weakness and atrophy. The primary feature of amyotrophic lateral sclerosis is the selective loss of motoneurons in the brain and spinal cord. However, changes in synaptic transmission and motoneuron excitability are among the first events that take place during development and accompany the relentless deterioration of motor circuitry. This chapter aims to summarize the current understanding of defects in intrinsic electrophysiological properties of motoneurons, local GABAergic and glycinergic inhibitory as well as cholinergic modulatory interneuron networks, and long-range glutamatergic excitatory input neurons that can precede disease onset or occur during the progression of the disease. We summarize evidence that therapeutic options that target synaptic transmission and intrinsic features of motoneurons might represent novel effective strategies for patients with amyotrophic lateral sclerosis.

Keywords: amyotrophic lateral sclerosis; inhibitory transmission; motoneuron excitability; neuromodulation; proprioception

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disorder affecting both upper motoneurons in the cerebral cortex and lower motoneurons in the brainstem and spinal cord. Upper motoneurons are glutamatergic descending neurons that synapse directly or indirectly via interneurons onto lower motoneurons typically through the corticobulbar and corticospinal tracts. Lower motoneurons are multipolar cholinergic neurons whose axons exit the central nervous system to innervate skeletal muscles to produce movement. Consequently, ALS leads to progressive muscle atrophy and weakness and eventual paralysis. Death occurs within 3 years of symptom onset, typically from respiratory failure. Sporadic ALS accounts for approximately 90% of cases; the remaining 10% are hereditary and referred to as familial (1). The discovery of ALS-causing mutations in the *superoxide dismutase-1* (*SOD1*) gene in 1993 led to the generation of transgenic mice that recapitulate the key features of the disease. *SOD1*-mutant mice, with other experimental models that have emerged following identification of a rapidly growing number of ALS-associated genes, have helped to learn about the molecular and cellular processes underlying the disease. Both cell- and non-cell-autonomous mechanisms contribute to the dysfunction and death of motoneurons. The expression of ALS-causing factors in glial cells, which include astrocytes, microglia, and oligodendrocytes, contribute to the selective death of motoneurons, which themselves present with a significant vulnerability due to these same determining factors (2–4). T lymphocytes infiltrating the central nervous system and peripheral macrophages are other cellular factors that participate in the pathogenesis of ALS (5–7). Non-cell-autonomous mechanisms may support dysfunction before the first clinical signs are evident or accompany motor decline during the symptomatic phase. The earliest signs, which are detected during embryonic and postnatal development and that will pave the way for the rest of the disease course in ALS mice, are linked to the electrophysiological properties and circuitry of motoneurons. Moreover, in humans, asymptomatic mutation carriers can exhibit electrophysiological abnormalities such as intracortical facilitation (ICF) transmission deficits, which can be observed 30 years before the onset of symptoms (8). Here, we review alterations of intrinsic electrophysiological features of motoneurons and synaptic transmission, including changes in inhibitory, excitatory, and modulatory signals, observed in patients with ALS and in mice. We discuss how these changes might be considered promising therapeutic targets for new and effective treatments for this devastating disease.

ELECTROPHYSIOLOGICAL PROPERTIES OF MOTONEURONS IN ALS

Motoneurons acquire molecular properties during their differentiation throughout embryonic development as a result of dynamic interplay between spatial and temporal expression of families of transcription factors and diffusible morphogens. In the terminal step of differentiation, the combinatorial activity of terminal effector genes defines the features of individual postmitotic motoneurons.

This battery of terminal identity genes governs the synthesis of neurotransmitters and expression of neurotransmitter receptors, ion channels, and axon guidance and synaptic adhesion molecules (9, 10). At these early stages of development, the first wirings of neuronal circuits proceed with axon outgrowth toward appropriate targets and by the complex interaction of both intrinsic genetic instructions and environmental cues. Spinal motoneurons are organized into motor columns along the rostrocaudal and ventrodorsal axes that project to a single muscle target in the periphery. Long descending premotor projection neurons from the spinal cord and supraspinal centers, as well as proprioceptive afferents, begin to establish the spinal motor circuitry during embryonic development (11, 12). The extensive dendritic arborization of motoneurons that integrates synaptic inputs critical for circuit formation and plasticity is shaped (13), and the diversity of local interneuron subtypes that direct early motor output enables further adaptive motor behavior (14). Among the developmental processes that build a coherent motor circuitry, the early calcium-mediated electrical activity and acquisition of intrinsic electrophysiological properties are critical factors. Expression of ion channels at the plasma membrane determines the intrinsic responses of motoneurons and undergoes dynamic changes from embryonic to postnatal development (15, 16). These multiple components of a developmental program represent a cornerstone establishing movement coordination, control, and skill that will be fundamental throughout the life span. The concept that alterations to these components can occur very early in the life of patients has been explored through animal models of the disease. Although the first clinical signs, and significant loss of motoneurons, appear in adults, early molecular and cellular signs have been documented in ALS mice. In *SOD1*^{G93A} mice, the first motor symptoms appear at around 90 days of age, but activation of cellular stress pathways can be observed in vulnerable motoneurons as early as postnatal day (P)12, and dysfunction of the neuromuscular junction is already noticeable at P50 (17, 18). However, the earliest alterations that evidence a functional defect are those observed during the developmental stages of the motor system and are associated with motoneurons' acquisition of electrophysiological properties and the integration of motoneurons into the motor circuitry.

Electrophysiological changes in motoneurons during embryonic and postnatal development

Some differences in the resting membrane potential (RMP), input resistance, capacitance, and rheobase have been observed in different experimental systems (isolated neurons from mouse or humans, spinal preparations, brainstem and spinal cord slices) at different stages (from embryonic day (E) 17.5 to P10, or after 11 weeks of differentiation *in vitro*) and in the presence of different ALS-causing mutations. However, changes in cell properties do not emerge as a common salient feature of ALS motoneurons (Table 1). The spike features, which include action potential (AP) threshold, delay to AP (i.e., the time interval between current injection and spike onset), AP amplitude, AP duration, rate of AP rise, and repolarization, as well as after-hyperpolarization (AHP) characteristics, are for the most part similar in experimental ALS models and controls. There have been discrepancies in the AP duration and the rate of AP rise between different studies in

TABLE 1 **Alterations of motoneuron electrophysiological features in ALS models**

	Embryonic primary culture	iPSC-derived neuron culture	Embryonic spinal cord preparation	Postnatal brainstem slice	Postnatal spinal cord slice	Postnatal spinal cord preparation
RMP	Unchanged (21–24)	Unchanged (28, 30, 31), Depolarized (27)	Unchanged (25, 34)	Unchanged (26)	Unchanged (20, 23), depolarized (19)	Unchanged (29)
Input resistance	Unchanged (21–23)	Unchanged (27, 28, 30, 31)	Increased (25, 34)	Unchanged (26)	Unchanged (19, 23)	Lower (29)
Capacitance	Unchanged (21)	Unchanged (28, 30), decreased (30), increased (27)	Decreased (25)		Unchanged (20)	Increased (29)
AP threshold	Unchanged (21, 22, 24)	Unchanged (28)	Unchanged (25)	Unchanged (26)	Unchanged (19, 20, 23)	
Delay to AP	Unchanged (21)		increased (25)		Unchanged (19)	
AP amplitude	Unchanged (21–24)	Unchanged (28)	Unchanged (25)	Increased (26)	Unchanged (23), decreased (19)	
AP duration	Unchanged (21–24)	Unchanged (28)	Unchanged (25)	Unchanged (26)	Unchanged (23), decreased (20), increased (19)	Decreased (29)
Rate of AP rise	Unchanged (21, 24)				Increased (20), decreased (19)	
Rate of repolarization	Increased (21)				Increased (20)	
AHP characteristics	Unchanged (22–24)	Unchanged (28)	Unchanged (25)	Unchanged (26)	Unchanged (19, 23), decreased τ (20)	Unchanged (29)

TABLE 1 **Alterations of motoneuron electrophysiological features in ALS models (Continued)**

	Embryonic primary culture	iPSC-derived neuron culture	Embryonic spinal cord preparation	Postnatal brainstem slice	Postnatal spinal cord slice	Postnatal spinal cord preparation
Firing frequency	Increased (21–24)	Increased (27, 28), decreased (27, 30, 31)	Increased (25)	Increased (26)	Unchanged (20), increased (23), decreased (19)	Decreased (29)
Maximum firing rate	Increased (23)		Unchanged (25)			Unchanged (29)
Na^+ current peak	Unchanged (250)	Unchanged (27, 28, 30), decreased (27, 30)				
K^+ current peak		Unchanged (27), decreased (27, 28), increased (30)				
Persistent Na^+ current	Increased (24)			Increased (26)	Increased (20)	
Persistent Ca^{2+} current	Increased (22)				Increased (20)	
HVA Ca^{2+} currents	Increased (22)					
Recovery from fast inactivation (Na^+ current)	Increased (250)					

TABLE 1 **Alterations of motoneuron electrophysiological features in ALS models (Continued)**

	Embryonic primary culture	iPSC-derived neuron culture	Embryonic spinal cord preparation	Postnatal brainstem slice	Postnatal spinal cord slice	Postnatal spinal cord preparation
Spontaneous motoneuron activity	Increased (24)	Increased (28), unchanged (30), reduced (30)				
Spontaneous locomotor outputs						Unchanged (36), Increased burst duration (37)
Evoked rhythmic activity			Slower rhythm period (34)			Unchanged (35), absent in lumbar but not in sacral roots (36)
Noradrenergic sensitivity						Increased (35)

The properties of motoneurons in different experimental ALS models were compared to their respective controls. Embryonic primary culture: neurons were isolated from E12–14 mice and cultured for 2–4 weeks before recording (23, 24), from E15 mice and cultured for 2–3 weeks (21), from E13 mice and cultured for 2–3 weeks (22), from E12–14 mice and cultured for 12–16 days (76), or from E15 and cultured for 8–13 days (250). Embryonic spinal cord preparations were obtained from E17.5 mice (25, 34). Postnatal brainstem slices were obtained from P4-P10 mice (26). Postnatal spinal cord slices were obtained from P0–P12 (20), P7 (23), or P6–P10 mice (19). Postnatal spinal cord preparations were obtained from P1–P3 (35), P3 (37), P3–P6 (36), and P6–P10 mice (29). Regarding iPSC-derived motoneurons, recordings were performed after 14 or 28 days (28), 66–79 days (31), 3–10 weeks (27) of neuronal differentiation. Of note, in (30), electrophysiological properties change with differentiation time: 3–4 weeks versus 7 weeks. RMP depends on ALS patient lines and time in culture, firing frequency varies between time of maturation (27). Of note, an increased amplitude of delay-rectifying potassium (K^+) current peaks can be observed in motoneurons harboring FUS and not SOD1 mutations (30). Evoked rhythmic patterns: the rhythmic activity induced by application of NMA and 5-HT is absent in lumbar (though it induced a tonic activity) but not in sacral segments (36). Noradrenergic sensitivity relates to the noradrenergic-induced amplification of lumbar ventral roots burst amplitude during evoked-fictive locomotion (35). AHP, after-hyperpolarization; AP, action potential; Ca^{2+}, calcium; E, embryonic day; HVA, high-voltage activated; iPSC, induced pluripotent stem cells; K^+, potassium; Na^+, sodium; NMA, N-methyl-D-, L-aspartate; P, postnatal day; RMP, resting membrane potential.

postnatal spinal cord slices. These discrepancies could be because the studies used different genetic models and controls (*SOD1*^{G85R} and *SOD1*^{G93A} mice with non-transgenic controls (19) and transgenic mice expressing the wildtype form of human SOD1 as controls (20)) and performed recordings at different ages (from P0 to P6 (20) and from P6 to P10 (19)). However, both isolated embryonic SOD1^{G93A}-expressing motoneurons (21) and those in slice preparation (20) consistently exhibit an increased rate of repolarization compared to controls.

Analysis of firing frequency-current intensity relationships reveals a common difference between ALS and control motoneurons (Table 1). AP frequency is increased in ALS embryonic motoneurons in culture (21–24), embryonic spinal cord preparation (25), postnatal spinal cord, and brainstem slices (23, 26), as well as in human motoneurons derived from induced pluripotent stem cells (iPSCs) obtained from patients with ALS (27, 28), relative to controls. A closer examination of the studies that show variations in this trend toward an increased AP frequency reveals the developmental dynamics that motoneurons are subject to and that can be altered by the presence of ALS-causing mutations. Indeed, spinal motoneurons from P6–P10 spinal cord slices or preparations show decreased firing frequency compared with wildtype, although by age, the gain is lower in motoneurons from P6–P7 transgenic mice and unchanged in motoneurons from older P8–10 transgenic mice versus those from wildtype mice (29). A broader study in spinal cord slices from P0 to P12 mice showed an overall unchanged frequency-current relationship (20). The maturation of iPSC-derived motoneurons and their progressive acquisition of electrical properties over time in culture also illustrates this differential susceptibility to ALS-causing mutations with respect to motoneuron excitability. A phenotypic switch from early hyperexcitability to late hypoexcitability observed in ALS patient iPSC-derived motoneurons (27, 30) explains the previously reported differences in the firing response of motoneurons (28, 31).

ALS motoneurons show other aberrant properties; elevated persistent sodium (Na^+) and calcium (Ca^{2+}) currents are consistently encountered in different experimental conditions (20, 22, 24, 26). Persistent Na^+ and Ca^{2+} currents that are resistant to inactivation by depolarization play an important role in spike initiation, amplification of synaptic inputs, and increasing firing rate (32, 33). It is noteworthy that Riluzole decreases persistent Na^+ currents in *SOD1*^{G93A} motoneurons and results in reduced excitability (24). Defects in inhibitory synaptic properties are also prominent early defects and are detailed in the next sections.

Analysis of chemically evoked locomotor outputs (rhythmic activity) that emerge from embryonic lumbar spinal cords revealed a slower rhythm period in *SOD1*^{G93A} versus wildtype spinal cords (34). Interestingly, this N-methyl-D-, L-aspartate (NMA)-, and serotonin (5-HT)-evoked locomotor-like slower rhythm period is not observed in *SOD1*^{G93A} postnatal spinal cord preparations. However, *SOD1*^{G93A} postnatal spinal networks display increased sensitivity to noradrenaline (NA)-induced enhancement of burst amplitude (35). Surprisingly, in *SOD1*^{G85R} P3-P6 mice, the rhythmic motor activity evoked by addition of NMA/5-HT was not observed in lumbar roots, whereas rhythmic patterns were observed in sacral roots and were similar to those observed in the sacral roots of wildtype controls (36). In terms of spontaneous rhythmic activity, motor output is similar in *SOD1*^{G85R} and wildtype postnatal spinal cords, while a longer burst duration is observed in *SOD1*^{G93A} spinal cords (36, 37). Interestingly, behavior analysis of postnatal ALS mice revealed early and transient defects in locomotor capacities (26, 36).

Discrepancies exist between different studies, which could be attributable to the use of different genetic models, recording approaches, and conditions, and/or to an effect of the experiment time window. However, altogether this evidence highlights altered motoneuron excitability, inhibitory imbalance, and changes in spinal locomotor networks as salient traits of the earliest origins of the pathology described to date.

Intrinsic features of adult motoneurons in ALS experimental models

To date, only three studies have reported the electrophysiological properties of adult motoneurons in ALS mouse models. In the first, whole-cell patch-clamp recordings were performed in ventral horn slices of 2.5-month-old transgenic mice that express green fluorescent protein (GFP) under the control of the choline acetyltransferase (ChAT) promoter. Based on 11 passive and active intrinsic properties of 42 lumbar motoneurons, the authors performed 11-dimensional cluster analysis from which they defined four clusters of motoneurons with similar properties (38). Table 2 displays the main electrophysiological characteristics.

TABLE 2 **Electrophysiological characteristics of motoneuron clusters in adult mice**

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Passive				
RMP (mV)	–70.3	–68.8	–74.2	–70.6
Input Resistance (mOhm)	95.4	73.4	48.0	43.8
Membrane time constant (ms)	8.9	6.9	4.9	2.2
SAG ratio (h current)	18.9	11.0	18.2	3.7
Active (500 ms square pulse)				
FIF (Hz) Instantaneous firing, beginning of the pulse	45.9	164.8 (doublet action potentials)	340.6	424.0
SSF (Hz) Steady-state firing end of the pulse	31.9	50.3	74.6	147.7
Muscle innervation				
Muscle	Soleus	Soleus	Tibialis Anterior	Tibialis Anterior
Fiber type	Slow twitch fiber	Slow twitch fiber	Fast twitch fiber	Fast twitch fiber

Motoneuron subtypes were defined using cluster analysis and functional identity was achieved with retrograde labeling of known muscle types (38). Soleus: slow-twitch fiber type and Tibialis anterior: fast-twitch fiber type. RMP, resting membrane potential; FIF, firing frequency; SSF, steady-state firing.

