

**INVITED REVIEW**

# Is there hope that transspinal direct current stimulation corrects motoneuron excitability and provides neuroprotection in amyotrophic lateral sclerosis?

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**Abstract**

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of largely unknown pathophysiology, characterized by the progressive loss of motoneurons (MNs). We review data showing that in presymptomatic ALS mice, MNs display reduced intrinsic excitability and impaired level of excitatory inputs. The loss of repetitive firing specifically affects the large MNs innervating fast contracting muscle fibers, which are the most vulnerable MNs in ALS. Interventions that aimed at restoring either the intrinsic excitability or the synaptic excitation result in a decrease of disease markers in MNs and delayed neuromuscular junction denervation. We then focus on trans-spinal direct current stimulation (tsDCS), a noninvasive tool, since it modulates the activity of spinal neurons and networks. Effects of tsDCS depend on the polarity of applied current. Recent work shows that anodal tsDCS induces long-lasting enhancement of MN excitability and synaptic excitation of spinal MNs. Moreover, we show preliminary results indicating that anodal tsDCS enhances the excitatory synaptic inputs to MNs in ALS mice. In conclusion, we suggest that chronic application of anodal tsDCS might be useful as a complementary method in the management of ALS patients.

**KEY WORDS**

excitability and excitation, intracellular recordings, neurodegenerative disease, spinal motoneurons

## 1 | INTRODUCTION

Recent data demonstrate that the excitability changes of spinal motoneurons (MNs) in amyotrophic lateral sclerosis (ALS, a prominent neurodegenerative disease of MNs) depend on the physiological type of motor unit and evolve with disease progression. Most interestingly, interventions with pharmacological or chemogenetic tools that aim at correcting the firing of the most vulnerable MNs prove to

have some beneficial impact on the disease. After reviewing these data, we focus on trans-spinal direct current stimulation (tsDCS) as a potential alternative therapeutic method in ALS. Indeed, electrical polarization by direct current is well-known to modify spinal networks. We review recent work suggesting that tsDCS could be used to compensate for the changes in intrinsic excitability of MNs, or synaptic excitation, and hopefully to deliver some neuroprotection in ALS.

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## 1.1 | Changes of electrical properties of MNs in ALS

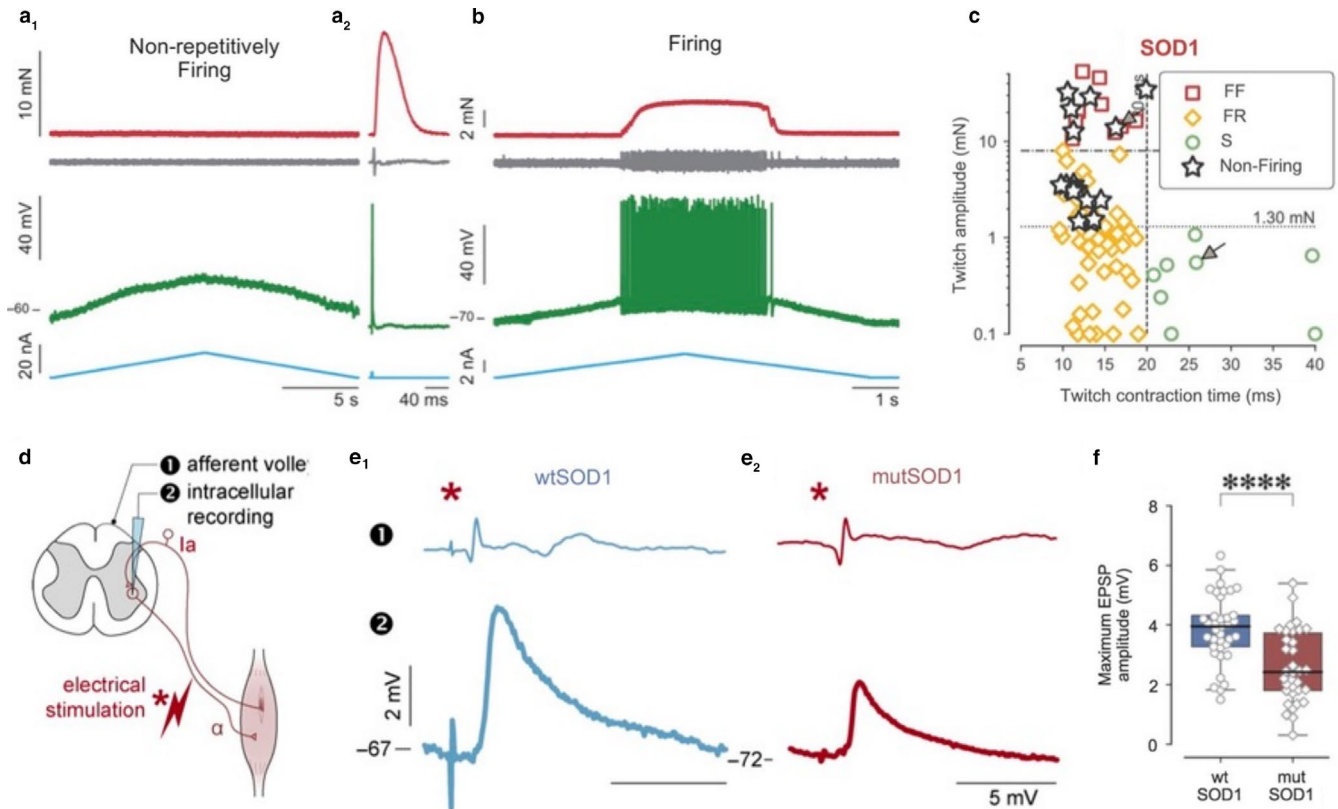
In ALS, some motor pools are more vulnerable than others (Kanning et al., 2010), but even within a given motor pool, the order of MN degeneration depends on MN type: fast contracting—fatigable (FF) motor units degenerate first, followed by fast contracting—fatigue-resistant units (FR), whereas slow contracting motor units (S) are resistant to degeneration (Hegedus et al., 2008; Pun et al., 2006). Despite more than 20 years of intense research, the pathophysiological mechanisms that lead to MN degeneration in ALS are still largely unknown. Among many others, the glutamate excitotoxic hypothesis has been proposed (Ilieva et al., 2009; Van Den Bosch et al., 2006), which relies on the assumption that excessive excitatory glutamatergic input may lead to an overload of cytosolic calcium, through calcium permeable- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) channels and voltage-dependent calcium channels activated by action potentials, which, in turn, triggers apoptosis.

