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SHORT REPORT

Neuroscience of Disease

Direct evidence of upper motor neuron excitability changes in a patient with ALS

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Abstract

A key feature of amyotrophic lateral sclerosis (ALS) pathophysiology is motor neuron hyperexcitability. However, the mechanisms of hyperexcitability are not well understood. Prior studies have used transcranial magnetic stimulation (TMS) to demonstrate increased motor cortex excitability and reduced intracortical inhibition in human ALS. Yet, interpretation of these findings is limited because measurement of muscle responses cannot disentangle the specific contribution of upper and lower motor neurons and of cortical interneurons to excitability changes. We had the rare opportunity to record directly the corticospinal output evoked by TMS upstream of the spinal circuitry in a patient with ALS who had undergone epidural electrode implantation for intractable pain. Single-pulse stimulation was performed both with a coil orientation inducing a current that activates corticospinal neurons directly, and with a coil orientation inducing a current that activates corticospinal neurons trans-synaptically. Short-interval intracortical inhibition (SICI) was also studied using paired-pulse stimulation. Data obtained from the patient were compared with those recorded in 10 conscious control subjects. Compared with control subjects, patient showed a reduced amplitude in response to direct corticospinal neuron activation, yet an enhanced amplitude of corticospinal output after trans-synaptic corticospinal neuron activation, together with a SICI reduction. Present findings provide direct evidence of hyperexcitability of monosynaptic glutamatergic inputs to corticospinal neurons that, in association with reduced intracortical inhibition, can trigger neurodegeneration. Taken together with the extensive body of evidence generated by noninvasive TMS studies, the findings from this single-case study may provide valuable insights into the pathophysiological mechanisms of the disease.

NEW & NOTEWORTHY The response evoked by direct activation of corticospinal neurons is reduced in human amyotrophic lateral sclerosis (ALS). In contrast, the response evoked by trans-synaptic activation of these cells is enhanced. The activity of inhibitory inputs to corticospinal neurons is reduced. These abnormalities related to abnormal excitatory and inhibitory input processing by corticospinal neurons may trigger neurodegeneration.

amyotrophic lateral sclerosis; epidural recording; excitotoxicity; motor neuron hyperexcitability

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease due to upper and lower motor neuron death. A key feature of ALS is the hyperexcitability of motor neurons, which has been reported in both human familial and sporadic ALS as well as in animal models of the disease (1, 2), evident in the presymptomatic phases (3), and proposed as a functional biomarker of disease progression (2, 4, 5). It has been hypothesized that enhanced corticospinal neuron (CSN) excitability is a pathophysiological mechanism of the neurodegenerative cascade in which motor neuron death is due to excessive glutamate receptor activity (6). Several studies have shown an impairment of inhibitory circuits at

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different levels. For instance, inhibitory GABAergic circuits connected to CSNs have been shown to be less effective, and the number of GABA receptors is reduced (7, 8). These findings have supported the hypothesis that ALS is a central motor network disorder rather than a pure motor neuron disorder, and that the pathophysiological mechanisms leading to motor neuron degeneration involve cortical interneurons (8–12). An early dysfunction of spinal interneurons is also suggested by some experimental studies (13). Thus, it is still unclear if hyperexcitability is caused by alterations in intrinsic excitability of motor neurons, in excitatory and inhibitory inputs to these cells, or by a combination of these factors.

Motor cortex excitability in human ALS has been evaluated by noninvasive transcranial magnetic stimulation (TMS). In early stages of ALS, motor cortex hyperexcitability has been reported (3, 14–19), while paired-pulse TMS has demonstrated a deficit in short-interval intracortical inhibition (SICI) and an enhancement in intracortical facilitation (3, 16, 17, 20–23). However, the interpretation of these studies is limited by the fact that evoked responses have been recorded from muscles. Thus, they cannot disentangle the specific contribution of upper and lower motor neurons to the evoked response. Furthermore, these methods cannot provide fine-grained information about excitatory and inhibitory circuits activated by TMS (24).

To circumvent this methodological limitation, some studies have recorded corticospinal outputs evoked by TMS upstream of spinal circuitry, in otherwise healthy subjects with spinal electrodes implanted for chronic pain (24) (Fig. 1). This method allows for analysis of the multiple components of corticospinal activity evoked by single-pulse TMS and modulated by paired-pulse TMS, giving a direct indication on the function of CSNs and of cortico-cortical excitatory and inhibitory circuits, uncontaminated by changes in spinal excitability (24). Specifically, TMS activates CSNs both directly and trans-synaptically, but it is possible to selectively evaluate direct and indirect activation of CSNs by changing the direction of induced current in the brain (24). TMS stimuli inducing a current flowing parallel to the central sulcus, in a lateral to medial direction (LM), preferentially activate CSNs directly, evoking an axonal response termed "D-wave," while an induced current flowing perpendicularly to the central sulcus, in a posterior to anterior direction (PA), activates CSNs trans-synaptically and evokes indirect responses or "I-waves" (24). The earliest I-wave (I1) evoked by PA TMS is not suppressed by drugs enhancing GABAergic transmission as opposed to the following waves (late I-waves) (26), indicating that I1 and late I-waves are produced by different sources of inputs to CSNs. The origin of these volleys was recently defined more precisely using computational modeling, showing that the I1-wave likely originates from monosynaptic activation of layer 5 CSNs by excitatory afferents originating in this layer, while later I-waves likely originate from the activation of CSNs by excitatory and inhibitory connections from interneurons in layers 2–3 (25) (Fig. 1).

Our aim was to provide more direct insight into motor cortex dysfunction in human ALS by invasively recording the corticospinal output evoked by TMS upstream of spinal circuitry, in a rare patient with ALS with an epidural electrode implanted in the high cervical cord for intractable cervico-dorso-lumbar pain (27), recordings were compared with those of subjects with no central nervous system abnormality, who also had cervical electrodes implanted for pain control.

