Published in final edited form as:

Neuroscience. 2017 October 24; 362: 33–46. doi:10.1016/j.neuroscience.2017.08.041.

Hyperexcitability in synaptic and firing activities of spinal motoneurons in an adult mouse model of amyotrophic lateral sclerosis

Mingchen C. Jiang^{a,*}, Adesoji Adimula^b, Derin Birch^d, and Charles J. Heckman^{a,c,d}

^aDepartment of Physiology, Northwestern University Feinberg School of Medicine, 303 E. Chicago Ave, Chicago, IL 60611, USA

^bDepartment of Biomedical Engineering, Northwestern University Feinberg School of Medicine, 303 E. Chicago Ave, Chicago, IL 60611, USA

^cDepartment of Physical Medicine and Rehabilitation, Northwestern University Feinberg School of Medicine, 303 E. Chicago Ave, Chicago, IL 60611, USA

^dDepartment of Physical Therapy and Human Movement Sciences, Northwestern University Feinberg School of Medicine, 303 E. Chicago Ave, Chicago, IL 60611, USA

Abstract

Hyperexcitability is hypothesized to contribute to the degeneration of spinal motoneurons (MNs) in amyotrophic lateral sclerosis (ALS). Studies, thus far, have not linked hyperexcitability to the intrinsic properties of MNs in the adult ALS mouse model with the G93A-mutated SOD1 protein (mSOD1^{G93A}). In this study, we obtained two types of measurements: ventral root recordings to assess motor output and intracellular recordings to assess synaptic properties of individual MNs. All studies were carried out in an *in vitro* preparation of the sacral spinal cords of mSOD1^{G93A} mice and their non-transgenic (NT) littermates, both in the age range of 50 – 90 days. Ventral root recordings revealed that maximum compound action potentials (coAPs) evoked by a short-train stimulation of corresponding dorsal roots were similar between the two types of mice. Although the progressive depression of coAPs was present during the train stimulation in all recordings, the coAP depression in mSOD1^{G93A} mice was to a lesser extent, which suggests an increased firing tendency in mSOD1^{G93A} MNs. Intracellular recordings showed no changes in fast excitatory postsynaptic potentials (EPSPs) in mSOD1^{G93A} MNs. However, recording did show that oscillating EPSPs (oEPSPs) were induced by poly-EPSPs at a higher frequency and by less-intense electrical stimulation in mSOD1^{G93A} MNs. These oEPSPs were dependent upon the activities of spinal network and N-methyl-D-aspartate receptors (NMDARs), and were subjected

*Corresponding author. Address: Department of Physiology, Northwestern University Feinberg School of Medicine, 303 E. Chicago Ave, Chicago, IL 60611, USA, Telephone: 312-503-4814, m-jiang@northwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest

The authors declare no conflicts of interest in relation to the publication of this work.

to riluzole modulation. Taken together, these findings revealed abnormal electrophysiology in mSOD1^{G93A} MNs that could underlie ALS excitotoxicity.

Keywords

Amyotrophic lateral Sclerosis; spinal motoneurons; hyperexcitability; synaptic transmission; short-term plasticity; NMDA receptor

Introduction

ALS is a neurodegenerative disease characterized by a massive loss of MNs. Several pathogenic mechanisms, such as glutamate-induced excitotoxicity, are recognized in this disease (Van Den Bosch et al., 2006; Ferraiuolo et al., 2011). Excitotoxicity could emerge either from changes in intrinsic electrical properties of MNs or from an increase in excitatory synaptic properties. Remarkably, the intrinsic excitability of MNs increases at a very early stage in mSOD1^{G93A} mice (Pieri et al., 2003; Kuo et al., 2004; Kuo et al., 2005) and even in the embryonic state (Martin et al., 2013). Once an animal is born, however, this intrinsic excitability tends to subside to near-normal levels. In mSOD1^{G93A} neonates, persistent sodium (Na⁺) currents continue to increase with age, but are offset by increases in cell input conductance (Quinlan et al., 2011). Consequently, net excitability stays normal. However, there is some variation in MN excitability among mice of different SOD1 mutations (Amendola et al., 2007). As an animal matures, normal excitability prevails, and hypo-excitability in firing properties begins to develop (Delestree et al., 2014). These findings suggest that excitotoxicity in adult mSOD1^{G93A} mice may primarily occur via synaptic activity.