One argument that supports the glutamate excitotoxic hypothesis is the fact that Riluzole (which was for a long time the only FDA-approved treatment for ALS) prolongs survival in patients with ALS, albeit modestly (a few months, Bensimon et al., 1994; Miller et al., 2003). Indeed, Riluzole has multiple actions, among those a decrease of glutamatergic transmission and intrinsic excitability—notably by blocking the persistent inward sodium current (Bellingham, 2013; Kuo et al., 2006; Lamanauskas & Nistri, 2008). However, it was recently shown that the survival benefit of Riluzole is achieved by extending the fourth stage of the disease, in which the motor functions are largely impaired and which immediately precedes death (Fang et al., 2018). Moreover, all other pharmacological interventions targeting the reduction of glutamate release or excitability have never worked in humans (Wobst et al., 2020). In particular, phase III clinical trials with Talampanel, an AMPA receptor antagonist, Memantine, an NMDA receptor antagonist, or Mexiletine, a sodium channel blocker, have not been conclusive (De Carvalho et al., 2010; Pascuzzi et al., 2010; Weiss et al., 2016). These results challenge the glutamatergic excitotoxicity hypothesis. However, intraspinal infusion of large doses of AMPA can induce excitotoxic MN degeneration *in vivo*, even in wild type animals (Netzahualcoyotzi & Tapia, 2015) but there is no evidence that such an intervention reproduces the action of synaptic glutamate release in ALS. Indeed, in ALS mouse models, AMPA receptor antagonists (Akamatsu et al., 2016; Van Damme et al., 2003) and NMDA receptor antagonists (Joo et al., 2007), delivered from symptom onset until death, elicit modest improvement of motor function and the survival time. This may not be so surprising, since the most vulnerable MNs have already degenerated at symptoms

onset (Hegedus et al., 2008; Pun et al., 2006). Saxena et al. (2013) started the administration of the drugs as early as P20 in the SOD1 G93A mice and under these conditions, AMPA (a) ameliorated cellular markers of the disease in MNs (less misfolded SOD1 proteins, reduced unfolded protein response and stress of the endoplasmic reticulum), (b) delayed the denervation of the less vulnerable motor units, (c) improved the force of contraction, and (d) extended the survival by 20–35 days while AMPA antagonists had opposite effects. These results are in contrast with previous studies and contradict the glutamate excitotoxicity theory in ALS and suggest that the time of drug application, the cumulative dose, and the peak CNS concentration may somehow account for these conflicting results.

Indeed, in ALS mouse models, time-dependent alterations of intrinsic MN excitability that start long before degeneration onset have been shown in both vulnerable and resistant MNs. In these studies it was found that MNs are hyperexcitable at embryonic stages in the SOD1<sup>G93A</sup> mice (input resistance is increased, rheobase is decreased and slope of the frequency–current relationship is increased; Martin et al., 2013; Pieri et al., 2003). When MNs were examined shortly after birth (P4–P10), contradictory results have been reported: in some works MN are hyperexcitable (van Zundert et al., 2008), in others they are hypoexcitable (Bories et al., 2007), or they do not display any change in excitability (Pambo-Pambo et al., 2009) suggesting an efficient excitability homeostasis (Quinlan et al., 2011). However none of these studies have sorted MNs according to their physiological type, which might explain the reported discrepancies. More recently, Leroy et al. (2014) classified spinal MNs according to their discharge pattern, their anatomy and the expression of specific molecular markers within F- and S-types in P6–P10 mice and found that only S-type MNs (the less vulnerable ones) are hyperexcitable (lower rheobase) at this age in the SOD1<sup>G93A</sup> mice while F-type MNs (the most vulnerable ones) display a normal excitability. A similar conclusion was reached by Venugopal et al. (2015) in trigeminal MNs.

However, the excitability pattern evolves during animal maturation. *In vivo* intracellular recordings in anesthetized mice allowed investigations in adults just prior to the degeneration of neuromuscular junctions of the most vulnerable motor units (P50–60 in SOD1<sup>G93A</sup> mice). In these conditions, Delestrée et al. (2014) showed that a fraction of MNs lose their ability to fire repetitively in response to a slow ramp of current despite being functionally connected to their muscle fibers (Figure 1a). This loss of firing is interpreted as a manifestation of hypoexcitability. The ability to type-identify motor units *in vivo* allowed Martínez-Silva et al. (2018) to demonstrate that the loss of repetitive firing in SOD1<sup>G93A</sup> mice (and also in an unrelated ALS model, FUS<sup>P525L</sup>; Sharma et al., 2016) occurred among the population of the most vulnerable MNs (innervating FF and largest FR motor units), while



**FIGURE 1** Intrinsic excitability and synaptic excitation are depressed in the  $SOD1^{G93A}$  mice. (a) MN that fails to display a repetitive discharge in response to a slow triangular ramp of current ( $A_1$ ) but that is still able to elicit a single spike in response to a short transient pulse ( $A_2$ ). The blue trace is the injected current, the green trace is the intracellular recording, the gray trace is the EMG recorded in the triceps surae, the red trace is the force recorded at the tendon of the triceps surae. (b) MN displaying a repetitive firing in response to a slow triangular ramp (same arrangement as in (a)). (c) Nonfiring MNs are found among the largest motor units (FF and FR with a twitch force larger than 1.3 mN). MNs indicated by an arrow correspond to the two examples in (a) and (b). Adapted from Martínez-Silva et al. (2018). (d) Experimental arrangement for testing the size of the maximal Ia Excitatory Post-Synaptic Potentials (EPSP). (e) Typical recordings in a wtSOD1 MN ( $E_1$ ) and a  $SOD1^{G93A}$  MN ( $E_2$ ). Lower traces are the intracellular recordings. Upper traces are the cord dorsum potentials showing the group I afferent volleys. (f) The maximal Ia EPSPs are significantly reduced in the MNs from  $SOD1^{G93A}$  mice (whereas resting potentials, input conductances and membrane time constants are unchanged). Adapted from Bączyk, Alami et al. (2020)