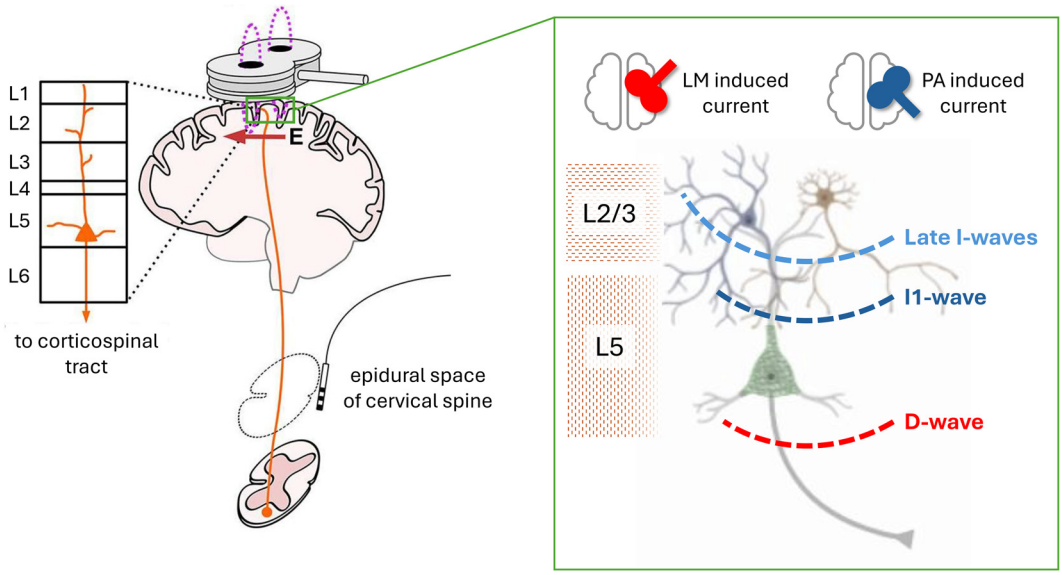

Figure 1. Diagrammatic representation of cortical circuits activated by transcranial magnetic stimulation. *Left*: corticospinal descending volleys generated by TMS activation of layer 5 pyramidal tract neurons can be recorded with an epidural electrode implanted at the cervical level [adapted from Yu et al. (25) used with permission under CC-BY license]. *Right*: TMS can activate layer 5 corticospinal neurons (CSNs) both directly and trans-synaptically. A coil orientation that induces a current flowing in a lateral to medial direction (LM), parallel to the central sulcus, preferentially activates CSNs directly, evoking a response of the axons of these cells termed "D-wave" (represented in red color), while an induced current flowing perpendicularly to the central sulcus, in a posterior to anterior direction (PA), activates CSNs trans-synaptically and evokes indirect responses or "I-waves" (represented in blue color). I1-wave likely originates from monosynaptic activation of layer 5 CSNs by excitatory afferents originating in this layer (dark blue), while later I-waves likely originate from the activation of CSNs by excitatory and inhibitory connections from interneurons in layers 2 and 3 (light blue).

METHODS

Patient and Controls

The patient was a 75-yr-old man with a diagnosis of definite ALS according to the El Escorial revised criteria (28). In November 2012, he presented with lower limb weakness and fasciculations, with walking difficulties. The electrode was implanted in February 2015. At that time, the patient had a spastic paraparesis, fasciculations and atrophy in the tongue, dysarthria, bilateral limb weakness, and bilateral Babinski sign. The revised ALS functional rating scale (ALSFRS-R) score was 34. He was taking riluzole since January 2014, but no other centrally acting medication.

An Octrode lead (Saint Jude Medical, St. Paul, MN) was implanted in the cervical epidural space at C1–C2 level, and recordings of motor cortex output were made 3 days after implantation during the trial screening period when the electrode connections were externalized.

Data obtained from this patient were compared with those recorded in five conscious control subjects [mean age 63.8 ± 11.9 (SD) yr] for single-pulse stimulation and six conscious control subjects for paired-pulse stimulation [mean age 61.7 ± 13.5 (SD) yr]. One subject of the latter control group is common with the control group for paired-pulse stimulation, resulting in a sample of 10 control subjects in total. These subjects, who had no abnormalities of the central nervous system, underwent implantation of high cervical epidural electrodes for the treatment of chronic intractable dorso-lumbar pain without spinal cord involvement. Recordings in control subjects were performed within 5 days after implantation during the trial screening period when the electrode connections were externalized. Control subjects were not taking centrally acting medications at the time of the experiments. This is because the trial screening period for epidural spinal cord stimulation, which precedes permanent implantation, is routinely conducted after a washout period of analgesic medications and other centrally acting drugs. This procedure is generally well tolerated because epidural stimulation is considered only in patients whose pain is refractory to medical treatment, and temporary discontinuation of medication for a few days usually does not cause significant discomfort. A washout period is necessary to allow an accurate evaluation of the efficacy of epidural stimulation on pain symptoms before permanent implantation. The experiments were undertaken with the understanding and written consent of each subject and were approved by the Ethical Committee of Campus Bio-Medico University, in accordance with the Declaration of Helsinki.

Transcranial Magnetic Stimulation

Recordings were made simultaneously from the epidural electrode and from the left first dorsal interosseous (FDI) muscle of the hand, both in the patient and in controls. In the patient, this muscle showed slight fibrillation on electromyographic study.

Motor evoked potentials (MEPs) were recorded with two 9-mm diameter Ag-AgCl surface electrodes with the active electrode over the motor point of the muscle and the reference on the metacarpophalangeal joint of the index finger. MEPs and epidural volleys were amplified and filtered (bandwidth 3 Hz to 3 kHz) by a D360 amplifier (Digitimer Ltd., Welwyn Garden City, UK), and digitized at 5 kHz using a CED 1401 A/D converter and associated Signal software (Cambridge Electronic Design Ltd, Cambridge, UK). TMS was performed with a Magstim 200^2 stimulator connected to a BiStim module (The Magstim Co. Ltd., Whitland, UK). Resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced an MEP $\geq$50 μV in five out of 10 trials. Active motor threshold (AMT) was defined as the minimum stimulus intensity that produced an MEP $\geq$200 μV in five of 10 trials during isometric contraction of the tested muscle at 10% maximum force (29).

Single-Pulse Stimulation

In present study, we stimulated the right motor cortex at the optimal scalp position to elicit MEPs in the contralateral FDI with both LM and PA TMS. The hotspot was defined independently for each direction of the induced current. Responses to TMS were recorded at an intensity of 120% RMT. Twenty-five pulses were delivered for each direction of the induced current in the brain. We measured the amplitude, the latency, and the number of the corticospinal volleys (D and I-waves). After averaging, wave amplitudes were measured from the negative to the following positive peak of each component, as in previous studies (26). In addition, to characterize more selectively the changes in the outputs evoked by activation of CSNs and of cortical interneurons, we calculated the ratios between the amplitudes of different components of the volley: the D-wave, the I1-wave, and the late I-waves (the I-waves following the I1-wave). Specifically, the following ratios were calculated: the sum of all I-waves/D-wave, I1-wave/D-wave, the sum of late I-waves/D-wave, and I1-wave/the sum of late I-waves. These ratios can indicate the relative contribution of pre- and postsynaptic components of motor cortex micro-columns to the observed changes in excitability (25). Corticospinal axon recording after intracortical stimulation in monkeys has shown that multiple descending waves corresponding to the D and I-waves can be recorded from individual axons (30). Because the same CSNs can respond with a D or an I-wave response, by recording D and I-waves, it is possible to evaluate selectively the intrinsic excitability of CSNs and their excitability in response to synaptic inputs. Specifically, the ratio between all I-waves and the D-wave can indicate abnormalities in trans-synaptic activation of CSNs by inhibitory and excitatory interneurons not influenced by any change in CSNs (e.g., reduction in the number of CSNs or changes in the intrinsic excitability of CSNs). The ratio between the I1-wave and the D-wave can indicate abnormalities in trans-synaptic activation of CSNs by excitatory layer 5 connections, not influenced by any change in CSNs. The ratio between the late I-waves and the D-wave can indicate abnormalities in trans-synaptic activation of CSNs by inhibitory and excitatory connections from layers 2 and 3 not influenced by any change in CSNs. Finally, the ratio between the I1-wave and the late I-waves can indicate abnormalities in trans-synaptic activation of CSNs by excitatory and inhibitory connections from layers II and III, not influenced by changes in excitatory layer 5 connections.