Retrograde labeling of motoneurons from slow-twitch muscle (Soleus) and fast-twitch muscle (Tibialis anterior) demonstrated that clusters 1 and 2 are representative of ALS-resistant slow motoneurons, while clusters 3 and 4 are representative of ALS-vulnerable fast motoneurons. The high input resistance of clusters 1 and 2 is consistent with the high excitability relative to the threshold recruitment of slow motoneurons. In this study, spinal cord slices were prepared from 2–3-month-old (asymptomatic) and ~4-month-old (symptomatic) *SOD1^{G85R}-YFP* transgenic mice (expressing mutant SOD1 fused with yellow fluorescent protein) to assess motoneuron electrophysiology both before and after the development of clinical signs. At 2–3 months, all four clusters were present in the mutant motoneurons, and their electrophysiological properties were similar to wildtype, except that in cluster 4, the RMP was hyperpolarized by 6 mV in mutant versus wildtype motoneurons. At 4 months, however, there was a decrease in the probability of recording mutant motoneurons from clusters 3 and 4, suggesting a loss of these populations. Interestingly, there was also a tendency toward hyperpolarization of the RMP of those motoneurons in clusters 1 and 2. This study suggests that RMP hyperpolarization could be a function of the pathogenic process in ALS mice. This observation supports the possibility that hypoexcitability arises from an increase in threshold current following RMP hyperpolarization.

In a study by Delestree et al., in vivo recordings in the sacrocaudal spinal cords of *SOD1^{G93A}* mice and their non-transgenic littermates from 34 to 82 days (presymptomatic to disease onset) allowed longitudinal analysis of motoneuron excitability during ALS progression in this ALS model (39). Intracellular recordings were performed on motoneurons that were identified by the antidromic APs observed in response to electrical stimulation of their axon in the sciatic nerve. In this study, no attempt was made to analyze according to clusters and so recorded values were distributed over a large range. For example, the recruitment current varied from 1 to 13 nA and the input conductance from 0.1 to 0.8 µS. In line with initial reports in cat motoneurons (40), the recruitment current highly correlated with the input conductance: the larger the input conductance, the higher the recruitment current. *SOD1^{G93A}* motoneurons behaved similarly to wildtype motoneurons, except that the mean input conductance was increased, which should induce increased excitability. As the mean recruitment threshold was not modified and no change in RMP occurred in either genotype, this expected hyperexcitability was probably compensated. As mentioned previously, an increase in persistent Na^+ current has been demonstrated in neonate mutant motoneurons; these results suggest that this increase could persist in the adult state. Remarkably, a greater proportion of mutant than wildtype motoneurons lost their ability to fire. The motoneurons that were unable to produce sustained firing were distributed along the full range of input conductance in *SOD1*-mutant mice, whereas in wildtype mice they were restricted to those with the highest input conductance. This study is in agreement with that of Hadzipasic et al.—it appears that in adult mice, pathogenic *SOD1* mutations lead to motoneuron hypoexcitability before muscle denervation (38).

In contrast to the above study, Jensen et al. showed that adult motoneurons in *SOD1^{G93A}* mice have an increased excitability attributed to a lower rheobase, higher input–output gains, and increased activation of persistent inward currents (41). Therefore, in vivo recordings in adult ALS mouse models lead to conflicting results concerning intrinsic electrical properties, which is presently attributed to

differences in experimental protocols. In any case, in vivo studies in ALS mice suggest that high electrical activity promotes endoplasmic reticulum stress, a marker of disease (42), while an increase in the recruitment threshold (i.e., a decrease in excitability) slows down disease onset and protects against muscle denervation (43). Therefore, the hypothesis that changes in motoneuron inputs could be a major factor in their vulnerability requires further evaluation.

ALS-ASSOCIATED INHIBITORY TRANSMISSION DEFECTS

Neuronal circuits called central pattern generators coordinate locomotion and control skilled movements. These neuronal networks comprise different cell types, such as motoneurons, interneurons, astrocytes, and microglial cells. Most interneurons use GABA or glycine as neurotransmitters and thus present an inhibitory phenotype. These interneurons are also the most abundant neurons in the spinal cord and play a major role in the regulation of neuronal excitability (44, 45).

GABAergic and glycinergic transmission

Among the numerous types of interneurons identified, different classes of inhibitory interneurons have been defined based on the expression of transcription factors. Among the V0 lineage made up of commissural interneurons projecting ipsilaterally or contralaterally (46, 47), inhibitory V0d interneurons participate in left-right alternation (48). The V1 interneuron population, including Renshaw cells and Ia inhibitory interneurons, project rostrally and ipsilaterally on motoneurons and reciprocal inhibitory neurons (49, 50). V2b inhibitory interneurons project ipsilaterally and caudally. Both V1 and V2b interneurons independently participate in alternation of extensor and flexor muscles (51). Neuronal activity is controlled by the balance between excitatory and inhibitory neurotransmission. While motoneuron pathology plays a large role in ALS pathogenesis, accumulating evidence highlights a relevant role for these inhibitory interneurons in the regulation of motoneuron excitability that might contribute to motoneuron pathology.

To date, mainly pharmacological approaches have been used to investigate the role of inhibitory neurotransmission in the control of motoneuron excitability. Both acute and chronic infusion of bicuculline (a $GABA_A$ receptor blocker) generates a dose-dependent and temporary muscular hyperexcitability, motor deficits, and loss of motoneurons, showing that inhibitory GABAergic blockade can generate hyperexcitability of the intraspinal neuronal circuits and motoneuron degeneration (52). In addition, increased motoneuron loss and total paralysis was observed when 4-amynopyridine or a low dose of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid was added (52, 53), suggesting a close functional link between glutamatergic transmission and GABAergic circuits in the regulation of motoneuron excitability. Considering that the use of strychnine (a glycine receptor blocker) has no significant effect and that glycinergic neurotransmission is mainly intersegmental (54), the GABAergic modulatory role appears to be intrasegmental, in line with evidence that ipsilateral flexor–extensor

alternations are governed by GABAergic neurons directly affecting motoneuron activity within each spinal segment.

Postmortem histological studies of ALS tissues have mainly described a decrease in inhibitory GABAergic and glycinergic interneurons. A layer-specific reduction of calbindin $(CB)^+$ neurons has been shown in cortical layers V and VI and in the ventral horn of spinal cords of patients with ALS (55–58). Analysis of the motor cortex of patients with ALS has also revealed a trend toward reduced calretinin $(CR)^+$ cells and a reduction in parvalbumin $(PV)^+$ cells (55, 59). Results from other studies also indicate that there are alterations in GABA homeostasis and transmission in cortical and spinal ALS inhibitory neurons. The motor cortices of patients with ALS exhibit a downregulation and an increase in the mRNA levels of the α1-subunit and the β1-subunit of the GABA receptor, respectively, versus control motor cortices, which could indicate altered receptor function (60). This correlates with the reduced binding of flumazenil (an α1-selective benzodiazepine antagonist) observed in positron emission tomography (PET) scanner studies (61) and the decrease in GABA levels observed by proton magnetic resonance spectroscopy (62) in the cortices of patients with ALS compared with controls.

As in humans, a reduction in CR^+ cells has been observed in the cortex, hippocampus (63), and the spinal cord (64) of $SOD1^{G93A}$ mice. Morrison *et al.* described a decrease in the number of interneurons in the spinal cord of $SOD1^{G86R}$ transgenic mice at early symptomatic stages versus age-matched control mice, with a parallel of onset motoneuron degeneration (65). Interestingly, an early increase in the population of PV^+ interneurons, observed in the motor and somatosensory cortices of ALS mice, could suggest that a transient increase in inhibitory neurotransmission acts as a compensatory mechanism (66).

Other approaches, such as high-resolution magnetic resonance spectroscopy, have revealed a decrease in $GABA_A$ receptors in $SOD1^{G93A}$ transgenic mice versus controls even at presymptomatic stages (67). Finally, gliosomes isolated from the spinal cord of presymptomatic ALS mice exhibit increased expression of the GABA transporter (GAT1) along with a reduction in GABA release versus gliosomes from control mice (68).

Electrophysiological whole-cell patch-clamp recordings in brainstem slices from postnatal $SOD1^{G93A}$ mice revealed an enhanced frequency of inhibitory transmission through an increased amplitude and frequency of miniature inhibitory postsynaptic currents (mIPSCs) mediated by GABA in superior colliculus interneurons (26). Whole-cell patch-clamp recordings of cultured glutamate decarboxylase Gad67-GFP-expressing interneurons from embryonic $SOD1^{G93A}$ mice revealed a significant decrease in the peak outward current and intrinsic hypoexcitability compared with wildtype Gad67-GFP interneurons (69), which could contribute to the attenuated inhibitory function observed in the disease. These results suggest early perturbation of inhibitory neuron populations but do not establish whether synaptic excitability alters to compensate for abnormal firing or whether this is a cause or consequence of perturbed excitatory neuron excitability in the disease (69). Interestingly, subtype-specific investigations have discovered that the largest PV^+ interneuron population in the cortex exhibits similar excitability in wildtype and presymptomatic $SOD1^{G93A}$ mice but that the population is hyperexcitable in symptomatic $SOD1^{G93A}$ mice (70). Interestingly, $SOD1^{G93A}$ PV^+ neurons were found to be more hyperexcitable neonatally than presymptomatically, suggesting that compensatory mechanisms take place at

some stage of the disease. Electromyography performed in end-stage *TDP-43^{A315T}* mice revealed fibrillation potentials and fasciculations (71). mIPSCs and evoked inhibitory postsynaptic currents (eIPSCs) were significantly reduced in layer V pyramidal neurons of 3-week-old *TDP-43^{A315T}* mice versus those of 3-week-old wildtype mice. These neurons also exhibited hyperexcitability that was abolished by picrotoxin (a $GABA_A$ receptor blocker), suggesting that impairments in GABAergic signaling contribute to cortical hyperexcitability (72). It was subsequently proposed that hyperactive somatostatin interneurons can inhibit PV^+ interneurons, inducing a disinhibition of cortical motoneurons (72), although there is yet no convincing evidence showing direct interactions between these interneurons in the motor cortex.

Nieto-Gonzales *et al.* (73) also demonstrated in the wobbler mouse model (74), through electrophysiological measurements of current threshold for input resistance and AP in the presence of picrotoxin, that cortical hyperexcitability could be related to a decrease in tonic GABAergic inhibition, which in turn was related to a reduction in $GABA_A$ receptor-mediated inhibitory currents in layer V pyramidal neurons of the motor cortex.

Electrophysiological studies performed in spinal cords revealed more discrepancies in impaired inhibition transmission: no significant differences in GABAergic mIPSCs and GABAergic currents were observed between *SOD1^{G93A}* and control spinal cord cultured motoneurons (75, 76). However, $GABA_A$ receptors had higher affinity and lower desensitization levels, and α1 subunit expression level were doubled in *SOD1^{G93A}* motoneurons. These differences could be the result of an adaptive process in response to reduced glycinergic inhibition but could also contribute to excitotoxic motoneuron death (75).

A more recent electrophysiological study also revealed impaired chloride homeostasis and a subsequent induction of a more depolarized reversal potential for $GABA_A$ receptors in *SOD1^{G93A}* embryonic motoneurons versus wildtype motoneurons (34). Also observed was a reduction in the frequency of inhibitory synaptic inputs in *SOD1^{G93A}* motoneurons, with less frequent and smaller amplitude mIPSCs. In addition, in *SOD1^{G93A}* motoneurons, inhibitory postsynaptic currents exhibited slower decay time than those in wildtype motoneurons, which correlated with a higher intracellular chloride concentration. Computer simulations projected that this slower relaxation of synaptic inhibitory events could, at the prenatal stage, act as a compensatory mechanism to strengthen GABA/glycine inhibition when E_{GABAAR} is more depolarized in order to maintain well-coordinated, although slightly slower, locomotor activity (34). These results also reinforce the hypothesis that very early inhibitory dysfunction may initiate pathogenesis in ALS motoneurons (77, 78) (Table 3).

Looking more specifically at glycine transmission, *in vitro* binding assays demonstrated reduced binding of strychnine to glycine receptors in the ventral horn of human ALS spinal cord versus controls (79, 80). Lumbar ventral and dorsal horns of patients with ALS were also found to exhibit significantly reduced glycine levels (81). Inhibitory synaptic changes, with reduced binding of strychnine to glycine receptors and a reduction in the inhibitory/excitatory synapse ratio of hypoglossal motoneurons, can be observed from the early symptomatic stages in *SOD1^{G93A}* transgenic mice (82). These changes may also contribute to motoneuron degeneration through both an increase in excitatory synapses and a decrease in inhibitory contacts.

TABLE 3 ALS-associated features of inhibitory currents

	Motoneuron (culture)	Cortical interneuron (culture)	Motoneuron (spinal cord preparation)	Hypoglossal motoneuron (brainstem slice)	Superior colliculus interneuron (midbrain slice)	PV+ interneuron (brain slice)	Layer V pyramidal neurons (brain slice)
Intrinsic excitability		Reduced (69)			Increased (26)	Increased (neonatal)-unchanged (pre-symptomatic)-increased (symptomatic) (70), Reduced (72, 233)	Increased ((72, 73)
GABA-induced current	Unchanged (75, 76)						
GABA-induced current decay τ	Unchanged (75, 76)						
GABAergic mIPSC	Unchanged (76)		Reduced (34)	Unchanged (26)	Unchanged (26)	Unchanged (72)	Reduced (72), Unchanged (73)
GABAergic mIPSC decay τ	Unchanged (75, 76)		Longer (34)				Longer (73)
GABAergic mIPSC frequency	Unchanged (75, 76)		Reduced (34)	Increased (26)	Increased (26)	Unchanged (72)	Reduced (72, 73)
GABAergic sIPSC frequency						Increased (72), Reduced (233)	Reduced (73)
$GABA_A$ receptor desensitization	Reduced (75)						

TABLE 3 **ALS-associated features of inhibitory currents (Continued)**

	Motoneuron (culture)	Cortical interneuron (culture)	Motoneuron (spinal cord preparation)	Hypoglossal motoneuron (brainstem slice)	Superior colliculus interneuron (midbrain slice)	PV+ interneuron (brain slice)	Layer V pyramidal neurons (brain slice)
Glycine-induced current	Reduced (76)						
Glycine-induced current decay τ	Unchanged (76)						
Glycinergic mIPSC	Reduced (76)		Reduced (34), Unchanged (Zebrafish) (85)	Unchanged (26)			
Glycinergic mIPSC decay τ	Unchanged (76)		Longer (34, 85)				
Glycinergic mIPSC frequency	Unchanged (75, 76)		Reduced (34, 85)	Increased (26)			
E_{GABAAR}			Depolarized (34)				
$[Cl^-]_i$			Increased (34)				

Spinal embryonic neurons were isolated from E12–14 mice and cultured for 12–16 days (76), or from E15 and cultured for 8–10 days (75). Embryonic spinal cord preparations were obtained from E17.5 mice (25, 34). Brainstem slices were obtained from P4-P10 mice and midbrain slices obtained from P10-P12 mice (26). Cortical interneurons were isolated from E15.5 mice and cultured for 12 days (69). VP^+ inhibitory interneurons (*Gad67-GFP*) were recorded in brain slices of $SOD1^{G93A}$ (and control) at 6 (neonatal), 26–35 days (pre-symptomatic) and 90–101 days (symptomatic) of age (70). Brain slices were obtained from P3 $TDP\text{-}43^{A315T}$ mice (74), or from P9 $SOD1^{G93A}$ mice (235). Layer 5 pyramidal neurons in brain slices from P15-P25 wobbler mice (76). Exposed spinal cord was prepared from 4 days post-fertilization larvae $Sod1^{G93R}$ zebrafish (87). $[Cl^-]_i$, intracellular chloride concentration; E_{GABAAR}, reversal potential for $GABA_AR$; mIPSC, miniature inhibitory postsynaptic current; PV, parvalbumin.

A progressive and presymptomatic loss of glycinergic synapses on lumbar motoneurons and of CB^+ cells has been observed in $SOD1^{G93A}$ mice (83). A decrease in glycine transporter 2 (GlyT2) and GAD65/67 expression has also been observed in the ventral horn of symptomatic $SOD1^{G93A}$ mice (64). Cell culture models also display a decrease in postsynaptic glycine receptor expression (76, 84). Electrophysiological whole-cell patch-clamp recordings of spinal cord motoneurons revealed an early and specific decrease in the densities of spontaneous glycinergic IPSCs and glycine-induced currents in large-sized $SOD1^{G93A}$ motoneurons compared with wildtype motoneurons (84). A similar decrease in glycinergic currents has been described in a mutant SOD1 zebrafish model; glycinergic neurotransmission is impaired in spinal motoneurons from mutant SOD1 zebrafish. This decrease has been shown to precede the onset of pathophysiological defects in motoneurons, suggesting that motoneuron hyperexcitability may be associated with their loss, or the loss of the recurrent inhibition (85).