the most resistant MNs (smallest FR and S-type motor units) display normal excitability (Figure 1c). Importantly, disease markers (p-eIF2 $\alpha$  and p62 aggregates) confirm that nonrepetitively firing MNs are in a more advanced stage of the disease than those that can still discharge normally (Martínez-Silva et al., 2018). It should be noted that the Meehan group has not reported such hypoexcitability either in the  $SOD1^{G127X}$  mice (Meehan et al., 2010) or in the  $SOD1^{G93A}$  mice (Jensen et al., 2020). This discrepancy may be due to the fact that they used older mice, in which the most vulnerable MNs have already started to degenerate, than in the previous studies (Delestrée, 2014; Martínez-Silva et al., 2018), as well as suboptimal discontinuous current-clamp switching rate, which may distort the firing of those cells (Manuel, 2020).

Remarkably, recordings in induced pluripotent stem cell (iPSC)-derived MNs from human patients have consistently reported the same time-dependent excitability changes as in ALS mice. After an initial hyperexcitability (Devlin et al.,

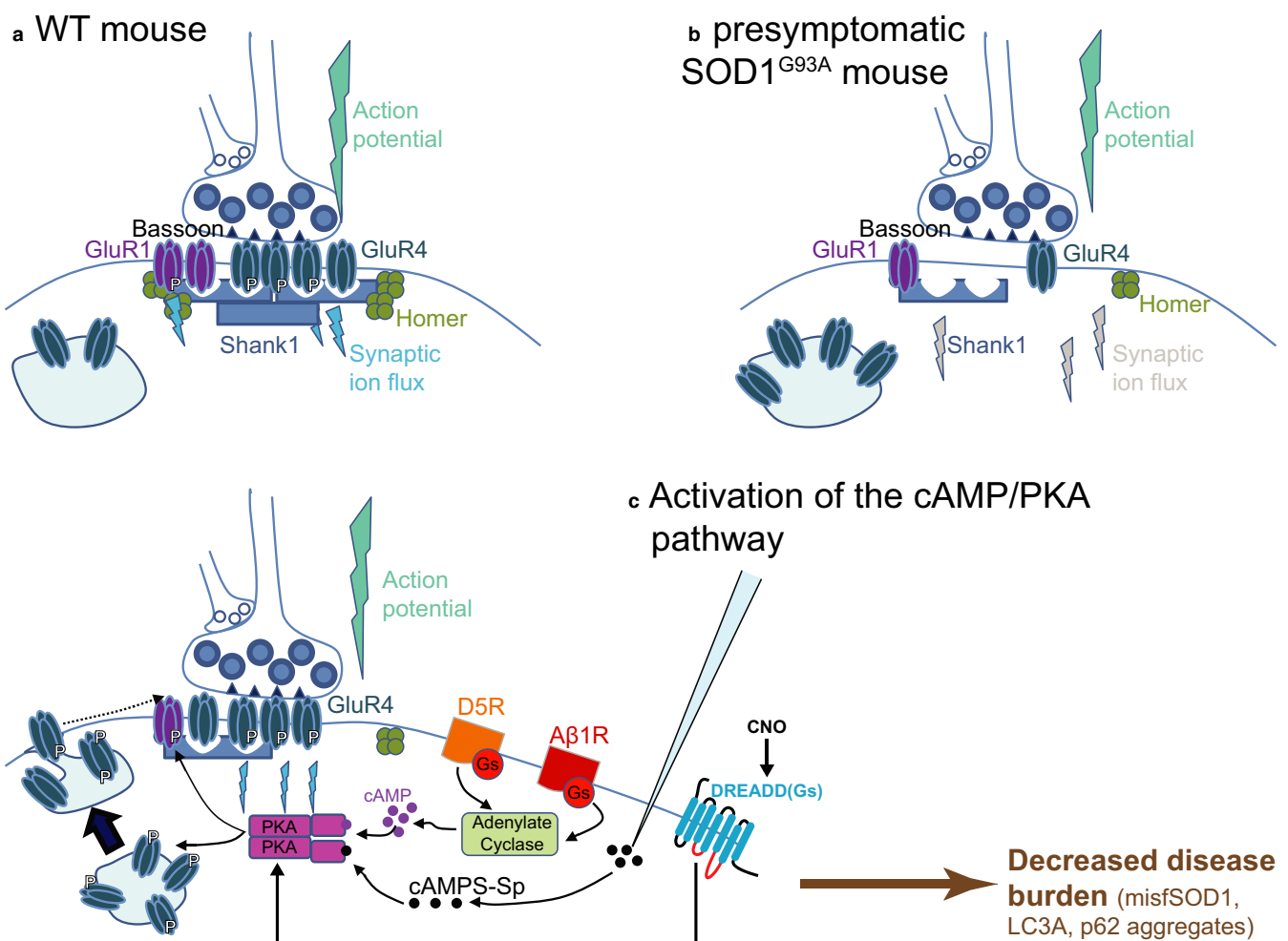
2015; Wainger et al., 2014) the cells turn into a hypoexcitable state as the cells mature (Devlin et al., 2015; Naujock et al., 2016; Sareen et al., 2013). A recent study found that the loss of repetitive discharge in iPSC-derived MNs occurs only in presence of mutant astrocytes, indicating that these processes involve nonneuronal cell-types (Zhao et al., 2020).