Paired-Pulse Stimulation (Short-Interval Intracortical Inhibition)

To study SICI, two magnetic stimuli were given through the same coil with a PA-induced current at an interstimulus interval (ISI) of 3 ms (31, 32). The conditioning stimulus was set at an intensity of 5% of maximum stimulator output below AMT, and test stimulus intensity was set at an intensity of 120% RMT.

The deviation of the patient's intracortical function measures from controls was estimated using z-scores. A z-score greater than 1.96 or less than −1.96 was considered statistically significant ($P < 0.05$, two-tailed) (33). Given the small sample size of the control population, normality of data distribution was checked on the distribution of single-trial wave amplitudes measured at the average peak latencies.

RESULTS

Figure 2 shows the averaged recordings in the patient and in one representative control subject.

Single-Pulse Magnetic Stimulation

Direct activation of corticospinal neurons (LM magnetic stimulation).

In control subjects, the mean RMT was 55.8% (SD 12.5) of maximum stimulator output using LM magnetic stimulation. In the patient, it was 49% of maximum stimulator output. At 120% RMT, single-pulse LM stimulation evoked a D-wave; in control subjects, this D response had an average amplitude of 21.0 μV (±4.9 μV SD) and an average latency of 2.4 ms (±0.8 ms SD). The patient's D-wave had a significantly reduced mean amplitude (10.1 μV; z-score: −2.21, $P < 0.05$) and a normal mean latency (2.2 ms; z-score: −0.29, $P > 0.05$) (Figs. 2 and 3, *A* and *E*).

Trans-synaptic activation of corticospinal neurons (PA magnetic stimulation).

In control subjects, mean RMT was 53.0% (SD 11.1) of maximum stimulator output using PA magnetic stimulation. In the patient, it was 45% of maximum stimulator output. In control subjects, mean AMT was 38.0% (SD 8.4) of maximum

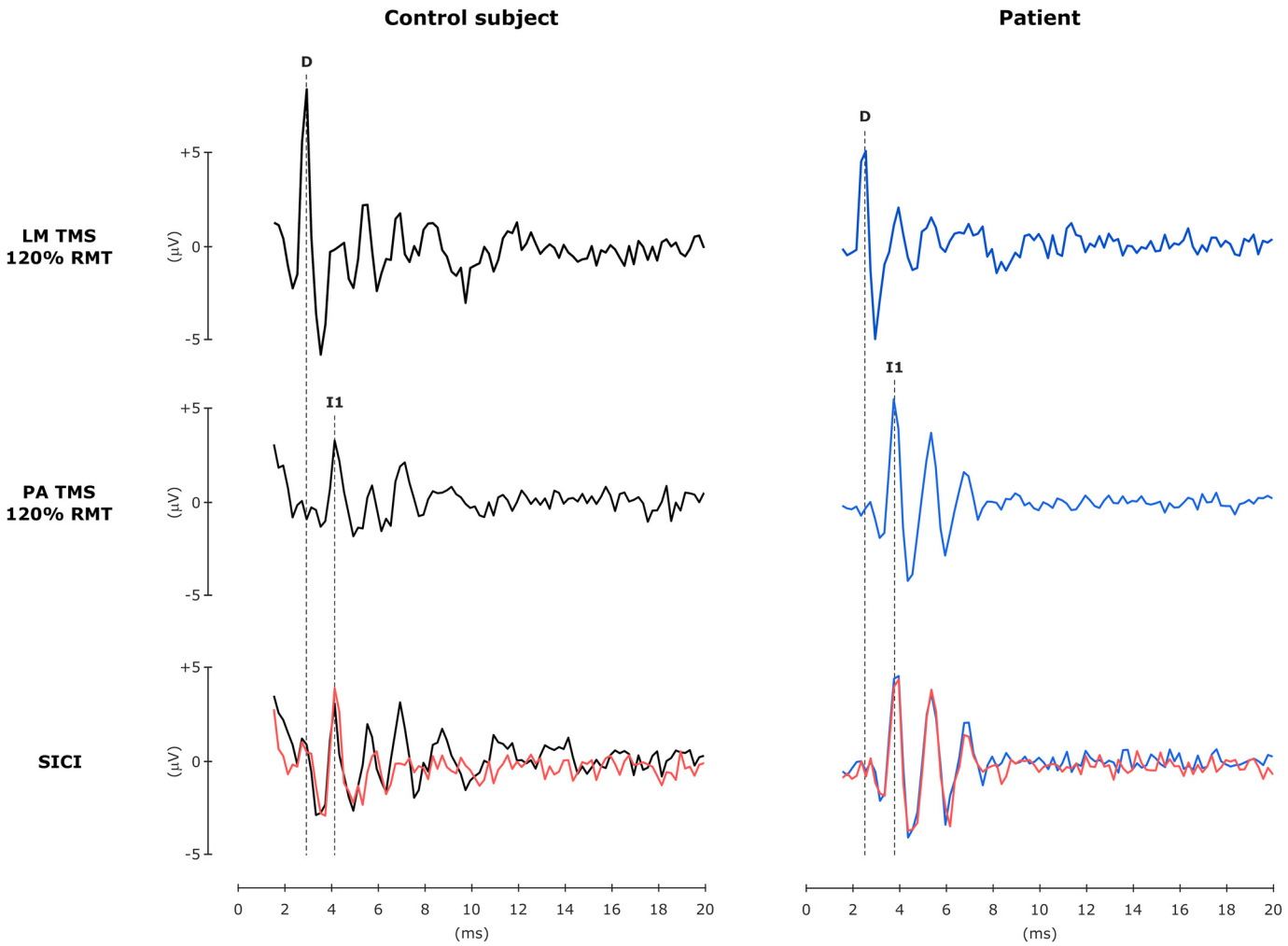

Figure 2. Corticospinal volleys recorded from the high cervical cord in the patient (*right*) and in one representative subject with no central nervous system abnormality (*left*). Corticospinal volleys were obtained after lateral to medial (LM) and posterior to anterior (PA) TMS at an intensity of 120% of the resting motor threshold (RMT). For the short-interval intracortical inhibition (SICI) protocol, the test response to PA TMS at 120% RMT (blue trace in the patient and black trace in the control) is compared with the conditioned response to PA TMS at an intensity below active motor threshold (red trace, see METHODS). Each trace is the average of at least 20 stimuli, sampled at 5 KHz. Vertical dashed lines indicate the latency of D- and I1-wave, respectively.