Recurrent and cortical inhibition

Motoneurons and Renshaw cells form a recurrent inhibitory circuit in order to adjust the motor output. Renshaw cells were first identified in cats by their high-frequency discharge in response to antidromic motor axon APs (86) and are located in the most ventral regions of laminae VII and IX of the spinal cord (87). They belong to the V1 interneuron subclass and can be identified by their medium to large size, expression of biochemical markers such as GlyT2, CB, and PV, location, and electrophysiological properties such as a high postsynaptic sensitivity to acetylcholine and large glycine- and GABA-evoked currents (88). Their inhibitory action is mediated by both GABA and glycinergic synapses, although synaptic boutons immunoreactive to glycine alone are more numerous than boutons that are immunoreactive to both GABA and glycine (89). Since Renshaw cells release both GABA and glycine, the recurrent inhibition they induce exerts a longer inhibitory synaptic action than the inhibition induced by Ia interneurons, in which neurotransmission is more phasic and solely glycinergic (90, 91). Renshaw cells are the only interneurons that receive direct excitatory synaptic inputs from motoneurons and, in turn, exert inhibitory feedback on them, known as recurrent inhibition (92). However, inhibitory synapses of Renshaw cells are located on dendrites rather than on the cell body (93) and the effectiveness of recurrent inhibition at reducing the motoneuron firing rate is limited (94). This is in keeping with the small amplitude of the postsynaptic inhibitory potential or current generated by Renshaw cells (95). In contrast, the synapses of Ia inhibitory interneurons are close to the motoneuron soma and have a more significant impact in counteracting the excitatory input arriving in the dendrites. Thus, Renshaw cells and Ia interneurons present distinct synaptic connectivity that serves different functions. Individual Renshaw cells receive inputs from particular motor pools and spread their inhibitory output to the same motoneurons, either directly or through inhibition of Ia inhibitory neurons mediating reciprocal inhibition of antagonistic muscles (flexor and extensor alternation activity), to γ-motoneurons controlling muscle spindle length, and to other Renshaw cells (88). Thus, recurrent inhibition is primarily generated by input from motor axon collaterals. However, it may also involve convergent signals from corticospinal origins (96, 97).

In humans, cortical excitability can be investigated using noninvasive procedures such as transcranial magnetic stimulation (TMS) or the nerve excitability test (NET). TMS consists of applying a local time-varying magnetic field that depolarizes neurons beyond their AP firing threshold and stimulates the primary motor cortex. The resulting evoked muscle response is then recorded using an electromyogram. The NET involves directly applying an electrical stimulus to a desired nerve and measuring the evoked response at the appropriate muscle. To differentiate between excitatory and inhibitory circuitries, different TMS stimulation protocols have been developed. To assess motor cortex excitability, this technique is associated with the measurement of motor evoked potentials (MEP), recorded from a contralateral innervated muscle (98), and an increased excitability which is detected following a conditioning stimulus (referred to as ICF). TMS procedures have shown that a transcranial subthreshold stimulus, activating low-threshold inhibitory circuits and thus increasing the stimulus threshold to elicit an evoked response (99), can suppress the response to a later suprathreshold stimulus (100). This inhibitory phenomenon, attributed to GABA-secreting inhibitory cortical interneurons via $GABA_A$ receptors, is referred to as short intracortical inhibition (SICI) (101, 102).

Electroneurography studies revealed marked variability in the hyperexcitability index scores of patients with ALS. The inhibitory effects of TMS on the corticospinal output of patients with ALS demonstrated that the threshold to elicit an MEP was significantly reduced after inhibitory stimuli (103–107). This was accompanied by a reduction in intracortical inhibition (103, 107–114), and lower and less effective SICI in ALS patients with limb-onset disease, suggesting either a dysfunction or a loss of inhibitory interneurons (107, 113, 115–120). However, it must be noted that at the cellular level, electrophysiological alterations, such as alterations of voltage-gated Na^+ and K^+ channels that affect motoneuron AP threshold (24, 121), may also contribute to reduced SICI in ALS.

TMS studies in humans have also demonstrated ICF (111–114, 122), and Vucic *et al*. reported that the measured reduction in SICI represented degeneration of inhibitory cortical circuits combined with hyperexcitation of high-threshold excitatory pathways (123). More recent work has shown that reduced and altered SICI affects motor cortical circuits in ALS; the study also showed that combining two parameters, short-interval ICF and SICI, increases the utility of SICI for identifying patients with ALS (124). Overall, these results demonstrate that in ALS the imbalance between excitatory and inhibitory circuits in the M1 cortex is based on a combination of increased excitability and decreased inhibition (125). One study reported more normal SICI values (126) and also demonstrated reduced late intracortical inhibition, attributed to $GABA_B$ receptors, in patients with ALS compared with control individuals (113, 126). Another showed more frequent and stronger inhibitory responses in cortices of patients with ALS versus those of control patients (127).

More evidence for cortical inhibition dysfunction came from analysis of the duration of the cortical silent period (CSP). Indeed, CSP is thought to reflect both inhibition of anterior horn cells from the spinal cord and cortical influences through $GABA_B$ receptors (128–132). Thus, the observed reduction in CSP duration, predominantly observed in patients with bulbar-onset disease (133), is likely to be associated with disinhibition of anterior horn cells (134, 135) and dysfunction of cortical inhibitory interneurons acting via $GABA_B$ receptors (120).

The late manifestation of overt cortical hyperexcitability (136) could be explained by the incredible capacity of inhibitory circuitry for compensation (137–140) and the high levels of brain reorganization observed in patients with ALS (141, 142). Indeed, these mechanisms of plasticity may slow disease progression. This hypothesis is supported by the observation that patients with preserved intracortical inhibitory circuitry display a slower disease progression (143). However, it remains unclear how interneuronal capacity may selectively fail in patients with ALS over time. As a loss of GABAergic populations is reported during aging in both human and murine studies (144, 145) and is associated with a decline in inhibition in a number of cortical regions (144, 146–149), it is also possible that although inhibitory circuitry can compensate for initial insults, an age-related decline of inhibition leads to failure of further compensation.

Overall, these observations demonstrate that loss of Renshaw cell function could be the result of degeneration of the corticospinal fibers directed to these cells and that the loss of cortical inhibitory influence, in association with ion channel alterations, may participate in increased motor network excitability (125). A better understanding and characterization of subtypes, inputs and outputs, morphology, and electrophysiological properties of the different cortical interneurons would be helpful to better dissect the mechanisms underlying cortical hyperexcitability in ALS.

Renshaw cell circuitry can be studied by combining TMS with the paired H-reflex technique, which produces a response whose amplitude inversely correlates with activity in recurrent inhibitory pathways (150, 151). Raynor *et al*. presented the first evidence for Renshaw cell impairment in patients with ALS (135), reporting an abnormal reduction in recurrent inhibition in patients with ALS compared with control individuals. The collision technique, used in motor axons, can be used to test recurrent inhibition by creating a relatively homogeneous population of motoneurons which are under the effect of both Renshaw inhibitory inputs and post-activation AHP that regulates the AP firing rate of the motoneurons themselves. One of the prerequisites therefore for the correct application of the paired H-reflex method is to produce results whereby the depression of motoneuron activity by Renshaw cells overcomes the depression produced by AHP (152, 153). Unfortunately, in this work (135), the paired H-reflex methodology was not fully implemented (153), and these findings were insufficient to conclude if it was recurrent inhibition, AHP, or both that was decreased. Indeed, even though no changes in AHP have been observed in motoneurons from the *SOD1*^{G93A} and the *SOD1*^{G85R} mouse models (23, 29), the shorter than normal AHP duration observed in earlier stages of ALS (154) could explain the results obtained by Raynor *et al*. (135).

In another study, Shefner and Logigian investigated the mixed nerve silent period (MNSP), the period of motor inhibition observed when the mixed nerve innervating a voluntary activated muscle is electrically stimulated, in patients with ALS and control individuals (155). Patients with ALS exhibited a longer MNSP duration, as well as less complete inhibition in the middle phases of the period, which may also reflect abnormalities in Renshaw cell function. However, the stimulated nerve fibers used in this study originated from the intrinsic muscles of the hand, which are devoid of recurrent inhibition (156).

A more recent study performed by Özyurt *et al*. compared spinal recurrent inhibition and postactivation depression (PAD) on the soleus muscle in

lumbar-affected and nonlumbar-affected ALS patients (157). PAD is another spinal circuit with an effective presynaptic network that tones down the output of the primary afferents on motoneurons. As in the previous studies, this work demonstrated a reduced duration of recurrent inhibition and reduced PAD of the H-reflex in patients with ALS compared with controls, which may lead to excessive excitation of motoneurons. Unfortunately, this work could not provide evidence of whether it is primarily Renshaw cells or motoneurons that are impaired.

Finally, it has been shown that both Renshaw cells and V1-derived Ia inhibitory interneurons, mediating recurrent and reciprocal inhibition of motoneurons, can be excited by V0c cholinergic interneurons to inhibit ipsilateral motoneuron excitability (158). Interestingly, early reduction of ChAT content in the presynaptic boutons of V0c interneurons on motoneuron somas and Renshaw cells has been observed in the *SOD1*^{G93A} mouse model (159). Similarly, it has been reported that cholinergic afferents from motoneurons to Renshaw cells are lost at early stages of ALS, by retraction of the motoneuron collateral (160). Inhibitory boutons from Renshaw cells on motoneurons and the number of Renshaw cells were unaffected at the same stage. In both studies, these changes occurred long before markers of motoneuron degeneration appeared. Therefore, according to these findings, cholinergic dysfunction can also trigger hyperexcitation and neurodegeneration processes in the spinal circuits through decreased excitatory action on inhibitory neurons.

Even though there is accumulating evidence to suggest that the inhibitory circuitry is affected and that interneuron populations are lost in ALS, controversies still exist about the evolution of this altered inhibition. Understanding these processes is of great interest considering that motoneuron hyperexcitability is observed at both the embryonic and presymptomatic stages in ALS models and patients (25, 26, 103) and that interneuron development is an activity-dependent process (161–164). Indeed, attenuating the activity of specific interneuron populations affects their migration and morphology during development (165) and their inhibitory synapse formation on excitatory cells (162, 166). In particular, the complexity of inhibitory innervation field is activity dependent. Thus, in ALS, where hyperexcitability is an early phenomenon (25, 167), aberrant inputs may be created at the motoneuron presynapse long before disease onset (168).

Two hypotheses have been proposed to explain how Renshaw cell alterations may lead to a hyperexcitable state and eventual degeneration of motoneurons. The first hypothesis postulates that the hyperexcitability is caused by loss of recurrent Renshaw cell-mediated inhibition and is based on electrophysiological findings suggesting an impairment of Renshaw cells in patients with ALS (135, 155). It is reinforced by the progressive loss of glycinergic boutons throughout the soma of the motoneurons and loss of CB^{+} cells observed in *SOD1*^{G93A} mice at an early symptomatic stage, before motoneuron degeneration. Since GABAergic terminals are only affected at the final stage, these changes can be assumed to be due to Renshaw cell loss (83). Another study reported early loss of Renshaw cells and revealed that lithium protects against Renshaw cell loss and delays disease progression, leading the authors to suggest that Renshaw cell loss may be the event that makes motoneurons more susceptible to glutamatergic toxicity in ALS (169). In addition, spinal motoneurons from *SOD1*-mutant zebrafish exhibited impaired glycinergic neurotransmission that preceded the onset of

pathophysiological defects in motoneurons, thus also suggesting that motoneuron hyperexcitability may be associated with the loss of these cells, or the loss of the mediated recurrent inhibition (85).

The second hypothesis proposes that the recurrent inhibitory circuit is altered ahead of motoneuron hyperexcitability and neurodegeneration but that this is not a consequence of Renshaw cell loss. Indeed, some studies suggest that the temporal onset of degeneration in motoneurons and interneurons may occur in parallel in patients with ALS (58) and in *SOD1*^{G86R} mice (65, 170). In agreement with the latter hypothesis is the observation that there is an early increase in the population of PV^{+} interneurons in the motor and somatosensory cortex of *SOD1*^{G93A} mice, which suggests that a transient increase in inhibitory neurotransmission could act as a compensatory mechanism to rescue motoneurons from glutamate excitotoxicity (66). Reinforcing this, activation of Renshaw cells has a poor effect on motoneuron soma activity (171) and interneurons are preserved in the symptomatic stage, indicating that progression of motoneuron degeneration is independent of Renshaw cell loss (160). In the same model, immunoreactivity experiments performed on vesicular inhibitory amino acid transporters (VIAATs) have shown a significantly reduced VIAAT expression in the ventral and dorsal horn neuropil, only at late stages, indicating that loss of inhibitory input (mostly Renshaw cells) does not precede but rather follows motoneuron death (172). In line with this, the finding that loss of inhibitory spinal interneurons occurs after loss of motoneurons (64) suggests that motoneuron degeneration may also trigger interneuronal pathology. Finally, as previously mentioned, V1 inhibitory neurons are thought to play a key role in modulating motor output, in part through recurrent and reciprocal inhibition. A more recent study on the fate of these neurons in the ventral spinal cord of *SOD1*^{G93A} mice (173) revealed increased V1 synaptic contacts with motoneuron cell bodies at an early stage of disease, followed by a 50% loss of V1 interneurons at a later stage. Since this loss is delayed compared with motoneurons and V2a excitatory neurons, this also supports the hypothesis that upregulation of inhibition is an early compensatory mechanism, followed by a substantial loss of V1 interneurons later in the disease (173).

These results may explain how Renshaw cell alterations may lead to hyperexcitability and eventually to motoneuron degeneration. However, there is still a debate about whether it is the selective loss of inhibitory interneuron regulation of motoneuron function, loss of inhibitory interneurons, or a combination of both, that contributes to motoneuron degeneration in ALS.

Therapeutic approaches

All these findings suggest that early impairment of GABAergic and glycinergic signaling occurs in ALS patients and animal models. As excitatory and inhibitory regulation are crucially linked from the presymptomatic stage of the disease, alterations in inhibitory circuitry may involve dynamic changes and determine the susceptibility and vulnerability of motoneurons. Therefore, new possible pharmacological neuroprotective strategies aiming to restore normal levels of excitability, potentially by preserving the integrity of inhibitory circuits or restoring inhibition in the spinal cord, may be appropriate for the treatment of ALS.

Therapeutic approaches using pharmaceutical compounds to target the inhibitory system have been successfully used to improve diseases in which

excitability and interneuronal alterations are present (174–176). In ALS, as spasticity, fasciculations, and cramps develop, GABA agonists such as diazepam and baclofen are prescribed to treat these features associated with the disease (177–179). Diazepam has been shown, using paired TMS, to reverse the hyperexcitability observed in patients with ALS compared with control individuals (115). The GABA analog gabapentin reduced fasciculations (180) with promising neuroprotective effects in a chronic model of glutamate toxicity (181) and reached clinical trials. However, later phase trials revealed no beneficial effects (182, 183), which could be explained by the fact that despite sharing structural similarity with GABA, gabapentin may not directly modulate GABA receptors and instead may selectively inhibit voltage-gated Ca^{2+} channels containing the α2δ-1 subunit (184, 185). In *SOD1*^{G93A} mice, administration of lithium prevented Renshaw cell loss and delayed the onset of symptoms (169, 186). Other therapeutic strategies using viral vectors to upregulate the production of GABA could also be employed (187).

To maintain physiological GABA concentrations, the use of drugs that block GABA uptake and catabolism at the synapse may be considered: tiagabine blocks the activity of the GABA transporter GAT1 (188), and vigabatrin blocks GABA transaminase and prevents the degradation of GABA (189). In addition, bumetanide, a drug that can inhibit the Na–K–Cl cotransporter NKCC1 and decreases intracellular chloride concentrations in immature $GABA_A$ receptors (190), and retigabine, which interacts with the KCNQ2/KCNQ3 subunits of K^+ channels and with $GABA_A$ receptors to weakly block sodium and calcium channels and thus decrease excitability (191), may also be considered.

In ALS, specific motoneurons are spared, such as the oculomotor and abducens populations (192), and gene expression studies have identified striking differences in genes responsible for the GABA and glutamate receptor subunits that may contribute to differential vulnerability. Indeed, in disease-resistant oculomotor neurons, α1, β1, β2, e, γ1, and θ $GABA_A$ receptor subunits are upregulated, whereas the α1 subunit is consistently reduced in vulnerable spinal and cortical motoneurons in patients with ALS (60, 62, 193). In addition, the specific vulnerability of ALS-resistant and ALS-vulnerable motoneurons correlates with the subunit composition of $GABA_A$ receptors, Gly/$GABA_A$ receptor density ratios, and the incidence of synaptic versus extrasynaptic $GABA_A$ receptors (194, 195). Considering that the subunit composition of $GABA_A$ receptors determines the location of the receptor as well as its specific pharmacological and electrophysiological properties, differential $GABA_A$ subunit expression will alter GABAergic receptor function (196–198). Thus, considering that an increase in $GABA_A$ receptors could generate a better GABAergic influence and protection, the development of GABA receptor subtype–selective compounds to counteract reduced inhibitory activity and modulate inhibition may be another interesting future therapeutic approach.

Finally, neural stem cell transplantation studies have shown strong evidence that restoration of the inhibitory drive can affect motoneuron survival (199, 200). More specifically, Xu *et al.* demonstrated that neural stem cells transplanted into *SOD1*^{G93A} mice differentiate into neurons presenting a GABAergic phenotype, which form local synapses and positively modify motoneuron survival (201–203), suggesting a future possible therapeutic use for these cells.