A major criticism that one may raise about in vivo pharmacological interventions is that such interventions act not only on MNs but also on all other neurons, including many classes of excitatory and inhibitory interneurons that provide inputs to MNs. Since MNs receive similar numbers of inhibitory and excitatory synapses (Bae et al., 1999), and since MNs were reported to be driven by balanced excitatory and inhibitory synaptic activity (Berg et al., 2007), the net effect of any drug on MN synaptic inputs is unpredictable and depends on the individual sensitivity of excitatory and inhibitory interneurons to the drug as well as to the synaptic composition and balance of each MN subpopulation.

Genetic interventions may also have an impact on both excitation and inhibition. For instance, Lalancette-Hebert et al. (2016) demonstrated, in an ALS mouse model, that a genetic ablation of gamma MNs, which reduced the spindle activation, increases the mouse survival time. However, while Ia proprioceptive spindle afferents excite MNs they also very efficiently excite inhibitory interneurons which act on MNs; in particular interneurons which mediate the so-called Ia reciprocal inhibition (Baldissera et al. 1981). The net effect on MNs (less excitation or less inhibition) of genetic ablation of gamma MNs is therefore unclear, and prevents a consistent conclusion for the mechanism responsible for the extended survival. To investigate the mechanism at work, interventions

must selectively target alpha MNs, or excitatory or inhibitory synapses acting onto them.

In this line, Saxena et al. (2013) performed *in vivo* chemo-genetic manipulations, using viral vectors specifically targeting lumbar MNs in adult presymptomatic double transgenic SOD1<sup>G93A</sup>/ChAT-cre mice. The virus expressed in MNs, the pharmacologically selective actuator module either coupled to 5HT3-transmembrane domain for neuronal depolarization (to enhance the excitability), or to glycine-receptor transmembrane domain for neuronal hyperpolarization (to decrease the excitability) (Magnus et al. 2011). Enhancing MN excitability reduced the amount of misfolded SOD1 protein, the endoplasmic reticulum stress in the FF-type MNs, and delayed the



**FIGURE 2** Excitatory synapses onto MNs are impaired in the SOD1<sup>G93A</sup> mice and are restored through activation of the cAMP/PKA signaling pathway. (a) Drawing illustrating a normal excitatory synapse in a WT mouse. (b) In the presymptomatic SOD1<sup>G93A</sup> mice (~ 50 days old), the GLUR subunits of the AMPA receptors and the scaffold proteins (Shank1, Homer) are less expressed in the postsynaptic side of excitatory synapses. At the same time, the presynaptic element does not seem affected. The postsynaptic disruption is responsible for a significant decrease of the EPSP amplitude (Bączyk, Alami et al., 2020). (c) Activation of the cAMP/PKA pathway, either through intracellular iontophoretic ejection of Sp-AMP (a cAMP agonist) or through CNO-activation of a DREADD G(s) specifically inserted in MN using an AAV9 vector, partially restores the synaptic impairment, entailing a firing increase and a burden decrease of disease markers such as misfSOD1, LC3A and p62 aggregates (Bączyk, Alami et al., 2020). The authors would like to thank Prof. Francesco Roselli who has drawn a preliminary draft of this figure and Dr. Marin Manuel who has prepared the final version



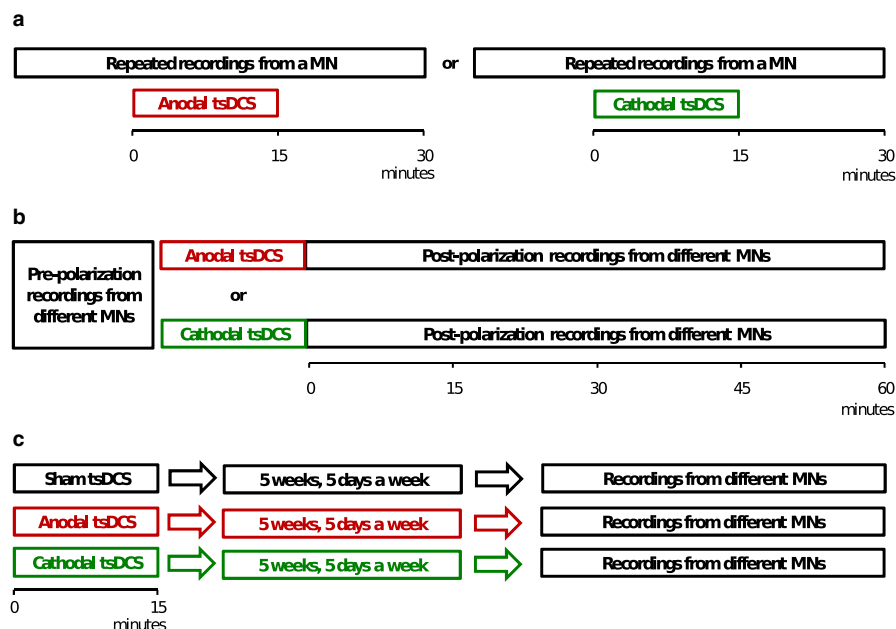
denervation of the neuromuscular junctions in the corresponding MUs. Conversely, reducing MN excitability had opposite effects (Saxena et al., 2013). This seminal work demonstrated a causal link between changes in MN intrinsic excitability and vulnerability. Furthermore, recent experiments targeting excitatory synapses on MNs of ALS mice (Bączyk, Alami et al., 2020) showed that in presymptomatic SOD1<sup>G93A</sup> mice, monosynaptic Excitatory Post-Synaptic Potentials (EPSPs) (either from Ia spindle afferents or from descending systems) are functionally depressed in spinal MN (EPSPs are about 30% smaller, Figure 1d–f). This depression is caused by a molecular disruption of the postsynaptic cell (reduced amount of GluR subunits and scaffolded proteins, Figure 2; Bączyk, Alami et al., 2020). Bączyk, Alami et al. (2020) also demonstrated that the synaptic impairment can be rescued using a viral vector to express a DREADD(Gs) specifically into MNs of the double transgenic SOD1<sup>G93A</sup>/ChAT-cre mice. Pharmacologic activation of DREADD(Gs) activated the cAMP/PKA signaling pathway eliciting membrane insertion of GluR4 subunits and restoration of excitatory synapses. This elicits an improvement of disease markers (misfolded SOD1 proteins, LC3A autophagic structures and P62 inclusions) through the enhancement of neuronal firing (Figure 2c; Bączyk, Alami et al., 2020). This work demonstrated that reduced excitation of MN at a presymptomatic stage contributes to the pathogenesis in ALS. On the contrary, increased excitation provides some neuroprotection of MNs.