stimulator output using PA magnetic stimulation. In the patient, it was 32% of maximum stimulator output. At 120% RMT, single-pulse PA stimulation evoked 3 I-waves in the patient and in 4 out of 5 control subjects (Fig. 2). In the remaining control subject, 2 I-waves were evoked. The first (I1) wave had an average latency of 3.8 ms (±0.7 ms SD) in control subjects and a latency of 3.8 ms in the patient (z-score: 0.03). In contrast, the amplitude of the I1-wave was 8.3 μV (±1.3 μV SD) in control subjects and significantly higher (12.1 μV) in the patient (z-score: 2.85, $P < 0.05$, Figs. 2 and 3, *B* and *E*).

The amplitude of late I-waves (i.e., the I-waves following I1) was similar in control subjects (9.9 ±3.2 μV SD) and in the patient (11.3 μV, z-score: 0.43, $P > 0.05$, Fig. 3, *C* and *E*).

Ratio between cortico-cortical and corticospinal outputs.

Ratios between corticospinal waves generated by cortico-cortical connections (I-waves) and direct upper motor neuron activation (D-wave) were calculated to reveal the different contributions of these mechanisms to corticospinal excitability changes. The ratio between total I-wave and D-wave amplitudes was 0.90 (±0.22 SD) in control subjects, and 2.32 in the patient (z-score: 6.46, $P < 0.0001$). The ratio between I1 and D-wave amplitudes was 0.41 (±0.08 SD) in control subjects and 1.20 in the patient (z-score: 10.23, $P < 0.0001$). The ratio between the late I-wave and D-wave amplitudes was 0.49 (±0.17 SD) in control subjects and 1.12 in the patient (z-score: 3.67, $P < 0.001$). The ratio between I1 and late I-wave amplitudes was 0.92 (±0.42 SD) in control subjects and 1.07 in the patient (z-score: 0.36, $P > 0.05$).

Taken together, these ratio changes suggest that there is an enhancement of the response of CSNs to excitatory inputs from layer 5 of the motor cortex, and a change in the balance between excitatory and inhibitory inputs from layers 2 and 3, in favor of greater excitatory drive.

Short-Interval Intracortical Inhibition

Similar to results previously reported (32), with paired cortical stimulation at the ISI of 3 ms, the I1-wave was not affected by the conditioning stimulus [amplitude of the I1-wave normalized to that measured after single-pulse TMS was 1.01 (±0.09 SD)], while the late I-waves were suppressed in all controls (Fig. 2). The amplitude of the late I-waves normalized to that measured after single-pulse TMS was 0.55 (±0.15 SD). A Wilcoxon signed-rank test in control subjects showed a significant effect of the conditioning stimulus for the total volley amplitude ($P = 0.031$) and for later volley amplitude ($P = 0.031$), while the I1-wave was not affected by the conditioning stimulus ($P = 1.000$).

In the patient, neither the I1 nor the late I-waves were suppressed. The normalized amplitude of the I1-wave (to that measured after single-pulse TMS) was 0.94 (z-score: −0.81 vs. controls, $P > 0.05$), and the normalized amplitude of the late I-waves was 0.90 (Fig. 3*D*). The z-score of this normalized late I-wave amplitude in the patient compared with controls (i.e., 0.90 vs. 0.55) was 2.31 ($P < 0.05$, Fig. 3*E*), demonstrating a lack of inhibition of these later I-waves. MEPs were also not suppressed by the conditioning stimulus [conditioned MEP amplitude normalized to that measured after single-pulse TMS in the FDI muscle was 1.006 (raw values: 0.855 ± 0.767 mV vs. 0.849 ± 0.724, $P = 0.768$, Mann–Whitney *U* test)].

DISCUSSION

Noninvasive brain stimulation studies using TMS have suggested that there are several functional abnormalities in the motor cortex of patients with ALS. The most consistent findings are the enhanced motor cortex excitability to single-pulse stimulation and the reduced cortical inhibition to paired-pulse stimulation. However, the indirect nature of these prior data provided by TMS studies in humans has not allowed for inference as to whether these abnormalities were caused by changes in intrinsic excitability of motor neurones (upper and/or lower), interneurons (cortical and/or spinal), or both. The present study, for the first time in a human patient with ALS, uses TMS with spinal cord recordings to provide direct insight into these mechanisms. Specifically, we demonstrate that the corticospinal output evoked by direct stimulation of CSNs is reduced, while, in contrast, the corticospinal output evoked by trans-synaptic activation of CSNs is enhanced. Furthermore, paired-pulse TMS demonstrated a reduction in intracortical inhibition in the patient compared with controls.

Reduced Corticospinal Output after Direct Activation of CSNs and Increased Corticospinal Output after Trans-Synaptic Activation of CSNs

In this study, our patient with ALS showed a reduction in the amplitude of the response evoked by direct activation of CSNs. In contrast, the patient demonstrated an enhanced

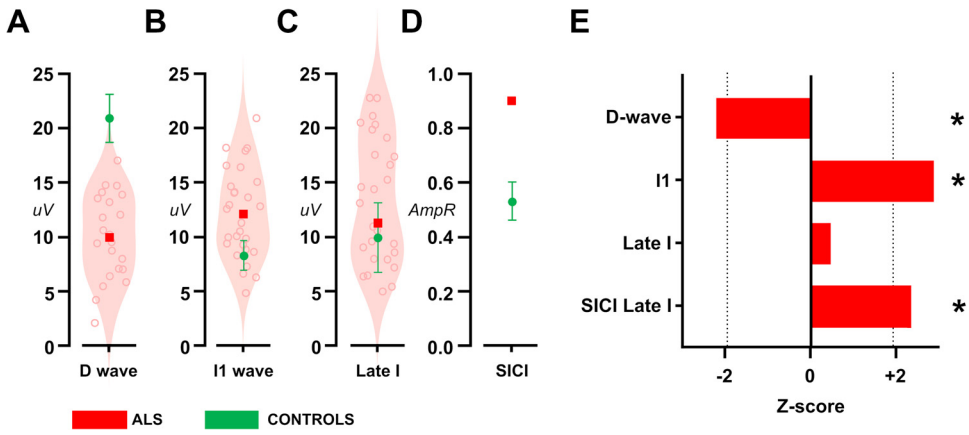

Figure 3. Comparison of corticospinal waves in the patient and in controls. *A*: D-wave amplitude based on LM stimulation at 120% RMT. *B*: I1-wave amplitude based on PA stimulation at 120% RMT. *C*: late I-wave amplitude based on PA stimulation at 120% RMT. *D*: late I-waves for short-interval intracortical inhibition (SICI) at the ISI of 3 ms. *E*: z-scores for (top to bottom) D-wave, I1-wave, late I-waves, SICI late I-waves for the ISI of 3 ms. Vertical dotted lines indicate z = ±1.96, *$P < 0.05$. Error bars correspond to SD in controls. Violin plots superimposed to mean data represent density distribution of single-trace data (empty circles) in the patient with ALS.