ALTERATIONS IN THE MODULATORY TRANSMISSION IN ALS

Neuromodulatory systems complement conventional neurotransmission by influencing neuronal excitability and synaptic efficacy. Abnormalities in this interneuronal signaling, where cholinergic and monoaminergic inputs modulate motor output, have been evidenced in ALS mice and patients.

Cholinergic transmission

Cholinergic C-synapses were identified several decades ago, mainly because of their unusual morphology. They form punctate large clusters (3–7 μm) primarily at the soma and proximal dendrites of α-motoneurons in the trigeminal, facial, and hypoglossal motor nuclei in the brainstem, as well as the α-motoneurons in the ventral horn of the spinal cord (204). C-bouton synapses originate from a small population of cholinergic *Pitx2*$^{+}$ interneurons, the V0c spinal neurons, found in the lamina X near the central canal (205, 206). Identifying that the V0c population forms the C-boutons allowed the *in vivo* function of these synapses to be addressed specifically. This major study revealed that these interneurons are involved in high task demands, such as swimming, that recruit the fast fatigable (FF) and fast fatigue-resistant (FR) motoneurons (206). Consistent with their role in task demand, these interneurons highly express the activity-dependent gene *c-Fos* following locomotion but also following painful sensory stimulation (207).

Interestingly, the motoneurons innervating fast-twitch muscles (those that are the first to degenerate in ALS) have a greater number of C-boutons than those innervating slow-twitch muscles (208). Moreover, C-boutons are not expressed among the motoneurons innervating the oculomotor, trochlear, abducens, and dorsal vagus nuclei, or the spinal gamma motoneurons and the autonomic motoneurons. Given the correlation between motoneurons without these terminals and survival, Ichikawa and Shimizu suggested that C-boutons might be involved in the neuron death that occurs in ALS (209). However, there is an exception to this correlation; the sphincteric motoneurons in Onuf's nucleus, a neuron type that survives in patients with ALS, are contacted by C-type terminals (210).

It is now well established that C-boutons increase the firing rate of motoneurons through a rather well-characterized sequence of cellular events involving activation of the postsynaptic muscarinic M2 receptors and inhibition of the Ca^{2+}-activated K^{+} current, SK channels (204, 211, 212). In addition, we recently demonstrated that muscarinic stimulation is dependent on motoneuron type, with a higher efficacy in the disease-vulnerable FF motoneurons; this further suggests that C-boutons may play a specific role in ALS (43).

Functional analysis of the role of C-boutons in ALS mouse models supports the hypothesis that the increased excitability mediated by C-boutons delays ALS progression, as does inhibition of ER stress (42). In addition, genetically silenced C-boutons in ALS mice exacerbates locomotor deficits (213). On the other hand, decreasing excitability through C-boutons-associated activity reduces motoneuron stress and denervation and thereby maintains muscle strength (43).

Monoaminergic systems

The developmental assembly and function of the locomotor circuits is subject to neuromodulation to provide adaptive behaviors (214). The monoaminergic system that encompasses NA, 5-HT, and dopamine (DA) has been shown to influence the rhythmic firing pattern of motoneurons and contribute to the flexibility of locomotor functions with premotor inputs and sensory afferents. A reduction in descending serotonergic fibers, linked to reduced levels of 5-HT in the spinal cord of *SOD1*^{G93A} mice, has been reported as early as E17.5. 5-HT hyperpolarizes E_{GABAAR} through 5-HT$_2$ receptors in embryonic motoneurons; similar intensities are observed in wildtype and *SOD1*^{G93A} mice (215). During postnatal development, while the levels of DA, NA, and 5-HT in the lumbar spinal cord increase between P1 and P10, only DA is increased in *SOD1*^{G93A} mice compared with wildtype mice, although this difference is mainly due to changes in the dorsal part of the spinal cord (35). DA, NA, and 5-HT increase all exerts marked modulatory activity, by potentiating fictive locomotion in spinal cord preparations. However, NA is the only biogenic amine to differentially enhance motor burst in ALS mice, potentially through modulation of excitatory inputs (35).

In the adult spinal cord, DA levels in patients with ALS were shown to be similar to those in control individuals; NA levels were found to be increased, as was the ratio of 5-HT to its metabolite 5-hydroxyindole-3-acetic (5-HIAA) (216). However, another study in patients with ALS documented a loss of dopaminergic neurons in the substantia nigra (217), which is consistent with the reduced dopaminergic function and nigrostriatal DA deficits observed in patients (218, 219). A study in ALS mice showed that reduced numbers of dopaminergic neurons in the substantia nigra pars compacta and ventral tegmental area were associated with reduced levels of DA (220). PET analysis showed a decrease in the binding of a selective 5-HT$_{1A}$ receptor in motor and extramotor regions of the brain in patients with ALS versus healthy volunteers (221). Another study reported that 5-HIAA/5-HT were elevated only in the lateral white matter of the cervical spinal cord of patients. A reduction in 5-HT$_2$ receptor binding, but not in the 5-HT$_{1A}$ receptor, was also observed in the motor and premotor cortex (222). A more recent study revealed a loss of serotonergic neurons in the brainstem and their projections in the hippocampus and spinal cord of patients with ALS (223). This loss is also found in *SOD1*^{G86R} mice and correlates with reduced levels of 5-HT in the cortex, brainstem, and spinal cord, even at the non-symptomatic stage (223). In ALS mice, reduced serotonergic innervation is associated with upregulation of the 5-HT$_{2B}$ receptor and development of spasticity, which can be abrogated by administration of a 5-HT$_{2B/C}$ receptor antagonist (223, 224). Treatment of *SOD1*^{G93A} neonates with fluoxetine, a selective serotonin reuptake inhibitor that acts at presynaptic terminals to increase 5-HT levels, decreased motor performance and weight of adult mice, without affecting disease onset (225). Analysis of P10 spinal cord slices revealed that 5-HT depolarizes RMP, hyperpolarizes the persistent inward current peak and increases motoneuron excitability, despite wildtype and *SOD1*^{G93A} motoneuron showing similar responses (225). Of note, treatment of adult ALS mice with fluoxetine has no effect on disease course. This underlines the importance of the monoaminergic, and in particular the serotonergic, system during critical stages of development; it will have long-term effects on the motor system. Monoaminergic system changes, which have a critical influence

on the assembly, maturation, and function of motor circuits, represent a pathological characteristic of ALS that remains largely understudied; these changes are therefore also a therapeutic target.

ALS-ASSOCIATED DEFECTS IN EXCITATORY TRANSMISSION

Glutamate is the major excitatory neurotransmitter for lower motoneurons that transmits information from upper motoneurons as well as proprioceptive sensory neurons. Glutamatergic dysfunction has been recognized as an important contributing factor to ALS.

Glutamatergic inputs

In humans, cortical hyperexcitability has been identified as an important pathogenic mechanism in ALS and is mediated through dysfunction of inhibitory and facilitatory intracortical circuits (114). The corticofugal hypothesis proposes that cortical hyperexcitability might cause motoneuron degeneration in ALS via trans-synaptic glutamate-induced excitotoxicity (226, 227). Decreased intracortical inhibition and cortical hyperexcitability can be seen in patients with *SOD1* gene mutations (112, 228). Moreover, cortical hyperexcitability appears to precede spinal motoneuron degeneration (103), supporting the dying-forward hypothesis that disease progression is mediated through glutamate-induced toxicity (229).

ALS mouse models are used to better understand the cellular basis of cortical hyperexcitability. In cultured cortical neurons baring the $SOD1^{G93A}$ mutant, hyperexcitability was attributed to a decrease in the threshold potential and time of the first AP and an increase in the firing frequency. This intrinsic hyperexcitability was attributed to an increase in the persistent inward Na^+ current density (230).

In situ whole-cell patch-clamp recordings of layer V cortical motoneurons in presymptomatic P26–31 $SOD1^{G93A}$ mice revealed increased excitability through increased frequency of spontaneous excitatory postsynaptic currents (231). This was accompanied by an increase in the expression of the vesicular glutamate transporter 2. Moreover, compared with controls, $SOD1^{G93A}$-expressing cortical neurons exhibited a higher output gain (slope of the frequency–current relationship) and lower rheobase.

These results have subsequently been mitigated by the observation that all neurons in $SOD1^{G93A}$ mice exhibit increased activity (whole-cell recordings in brain slices from P90 to P129 versus controls). This result is from a study in which corticospinal and corticocortical neurons were identified following injection of neuronal tracers at specific sites, and inhibitory GABAergic PV^+ neurons were identified by use of cells from *Gad67-GFP* mice (70). Interestingly, the cellular mechanisms leading to hyperactivity varied among the different neuronal populations. Corticospinal neurons exhibited an increase in the output gain, without changes in the rheobase, while corticocortical neurons displayed a decrease in rheobase and an increase in the output gain. It is well established that the activity of layer V pyramidal neurons is strongly inhibited in the perisomatic compartment by PV^+ GABAergic interneurons, which represent 40–50% of layer V

interneurons (232). It was thus unexpected that, in symptomatic *SOD1*^{G93A} mice, inhibitory PV$^+$ neurons became hyperexcitable, with a decrease in rheobase and a leftward shift in their output gain (without change in the maximal frequency of firing). However, this study did not address whether there was a partial loss of these inhibitory interneurons. This longitudinal analysis of cortical excitability highlights that neuronal plasticity occurs during disease progression, beginning with hyperexcitability at the neonatal stage, followed by normal excitability and a return to hyperexcitability at symptomatic stages of ALS. These results supporting an overall hyperexcitability of excitatory and inhibitory cortical neurons were further confirmed and suggested to involve compensatory mechanisms occurring all along disease progression in *SOD1*^{G93A} mice (70). It is interesting to note that spinal motoneurons also display hyperexcitability at embryonic and neonatal stages, which is followed by hypoexcitability in adults without reemergence of hyperexcitability. To further investigate the overall effects of neuronal hyperexcitability on the homeostasis of layer V neurons, intracellular Ca^{2+} levels were assessed using two-photon imaging of GCaMP6s-infected neurons (70). The main conclusion was that basal levels of intracellular Ca^{2+} are increased in SOD1-mutant layer V neurons, supporting a net hyperexcitability and/or an inability to maintain Ca^{2+} homeostasis, a factor that is known to be responsible for neuronal toxicity. It should be noted that spinal motoneurons have a poor capacity to buffer intracellular Ca^{2+} and are thus very sensitive to Ca^{2+}-induced toxicity. Consequently, the hypoexcitability reported at symptomatic stages could be a compensatory mechanism to prevent Ca^{2+} overload.

Similarly, hyperexcitability of layer V pyramidal neurons in 3-week-old *TDP-43*^{A315T} mice (a mouse model of ALS and frontotemporal dementia with profound cortical pathology) was found to be due to reduced mIPSCs, indicative of a reduced GABAergic tone, versus wildtype mice. Consistent with this, PV$^+$ GABAergic interneurons of these mice were hypoexcitable; this was due to hyperactivity of somatostatin interneurons located in the M1 cortex. Interestingly, ablation of somatostatin interneurons restores the PV$^+$ GABAergic inhibition of layer V neurons and protects against excitotoxicity induced by L5 neurons (72). A recent study in late presymptomatic *SOD1*^{G93A} mice confirmed the hypoactivity of PV$^+$ neurons (233). Altogether, these studies support the idea that different cell types contribute to the control of corticospinal layer V neuron activity during ALS progression.

In vivo genetic manipulation is now emerging as a technique that can help us understand the overall effects of changes in cortical activity on ALS onset and progression by allowing modulation of neuronal activity. Chemogenetics—specifically, the chemogenetic tool designer receptors exclusively activated by designer drugs (DREADD) (234)—has been used to increase PV$^+$ neuron activity. Chronic activation of PV$^+$ interneurons at the presymptomatic stage or at symptom onset delays the cortical neurodegeneration observed at the symptomatic P117 stage and delays motor deficits in the *SOD1*^{G93A} ALS model (233).

In addition, genetic ablation of subcerebral projection neurons, including the layer V neurons, has been used to assess the *in vivo* contribution of the cerebral cortex to ALS. This was achieved through ablation of the transcription factor *Fezf2*, which is necessary and sufficient to instruct birth and specification of corticospinal neurons and subcerebral projection neurons. Crossing *SOD1*^{G86R} mice with *Fezf2*$^{-/-}$ mice generates ALS mice entirely lacking both these

neuron populations. The loss of subcerebral projection neurons delayed disease onset and improved motor function in ALS mice (235).

Proprioceptive system

Proprioception is defined as our sense of body position and movement. We are constantly receiving signals from our moving body that allow us to interact with the environment and rapidly adapt to changing circumstances. It is largely the proprioceptors that tell us about the position and movement of our limbs and trunk. The information they provide allows us to bypass obstacles in the dark and handle objects without needing to see them (236, 237). Several types of proprioceptors inform us about different aspects of our body shape.

As an example, in skeletal muscles the spindles associated with the Ia/II afferent fibers encode the muscle length and the velocity of muscle length. These muscle spindles also receive γ-motoneuron efferent innervation that regulates the tension of the spindle. At the junction between muscle and bone, the Golgi tendon organs innervated by Ib afferent neurons encode for muscle strength to control α-motoneuron activity when the strength of contraction may damage the muscle. Consequently, people suffering from major proprioceptive deficits are not able to coordinate movements and become unable to move. They must learn how to use another sensory modality, usually sight, to provide sensitive feedback of movements.

The preceding sections have illustrated that the pathophysiological processes leading to ALS are not circumscribed to motoneuron cell-autonomous features but also affect the motor circuitry in which motoneurons are integrated. The proprioceptive system is part of these sensory motor networks and is thought be one of the systems involved in the pathophysiology of ALS; it also seems to be part of the process of neuronal degeneration (3, 238, 239).

Growing evidence supports the involvement of the somatosensory system in patients with ALS. Most studies have demonstrated the presence of sensory symptoms that can be associated with sensory neuropathy and loss of large-diameter myelinated fibers (240). Interestingly, spinal diffusion tensor imaging coupled with electrophysiological measurements revealed sensory defects in 85% of patients with ALS who had moderate impairment and no sensory symptoms (241). The implications of sensory deficits in the pathophysiology of ALS may have been underestimated; this work provides additional evidence of early degeneration of sensory pathways in patients with ALS.

ALS mouse models confirm the involvement of peripheral sensory abnormalities; in most, sensory deficiencies occur during early stages of the disease (240, 242). Studies in $SOD1^{G93A}$ and $TDP\text{-}43^{A315T}$ mice analyzing proprioceptive nerve ending in muscles reported that peripheral innervation of spindles by Ia and II afferent fibers is diminished in the presymptomatic stages of the disease. The sensory neuron somata are unaffected (243, 244), and central synapses are affected only late in the disease process. Furthermore, $TDP\text{-}43^{A315T}$ mice develop sensory abnormalities even in the absence of α-motoneuron axon lesions (244).

In recent years, several investigators have attempted to address whether degeneration starts with the spinal motoneurons or in other cells of the sensory motor networks. Only two studies have addressed this point through an electrophysiological approach. The first was carried out in a Drosophila $dSod1^{G85R}$ knock-in model

(245) and used structural and electrophysiological measures to reveal early larvae motor deficits. This early reduced locomotion was not due to neuromuscular junction dysfunction, deficiencies in muscle contraction, or to alterations in motoneuron properties. On the contrary, dysfunction of peripheral sensory feedback occurred before any evidence of motoneuron degeneration. These results suggested that the proprioceptors could be affected first in ALS and that their dysfunction could explain the altered motor activity and could ultimately lead to motoneuron degeneration. The second study used the jaw reflex in *SOD1*^{G93A} mice as a model and showed that proprioceptive Ia afferent sensory neurons display electrical abnormalities in the postnatal stage at the beginning of the disease process (246). Proprioceptive neurons were hypoexcitable and more likely to discharge phasically (bursting neurons). Moreover, bursting properties were abnormal and led to an irregular burst pattern. In addition, the existence of a Nav1.6 Na^+ channel deficiency contributed to the arrhythmic burst discharge. Interestingly, examination of other brainstem sensory neurons (tactile, nociceptive, and visual) at 2 weeks of age confirmed that changes in excitability had occurred exclusively in proprioceptive neurons. The authors concluded that such sensory arrhythmia could lead to a disturbance of reflexes causing the muscle fasciculations that are encountered in ALS.

As the disease progresses, sensory motor network dysfunction occurs in an attempt to maintain contractile force for as long as possible, but this ultimately leads to excitotoxicity and death of motoneurons. New therapies targeted toward sensory motor network dysfunction might therefore positively influence disease progression (247, 248).