Altogether, recent experiments, specifically targeting MNs, show that restoring MN intrinsic excitability or synaptic excitation has a beneficial impact on the disease pathobiochemistry. Gene therapy might then be envisioned to produce such a

restoration. However, human gene therapy still requires a lot of development in order to (a) massively and specifically target MN, (b) control the cellular and immune responses, and (c) avoid side effects such as neoplastic tumors. There is evidently a real interest to develop noninvasive methods that could restore intrinsic excitability or synaptic excitation of MNs. In this framework, we will present new data showing that tsDCS may actually induce long-lasting restoration of MN excitability and synaptic inputs, with the hope that tsDCS has the potential to deliver some neuroprotection in ALS.

## 1.2 | Translational implications of direct current stimulation

The idea that electrical fields can influence the activity of spinal networks was introduced quite early. In the classical experiments it was already clear that the membrane of neurons is traversed by ionic currents that are responsible not only for action potentials (Hodgkin & Huxley, 1952), but also for excitatory and inhibitory postsynaptic potentials (Coombs et al., 1957). Spinal polarization was soon shown to modify the effectiveness of synaptic activation (Eccles et al., 1962) indicating that direct current stimulation can alter membrane ionic currents. Externally applied electrical currents gained further recognition as a neuromodulatory technique at the turn of the new century with the introduction of trans-cranial direct current stimulation (tDCS) (Nitsche & Paulus, 2000). In this technique, direct current applied by electrodes located on the scalp modifies the activity of both cortical (Nitsche & Paulus, 2000)



**FIGURE 3** Schematic diagram of the experimental designs to investigate short-term (a), persistent, long-lasting (b) effects of acute tsDCS, and adaptive changes in response to chronic tsDCS application (c) in rat MNs

**TABLE 1** Summary of relative changes of membrane properties of MNs in response to acute or chronic application of tsDCS in rats

	1	2	3	4	5
	During polarization	15-min postpolarization period	30-min postpolarization period	60-min postpolarization period	5-week chronic polarization
<b>Anodal polarization</b>					
RMP (mV)	↑ 10%	–	–	–	–
RIN (MΩ)	–	–	–	–	↑ 27%
Rheo (nA)	↓ 23%	↓ 38%	↓ 31%	–	–
VT (mV)	–	↓ 15%	↓ 13%	–	↓ 8%
<b>Cathodal polarization</b>					
RMP (mV)	↓ 12%	–	–	–	–
RIN (MΩ)	–	–	–	–	–
Rheo (nA)	–	↓ 28%	–	–	–
VT (mV)	↓ 20%	–	–	–	–

Columns 1 and 2 indicate short-term effects (see Figure 3a), based on data recorded from single MNs during, and 15 min after tsDCS (0.1 mA) application, compared to control recordings before the onset of polarization (averaged across MNs in anodal [ $N = 10$ ] or cathodal [ $N = 10$ ] polarization groups, Bączyk et al., 2019); columns 3 and 4 present long-lasting effects (see Figure 3b), based on data averaged across separate groups of neurons, recorded during the first 30 min ( $N = 22$  for anodal tsDCS,  $N = 21$ , for cathodal tsDCS), and between 30 and 60 min ( $N = 21$  for anodal tsDCS,  $N = 22$ , for cathodal tsDCS) after the offset of tsDCS (0.1 mA), respectively, compared to the prepolarization group ( $N = 36$ ) from which records were made prior to the onset of tsDCS (Bączyk et al., 2020a); column 5 shows chronic effects (see Figure 3c), based on data averaged for MNs recorded after repeated transcutaneous application of anodal ( $N = 39$ ) or cathodal ( $N = 43$ ) tsDCS (0.5 mA, 15 min daily, for 5 weeks), compared to the sham control group ( $N = 41$ ; Bączyk et al., 2020b). Statistically significant changes of respective parameters (an increase or a decrease) are expressed in percentages in regard to prepolarization or sham control values at  $p < 0.05$ . Columns 1 and 2, RM ANOVA with a post hoc Tukey's test (for data with normal distribution and equal variance) or the Friedman tests with post hoc analysis of data with paired Kruskal–Wallis or Student's  $t$ -test (for repeated nonparametric comparisons). Columns 3 and 4, two-way ANOVA with a post hoc Tukey's test. Column 5, one-way ANOVA with a post hoc Tukey's test. RMP, resting membrane potential;  $R_{IN}$ , input resistance; Rheo, rheobase current; VT, voltage threshold for spike generation

and subcortical (Bączyk & Jankowska, 2014; Bolzoni et al., 2013) regions. Therapeutic benefits of tDCS have recently been reported in motor rehabilitation (Bai et al., 2019), pain management (Ramger et al., 2019) and even psychiatric disorders (Kuo et al., 2017).

tsDCS was introduced by Cogiamanian et al. (2008) to induce long-lasting alterations of the conduction velocity in the human lemniscal pathway. Excitability alteration of neuronal tracts by tsDCS was then shown to reduce nociception (Cogiamanian et al., 2011; Truini et al., 2011) or to modify H-reflex (Lamy et al., 2012; Winkler et al., 2010). Subsequently tsDCS was used for a variety of treatments including to ameliorate idiopathic restless leg symptoms (Heide et al., 2014), modulate cortico-spinal excitability (Bocci et al., 2015; Knikou et al., 2015;