response to indirect, trans-synaptic activation by cortico-cortical cells. Specifically, magnetic stimulation with LM-induced current in the brain directly activates the corticospinal axons (24), and this evoked a lower amplitude D-wave, while magnetic stimulation with PA-induced current in the brain activates CSNs trans-synaptically, and this stimulation evoked an enhanced I1-wave, the response produced by monosynaptic activation of these CSNs. According to modeling studies, the I1-wave is the response of CSNs to excitatory inputs originating in motor cortex layer 5 (25), and according to pharmaco-TMS studies, the I1-wave is produced by pure excitatory connections without involvement of inhibitory GABAergic inputs (26). Because the same CSNs respond with a D and I-wave response (30), our findings suggest an impairment of CSN output after direct stimulation of these neurons associated with hyperexcitability of CSNs in response to monosynaptic glutamatergic inputs in ALS. It should be considered that the epidurally recorded corticospinal volleys represent the activity of a large population of CSNs; thus, the reduced amplitude of the D-wave in our patient might be due either to a reduced excitability of CSNs in response to direct activation or to the ALS-related neurodegeneration of a portion of the CSN population. The evidence of a threshold to LM magnetic stimulation comparable to that of healthy subjects suggests that the excitability of surviving corticospinal axons is not impaired and that CSN loss is more likely to be the cause of D-wave amplitude reduction than a corticospinal axon excitability change.

We also observed a relative increase of the late I-waves (with respect to the amplitude of the D-wave), the responses produced by trans-synaptic activation of CSNs by cortico-cortical excitatory and inhibitory inputs from layers 2–3 of the motor cortex (25). Because late I-waves are produced by a complex circuit composed of excitatory and inhibitory neurons (34), their enhancement can be due to increased excitatory input, decreased inhibitory input, or abnormal processing of the excitatory and/or inhibitory inputs. In our patient, there was also a substantial reduction in the intrinsic inhibitory activity of the motor cortex as evaluated by the study of SICI with paired-pulse TMS. This finding suggests that loss of GABAergic inputs contributes to the enhanced (late I-wave) response of CSNs to excitatory inputs. It should be noted that the patient was taking riluzole, which has been reported to affect SICI (35). However, these effects have been shown to be transient, with cortical excitability returning to baseline levels within few weeks of treatment initiation (36). Therefore, we believe that it is unlikely that riluzole significantly influenced cortical excitability measures assessed by TMS in our patient; however, this cannot be completely ruled out.

Cumulatively, our findings reveal multifaceted excitability changes: *1*) a reduction of the response evoked by direct activation of CSNs; *2*) a pronounced enhancement of the response to monosynaptic glutamatergic inputs to CSNs from layer 5; and *3*) a relative enhancement in layer 2–3 inputs to CSNs, which, due to our finding of a loss of SICI using a paired-pulse TMS protocol, can be at least partly attributed to a reduced activity of inhibitory cells within this circuit. Although the first change can well be explained by the ALS-related motor neuron loss, resulting in a reduced corticospinal output, the enhancement of the output produced by cortico-cortical activation and the reduction in intracortical inhibition might be explained either by abnormalities in cortical excitatory and inhibitory interneurons, as suggested by several studies (37), or by an abnormal response of CSNs to synaptic inputs. In favor of a dysfunction of interneurons, a recent study by van den Bos and colleagues (37) provided evidence of impaired intracortical inhibition in patients with ALS, leading to the interneuron hypothesis of ALS (12), suggesting that inhibitory interneurons are the drivers of CSN degeneration. However, an inhibitory interneuron abnormality can explain only part of the changes observed in our patient, in that the I1-wave enhancement cannot be caused by an impairment in inhibitory interneurons because this wave is not affected by GABAergic inputs (26). Therefore, the hypothesis of abnormal processing of synaptic inputs might better explain all our findings, because this would affect both excitatory and inhibitory transmission with the same unique mechanism.

This unique pathophysiological mechanism impacting both excitatory and inhibitory neurotransmission could be driven by the defects in voltage-gated channels reported in ALS (38).

Interestingly, a recent study using induced pluripotent stem cell-derived neurons and postmortem ALS brain and spinal cord tissue showed that TAR DNA-binding protein 43 (TDP-43) aggregation, the hallmark of ALS, causes mis-splicing of potassium voltage-gated channel subfamily Q member 2 (KCNQ2) mRNA with severely reduced ion conductance, causing motor neuron hyperexcitability (39). KCNQ2 produces the Kv7.2 ion channel responsible for the neuronal M-current that regulates resting membrane potentials, shapes action potentials, and impedes excessive repetitive neuronal firing (38, 40). An M-current impairment can well explain the enhanced corticospinal output produced by cortico-cortical activation evoked by transcranial stimulation in our patient. As M-current impairment specifically enhances pyramidal neuron bursting (41), a change in potassium currents can also explain why paired-pulse stimulation is not effective in suppressing later discharges in patients with ALS. This abnormality can also cause the hyperexcitability of peripheral axons reported in patients with ALS by Kanai and co-workers, who investigated multiple axonal excitability properties and suggested that the observed changes were consistent with an abnormality of the M-current associated with the KCNQ2 channel (42).

Interestingly, a recent study has shown that retigabine, a KCNQ channel activator approved as an antiepileptic drug, reduces both upper (43) and lower motor neuron excitability in human ALS (44). Moreover, it has been shown that the compound N-(6-Chloro-pyridin-3-yl)-3,4-difluorobenzamide, a highly selective Kv7.2/7.3 opener mainly used in epilepsy, prevents motor neuron degeneration in spinal cord organotypic cultures exposed to acute excitotoxicity and reduces disease progression in SOD1G93A ALS mice (45).

CSNs are the neurons showing an extremely high-bursting frequency (>600 Hz) (46). This characteristic might explain why CSNs are selectively involved in alterations further enhancing neuron bursting. High-frequency CSN bursting is evoked by single-pulse TMS (24), and this might be the reason why this technique is well suited to revealing motor

cortex excitability changes in ALS (2). On the other hand, the enhanced bursting of CSNs by overstimulating the lower motor neuron, made hyperexcitable by M-current impairment, can trigger excitotoxic damage of these cells in line with the dying forward hypothesis of ALS (47).