CONCLUSION

Intrinsic neuronal hyperexcitability in upper and lower motoneurons is the earliest pathogenic defect of ALS to have been identified. Whether this is causative, or aggravating remains to be definitively established, although identification of causative genes in ALS rather supports an aggravating role. The hypoexcitability that emerges during disease progression could be an adaptive process to protect against cell death. A question remains concerning whether the synaptic propagation of aberrant activity could arise from the relationship between upper and lower motoneurons—the so-called forward propagation of excitotoxicity. *In vivo* studies in rodents support a functional correlation between upper and lower motoneurons in disease aggravation (233, 235). However, it should be mentioned that unlike in humans, direct cortical-motoneurons synapses disappear in rodents at postnatal ages (249). The cortical influence on motoneuron excitability in rodents could be more pronounced at early developmental stages, while defects of local circuitry in spinal cord could become more predominant during later stages. In humans, the corticospinal tract could have an even greater influence in ALS progression than it does in rodents. Likewise, the higher sensitivity of lower motoneurons than upper motoneurons to excitotoxicity could explain their earlier death. Therapeutic intervention in circuit dynamic and motoneuron electrophysiological features hold promise of successful therapy for ALS, although it still requires improving knowledge of the complex adaptive changes that occur during development and adulthood.

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REFERENCES

1. Brown RH, Al-Chalabi A. Amyotrophic Lateral Sclerosis. N Engl J Med. 2017;377(2):162–72. https://doi.org/10.1056/NEJMra1603471
2. Karagiannis P, Inoue H. ALS, a cellular whodunit on motor neuron degeneration. Mol Cell Neurosci. 2020;107:103524. https://doi.org/10.1016/j.mcn.2020.103524
3. Crabe R, Aimond F, Gosset P, Scamps F, Raoul C. How Degeneration of Cells Surrounding Motoneurons Contributes to Amyotrophic Lateral Sclerosis. Cells. 2020;9(12). https://doi.org/10.3390/cells9122550
4. Nijssen J, Comley LH, Hedlund E. Motor neuron vulnerability and resistance in amyotrophic lateral sclerosis. Acta neuropathologica. 2017;133(6):863–85. https://doi.org/10.1007/s00401-017-1708-8
5. Chiot A, Zaidi S, Iltis C, Ribon M, Berriat F, Schiaffino L, et al. Modifying macrophages at the periphery has the capacity to change microglial reactivity and to extend ALS survival. Nat Neurosci. 2020;23(11):1339–51. https://doi.org/10.1038/s41593-020-00718-z
6. Coque E, Salsac C, Espinosa-Carrasco G, Varga B, Degauque N, Cadoux M, et al. Cytotoxic CD8(+) T lymphocytes expressing ALS-causing SOD1 mutant selectively trigger death of spinal motoneurons. Proc Natl Acad Sci U S A. 2019;116(6):2312–7. https://doi.org/10.1073/pnas.1815961116
7. Thonhoff JR, Simpson EP, Appel SH. Neuroinflammatory mechanisms in amyotrophic lateral sclerosis pathogenesis. Curr Opin Neurol. 2018;31(5):635–9. https://doi.org/10.1097/WCO.0000000000000599
8. Chipika RH, Siah WF, McKenna MC, Li Hi Shing S, Hardiman O, Bede P. The presymptomatic phase of amyotrophic lateral sclerosis: are we merely scratching the surface? J Neurol. 2020. https://doi.org/10.1007/s00415-020-10289-5
9. Deneris ES, Hobert O. Maintenance of postmitotic neuronal cell identity. Nat Neurosci. 2014;17(7):899–907. https://doi.org/10.1038/nn.3731
10. Li Y, Osuma A, Correa E, Okebalama MA, Dao P, Gaylord O, et al. Establishment and maintenance of motor neuron identity via temporal modularity in terminal selector function. Elife. 2020;9. https://doi.org/10.7554/eLife.59464
11. Glover JC. Development of specific connectivity between premotor neurons and motoneurons in the brain stem and spinal cord. Physiol Rev. 2000;80(2):615–47. https://doi.org/10.1152/physrev.2000.80.2.615
12. Arber S. Organization and function of neuronal circuits controlling movement. EMBO molecular medicine. 2017;9(3):281–4. https://doi.org/10.15252/emmm.201607226
13. Dong X, Shen K, Bulow HE. Intrinsic and extrinsic mechanisms of dendritic morphogenesis. Annu Rev Physiol. 2015;77:271–300. https://doi.org/10.1146/annurev-physiol-021014-071746
14. Berg EM, Bjornfors ER, Pallucchi I, Picton LD, El Manira A. Principles Governing Locomotion in Vertebrates: Lessons From Zebrafish. Front Neural Circuits. 2018;12:73. https://doi.org/10.3389/fncir.2018.00073

15. Perrier JF, Hounsgaard J. Development and regulation of response properties in spinal cord motoneurons. Brain Res Bull. 2000;53(5):529–35. https://doi.org/10.1016/S0361-9230(00)00386-5
16. Borodinsky LN, Belgacem YH, Swapna I. Electrical activity as a developmental regulator in the formation of spinal cord circuits. Curr Opin Neurobiol. 2012;22(4):624–30. https://doi.org/10.1016/j.conb.2012.02.004
17. Pun S, Santos AF, Saxena S, Xu L, Caroni P. Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. Nat Neurosci. 2006;9(3):408–19. https://doi.org/10.1038/nn1653
18. Saxena S, Cabuy E, Caroni P. A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. Nat Neurosci. 2009;12(5):627–36. https://doi.org/10.1038/nn.2297
19. Pambo-Pambo A, Durand J, Gueritaud JP. Early excitability changes in lumbar motoneurons of transgenic SOD1G85R and SOD1G(93A-Low) mice. J Neurophysiol. 2009;102(6):3627–42. https://doi.org/10.1152/jn.00482.2009
20. Quinlan KA, Schuster JE, Fu R, Siddique T, Heckman CJ. Altered postnatal maturation of electrical properties in spinal motoneurons in a mouse model of amyotrophic lateral sclerosis. The Journal of physiology. 2011;589(Pt 9):2245–60. https://doi.org/10.1113/jphysiol.2010.200659
21. Pieri M, Albo F, Gaetti C, Spalloni A, Bengtson CP, Longone P, et al. Altered excitability of motor neurons in a transgenic mouse model of familial amyotrophic lateral sclerosis. Neurosci Lett. 2003;351(3):153–6. https://doi.org/10.1016/j.neulet.2003.07.010
22. Chang Q, Martin LJ. Voltage-gated calcium channels are abnormal in cultured spinal motoneurons in the G93A-SOD1 transgenic mouse model of ALS. Neurobiol Dis. 2016;93:78–95. https://doi.org/10.1016/j.nbd.2016.04.009
23. Kuo JJ, Schonewille M, Siddique T, Schults AN, Fu R, Bar PR, et al. Hyperexcitability of cultured spinal motoneurons from presymptomatic ALS mice. J Neurophysiol. 2004;91(1):571–5. https://doi.org/10.1152/jn.00665.2003
24. Kuo JJ, Siddique T, Fu R, Heckman CJ. Increased persistent Na(+) current and its effect on excitability in motoneurones cultured from mutant SOD1 mice. The Journal of physiology. 2005;563(Pt 3):843–54. https://doi.org/10.1113/jphysiol.2004.074138
25. Martin E, Cazenave W, Cattaert D, Branchereau P. Embryonic alteration of motoneuronal morphology induces hyperexcitability in the mouse model of amyotrophic lateral sclerosis. Neurobiol Dis. 2013;54:116–26. https://doi.org/10.1016/j.nbd.2013.02.011
26. van Zundert B, Peuscher MH, Hynynen M, Chen A, Neve RL, Brown RH, Jr., et al. Neonatal neuronal circuitry shows hyperexcitable disturbance in a mouse model of the adult-onset neurodegenerative disease amyotrophic lateral sclerosis. J Neurosci. 2008;28(43):10864–74. https://doi.org/10.1523/JNEUROSCI.1340-08.2008
27. Devlin AC, Burr K, Borooah S, Foster JD, Cleary EM, Geti I, et al. Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability. Nature communications. 2015;6:5999. https://doi.org/10.1038/ncomms6999
28. Wainger BJ, Kiskinis E, Mellin C, Wiskow O, Han SS, Sandoe J, et al. Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. Cell Rep. 2014;7(1):1–11. https://doi.org/10.1016/j.celrep.2014.03.019
29. Bories C, Amendola J, Lamotte d'Incamps B, Durand J. Early electrophysiological abnormalities in lumbar motoneurons in a transgenic mouse model of amyotrophic lateral sclerosis. Eur J Neurosci. 2007;25(2):451–9. https://doi.org/10.1111/j.1460-9568.2007.05306.x
30. Naujock M, Stanslowsky N, Bufler S, Naumann M, Reinhardt P, Sterneckert J, et al. 4-Aminopyridine Induced Activity Rescues Hypoexcitable Motor Neurons from Amyotrophic Lateral Sclerosis Patient-Derived Induced Pluripotent Stem Cells. Stem cells (Dayton, Ohio). 2016;34(6):1563–75. https://doi.org/10.1002/stem.2354
31. Sareen D, O'Rourke JG, Meera P, Muhammad AK, Grant S, Simpkinson M, et al. Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion. Sci Transl Med. 2013;5(208):208ra149. https://doi.org/10.1126/scitranslmed.3007529
32. Kuo JJ, Lee RH, Zhang L, Heckman CJ. Essential role of the persistent sodium current in spike initiation during slowly rising inputs in mouse spinal neurones. The Journal of physiology. 2006;574(Pt 3):819–34. https://doi.org/10.1113/jphysiol.2006.107094

33. Binder MD, Powers RK, Heckman CJ. Nonlinear Input-Output Functions of Motoneurons. Physiology (Bethesda). 2020;35(1):31–9. https://doi.org/10.1152/physiol.00026.2019
34. Branchereau P, Martin E, Allain AE, Cazenave W, Supiot L, Hodeib F, et al. Relaxation of synaptic inhibitory events as a compensatory mechanism in fetal SOD spinal motor networks. Elife. 2019;8. https://doi.org/10.7554/eLife.51402
35. Milan L, Barriere G, De Deurwaerdere P, Cazalets JR, Bertrand SS. Monoaminergic control of spinal locomotor networks in SOD1G93A newborn mice. Front Neural Circuits. 2014;8:77. https://doi.org/10.3389/fncir.2014.00077
36. Amendola J, Verrier B, Roubertoux P, Durand J. Altered sensorimotor development in a transgenic mouse model of amyotrophic lateral sclerosis. Eur J Neurosci. 2004;20(10):2822–6. https://doi.org/10.1111/j.1460-9568.2004.03745.x
37. Medelin M, Rancic V, Cellot G, Laishram J, Veeraraghavan P, Rossi C, et al. Altered development in GABA co-release shapes glycinergic synaptic currents in cultured spinal slices of the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. The Journal of physiology. 2016;594(13):3827–40. https://doi.org/10.1113/JP272382
38. Hadzipasic M, Tahvildari B, Nagy M, Bian M, Horwich AL, McCormick DA. Selective degeneration of a physiological subtype of spinal motor neuron in mice with SOD1-linked ALS. Proc Natl Acad Sci U S A. 2014;111(47):16883–8. https://doi.org/10.1073/pnas.1419497111
39. Delestree N, Manuel M, Iglesias C, Elbasiouny SM, Heckman CJ, Zytnicki D. Adult spinal motoneurones are not hyperexcitable in a mouse model of inherited amyotrophic lateral sclerosis. The Journal of physiology. 2014;592(7):1687–703. https://doi.org/10.1113/jphysiol.2013.265843
40. Gustafsson B, Pinter MJ. An investigation of threshold properties among cat spinal alpha-motoneurones. The Journal of physiology. 1984;357:453–83. https://doi.org/10.1113/jphysiol.1984.sp015511
41. Jensen DB, Kadlecova M, Allodi I, Meehan CF. Spinal motoneurones are intrinsically more responsive in the adult G93A SOD1 mouse model of amyotrophic lateral sclerosis. The Journal of physiology. 2020;598(19):4385–403. https://doi.org/10.1113/JP280097
42. Saxena S, Roselli F, Singh K, Leptien K, Julien JP, Gros-Louis F, et al. Neuroprotection through Excitability and mTOR Required in ALS Motoneurons to Delay Disease and Extend Survival. Neuron. 2013;80(1):80–96. https://doi.org/10.1016/j.neuron.2013.07.027
43. Soulard C, Salsac C, Mouzat K, Hilaire C, Roussel J, Mezghrani A, et al. Spinal Motoneuron TMEM16F Acts at C-boutons to Modulate Motor Resistance and Contributes to ALS Pathogenesis. Cell Rep. 2020;30(8):2581–93 e7. https://doi.org/10.1016/j.celrep.2020.02.001
44. Ramirez-Jarquin UN, Lazo-Gomez R, Tovar YRLB, Tapia R. Spinal inhibitory circuits and their role in motor neuron degeneration. Neuropharmacology. 2014;82:101–7. https://doi.org/10.1016/j.neuropharm.2013.10.003
45. Ramirez-Jarquin UN, Tapia R. Excitatory and Inhibitory Neuronal Circuits in the Spinal Cord and Their Role in the Control of Motor Neuron Function and Degeneration. ACS Chem Neurosci. 2018;9(2):211–6. https://doi.org/10.1021/acschemneuro.7b00503
46. Talpalar AE, Bouvier J, Borgius L, Fortin G, Pierani A, Kiehn O. Dual-mode operation of neuronal networks involved in left-right alternation. Nature. 2013;500(7460):85–8. https://doi.org/10.1038/nature12286
47. Talpalar AE, Kiehn O. Glutamatergic mechanisms for speed control and network operation in the rodent locomotor CpG. Front Neural Circuits. 2010;4. https://doi.org/10.3389/fncir.2010.00019
48. Lanuza GM, Gosgnach S, Pierani A, Jessell TM, Goulding M. Genetic identification of spinal interneurons that coordinate left-right locomotor activity necessary for walking movements. Neuron. 2004;42(3):375–86. https://doi.org/10.1016/S0896-6273(04)00249-1
49. Alvarez FJ, Jonas PC, Sapir T, Hartley R, Berrocal MC, Geiman EJ, et al. Postnatal phenotype and localization of spinal cord V1 derived interneurons. J Comp Neurol. 2005;493(2):177–92. https://doi.org/10.1002/cne.20711
50. Gosgnach S, Lanuza GM, Butt SJ, Saueressig H, Zhang Y, Velasquez T, et al. V1 spinal neurons regulate the speed of vertebrate locomotor outputs. Nature. 2006;440(7081):215–9. https://doi.org/10.1038/nature04545

51. Zhang J, Lanuza GM, Britz O, Wang Z, Siembab VC, Zhang Y, et al. V1 and v2b interneurons secure the alternating flexor-extensor motor activity mice require for limbed locomotion. Neuron. 2014;82(1):138–50. https://doi.org/10.1016/j.neuron.2014.02.013
52. Ramirez-Jarquin UN, Tapia R. Chronic GABAergic blockade in the spinal cord in vivo induces motor alterations and neurodegeneration. Neuropharmacology. 2017;117:85–92. https://doi.org/10.1016/j.neuropharm.2017.01.040
53. Lazo-Gomez R, Tapia R. Motor Alterations Induced by Chronic 4-Aminopyridine Infusion in the Spinal Cord In vivo: Role of Glutamate and GABA Receptors. Front Neurosci. 2016;10:200. https://doi.org/10.3389/fnins.2016.00200
54. Hanson MG, Landmesser LT. Characterization of the circuits that generate spontaneous episodes of activity in the early embryonic mouse spinal cord. J Neurosci. 2003;23(2):587–600. https://doi.org/10.1523/JNEUROSCI.23-02-00587.2003
55. Maekawa S, Al-Sarraj S, Kibble M, Landau S, Parnavelas J, Cotter D, et al. Cortical selective vulnerability in motor neuron disease: a morphometric study. Brain. 2004;127(Pt 6):1237–51. https://doi.org/10.1093/brain/awh132
56. Ince P, Stout N, Shaw P, Slade J, Hunziker W, Heizmann CW, et al. Parvalbumin and calbindin D-28k in the human motor system and in motor neuron disease. Neuropathol Appl Neurobiol. 1993;19(4):291–9. https://doi.org/10.1111/j.1365-2990.1993.tb00443.x
57. Oyanagi K, Ikuta F, Horikawa Y. Evidence for sequential degeneration of the neurons in the intermediate zone of the spinal cord in amyotrophic lateral sclerosis: a topographic and quantitative investigation. Acta neuropathologica. 1989;77(4):343–9. https://doi.org/10.1007/BF00687368
58. Stephens B, Guiloff RJ, Navarrete R, Newman P, Nikhar N, Lewis P. Widespread loss of neuronal populations in the spinal ventral horn in sporadic motor neuron disease. A morphometric study. J Neurol Sci. 2006;244(1–2):41–58. https://doi.org/10.1016/j.jns.2005.12.003
59. Nihei K, McKee AC, Kowall NW. Patterns of neuronal degeneration in the motor cortex of amyotrophic lateral sclerosis patients. Acta neuropathologica. 1993;86(1):55–64. https://doi.org/10.1007/BF00454899
60. Petri S, Krampfl K, Hashemi F, Grothe C, Hori A, Dengler R, et al. Distribution of GABAA receptor mRNA in the motor cortex of ALS patients. J Neuropathol Exp Neurol. 2003;62(10):1041–51. https://doi.org/10.1093/jnen/62.10.1041
61. Lloyd CM, Richardson MP, Brooks DJ, Al-Chalabi A, Leigh PN. Extramotor involvement in ALS: PET studies with the GABA(A) ligand [(11)C]flumazenil. Brain. 2000;123(Pt 11):2289–96. https://doi.org/10.1093/brain/123.11.2289
62. Foerster BR, Callaghan BC, Petrou M, Edden RA, Chenevert TL, Feldman EL. Decreased motor cortex gamma-aminobutyric acid in amyotrophic lateral sclerosis. Neurology. 2012;78(20):1596–600. https://doi.org/10.1212/WNL.0b013e3182563b57
63. Chung YH, Joo KM, Nam RH, Cho MH, Kim DJ, Lee WB, et al. Decreased expression of calretinin in the cerebral cortex and hippocampus of SOD1G93A transgenic mice. Brain Res. 2005;1035(1):105–9. https://doi.org/10.1016/j.brainres.2004.12.022
64. Hossaini M, Cano SC, van Dis V, Haasdijk ED, Hoogenraad CC, Holstege JC, et al. Spinal Inhibitory Interneuron Pathology Follows Motor Neuron Degeneration Independent of Glial Mutant Superoxide Dismutase 1 Expression in SOD1-ALS Mice. J Neuropathol Exp Neurol. 2011;70(8):662–77. https://doi.org/10.1097/NEN.0b013e31822581ac
65. Morrison BM, Janssen WG, Gordon JW, Morrison JH. Time course of neuropathology in the spinal cord of G86R superoxide dismutase transgenic mice. J Comp Neurol. 1998;391(1):64–77. https://doi.org/10.1002/(SICI)1096-9861(19980202)391:1<64::AID-CNE6>3.0.CO;2-P
66. Minciacchi D, Kassa RM, Del Tongo C, Mariotti R, Bentivoglio M. Voronoi-based spatial analysis reveals selective interneuron changes in the cortex of FALS mice. Exp Neurol. 2009;215(1):77–86. https://doi.org/10.1016/j.expneurol.2008.09.005
67. Niessen HG, Debska-Vielhaber G, Sander K, Angenstein F, Ludolph AC, Hilfert L, et al. Metabolic progression markers of neurodegeneration in the transgenic G93A-SOD1 mouse model of amyotrophic lateral sclerosis. Eur J Neurosci. 2007;25(6):1669–77. https://doi.org/10.1111/j.1460-9568.2007.05415.x