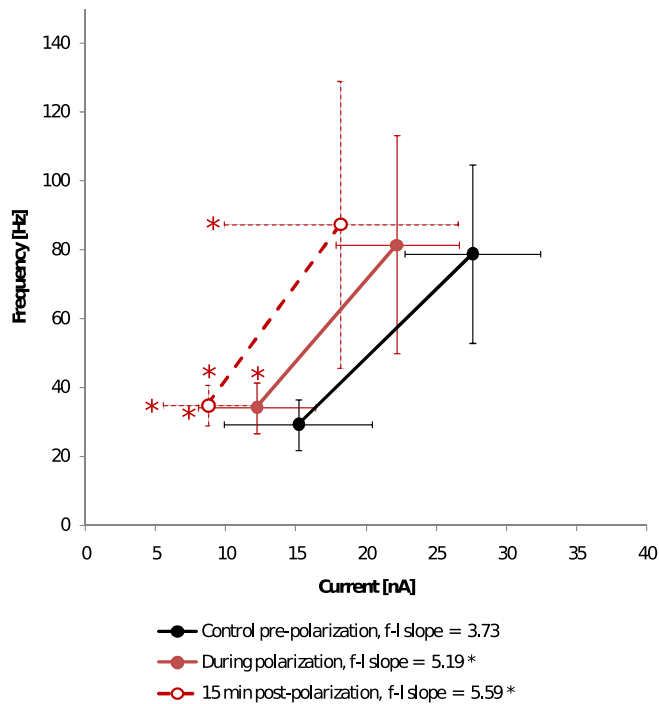
Murray et al., 2018), improve motor unit recruitment (Bocci et al., 2014), aid motor rehabilitation (Hubli et al., 2013), reduce spasticity (Ardolino et al., 2018; Paget-Blanc et al., 2019), and reduce pain (Berra et al., 2019; Choi et al., 2019).

### 1.3 | Effects of TSDCS on spinal neuronal networks

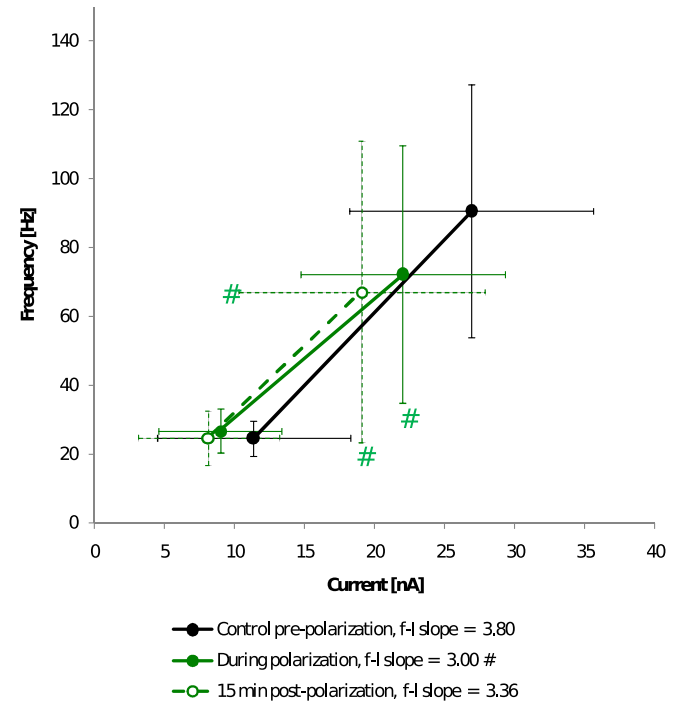
Despite a large number of translational studies, our basic knowledge of how tsDCS affects neuronal networks remains limited. It is already clear that tsDCS affects not only nerve fibers and spinal tract excitability, but it can also modulate intraspinal connectivity (Lenoir et al., 2018). Extensive

**FIGURE 4** Summary of the changes in the frequency–current ( $f$ – $I$ ) relationship during rhythmic steady-state firing (SSF) for MNs subjected to various polarization protocols. The linear relationship between the discharge frequency and injected current was assessed for each MN on the equation  $y = ax + b$ , where  $a$  determines the slope of the relationship in the primary range. Short-term effects of anodal (a) and cathodal (b) polarization as in Figure 3a and Table 1 (columns 1 and 2) (Bączyk et al., 2019). (c) Long-lasting effects of polarization as in Figure 3b and Table 1 (columns 3 and 4) (Bączyk et al., 2020a). (d) Chronic effects of polarization as in Figure 3c and Table 1 (column 5) (Bączyk et al., 2020b). Filled and open circles represent the average values for each group, while horizontal and vertical whiskers represent the SD values. “\*” indicates significant effect of anodal polarization regarding the minimum and the maximum SSF current, the minimum and the maximum SSF frequency, and the  $f$ – $I$  slope, at  $p < 0.05$ . “#” indicates significant effect of cathodal polarization for respective parameters, at  $p < 0.05$ . (a) and (b) RM ANOVA with a post hoc Tukey's test (for data with normal distribution and equal variance) or the Friedman tests with post hoc analysis of data with paired Kruskal–Wallis or Student's  $t$ -test (for repeated nonparametric comparisons). (c) Two-way ANOVA with a post hoc Tukey's test. (d) One-way ANOVA with a post hoc Tukey's test

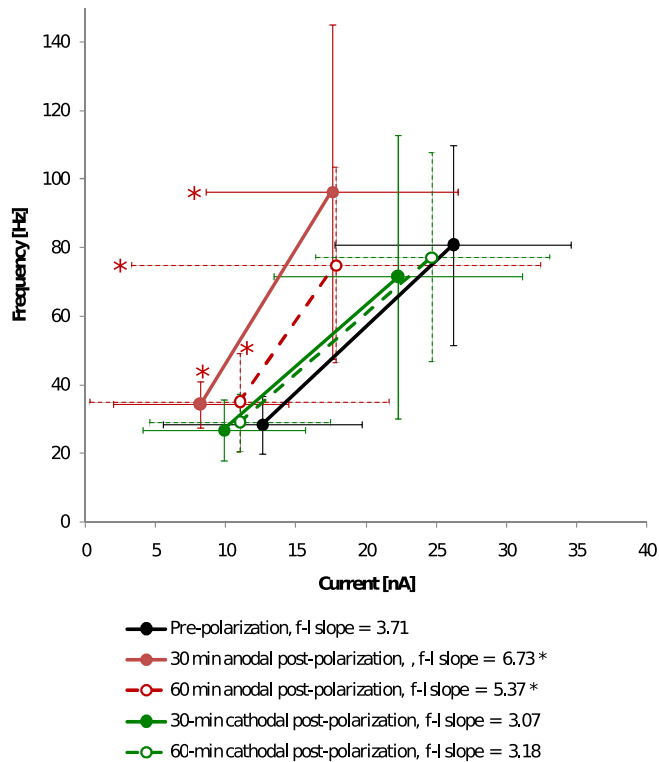
**a** Short-term effects of anodal polarization