Preliminary studies have explored neuromodulation protocols suppressing CSN excitability as a possible tool to reduce excitotoxicity (48). The insights provided by this single case study suggest that protocols capable of suppressing CSN bursting might represent a therapeutic strategy worthy of further investigation in ALS.

Interestingly, the most promising preliminary results were observed in studies using the protocol of repetitive TMS termed continuous theta burst stimulation that selectively suppresses the monosynaptic excitatory inputs to CSNs (i.e., the I1 wave) (24), which were hyperexcitable in our patient, as well as the technique of static magnetic stimulation (49), which has been shown to reduce bursting of pyramidal neurons by enhancing chloride current (50).

Limitations of the Study

The main limitation of the present study is the generalizability of findings from a single patient, considering the heterogeneity of ALS. However, this is quite a unique observation, and the opportunities to study additional patients with ALS with these methods may be scarce.

Given the exploratory nature of this study in which we assessed the activation of different cortical sites with predetermined comparisons, we did not correct for multiple comparisons, therefore, results should be interpreted in this light. Nonetheless, this limitation is partially overcome by the fact that the observed changes are consistent with those reported by many noninvasive studies in humans, revealing specific features of the excitability changes that cannot be inferred by muscle response recording.

Another relevant limitation is that this is a single timepoint physiology in a disease known to vary in TMS metrics across disease stage (5, 51).

Finally, it should be considered that both the patient and the controls had chronic pain. Because noxious stimuli affect motor cortex excitability (52), we cannot exclude the possibility that the presence of pain influenced our measures of cortical excitability. However, this limitation is tempered by the fact that both the patient and controls had chronic pain, making the comparison still informative.

Conclusions

In this study, we had the rare opportunity to measure direct responses to TMS in a patient with ALS who had undergone an epidural electrode implantation for intractable pain. These recordings allowed us to disentangle the specific contributions of upper and lower motor neurons to evoked responses in ALS, and the selective activity of excitatory and inhibitory cell populations. Our findings demonstrated a reduced amplitude of the response to direct activation of CSNs, an enhanced excitability of CSNs in response to monosynaptic glutamatergic inputs from layer 5, and a relative enhancement in layer 2–3 cortico-cortical inputs to CSNs, which can be at least partly attributed to a reduction in the activity of GABAergic cells within this circuit. With the obvious limitations of a single case study, the present findings suggest an abnormal processing of excitatory and inhibitory synaptic inputs by CSNs in ALS that might trigger degeneration of these cells.

DATA AVAILABILITY

Data will be made available upon reasonable request.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

V.D.L., F.C., F.P., P.M., and F.R. conceived and designed research; V.D.L., G.M., F.C., F.P., A.I., and F.R. performed experiments; V.D.L., G.P., and F.R. analyzed data; V.D.L., G.P., D.T.C., and F.R. interpreted results of experiments; F.R. prepared figures; V.D.L. drafted manuscript; G.P., D.T.C., G.M., F.C., F.P., P.M., A.I., and F.R. edited and revised manuscript; V.D.L., G.P., D.T.C., G.M., F.C., F.P., P.M., A.I., and F.R. approved final version of manuscript.

REFERENCES

1. **Vucic S**, **Kiernan MC.** Transcranial magnetic stimulation for the assessment of neurodegenerative disease. *Neurotherapeutics* 14: 91–106, 2017. doi:10.1007/s13311-016-0487-6.
2. **Vucic S**, **Pavey N**, **Haidar M**, **Turner BJ**, **Kiernan MC.** Cortical hyperexcitability: diagnostic and pathogenic biomarker of ALS. *Neurosci Lett* 759: 136039, 2021. doi:10.1016/j.neulet.2021.136039.
3. **Vucic S**, **Nicholson GA**, **Kiernan MC.** Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. *Brain* 131: 1540–1550, 2008 [Erratum in *Brain* 131: 2234, 2008]. doi:10.1093/brain/awn071.
4. **Goutman SA**, **Hardiman O**, **Al-Chalabi A**, **Chió A**, **Savelieff MG**, **Kiernan MC**, **Feldman EL.** Emerging insights into the complex genetics and pathophysiology of amyotrophic lateral sclerosis. *Lancet Neurol* 21: 465–479, 2022. doi:10.1016/S1474-4422(21)00414-2.
5. **Ranieri F**, **Senerchia G**, **Bonan L**, **Casali S**, **Cabona C**, **Cantone M**; **et al.** Cortical excitability as a prognostic and phenotypic stratification biomarker in amyotrophic lateral sclerosis. *Ann Neurol* 98: 801–813, 2025. doi:10.1002/ana.27305.
6. **Rothstein JD**, **Van Kammen M**, **Levey AI**, **Martin LJ**, **Kuncl RW.** Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol* 38: 73–84, 1995. doi:10.1002/ana.410380114.
7. **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* 21: 625–635, 2011. doi:10.1093/cercor/bhq134.
8. **Dey S**, **Quintanilla C**, **Bitlis D**, **Gautam M**, **Ozdinler PH**, **Martina M.** Cell type-specific alterations in excitability and inhibition of upper motor neurons in AlsinKO mice, a model of juvenile onset ALS. *J Neurosci* 45: e2409242025, 2025. doi:10.1523/JNEUROSCI.2409-24.2025.
9. **Odierna GL**, **Vucic S**, **Dyer M**, **Dickson T**, **Woodhouse A**, **Blizzard C.** How do we get from hyperexcitability to excitotoxicity in amyotrophic lateral sclerosis? *Brain* 147: 1610–1621, 2024. doi:10.1093/brain/awae039.
10. **Khademullah CS**, **Aqrabawi AJ**, **Place KM**, **Dargaei Z**, **Liang X**, **Pressey JC**, **Bedard S**, **Yang JW**, **Garand D**, **Keramidis I**, **Gasecka**

A, **Côté D**, **De Koninck Y**, **Keith J**, **Zinman L**, **Robertson J**, **Kim JC**, **Woodin MA.** Cortical interneuron-mediated inhibition delays the onset of amyotrophic lateral sclerosis. *Brain* 143: 800–810, 2020. doi:10.1093/brain/awaa034.