68. Milanese M, Zappettini S, Jacchetti E, Bonifacino T, Cervetto C, Usai C, et al. In vitro activation of GAT1 transporters expressed in spinal cord gliosomes stimulates glutamate release that is abnormally elevated in the SOD1/G93A(+) mouse model of amyotrophic lateral sclerosis. J Neurochem. 2010;113(2):489–501. https://doi.org/10.1111/j.1471-4159.2010.06628.x
69. Clark RM, Brizuela M, Blizzard CA, Dickson TC. Reduced Excitability and Increased Neurite Complexity of Cortical Interneurons in a Familial Mouse Model of Amyotrophic Lateral Sclerosis. Front Cell Neurosci. 2018;12:328. https://doi.org/10.3389/fncel.2018.00328
70. Kim J, Hughes EG, Shetty AS, Arlotta P, Goff LA, Bergles DE, et al. Changes in the Excitability of Neocortical Neurons in a Mouse Model of Amyotrophic Lateral Sclerosis Are Not Specific to Corticospinal Neurons and Are Modulated by Advancing Disease. J Neurosci. 2017;37(37):9037–53. https://doi.org/10.1523/JNEUROSCI.0811-17.2017
71. Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH. TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci U S A. 2009;106(44): 18809–14. https://doi.org/10.1073/pnas.0908767106
72. Zhang W, Zhang L, Liang B, Schroeder D, Zhang ZW, Cox GA, et al. Hyperactive somatostatin interneurons contribute to excitotoxicity in neurodegenerative disorders. Nat Neurosci. 2016;19(4):557–9. https://doi.org/10.1038/nn.4257
73. Nieto-Gonzalez JL, Moser J, Lauritzen M, Schmitt-John T, Jensen K. Reduced GABAergic inhibition explains cortical hyperexcitability in the wobbler mouse model of ALS. Cereb Cortex. 2011;21(3):625–35. https://doi.org/10.1093/cercor/bhq134
74. Moser JM, Bigini P, Schmitt-John T. The wobbler mouse, an ALS animal model. Mol Genet Genomics. 2013;288(5–6):207–29. https://doi.org/10.1007/s00438-013-0741-0
75. Carunchio I, Mollinari C, Pieri M, Merlo D, Zona C. GAB(A) receptors present higher affinity and modified subunit composition in spinal motor neurons from a genetic model of amyotrophic lateral sclerosis. Eur J Neurosci. 2008;28(7):1275–85. https://doi.org/10.1111/j.1460-9568.2008.06436.x
76. Chang Q, Martin LJ. Glycine receptor channels in spinal motoneurons are abnormal in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurosci. 2011;31(8):2815–27. https://doi.org/10.1523/JNEUROSCI.2475-10.2011
77. Clark R, Blizzard C, Dickson T. Inhibitory dysfunction in amyotrophic lateral sclerosis: future therapeutic opportunities. Neurodegener Dis Manag. 2015;5(6):511–25. https://doi.org/10.2217/nmt.15.49
78. van Zundert B, Izaurieta P, Fritz E, Alvarez FJ. Early pathogenesis in the adult-onset neurodegenerative disease amyotrophic lateral sclerosis. J Cell Biochem. 2012;113(11):3301–12. https://doi.org/10.1002/jcb.24234
79. Hayashi H, Suga M, Satake M, Tsubaki T. Reduced glycine receptor in the spinal cord in amyotrophic lateral sclerosis. Ann Neurol. 1981;9(3):292–4. https://doi.org/10.1002/ana.410090313
80. Whitehouse PJ, Wamsley JK, Zarbin MA, Price DL, Tourtellotte WW, Kuhar MJ. Amyotrophic lateral sclerosis: alterations in neurotransmitter receptors. Ann Neurol. 1983;14(1):8–16. https://doi.org/10.1002/ana.410140103
81. Malessa S, Leigh PN, Bertel O, Sluga E, Hornykiewicz O. Amyotrophic lateral sclerosis: glutamate dehydrogenase and transmitter amino acids in the spinal cord. J Neurol Neurosurg Psychiatry. 1991;54(11):984–8. https://doi.org/10.1136/jnnp.54.11.984
82. Sunico CR, Dominguez G, Garcia-Verdugo JM, Osta R, Montero F, Moreno-Lopez B. Reduction in the motoneuron inhibitory/excitatory synaptic ratio in an early-symptomatic mouse model of amyotrophic lateral sclerosis. Brain pathology (Zurich, Switzerland). 2011;21(1):1–15. https://doi.org/10.1111/j.1750-3639.2010.00417.x
83. Chang Q, Martin LJ. Glycinergic innervation of motoneurons is deficient in amyotrophic lateral sclerosis mice: a quantitative confocal analysis. Am J Pathol. 2009;174(2):574–85. https://doi.org/10.2353/ajpath.2009.080557
84. Chang Q, Martin LJ. Motoneuron subtypes show specificity in glycine receptor channel abnormalities in a transgenic mouse model of amyotrophic lateral sclerosis. Channels (Austin). 2011;5(4):299–303. https://doi.org/10.4161/chan.5.4.16206

85. McGown A, McDearmid JR, Panagiotaki N, Tong H, Al Mashhadi S, Redhead N, et al. Early interneuron dysfunction in ALS: insights from a mutant sod1 zebrafish model. Ann Neurol. 2013;73(2):246–58. https://doi.org/10.1002/ana.23780
86. Willis WD. The case for the Renshaw cell. Brain Behav Evol. 1971;4(1):5–52. https://doi.org/10.1159/000125422
87. Thomas RC, Wilson VJ. Precise localization of Renshaw cells with a new marking technique. Nature. 1965;206(980):211–3. https://doi.org/10.1038/206211b0
88. Alvarez FJ, Fyffe RE. The continuing case for the Renshaw cell. The Journal of physiology. 2007;584(Pt 1):31–45. https://doi.org/10.1113/jphysiol.2007.136200
89. Shigenaga Y, Moritani M, Oh SJ, Park KP, Paik SK, Bae JY, et al. The distribution of inhibitory and excitatory synapses on single, reconstructed jaw-opening motoneurons in the cat. Neuroscience. 2005;133(2):507–18. https://doi.org/10.1016/j.neuroscience.2005.02.022
90. Bhumbra GS, Moore NJ, Moroni M, Beato M. Co-Release of GABA Does Not Occur at Glycinergic Synapses onto Lumbar Motoneurons in Juvenile Mice. Front Cell Neurosci. 2012;6:8. https://doi.org/10.3389/fncel.2012.00008
91. Kiehn O. Decoding the organization of spinal circuits that control locomotion. Nat Rev Neurosci. 2016;17(4):224–38. https://doi.org/10.1038/nrn.2016.9
92. Alvarez FJ, Benito-Gonzalez A, Siembab VC. Principles of interneuron development learned from Renshaw cells and the motoneuron recurrent inhibitory circuit. Annals of the New York Academy of Sciences. 2013;1279:22–31. https://doi.org/10.1111/nyas.12084
93. Fyffe RE. Spatial distribution of recurrent inhibitory synapses on spinal motoneurons in the cat. J Neurophysiol. 1991;65(5):1134–49. https://doi.org/10.1152/jn.1991.65.5.1134
94. Mattei B, Schmied A, Mazzocchio R, Decchi B, Rossi A, Vedel JP. Pharmacologically induced enhancement of recurrent inhibition in humans: effects on motoneurone discharge patterns. The Journal of physiology. 2003;548(Pt 2):615–29. https://doi.org/10.1113/jphysiol.2002.033126
95. Windhorst U. On the role of recurrent inhibitory feedback in motor control. Prog Neurobiol. 1996;49(6):517–87. https://doi.org/10.1016/0301-0082(96)00023-8
96. Katz R, Pierrot-Deseilligny E. Recurrent inhibition in humans. Prog Neurobiol. 1999;57(3):325–55. https://doi.org/10.1016/S0301-0082(98)00056-2
97. Mazzocchio R, Rossi A. Involvement of spinal recurrent inhibition in spasticity. Further insight into the regulation of Renshaw cell activity. Brain. 1997;120(Pt 6):991–1003. https://doi.org/10.1093/brain/120.6.991
98. Vucic S, Ziemann U, Eisen A, Hallett M, Kiernan MC. Transcranial magnetic stimulation and amyotrophic lateral sclerosis: pathophysiological insights. J Neurol Neurosurg Psychiatry. 2013;84(10):1161–70. https://doi.org/10.1136/jnnp-2012-304019
99. McClintock SM, Freitas C, Oberman L, Lisanby SH, Pascual-Leone A. Transcranial magnetic stimulation: a neuroscientific probe of cortical function in schizophrenia. Biol Psychiatry. 2011;70(1):19–27. https://doi.org/10.1016/j.biopsych.2011.02.031
100. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. The Journal of physiology. 1993;471:501–19. https://doi.org/10.1113/jphysiol.1993.sp019912
101. Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. The effect of lorazepam on the motor cortical excitability in man. Experimental brain research Experimentelle Hirnforschung. 1996;109(1):127–35. https://doi.org/10.1007/BF00228633
102. Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strafella A, Badawy R, et al. TMS and drugs revisited 2014. Clin Neurophysiol. 2015;126(10):1847–68. https://doi.org/10.1016/j.clinph.2014.08.028
103. Menon P, Kiernan MC, Vucic S. Cortical hyperexcitability precedes lower motor neuron dysfunction in ALS. Clin Neurophysiol. 2015;126(4):803–9. https://doi.org/10.1016/j.clinph.2014.04.023
104. Vucic S, Kiernan MC. Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. Brain. 2006;129(Pt 9):2436–46. https://doi.org/10.1093/brain/awl172
105. Wagle-Shukla A, Ni Z, Gunraj CA, Bahl N, Chen R. Effects of short interval intracortical inhibition and intracortical facilitation on short interval intracortical facilitation in human primary motor cortex. The Journal of physiology. 2009;587(Pt 23):5665–78. https://doi.org/10.1113/jphysiol.2009.181446

106. Williams KL, Fifita JA, Vucic S, Durnall JC, Kiernan MC, Blair IP, et al. Pathophysiological insights into ALS with C9ORF72 expansions. J Neurol Neurosurg Psychiatry. 2013;84(8):931–5. https://doi.org/10.1136/jnnp-2012-304529
107. Ziemann U, Winter M, Reimers CD, Reimers K, Tergau F, Paulus W. Impaired motor cortex inhibition in patients with amyotrophic lateral sclerosis. Evidence from paired transcranial magnetic stimulation. Neurology. 1997;49(5):1292–8. https://doi.org/10.1212/WNL.49.5.1292
108. Cengiz B, Fidanci H, Kiyak Keceli Y, Baltaci H, KuruoGlu R. Impaired short- and long-latency afferent inhibition in amyotrophic lateral sclerosis. Muscle Nerve. 2019;59(6):699–704. https://doi.org/10.1002/mus.26464
109. Shibuya K, Simon NG, Geevasinga N, Menon P, Howells J, Park SB, et al. The evolution of motor cortical dysfunction in amyotrophic lateral sclerosis. Clin Neurophysiol. 2017;128(6):1075–82. https://doi.org/10.1016/j.clinph.2017.03.004
110. Turner MR, Hammers A, Al-Chalabi A, Shaw CE, Andersen PM, Brooks DJ, et al. Distinct cerebral lesions in sporadic and 'D90A' SOD1 ALS: studies with [11C]flumazenil PET. Brain. 2005;128(Pt 6):1323–9. https://doi.org/10.1093/brain/awh509
111. Vucic S, Kiernan MC. Upregulation of persistent sodium conductances in familial ALS. J Neurol Neurosurg Psychiatry. 2010;81(2):222–7. https://doi.org/10.1136/jnnp.2009.183079
112. Vucic S, Nicholson GA, Kiernan MC. Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. Brain. 2008;131(Pt 6):1540–50. https://doi.org/10.1093/brain/awn071
113. Zanette G, Tamburin S, Manganotti P, Refatti N, Forgione A, Rizzuto N. Changes in motor cortex inhibition over time in patients with amyotrophic lateral sclerosis. J Neurol. 2002;249(12):1723–8. https://doi.org/10.1007/s00415-002-0926-7
114. Van den Bos MAJ, Higashihara M, Geevasinga N, Menon P, Kiernan MC, Vucic S. Imbalance of cortical facilitatory and inhibitory circuits underlies hyperexcitability in ALS. Neurology. 2018;91(18):e1669-e76. https://doi.org/10.1212/WNL.0000000000006438
115. Caramia MD, Palmieri MG, Desiato MT, Iani C, Scalise A, Telera S, et al. Pharmacologic reversal of cortical hyperexcitability in patients with ALS. Neurology. 2000;54(1):58–64. https://doi.org/10.1212/WNL.54.1.58
116. Desiato MT, Palmieri MG, Giacomini P, Scalise A, Arciprete F, Caramia MD. The effect of riluzole in amyotrophic lateral sclerosis: a study with cortical stimulation. J Neurol Sci. 1999;169(1–2):98–107. https://doi.org/10.1016/S0022-510X(99)00225-7
117. Hanajima R, Ugawa Y, Terao Y, Ogata K, Kanazawa I. Ipsilateral cortico-cortical inhibition of the motor cortex in various neurological disorders. J Neurol Sci. 1996;140(1–2):109–16. https://doi.org/10.1016/0022-510X(96)00100-1
118. Sommer M, Tergau F, Wischer S, Reimers CD, Beuche W, Paulus W. Riluzole does not have an acute effect on motor thresholds and the intracortical excitability in amyotrophic lateral sclerosis. J Neurol. 1999;246 Suppl 3:III22–6. https://doi.org/10.1007/BF03161086
119. Yokota T, Yoshino A, Inaba A, Saito Y. Double cortical stimulation in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 1996;61(6):596–600. https://doi.org/10.1136/jnnp.61.6.596
120. Zanette G, Tamburin S, Manganotti P, Refatti N, Forgione A, Rizzuto N. Different mechanisms contribute to motor cortex hyperexcitability in amyotrophic lateral sclerosis. Clin Neurophysiol. 2002;113(11):1688–97. https://doi.org/10.1016/S1388-2457(02)00288-2
121. Kanai K, Kuwabara S, Misawa S, Tamura N, Ogawara K, Nakata M, et al. Altered axonal excitability properties in amyotrophic lateral sclerosis: impaired potassium channel function related to disease stage. Brain. 2006;129(Pt 4):953–62. https://doi.org/10.1093/brain/awl024
122. Menon P, Geevasinga N, van den Bos M, Yiannikas C, Kiernan MC, Vucic S. Cortical hyperexcitability and disease spread in amyotrophic lateral sclerosis. Eur J Neurol. 2017;24(6):816–24. https://doi.org/10.1111/ene.13295
123. Vucic S, Cheah BC, Kiernan MC. Defining the mechanisms that underlie cortical hyperexcitability in amyotrophic lateral sclerosis. Exp Neurol. 2009;220(1):177–82. https://doi.org/10.1016/j.expneurol.2009.08.017
124. Cengiz B, Kuruoglu R. A new parameter to discriminate amyotrophic lateral sclerosis patients from healthy participants by motor cortical excitability changes. Muscle Nerve. 2020;61(3):354–62. https://doi.org/10.1002/mus.26786