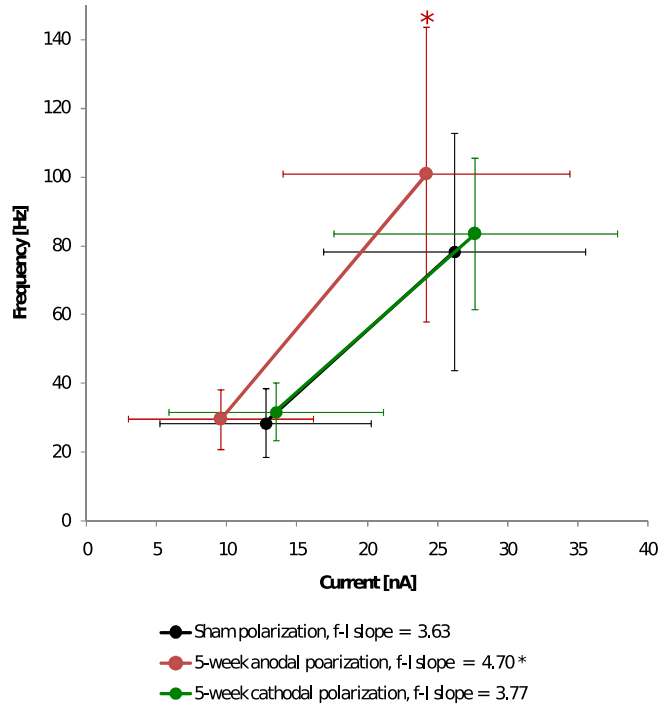
**b** Short-term effects of cathodal polarization



**c** Long-lasting effects of polarization



**d** Chronic effects of polarization



animal studies have provided further evidence that tDCS can directly affect spinal MN activation through both synaptic and axonal mechanisms (Ahmed, 2014). Significant improvement of our understanding of tDCS actions came from the works of the E. Jankowska group who methodologically investigated how tDCS influences the excitability

of cutaneous and Ia afferents (Bolzoni & Jankowska, 2015), activity-independent plasticity (Jankowska et al., 2016), myelinated nerve fibers activity (Jankowska, 2017), postactivation depression and presynaptic inhibition (Kaczmarek et al., 2017), as well as excitability of nerve fibers and their terminal branches in the presence of 4-aminopyridine

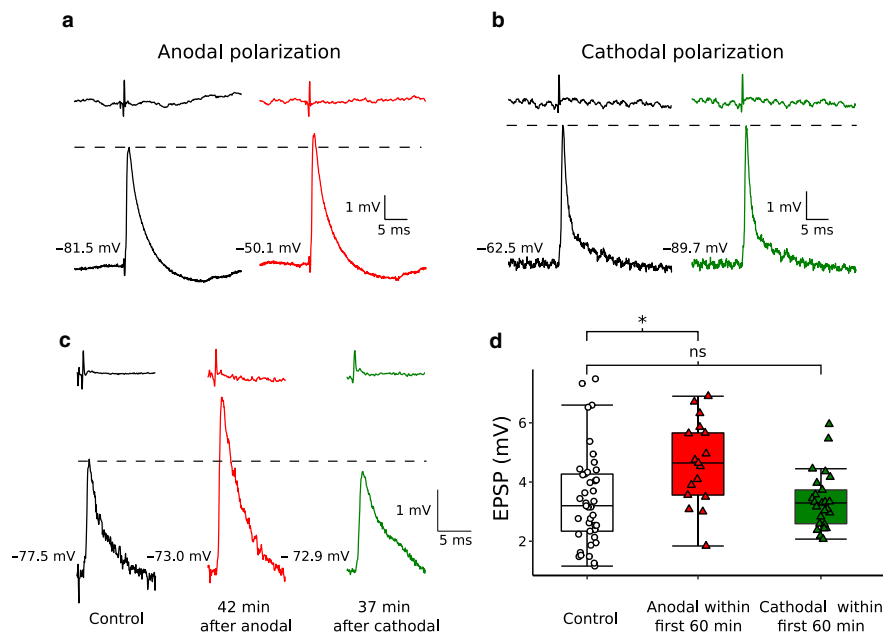
(Kaczmarek & Jankowska, 2018). The most recent study from the group suggests that branching points of primary afferent fibers are especially sensitive to DC current (Li et al., 2020).

One may wonder whether tsDCS mimics the actions of the spinal cord polarization produced by the electrical fields present around active neurons. Some electrical fields have amplitudes on the order of several millivolts, and can be detected at significant distances from cells active during locomotion (Noga et al., 1995). It is therefore possible that these fields influence the activity of neighboring cells and this concept was tested by Nelson (1966) in an elegant experiment that demonstrated MN activity can be influenced by subthreshold activation of synergistic neurons. More recently a similar concept was tested by Bączyk and Jankowska (2018) who showed that the excitability of myelinated nerve fibers can be modified by local field potentials evoked by stimulation of peripheral afferents.

The effects of tsDCS depend on the polarity of the applied current. In mice, spinal cord excitability was increased following cathodal polarization, but reduced during anodal tsDCS (Ahmed, 2014). In rats, the electromyographic responses from reticulospinal and

rubrospinal pathways were facilitated by cathodal tDCS and depressed by anodal tDCS (Bolzoni et al., 2013). Furthermore, intraspinally applied cathodal current replicated the effects of tsDCS and strongly increased MN synaptic excitation by acting on the afferents to MNs, and these actions were consistently facilitatory with cathodal DC and depressive with anodal DC (Bolzoni & Jankowska, 2015; Kaczmarek & Jankowska, 2018). On the other hand, presynaptic inhibition and post activation depression were both facilitated by tsDCS in a polarity-independent fashion (Kaczmarek et al., 2017). In the cat, in contrast to rats, anodal tDCS facilitated the activation of reticulospinal neurons (Bolzoni et al., 2013) and the actions of pyramidal tracts on MNs (Bączyk et al., 2014).