Indeed, several lines of evidence support the involvement of synaptic mechanisms in excitotoxicity. Extracellular glutamate can be enhanced by the loss of glutamate transporters in glial cells, which has been found in both ALS patients (Rothstein et al., 1995) and mouse models (Bruijn et al., 1997; Bendotti et al., 2001). An increase in synaptic transmission was reported in hypoglossal MNs of neonatal mSOD1^{G93A} mice and in cortical MNs of young adult mSOD1^{G93A} mice (van Zundert et al., 2008; Saba et al., 2015). A disinhibitory synaptic mechanism may be involved in ALS due to breakdowns in glycinergic transmission (Chang and Martin, 2009; McGown et al., 2013) and the synaptic connections between MNs and Renshaw cells (Wootz et al., 2013). For instance, data from an *in vitro* sacral cord preparation of an mSOD1^{G93A} mouse, exhibiting overt symptoms in late ALS, confirmed an increased tendency for spontaneous motor output due to increased excitatory synaptic inputs to mSOD1^{G93A} MNs (Jiang et al., 2009). In MNs, inefficient RNA editing of the GluR2 mRNA is found in both ALS patients and mSOD1^{G93A} mice (Tortarolo et al., 2006; Kwak et al., 2010), which results in increased calcium (Ca²⁺) permeability of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptors). NMDARs also play an important role in ALS excitotoxicity based on their Ca²⁺ permeability and altered levels of their endogenous co-agonists/inhibitors (Tokuda et al., 2007; Paul and de Belleruche, 2014, 2015). Although these findings imply that ALS hyperexcitability is imparted by synaptic properties, detailed measurements of these changes in these adult MNs are lacking.

In this study, we applied two recording techniques to investigate possible malfunctions involving synaptic activities in adult mSOD1^{G93A} MNs. With ventral root recordings to analyze overall motor output, we found enhancements in repetitive motor firings during train stimuli. Conversely, in intracellular recordings, we observed normal fast EPSPs and hyperexcitable oEPSPs in mSOD1^{G93A} MNs. Such findings further suggest the involvement of several electrical activities in ALS excitotoxicity.

Material and Methods

Transgenic mSOD1^{G93A} mice with B6SJL background and their non-transgenic (NT) littermates were bred and maintained at the Center for Comparative Medicine of Northwestern University Medical Center. All experimental procedures were reviewed and approved by the University Animal Research Committee and were in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Excitability of spinal MNs was studied at both system and single neuronal levels. Bipolar, stainless-steel wire electrodes were used to investigate motor output (i.e. the evoked coAPs) on ventral roots at sacral segments 2 – 3 (S2 – 3) while sharp, glass electrodes were used to record synaptic and intrinsic properties of single MNs in the same segments. In total, 61 adult mice of ages between P50 – P90 were tested in the experiments. Of those 61 adult mice, 33 were mSOD1^{G93A} mice, and 28 were NT littermates. The age range was determined by pathological changes in the mSOD1^{G93A} mouse model (Pun et al., 2006; Jiang et al., 2009).