125. Brunet A, Stuart-Lopez G, Burg T, Scekic-Zahirovic J, Rouaux C. Cortical Circuit Dysfunction as a Potential Driver of Amyotrophic Lateral Sclerosis. Front Neurosci. 2020;14:363. https://doi.org/10.3389/fnins.2020.00363
126. Salerno A, Georgesco M. Double magnetic stimulation of the motor cortex in amyotrophic lateral sclerosis. Electroencephalogr Clin Neurophysiol. 1998;107(2):133–9. https://doi.org/10.1016/S0013-4694(98)00054-6
127. Attarian S, Pouget J, Schmied A. Changes in cortically induced inhibition in amyotrophic lateral sclerosis with time. Muscle Nerve. 2009;39(3):310–7. https://doi.org/10.1002/mus.21137
128. Cantello R, Gianelli M, Civardi C, Mutani R. Magnetic brain stimulation: the silent period after the motor evoked potential. Neurology. 1992;42(10):1951–9. https://doi.org/10.1212/WNL.42.10.1951
129. Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Experimental brain research Experimentelle Hirnforschung. 1999;128(4):539–42. https://doi.org/10.1007/s002210050878
130. Inghilleri M, Berardelli A, Cruccu G, Manfredi M. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. The Journal of physiology. 1993;466:521–34.
131. Sanger TD, Garg RR, Chen R. Interactions between two different inhibitory systems in the human motor cortex. The Journal of physiology. 2001;530(Pt 2):307–17. https://doi.org/10.1111/j.1469-7793.2001.0307l.x
132. Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. The Journal of physiology. 1999;517 (Pt 2):591–7. https://doi.org/10.1111/j.1469-7793.1999.0591t.x
133. Desiato MT, Bernardi G, Hagi HA, Boffa L, Caramia MD. Transcranial magnetic stimulation of motor pathways directed to muscles supplied by cranial nerves in amyotrophic lateral sclerosis. Clin Neurophysiol. 2002;113(1):132–40. https://doi.org/10.1016/S1388-2457(01)00724-6
134. Drory VE, Kovach I, Groozman GB. Electrophysiologic evaluation of upper motor neuron involvement in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2001;2(3):147–52. https://doi.org/10.1080/146608201753275616
135. Raynor EM, Shefner JM. Recurrent inhibition is decreased in patients with amyotrophic lateral sclerosis. Neurology. 1994;44(11):2148–53. https://doi.org/10.1212/WNL.44.11.2148
136. Eisen A, Kiernan M, Mitsumoto H, Swash M. Amyotrophic lateral sclerosis: a long preclinical period? J Neurol Neurosurg Psychiatry. 2014;85(11):1232–8. https://doi.org/10.1136/jnnp-2013-307135
137. Blizzard CA, Chuckowree JA, King AE, Hosie KA, McCormack GH, Chapman JA, et al. Focal damage to the adult rat neocortex induces wound healing accompanied by axonal sprouting and dendritic structural plasticity. Cereb Cortex. 2011;21(2):281–91. https://doi.org/10.1093/cercor/bhq091
138. Mendez P, Bacci A. Assortment of GABAergic plasticity in the cortical interneuron melting pot. Neural Plast. 2011;2011:976856. https://doi.org/10.1155/2011/976856
139. Nissen W, Szabo A, Somogyi J, Somogyi P, Lamsa KP. Cell type-specific long-term plasticity at glutamatergic synapses onto hippocampal interneurons expressing either parvalbumin or CB1 cannabinoid receptor. J Neurosci. 2010;30(4):1337–47. https://doi.org/10.1523/JNEUROSCI.3481-09.2010
140. Strigaro G, Hamada M, Murase N, Cantello R, Rothwell JC. Interaction between different interneuron networks involved in human associative plasticity. Brain Stimul. 2014;7(5):658–64. https://doi.org/10.1016/j.brs.2014.05.010
141. Agosta F, Canu E, Valsasina P, Riva N, Prelle A, Comi G, et al. Divergent brain network connectivity in amyotrophic lateral sclerosis. Neurobiology of aging. 2013;34(2):419–27. https://doi.org/10.1016/j.neurobiolaging.2012.04.015
142. Chio A, Pagani M, Agosta F, Calvo A, Cistaro A, Filippi M. Neuroimaging in amyotrophic lateral sclerosis: insights into structural and functional changes. Lancet Neurol. 2014;13(12):1228–40. https://doi.org/10.1016/S1474-4422(14)70167-X
143. Weber M, Eisen A, Stewart HG, Andersen PM. Preserved slow conducting corticomotoneuronal projections in amyotrophic lateral sclerosis with autosomal recessive D90A CuZn-superoxide dismutase mutation. Brain. 2000;123 (Pt 7):1505–15. https://doi.org/10.1093/brain/123.7.1505
144. Bu J, Sathyendra V, Nagykery N, Geula C. Age-related changes in calbindin-D28k, calretinin, and parvalbumin-immunoreactive neurons in the human cerebral cortex. Exp Neurol. 2003;182(1):220–31. https://doi.org/10.1016/S0014-4886(03)00094-3

145. Ling LL, Hughes LF, Caspary DM. Age-related loss of the GABA synthetic enzyme glutamic acid decarboxylase in rat primary auditory cortex. Neuroscience. 2005;132(4):1103–13. https://doi.org/10.1016/j.neuroscience.2004.12.043
146. Burianova J, Ouda L, Profant O, Syka J. Age-related changes in GAD levels in the central auditory system of the rat. Exp Gerontol. 2009;44(3):161–9. https://doi.org/10.1016/j.exger.2008.09.012
147. Miettinen R, Sirvio J, Riekkinen P, Sr., Laakso MP, Riekkinen M, Riekkinen P, Jr. Neocortical, hippocampal and septal parvalbumin- and somatostatin-containing neurons in young and aged rats: correlation with passive avoidance and water maze performance. Neuroscience. 1993;53(2):367–78. https://doi.org/10.1016/0306-4522(93)90201-P
148. Potier B, Jouvenceau A, Epelbaum J, Dutar P. Age-related alterations of GABAergic input to CA1 pyramidal neurons and its control by nicotinic acetylcholine receptors in rat hippocampus. Neuroscience. 2006;142(1):187–201. https://doi.org/10.1016/j.neuroscience.2006.06.040
149. Stanley EM, Fadel JR, Mott DD. Interneuron loss reduces dendritic inhibition and GABA release in hippocampus of aged rats. Neurobiology of aging. 2012;33(2):431 e1–13. https://doi.org/10.1016/j.neurobiolaging.2010.12.014
150. Mazzocchio R, Rossi A, Rothwell JC. Depression of Renshaw recurrent inhibition by activation of corticospinal fibres in human upper and lower limb. The Journal of physiology. 1994;481 (Pt 2):487–98. https://doi.org/10.1113/jphysiol.1994.sp020457
151. Wada N, Yawashima Y, Nakajima Y. Effect of nucleus raphe magnus stimulation on recurrent inhibition of the monosynaptic reflex in the cat. Neurosci Lett. 1989;107(1–3):94–8. https://doi.org/10.1016/0304-3940(89)90797-0
152. Mazzocchio R, Rossi A. Further evidence for Renshaw inhibition in man. A combined electrophysiological and pharmacological approach. Neurosci Lett. 1989;106(1–2):131–6. https://doi.org/10.1016/0304-3940(89)90214-0
153. Mazzocchio R, Rossi A. Recurrent inhibition. Neurology. 1996;47(6):1606–8. https://doi.org/10.1212/WNL.47.6.1606-b
154. Piotrkiewicz M, Hausmanowa-Petrusewicz I. Motoneuron afterhyperpolarisation duration in amyotrophic lateral sclerosis. The Journal of physiology. 2011;589(Pt 11):2745–54. https://doi.org/10.1113/jphysiol.2011.204891
155. Shefner JM, Logigian EL. The mixed nerve silent period in normal subjects and patients with amyotrophic lateral sclerosis. Electromyogr Clin Neurophysiol. 1998;38(8):505–10.
156. Katz R, Mazzocchio R, Penicaud A, Rossi A. Distribution of recurrent inhibition in the human upper limb. Acta Physiol Scand. 1993;149(2):183–98. https://doi.org/10.1111/j.1748-1716.1993.tb09611.x
157. Ozyurt MG, Topkara B, Isak B, Turker KS. Amyotrophic lateral sclerosis weakens spinal recurrent inhibition and post-activation depression. Clin Neurophysiol. 2020;131(12):2875–86. https://doi.org/10.1016/j.clinph.2020.09.021
158. Siembab VC, Smith CA, Zagoraiou L, Berrocal MC, Mentis GZ, Alvarez FJ. Target selection of proprioceptive and motor axon synapses on neonatal V1-derived Ia inhibitory interneurons and Renshaw cells. J Comp Neurol. 2010;518(23):4675–701. https://doi.org/10.1002/cne.22441
159. Casas C, Herrando-Grabulosa M, Manzano R, Mancuso R, Osta R, Navarro X. Early presymptomatic cholinergic dysfunction in a murine model of amyotrophic lateral sclerosis. Brain Behav. 2013;3(2):145–58. https://doi.org/10.1002/brb3.104
160. Wootz H, Fitzsimons-Kantamneni E, Larhammar M, Rotterman TM, Enjin A, Patra K, et al. Alterations in the motor neuron-renshaw cell circuit in the Sod1(G93A) mouse model. J Comp Neurol. 2013;521(7):1449–69. https://doi.org/10.1002/cne.23266
161. Allene C, Cossart R. Early NMDA receptor-driven waves of activity in the developing neocortex: physiological or pathological network oscillations? The Journal of physiology. 2010;588(Pt 1):83–91. https://doi.org/10.1113/jphysiol.2009.178798
162. Chattopadhyaya B, Di Cristo G, Wu CZ, Knott G, Kuhlman S, Fu Y, et al. GAD67-mediated GABA synthesis and signaling regulate inhibitory synaptic innervation in the visual cortex. Neuron. 2007;54(6):889–903. https://doi.org/10.1016/j.neuron.2007.05.015
163. Dupont E, Hanganu IL, Kilb W, Hirsch S, Luhmann HJ. Rapid developmental switch in the mechanisms driving early cortical columnar networks. Nature. 2006;439(7072):79–83. https://doi.org/10.1038/nature04264

164. Garaschuk O, Linn J, Eilers J, Konnerth A. Large-scale oscillatory calcium waves in the immature cortex. Nat Neurosci. 2000;3(5):452–9. https://doi.org/10.1038/74823
165. De Marco Garcia NV, Karayannis T, Fishell G. Neuronal activity is required for the development of specific cortical interneuron subtypes. Nature. 2011;472(7343):351–5. https://doi.org/10.1038/nature09865
166. Chattopadhyaya B, Di Cristo G, Higashiyama H, Knott GW, Kuhlman SJ, Welker E, et al. Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period. J Neurosci. 2004;24(43):9598–611. https://doi.org/10.1523/JNEUROSCI.1851-04.2004
167. Fogarty MJ, Noakes PG, Bellingham MC. Motor cortex layer V pyramidal neurons exhibit dendritic regression, spine loss, and increased synaptic excitation in the presymptomatic hSOD1(G93A) mouse model of amyotrophic lateral sclerosis. J Neurosci. 2015;35(2):643–7. https://doi.org/10.1523/JNEUROSCI.3483-14.2015
168. Kiernan MC, Ziemann U, Eisen A. Amyotrophic lateral sclerosis: Origins traced to impaired balance between neural excitation and inhibition in the neonatal period. Muscle Nerve. 2019;60(3):232–5. https://doi.org/10.1002/mus.26617
169. Fornai F, Longone P, Cafaro L, Kastsiuchenka O, Ferrucci M, Manca ML, et al. Lithium delays progression of amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2008;105(6):2052–7. https://doi.org/10.1073/pnas.0708022105
170. Morrison BM, Gordon JW, Ripps ME, Morrison JH. Quantitative immunocytochemical analysis of the spinal cord in G86R superoxide dismutase transgenic mice: neurochemical correlates of selective vulnerability. J Comp Neurol. 1996;373(4):619–31. https://doi.org/10.1002/(SICI)1096-9861(19960930)373:4<619::AID-CNE9>3.0.CO;2-4
171. Mazzocchio R, Rossi A. Role of Renshaw cells in amyotrophic lateral sclerosis. Muscle Nerve. 2010;41(4):441–3. https://doi.org/10.1002/mus.21602
172. Schutz B. Imbalanced excitatory to inhibitory synaptic input precedes motor neuron degeneration in an animal model of amyotrophic lateral sclerosis. Neurobiol Dis. 2005;20(1):131–40. https://doi.org/10.1016/j.nbd.2005.02.006
173. Salamatina A, Yang JH, Brenner-Morton S, Bikoff JB, Fang L, Kintner CR, et al. Differential Loss of Spinal Interneurons in a Mouse Model of ALS. Neuroscience. 2020;450:81–95. https://doi.org/10.1016/j.neuroscience.2020.08.011
174. Rudolph U, Mohler H. GABAA receptor subtypes: Therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. Annu Rev Pharmacol Toxicol. 2014;54:483–507. https://doi.org/10.1146/annurev-pharmtox-011613-135947
175. Schousboe A, Madsen KK, Barker-Haliski ML, White HS. The GABA synapse as a target for antiepileptic drugs: a historical overview focused on GABA transporters. Neurochemical research. 2014;39(10):1980–7. https://doi.org/10.1007/s11064-014-1263-9
176. Uusi-Oukari M, Korpi ER. Regulation of GABA(A) receptor subunit expression by pharmacological agents. Pharmacol Rev. 2010;62(1):97–135. https://doi.org/10.1124/pr.109.002063
177. Forshew DA, Bromberg MB. A survey of clinicians' practice in the symptomatic treatment of ALS. Amyotroph Lateral Scler Other Motor Neuron Disord. 2003;4(4):258–63. https://doi.org/10.1080/14660820310017344
178. Kita M, Goodkin DE. Drugs used to treat spasticity. Drugs. 2000;59(3):487–95. https://doi.org/10.2165/00003495-200059030-00006
179. McClelland S, 3rd, Bethoux FA, Boulis NM, Sutliff MH, Stough DK, Schwetz KM, et al. Intrathecal baclofen for spasticity-related pain in amyotrophic lateral sclerosis: efficacy and factors associated with pain relief. Muscle Nerve. 2008;37(3):396–8. https://doi.org/10.1002/mus.20900
180. Romano JG. Reduction of fasciculations in patients with amyotrophic lateral sclerosis with the use of gabapentin. Arch Neurol. 1996;53(8):716. https://doi.org/10.1001/archneur.1996.00550080022006
181. Rothstein JD, Kuncl RW. Neuroprotective strategies in a model of chronic glutamate-mediated motor neuron toxicity. J Neurochem. 1995;65(2):643–51. https://doi.org/10.1046/j.1471-4159.1995.65020643.x
182. Kalra S, Cashman NR, Caramanos Z, Genge A, Arnold DL. Gabapentin therapy for amyotrophic lateral sclerosis: lack of improvement in neuronal integrity shown by MR spectroscopy. AJNR Am J Neuroradiol. 2003;24(3):476–80.