11. **Lorenc F**, **Dupuis L**, **Cassel R.** Impairments of inhibitory neurons in amyotrophic lateral sclerosis and frontotemporal dementia. *Neurobiol Dis* 203: 106748, 2024. doi:10.1016/j.nbd.2024.106748.
12. **Traynor BJ.** The interneuron hypothesis of amyotrophic lateral sclerosis. *Brain* 148: 1045–1046, 2025. doi:10.1093/brain/awaf104.
13. **Soares-Neto R**, **Calixtro J**, **Ribeiro FC.** Early dysfunction of spinal cord interneurons in a mouse model of amyotrophic lateral sclerosis. *J Physiol* 601: 1057–1058, 2023. doi:10.1113/JP284365.
14. **Mills KR**, **Nithi KA.** Corticomotor threshold is reduced in early sporadic amyotrophic lateral sclerosis. *Muscle Nerve* 20: 1137–1141, 1997. doi:10.1002/(sici)1097-4598(199709)20:9<1137::aid-mus7>3.0.co;2-9.
15. **Eisen A**, **Weber M.** The motor cortex and amyotrophic lateral sclerosis. *Muscle Nerve* 24: 564–573, 2001. doi:10.1002/mus.1042.
16. **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* 113: 1688–1697, 2002. doi:10.1016/s1388-2457(02)00288-2.
17. **Vucic S**, **Kiernan MC.** Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain* 129: 2436–2446, 2006. doi:10.1093/brain/awl172.
18. **Menon P**, **Kiernan MC**, **Vucic S.** Cortical hyperexcitability precedes lower motor neuron dysfunction in ALS. *Clin Neurophysiol* 126: 803–809, 2015. doi:10.1016/j.clinph.2014.04.023.
19. **Timmins HC**, **Vucic S**, **Kiernan MC.** Cortical hyperexcitability in amyotrophic lateral sclerosis: from pathogenesis to diagnosis. *Curr Opin Neurol* 36: 353–359, 2023. doi:10.1097/WCO.0000000000001162.
20. **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* 49: 1292–1298, 1997. doi:10.1212/wnl.49.5.1292.
21. **Calma AD**, **Pavey N**, **Silva CS**, **van den Bos MAJ**, **Yiannikas C**, **Farrar MA**, **Kiernan MC**, **Menon P**, **Vucic S.** Diagnostic utility of threshold tracking TMS paradigms in early amyotrophic lateral sclerosis. *Clin Neurophysiol* 174: 105–113, 2025. doi:10.1016/j.clinph.2025.03.044.
22. **Krishnan D**, **Talbot D-L**, **Ashhurst JF**, **Park SB**, **Vucic S**, **Timmins HC**, **Kiernan MC.** Longitudinal assessment of cortical motor function in amyotrophic lateral sclerosis. *Sci Rep* 15: 16978, 2025. doi:10.1038/s41598-025-01570-6.
23. **Santos Silva C**, **Pavey N**, **Calma AD**, **Kiernan MC**, **Menon P**, **van den Bos M**, **Vucic S.** Utility of cortical inhibitory and facilitatory neuronal circuits in amyotrophic lateral sclerosis diagnosis. *Eur J Neurol* 32: e70203, 2025. doi:10.1111/ene.70203.
24. **Di Lazzaro V**, **Rothwell J**, **Capogna M.** Noninvasive stimulation of the human brain: activation of multiple cortical circuits. *Neuroscientist* 24: 246–260, 2018. doi:10.1177/1073858417717660.
25. **Yu GJ**, **Ranieri F**, **Di Lazzaro V**, **Sommer MA**, **Peterchev AV**, **Grill WM.** Circuits and mechanisms for TMS-induced corticospinal waves: connecting sensitivity analysis to the network graph. *PLoS Comput Biol* 20: e1012640, 2024. doi:10.1371/journal.pcbi.1012640.
26. **Di Lazzaro V**, **Oliviero A**, **Meglio M**, **Cioni B**, **Tamburrini G**, **Tonali P**, **Rothwell JC.** Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. *Clin Neurophysiol* 111: 794–799, 2000. doi:10.1016/s1388-2457(99)00314-4.
27. **Di Lazzaro V**, **Mazzone P**, **Insola A**, **Florio L**, **Capone F**, **Ranieri F.** Spinal cord stimulation for pain treatment failed to modify disease progression in a patient with amyotrophic lateral sclerosis. *Clin Neurol Neurosurg* 163: 108–109, 2017. doi:10.1016/j.clineuro.2017.10.010.
28. **Brooks BR**, **Miller RG**, **Swash M**, **Munsat TL**; **World Federation of Neurology Research Group on Motor Neuron Diseases.** El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 1: 293–299, 2000. doi:10.1080/146608200300079536.
29. **Groppa S**, **Oliviero A**, **Eisen A**, **Quartarone A**, **Cohen LG**, **Mall V**, **Kaelin-Lang A**, **Mima T**, **Rossi S**, **Thickbroom GW**, **Rossini PM**, **Ziemann U**, **Valls-Solé J**, **Siebner HR.** A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol* 123: 858–882, 2012. doi:10.1016/j.clinph.2012.01.010.
30. **Maier MA**, **Kirkwood PA**, **Brochier T**, **Lemon RN.** Responses of single corticospinal neurons to intracortical stimulation of primary motor and premotor cortex in the anesthetized macaque monkey. *J Neurophysiol* 109: 2982–2998, 2013. doi:10.1152/jn.01080.2012.
31. **Kujirai T**, **Caramia MD**, **Rothwell JC**, **Day BL**, **Thompson PD**, **Ferbert A**, **Wroe S**, **Asselman P**, **Marsden CD.** Corticocortical inhibition in human motor cortex. *J Physiol* 471: 501–519, 1993. doi:10.1113/jphysiol.1993.sp019912.
32. **Di Lazzaro V**, **Restuccia D**, **Oliviero A**, **Profice P**, **Ferrara L**, **Insola A**, **Mazzone P**, **Tonali P**, **Rothwell JC.** Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. *Exp Brain Res* 119: 265–268, 1998. doi:10.1007/s002210050341.
33. **Crawford JR**, **Howell DC.** Comparing an individual's test score against norms derived from small samples. *Clin Neuropsychol* 12: 482–486, 1998. doi:10.1076/clin.12.4.482.7241.
34. **Pavey N**, **Hannaford A**, **van den Bos M**, **Kiernan MC**, **Menon P**, **Vucic S.** Distinct neuronal circuits mediate cortical hyperexcitability in amyotrophic lateral sclerosis. *Brain* 147: 2344–2356, 2024. doi:10.1093/brain/awae049.
35. **Vucic S**, **Lin CS-Y**, **Cheah BC**, **Murray J**, **Menon P**, **Krishnan AV**, **Kiernan MC.** Riluzole exerts central and peripheral modulating effects in amyotrophic lateral sclerosis. *Brain* 136: 1361–1370, 2013. doi:10.1093/brain/awt085.
36. **Geevasinga N**, **Menon P**, **Ng K**, **Van Den Bos M**, **Byth K**, **Kiernan MC**, **Vucic S.** Riluzole exerts transient modulating effects on cortical and axonal hyperexcitability in ALS. *Amyotroph Lateral Scler Frontotemporal Degener* 17: 580–588, 2016. doi:10.1080/21678421.2016.1188961.
37. **van den Bos MAJ**, **Menon P**, **Pavey N**, **Higashihara M**, **Kiernan MC**, **Vucic S.** Direct interrogation of cortical interneuron circuits in amyotrophic lateral sclerosis. *Brain* 148: 1169–1179, 2025. doi:10.1093/brain/awae317.
38. **Guo C**, **Chen K**, **Vatsavayai SC**, **Akiyama T**, **Zeng Y**, **Liu C**, **Sianto O**, **Yang E**, **Bombosch J**, **Powell R**, **Zhen S**, **Mekhoubad S**, **Morrie RD**, **Miller G**, **Green EM**, **Petrucelli L**, **Seeley WW**, **Gitler AD.** Cryptic splicing in synaptic and membrane excitability genes links TDP-43 loss to neuronal dysfunction. *Sci Transl Med* 18: eaeb8517, 2026. doi:10.1126/scitranslmed.aeb8517.
39. **Joseph BJ**, **Marshall KA**, **Harley P**, **Mann JR**, **Alessandrini F**, **Vanoye CG**, **et al.** TDP-43-dependent mis-splicing of KCNQ2 triggers intrinsic neuronal hyperexcitability in ALS/FTD. *Nat Neurosci* 28: 2476–2492, 2025. doi:10.1038/s41593-025-02096-w.
40. **Hinojo-Perez A**, **Eldstrom J**, **Dou Y**, **Marinho-Alcara A**, **Edmond MA**, **de la Cruz A**, **Perez Rodriguez ME**, **Diaz-Solares M**, **Dykxhoorn DM**, **Fedida D**, **Barro-Soria R.** The conductance of KCNQ2 and its pathogenic variants is determined by individual subunit gating. *Sci Adv* 11: eadr7012, 2025. doi:10.1126/sciadv.adr7012.
41. **Simkin D**, **Marshall KA**, **Vanoye CG**, **Desai RR**, **Bustos BI**, **Piyevsky BN**, **Ortega JA**, **Forrest M**, **Robertson GL**, **Penzes P**, **Laux LC**, **Lubbe SJ**, **Millichap JJ**, **George AL**, **Kiskinis E.** Dyshomeostatic modulation of Ca^{2+}-activated K^{+} channels in a human neuronal model of KCNQ2 encephalopathy. *eLife* 10: e64434, 2021. doi:10.7554/eLife.64434.
42. **Kanai K**, **Kuwabara S**, **Misawa S**, **Tamura N**, **Ogawara K**, **Nakata M**, **Sawai S**, **Hattori T**, **Bostock H.** Altered axonal excitability properties in amyotrophic lateral sclerosis: impaired potassium channel function related to disease stage. *Brain* 129: 953–962, 2006. doi:10.1093/brain/awl024.
43. **Wainger BJ**, **Macklin EA**, **Vucic S**, **McIlduff CE**, **Paganoni S**, **Maragakis NJ**, **et al.** Effect of ezogabine on cortical and spinal motor neuron excitability in amyotrophic lateral sclerosis: a randomized clinical trial. *JAMA Neurol* 78: 186–196, 2021. doi:10.1001/jamaneurol.2020.4300.
44. **Kovalchuk MO**, **Heuberger JAAC**, **Sleutjes BTHM**, **Ziagkos D**, **van den Berg LH**, **Ferguson TA**, **Franssen H**, **Groeneveld GJ.** Acute effects of riluzole and retigabine on axonal excitability in patients with amyotrophic lateral sclerosis: a randomized, double-blind, placebo-controlled, crossover trial. *Clin Pharmacol Ther* 104: 1136–1145, 2018. doi:10.1002/cpt.1096.
45. **Masegosa VM**, **Navarro X**, **Herrando-Grabulosa M.** ICA-27243 improves neuromuscular function and preserves motoneurons in