One important feature of tsDCS is its long-term effects. Multiple studies performed both in human (Berry et al., 2017; Bolzoni et al., 2017; Kuck et al., 2018) and animal preparations (Bączyk & Jankowska, 2018; Bolzoni & Jankowska, 2015; Jankowska, 2017) indicate that the effects of polarization last up to several hours after the cessation of the stimulation. This phenomenon is of crucial importance when designing tsDCS interventions aimed at inducing long-term neuromodulation.



**FIGURE 5** Short-term and long-lasting effects of polarization in SOD<sup>G93A</sup> mice. (a) Monosynaptic EPSPs, evoked by stimulation of the triceps surae nerve, recorded from the same MN, before, and during anodal polarization. (b) as in (a), but records made in a different animal before and during cathodal polarization. (c) Examples of EPSPs recorded in the control (white), long-lasting anodal (red), and long-lasting cathodal (green) polarization groups. (d) Distribution of EPSP amplitudes within control, long-lasting anodal, and long-lasting cathodal polarization groups. Each data point represents a single MN, while box-plots cover 25% of the upper and lower data range with horizontal lines showing the median. Notice a strong, 32% increase in EPSP amplitude following anodal polarization (without any change in input resistance). Difference in mean EPSPs amplitude is significant between control and long-lasting anodal polarization groups ( $p < 0.01$ , Mann-Whitney test)



## 1.4 | Effects of tsDCS on intrinsic and synaptic properties of MNS

How does spinal polarization act at the level of a single spinal MN? The recent development of intracellular MN recordings coupled with spinal polarization *in vivo* (Bączyk & Krutki, 2020), enabled the direct investigation of short-term, long-lasting and chronic tsDCS-induced alterations of MN electrophysiological properties (Figure 3).

Both anodal and cathodal tsDCS elicit polarity-dependent changes in threshold and firing properties of MNs which appear immediately after onset of polarization and outlast the duration of tsDCS application by at least 15 min (Bączyk et al., 2019; Table 1, Figure 4a,b). The major effects of anodal intervention act toward potentiation of MN firing, whereas cathodal polarization acts mainly toward firing inhibition. Moreover, the effects of anodal polarization are generally more pronounced and uniform than those evoked by cathodal polarization.

Significant long-lasting effects of anodal tsDCS causing potentiation of firing of MNs were shown to persist in MNs up to 60 min after the offset of polarization (Bączyk et al., 2020a; Table 1, Figure 4c). The effects of cathodal polarization were less prominent and shorter-lasting, and were not observed 30 min after the offset of tsDCS. These observations are consistent with several other studies in rats and cats reporting that the effects of polarization last up to 2 hr after cessation of the stimuli (Bączyk et al., 2014; Bolzoni & Jankowska, 2015; Bolzoni, Bączyk, et al., 2013; Bolzoni, Pettersson, et al., 2013). The larger impact of anodal tsDCS compared to cathodal tsDCS may appear surprising but marginal effects of cathodal polarization on activity of MNs were also observed in other animal studies (Bolzoni et al., 2013). *In vitro* experiments combined with computational neuron models (Lafon et al., 2017) suggest that these differences might be explained by the fact that anodal polarization has a synergistic effect on somatic and dendritic compartments, whereas under cathodal polarization the effects on the two compartments tend to cancel each other.

Chronic tsDCS elicits adaptive changes in electrophysiological properties of lumbar spinal MNs due to repeated and consistent alterations in activity of spinal circuitry (Bączyk et al., 2020b). Anodal polarization evokes adaptations in MN properties in such a way that excitability is increased and firing is facilitated, whereas chronic cathodal polarization has no significant effects (Table 1, Figure 4d). Chronic DC polarization can increase the MN excitability and therefore suggests that this technique may be used to deliver neuroprotection in ALS (Bączyk, Alami et al., 2020; Saxena et al., 2013).

The main advantage of DC polarization is that it is not invasive and can be easily used in humans. However preclinical animal studies have to be performed before starting clinical

trials. Preliminary results indicate that anodal tsDCS can enhance the excitatory synaptic inputs to MNs in the SOD1<sup>G93A</sup> mouse model of ALS (Figure 5a,b). Interestingly this effect persists up to 60 min after the end of polarization (Figure 5c,d). Moreover, when ALS mice were chronically treated with daily anodal or cathodal polarization for 2 weeks, EPSP amplitudes of DC-treated SOD1<sup>G93A</sup> mice were significantly larger in the anodal polarization group than in the nonpolarized group, whereas no change was seen in the cathodal polarization group (not shown). Altogether these results indicate that in SOD1<sup>G93A</sup> mice tsDCS evokes polarity-dependent MN plasticity. Although the functional and survival analysis of tsDCS effects on ALS mice are ongoing, these preliminary findings already provide a proof of concept for further tsDCS application in ALS management.

## 2 | CONCLUSION

Recent experiments suggest that vulnerable MNs become intrinsically hypoexcitable (as seen in a loss of their ability to discharge repetitively) and that their excitatory synapses are impaired in the SOD1<sup>G93A</sup> mice. Pharmacological and chemogenetics interventions that aimed at restoring either the intrinsic excitability or the synaptic strength were shown to ameliorate the disease phenotype. In parallel, other experiments showed that anodal tsDCS enhances the intrinsic excitability of spinal MNs and the effect outlasts the stimulation period. Moreover, preliminary results show that anodal tsDCS also elicits long-lasting enhancement of EPSPs in SOD1<sup>G93A</sup> mice, compensating the EPSP impairment observed in these mice. We suggest that chronic anodal tsDCS may be useful in the management of ALS patients.

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