183. Miller RG, Moore DH, 2nd, Gelinas DF, Dronsky V, Mendoza M, Barohn RJ, et al. Phase III randomized trial of gabapentin in patients with amyotrophic lateral sclerosis. Neurology. 2001;56(7):843–8. https://doi.org/10.1212/WNL.56.7.843
184. Cheng JK, Chen CC, Yang JR, Chiou LC. The antiallodynic action target of intrathecal gabapentin: Ca2+ channels, KATP channels or N-methyl-d-aspartic acid receptors? Anesth Analg. 2006;102(1):182–7. https://doi.org/10.1213/01.ane.0000189550.97536.83
185. Sills GJ. The mechanisms of action of gabapentin and pregabalin. Curr Opin Pharmacol. 2006;6(1):108–13. https://doi.org/10.1016/j.coph.2005.11.003
186. Pasquali L, Longone P, Isidoro C, Ruggieri S, Paparelli A, Fornai F. Autophagy, lithium, and amyotrophic lateral sclerosis. Muscle Nerve. 2009;40(2):173–94. https://doi.org/10.1002/mus.21423
187. Handy CR, Krudy C, Boulis N, Federici T. Pain in amyotrophic lateral sclerosis: a neglected aspect of disease. Neurol Res Int. 2011;2011:403808. https://doi.org/10.1155/2011/403808
188. Madsen KK, White HS, Schousboe A. Neuronal and non-neuronal GABA transporters as targets for antiepileptic drugs. Pharmacol Ther. 2010;125(3):394–401. https://doi.org/10.1016/j.pharmthera.2009.11.007
189. Rowley NM, Madsen KK, Schousboe A, Steve White H. Glutamate and GABA synthesis, release, transport and metabolism as targets for seizure control. Neurochem Int. 2012;61(4):546–58. https://doi.org/10.1016/j.neuint.2012.02.013
190. Kahle KT, Staley KJ. The bumetanide-sensitive Na-K-2Cl cotransporter NKCC1 as a potential target of a novel mechanism-based treatment strategy for neonatal seizures. Neurosurg Focus. 2008;25(3):E22. https://doi.org/10.3171/FOC/2008/25/9/E22
191. Harris JA, Murphy JA. Retigabine (ezogabine) as add-on therapy for partial-onset seizures: an update for clinicians. Ther Adv Chronic Dis. 2011;2(6):371–6. https://doi.org/10.1177/2040622311421542
192. Mannen T, Iwata M, Toyokura Y, Nagashima K. Preservation of a certain motoneurone group of the sacral cord in amyotrophic lateral sclerosis: its clinical significance. J Neurol Neurosurg Psychiatry. 1977;40(5):464–9. https://doi.org/10.1136/jnnp.40.5.464
193. Brockington A, Ning K, Heath PR, Wood E, Kirby J, Fusi N, et al. Unravelling the enigma of selective vulnerability in neurodegeneration: motor neurons resistant to degeneration in ALS show distinct gene expression characteristics and decreased susceptibility to excitotoxicity. Acta neuropathologica. 2013;125(1):95–109. https://doi.org/10.1007/s00401-012-1058-5
194. Lorenzo LE, Barbe A, Portalier P, Fritschy JM, Bras H. Differential expression of GABAA and glycine receptors in ALS-resistant vs. ALS-vulnerable motoneurons: possible implications for selective vulnerability of motoneurons. Eur J Neurosci. 2006;23(12):3161–70. https://doi.org/10.1111/j.1460-9568.2006.04863.x
195. Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. Orphanet J Rare Dis. 2009;4:3. https://doi.org/10.1186/1750-1172-4-3
196. Labrakakis C, Rudolph U, De Koninck Y. The heterogeneity in GABAA receptor-mediated IPSC kinetics reflects heterogeneity of subunit composition among inhibitory and excitatory interneurons in spinal lamina II. Front Cell Neurosci. 2014;8:424. https://doi.org/10.3389/fncel.2014.00424
197. Sieghart W, Sperk G. Subunit composition, distribution and function of GABA(A) receptor subtypes. Curr Top Med Chem. 2002;2(8):795–816. https://doi.org/10.2174/1568026023393507
198. Sigel E, Steinmann ME. Structure, function, and modulation of GABA(A) receptors. J Biol Chem. 2012;287(48):40224–31. https://doi.org/10.1074/jbc.R112.386664
199. Gowing G, Svendsen CN. Stem cell transplantation for motor neuron disease: current approaches and future perspectives. Neurotherapeutics. 2011;8(4):591–606. https://doi.org/10.1007/s13311-011-0068-7
200. Thomsen GM, Gowing G, Svendsen S, Svendsen CN. The past, present and future of stem cell clinical trials for ALS. Exp Neurol. 2014;262 Pt B:127–37. https://doi.org/10.1016/j.expneurol.2014.02.021
201. Xu L, Ryugo DK, Pongstaporn T, Johe K, Koliatsos VE. Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the segmental motor circuitry. J Comp Neurol. 2009;514(4):297–309. https://doi.org/10.1002/cne.22022
202. Xu L, Shen P, Hazel T, Johe K, Koliatsos VE. Dual transplantation of human neural stem cells into cervical and lumbar cord ameliorates motor neuron disease in SOD1 transgenic rats. Neurosci Lett. 2011;494(3):222–6. https://doi.org/10.1016/j.neulet.2011.03.017

203. Xu L, Yan J, Chen D, Welsh AM, Hazel T, Johe K, et al. Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. Transplantation. 2006;82(7):865–75. https://doi.org/10.1097/01.tp.0000235532.00920.7a
204. Witts EC, Zagoraiou L, Miles GB. Anatomy and function of cholinergic C bouton inputs to motor neurons. J Anat. 2014;224(1):52–60. https://doi.org/10.1111/joa.12063
205. Enjin A, Rabe N, Nakanishi ST, Vallstedt A, Gezelius H, Memic F, et al. Identification of novel spinal cholinergic genetic subtypes disclose Chodl and Pitx2 as markers for fast motor neurons and partition cells. J Comp Neurol. 2010;518(12):2284–304. https://doi.org/10.1002/cne.22332
206. Zagoraiou L, Akay T, Martin JF, Brownstone RM, Jessell TM, Miles GB. A cluster of cholinergic premotor interneurons modulates mouse locomotor activity. Neuron. 2009;64(5):645–62. https://doi.org/10.1016/j.neuron.2009.10.017
207. Sathyamurthy A, Johnson KR, Matson KJE, Dobrott CI, Li L, Ryba AR, et al. Massively Parallel Single Nucleus Transcriptional Profiling Defines Spinal Cord Neurons and Their Activity during Behavior. Cell Rep. 2018;22(8):2216–25. https://doi.org/10.1016/j.celrep.2018.02.003
208. Hellstrom J, Oliveira AL, Meister B, Cullheim S. Large cholinergic nerve terminals on subsets of motoneurons and their relation to muscarinic receptor type 2. J Comp Neurol. 2003;460(4):476–86. https://doi.org/10.1002/cne.10648
209. Ichikawa T, Shimizu T. Organization of choline acetyltransferase-containing structures in the cranial nerve motor nuclei and spinal cord of the monkey. Brain Res. 1998;779(1–2):96–103. https://doi.org/10.1016/S0006-8993(97)01090-1
210. Pullen AH, Martin JE, Swash M. Ultrastructure of pre-synaptic input to motor neurons in Onuf's nucleus: controls and motor neuron disease. Neuropathol Appl Neurobiol. 1992;18(3):213–31. https://doi.org/10.1111/j.1365-2990.1992.tb00784.x
211. Miles GB, Hartley R, Todd AJ, Brownstone RM. Spinal cholinergic interneurons regulate the excitability of motoneurons during locomotion. Proc Natl Acad Sci U S A. 2007;104(7):2448–53. https://doi.org/10.1073/pnas.0611134104
212. Deardorff AS, Romer SH, Sonner PM, Fyffe RE. Swimming against the tide: investigations of the C-bouton synapse. Front Neural Circuits. 2014;8:106. https://doi.org/10.3389/fncir.2014.00106
213. Landoni LM, Myles JR, Wells TL, Mayer WP, Akay T. Cholinergic modulation of motor neurons through the C-boutons are necessary for the locomotor compensation for severe motor neuron loss during amyotrophic lateral sclerosis disease progression. Behav Brain Res. 2019;369:111914. https://doi.org/10.1016/j.bbr.2019.111914
214. Miles GB, Sillar KT. Neuromodulation of vertebrate locomotor control networks. Physiology (Bethesda). 2011;26(6):393–411. https://doi.org/10.1152/physiol.00013.2011
215. Martin E, Cazenave W, Allain AE, Cattaert D, Branchereau P. Implication of 5-HT in the Dysregulation of Chloride Homeostasis in Prenatal Spinal Motoneurons from the G93A Mouse Model of Amyotrophic Lateral Sclerosis. Int J Mol Sci. 2020;21(3). https://doi.org/10.3390/ijms21031107
216. Bertel O, Malessa S, Sluga E, Hornykiewicz O. Amyotrophic lateral sclerosis: changes of noradrenergic and serotonergic transmitter systems in the spinal cord. Brain Res. 1991;566(1–2):54–60. https://doi.org/10.1016/0006-8993(91)91680-Y
217. Kato S, Oda M, Tanabe H. Diminution of dopaminergic neurons in the substantia nigra of sporadic amyotrophic lateral sclerosis. Neuropathol Appl Neurobiol. 1993;19(4):300–4. https://doi.org/10.1111/j.1365-2990.1993.tb00444.x
218. Borasio GD, Linke R, Schwarz J, Schlamp V, Abel A, Mozley PD, et al. Dopaminergic deficit in amyotrophic lateral sclerosis assessed with [I-123] IPT single photon emission computed tomography. J Neurol Neurosurg Psychiatry. 1998;65(2):263–5. https://doi.org/10.1136/jnnp.65.2.263
219. Takahashi H, Snow BJ, Bhatt MH, Peppard R, Eisen A, Calne DB. Evidence for a dopaminergic deficit in sporadic amyotrophic lateral sclerosis on positron emission scanning. Lancet. 1993;342(8878):1016–8. https://doi.org/10.1016/0140-6736(93)92878-W
220. Kostic V, Gurney ME, Deng HX, Siddique T, Epstein CJ, Przedborski S. Midbrain dopaminergic neuronal degeneration in a transgenic mouse model of familial amyotrophic lateral sclerosis. Ann Neurol. 1997;41(4):497–504. https://doi.org/10.1002/ana.410410413

221. Turner MR, Rabiner EA, Hammers A, Al-Chalabi A, Grasby PM, Shaw CE, et al. [11C]-WAY100635 PET demonstrates marked 5-HT1A receptor changes in sporadic ALS. Brain. 2005;128(Pt 4):896–905. https://doi.org/10.1093/brain/awh428
222. Forrest V, Ince P, Leitch M, Marshall EF, Shaw PJ. Serotonergic neurotransmission in the spinal cord and motor cortex of patients with motor neuron disease and controls: quantitative autoradiography for 5-HT1a and 5-HT2 receptors. J Neurol Sci. 1996;139 Suppl:83–90. https://doi.org/10.1016/0022-510X(96)00109-8
223. Dentel C, Palamiuc L, Henriques A, Lannes B, Spreux-Varoquaux O, Gutknecht L, et al. Degeneration of serotonergic neurons in amyotrophic lateral sclerosis: a link to spasticity. Brain. 2013;136(Pt 2):483–93. https://doi.org/10.1093/brain/aws274
224. El Oussini H, Scekic-Zahirovic J, Vercruysse P, Marques C, Dirrig-Grosch S, Dieterle S, et al. Degeneration of serotonin neurons triggers spasticity in amyotrophic lateral sclerosis. Ann Neurol. 2017;82(3):444–56. https://doi.org/10.1002/ana.25030
225. Koschnitzky JE, Quinlan KA, Lukas TJ, Kajtaz E, Kocevar EJ, Mayers WF, et al. Effect of fluoxetine on disease progression in a mouse model of ALS. J Neurophysiol. 2014;111(11):2164–76. https://doi.org/10.1152/jn.00425.2013
226. Eisen A, Kim S, Pant B. Amyotrophic lateral sclerosis (ALS): a phylogenetic disease of the corticomotoneuron? Muscle Nerve. 1992;15(2):219–24. https://doi.org/10.1002/mus.880150215
227. de Carvalho M, Eisen A, Krieger C, Swash M. Motoneuron firing in amyotrophic lateral sclerosis (ALS). Front Hum Neurosci. 2014;8:719. https://doi.org/10.3389/fnhum.2014.00719
228. Bae JS, Simon NG, Menon P, Vucic S, Kiernan MC. The puzzling case of hyperexcitability in amyotrophic lateral sclerosis. J Clin Neurol. 2013;9(2):65–74. https://doi.org/10.3988/jcn.2013.9.2.65
229. Geevasinga N, Menon P, Ozdinler PH, Kiernan MC, Vucic S. Pathophysiological and diagnostic implications of cortical dysfunction in ALS. Nat Rev Neurol. 2016;12(11):651–61. https://doi.org/10.1038/nrneurol.2016.140
230. Pieri M, Carunchio I, Curcio L, Mercuri NB, Zona C. Increased persistent sodium current determines cortical hyperexcitability in a genetic model of amyotrophic lateral sclerosis. Exp Neurol. 2009;215(2):368–79. https://doi.org/10.1016/j.expneurol.2008.11.002
231. Saba L, Viscomi MT, Caioli S, Pignataro A, Bisicchia E, Pieri M, et al. Altered Functionality, Morphology, and Vesicular Glutamate Transporter Expression of Cortical Motor Neurons from a Presymptomatic Mouse Model of Amyotrophic Lateral Sclerosis. Cereb Cortex. 2016;26(4):1512–28. https://doi.org/10.1093/cercor/bhu317
232. Kepecs A, Fishell G. Interneuron cell types are fit to function. Nature. 2014;505(7483):318–26. https://doi.org/10.1038/nature12983
233. Khademullah CS, Aqrabawi AJ, Place KM, Dargaei Z, Liang X, Pressey JC, et al. Cortical interneuron-mediated inhibition delays the onset of amyotrophic lateral sclerosis. Brain. 2020;143(3):800–10. https://doi.org/10.1093/brain/awaa034
234. Roth BL. DREADDs for Neuroscientists. Neuron. 2016;89(4):683–94. https://doi.org/10.1016/j.neuron.2016.01.040
235. Burg T, Bichara C, Scekic-Zahirovic J, Fischer M, Stuart-Lopez G, Brunet A, et al. Absence of Subcerebral Projection Neurons Is Beneficial in a Mouse Model of Amyotrophic Lateral Sclerosis. Ann Neurol. 2020;88(4):688–702. https://doi.org/10.1002/ana.25833
236. Proske U, Gandevia SC. The proprioceptive senses: their roles in signaling body shape, body position and movement, and muscle force. Physiol Rev. 2012;92(4):1651–97. https://doi.org/10.1152/physrev.00048.2011
237. Tuthill JC, Azim E. Proprioception. Curr Biol. 2018;28(5):R194-R203. https://doi.org/10.1016/j.cub.2018.01.064
238. Falgairolle M, O'Donovan MJ. Motoneuronal Spinal Circuits in Degenerative Motoneuron Disease. Front Mol Neurosci. 2020;13:74. https://doi.org/10.3389/fnmol.2020.00074
239. Ruffoli R, Biagioni F, Busceti CL, Gaglione A, Ryskalin L, Gambardella S, et al. Neurons other than motor neurons in motor neuron disease. Histology and histopathology. 2017;32(11):1115–23.
240. Riancho J, Paz-Fajardo L, Lopez de Munain A. Clinical and preclinical evidence of somatosensory involvement in amyotrophic lateral sclerosis. Br J Pharmacol. 2020. https://doi.org/10.1111/bph.15202

241. Iglesias C, Sangari S, El Mendili MM, Benali H, Marchand-Pauvert V, Pradat PF. Electrophysiological and spinal imaging evidences for sensory dysfunction in amyotrophic lateral sclerosis. BMJ open. 2015;5(2):e007659. https://doi.org/10.1136/bmjopen-2015-007659
242. Tao QQ, Wei Q, Wu ZY. Sensory nerve disturbance in amyotrophic lateral sclerosis. Life Sci. 2018;203:242–5. https://doi.org/10.1016/j.lfs.2018.04.052
243. Dal Canto MC, Gurney ME. Neuropathological changes in two lines of mice carrying a transgene for mutant human Cu,Zn SOD, and in mice overexpressing wild type human SOD: a model of familial amyotrophic lateral sclerosis (FALS). Brain Res. 1995;676(1):25–40. https://doi.org/10.1016/0006-8993(95)00063-V
244. Vaughan SK, Kemp Z, Hatzipetros T, Vieira F, Valdez G. Degeneration of proprioceptive sensory nerve endings in mice harboring amyotrophic lateral sclerosis-causing mutations. J Comp Neurol. 2015;523(17):2477–94. https://doi.org/10.1002/cne.23848
245. Held A, Major P, Sahin A, Reenan RA, Lipscombe D, Wharton KA. Circuit Dysfunction in SOD1-ALS Model First Detected in Sensory Feedback Prior to Motor Neuron Degeneration Is Alleviated by BMP Signaling. J Neurosci. 2019;39(12):2347–64. https://doi.org/10.1523/JNEUROSCI.1771-18.2019
246. Seki S, Yamamoto T, Quinn K, Spigelman I, Pantazis A, Olcese R, et al. Circuit-Specific Early Impairment of Proprioceptive Sensory Neurons in the SOD1(G93A) Mouse Model for ALS. J Neurosci. 2019;39(44):8798–815. https://doi.org/10.1523/JNEUROSCI.1214-19.2019
247. Lalancette-Hebert M, Sharma A, Lyashchenko AK, Shneider NA. Gamma motor neurons survive and exacerbate alpha motor neuron degeneration in ALS. Proc Natl Acad Sci U S A. 2016;113(51):E8316-E25. https://doi.org/10.1073/pnas.1605210113
248. Brownstone RM, Lancelin C. Escape from homeostasis: spinal microcircuits and progression of amyotrophic lateral sclerosis. J Neurophysiol. 2018;119(5):1782–94. https://doi.org/10.1152/jn.00331.2017
249. Gu Z, Kalambogias J, Yoshioka S, Han W, Li Z, Kawasawa YI, et al. Control of species-dependent cortico-motoneuronal connections underlying manual dexterity. Science. 2017;357(6349):400–4. https://doi.org/10.1126/science.aan3721
250. Zona C, Pieri M, Carunchio I. Voltage-dependent sodium channels in spinal cord motor neurons display rapid recovery from fast inactivation in a mouse model of amyotrophic lateral sclerosis. J Neurophysiol. 2006;96(6):3314–22. https://doi.org/10.1152/jn.00566.2006