the transgenic SOD1G93A mice. *Neurotherapeutics* 21: e00319, 2024. doi:10.1016/j.neurot.2024.e00319.

46. **Adrian ED**, **Moruzzi G.** Impulses in the pyramidal tract. *J Physiol* 97: 153–199, 1939. doi:10.1113/jphysiol.1939.sp003798.
47. **Haidar M**, **Viden A**, **Daniel C**, **Cuic B**, **Wang T**, **Rosier M**, **Tomas D**, **Mills SA**, **Govier-Cole A**, **Djouma E**, **Perera ND**, **Luikinga S**, **Rytova V**, **Barton SK**, **Gonsalvez DG**, **Palmer LM**, **McLean C**, **Kiernan MC**, **Vucic S**, **Turner BJ.** Cortical hyperexcitability drives dying forward amyotrophic lateral sclerosis symptoms and pathology in mice. *Prog Neurobiol* 252: 102809, 2025 [Erratum in *Prog Neurobiol* 260: 102910, 2026]. doi:10.1016/j.pneurobio.2025.102809.
48. **Di Lazzaro V**, **Ranieri F**, **Bączyk M**, **de Carvalho M**, **Dileone M**, **Dubbioso R**, **Fernandes S**, **Kozak G**, **Motolese F**, **Ziemann U.** Novel approaches to motoneuron disease/ALS treatment using non-invasive brain and spinal stimulation: IFCN handbook chapter. *Clin Neurophysiol* 158: 114–136, 2024. doi:10.1016/j.clinph.2023.12.012.
49. **Di Lazzaro V**, **Ranieri F**, **Doretti A**, **Boscarino M**, **Maderna L**, **Colombo E**, **Soranna D**, **Zambon A**, **Ticozzi N**, **Musumeci G**, **Capone F**, **Silani V.** Transcranial static magnetic stimulation for amyotrophic lateral sclerosis: a bicentric, randomised, double-blind placebo-controlled phase 2 trial. *Lancet Reg Health Eur* 45: 101019, 2024. doi:10.1016/j.lanepe.2024.101019.
50. **Sinha AS**, **Shibata S**, **Takamatsu Y**, **Akita T**, **Fukuda A**, **Mima T.** Static magnetic field stimulation enhances shunting inhibition via a SLC26 family Cl^- channel, inducing intrinsic plasticity. *J Neurosci* 44: e1324222024, 2024. doi:10.1523/JNEUROSCI.1324-22.2024.
51. **De Carvalho M**, **Swash M.** Transcranial magnetic stimulation to monitor disease progression in ALS: a review. *Amyotroph Lateral Scler Frontotemporal Degener* 24: 362–368, 2023. doi:10.1080/21678421.2022.2160649.
52. **Valeriani M**, **Restuccia D**, **Di Lazzaro V**, **Oliviero A**, **Profice P**, **Le Pera D**, **Saturno E**, **Tonali P.** Inhibition of the human primary motor area by painful heat stimulation of the skin. *Clin Neurophysiol* 110: 1475–1480, 1999. doi:10.1016/S1388-2457(99)00075-9.