Sacral cord preparation

The spinal cord at S2 – 3 was chosen in this study because ALS-resistant MNs are not located in this area (Hamson et al., 2002). Furthermore, the response of S2 – 3 ventral roots can be readily evoked by stimulating the corresponding dorsal roots. The surgical procedure was similar to previous publications (Jiang and Heckman, 2006; Jiang et al., 2009) with some modifications. Briefly, animals were deeply anesthetized with intraperitoneal injections of urethane at 0.18 g/100 g for NT mice and at 0.15 g/100 g for mSOD1^{G93A} mice. Supplemental anesthesia (0.01 – 0.05 g/ 100 g) was determined by each animal's response to foot pinching with forceps. After the vertebral column was opened below thoracic region, the spinal cord was immediately superfused with modified artificial cerebrospinal fluid (mACSF) at a flow rate of 5–7 ml/min. The mACSF was composed of the following (in mM): 120 NaCl, 3 KCl, 1 NaH₂PO₄, 5MgSO₄, 1 CaCl₂, 26 NaHCO₃, 10 glucose, and 1 kynurenic acid. Also, the mACSF was adjusted to a pH value of 7.4 by carefully adding NaHCO₃ and bubbled with 95 % O₂-5 % CO₂. The mouse was then decapitated, and the cord was transected at the rostral edge of lumbar enlargement. Dorsal and ventral roots of the caudal region of the cord were cut at their spinal outlets. The transected spinal cord with its attached roots was quickly transferred to a 100mm Petri dish filled with the mACSF, bubbled with 95%O₂/5% CO₂. After separating the S2 – 3 ventral roots and the dorsal roots that enter at the S3 segment, the cord was cut at the caudal edge of the lumbar enlargement and transferred to a recording chamber comprised of a 55mm Petri dish filled with silicone elastomer (Sylgard) at the bottom. The cord was pinned at its rostral/caudal edges and positioned with its ventral side facing up at the center of the plate. Two homemade electrode plates, containing six bipolar, stainless-steel wire electrodes (three on

each side), were placed on the lateral sides of the cord (Jiang et al., 2015). Electrodes, at a distal distance of about 1.5 mm from the cord, were mounted on the dorsal and ventral roots, which were covered with a mineral oil/petroleum jelly (2:1) mixture. It should be noted that the quality of extracellular recording depends on both the electrical properties of the electrode plate and the contacts between the electrodes and roots. To ensure quality of the recording, the electrodes were newly made for this study and the same surgery protocol was used. The cord was submerged in ~0.2 ml normal ACSF, which was circulated at a flow rate of 2.5–3 ml/min and bubbled with 95% O₂/5% CO₂. The normal ACSF was composed of the following (in mM): 126 NaCl, 3 KCl, 1 NaH₂PO₄, 1.5 MgSO₄, 2.5 CaCl₂, 26 NaHCO₃, and 10 glucose. Also, the normal ACSF was bubbled with 95% O₂/5% CO₂ and adjusted to a pH value of 7.4. For the ACSF containing low Ca²⁺ (1 mM), supplemental NaCl was added to compensate osmolality.

Ventral root recording and protocols

CoAPs were produced by stimulating dorsal roots and recording from their corresponding ventral roots. The stimulating pulse was provided by an isolation unit (PSIU6E, Grass Instruments) that was connected to a stimulator (S88, Grass Instruments). In order to evoke repetitive coAPs, five electrical pulses at 0.2 ms/pulse in duration, 1 – 25 Hz in ranging frequency, and 1X, 1.5X, 2X and 5X threshold in intensity were employed. The four recording electrodes were individually connected to four differential amplifiers (DAM 50, WPI) with gain at 1000X, high-pass filtering at 300 Hz, and low-pass filtering at 20 kHz. The analog signals were digitized into a Dell computer using a Spike2/1401Plus system (CED).

Intracellular recordings and protocols

Synaptic and intrinsic activities of MNs were recorded by intracellular sharp electrodes. Glass pipette electrodes (603000, WPI) were pulled with a Sutter puller (P-97, Sutter Instrument Co.) and filled with 3M KCl in order to have a resistance of 20 – 25MΩ. KCl was used as an internal solution because it produced lower resistance electrodes than those of potassium acetate. The electrodes were driven into the ventral horn using a stepper-motor (Model 2660 Micropositioner, David Kopf Instruments). The recorded electrical signal was amplified using an Axoclamp 2B amplifier (Axon Instruments) in the bridge or DCC (discontinuous current clamp mode) mode and digitized at 20 kHz into a Dell computer using a CED Micro 1401 and Spike2 software. Upon penetration, neurons were hyperpolarized with a 1 – 3 nA negative DC current that was gradually withdrawn within 3 minutes depending on their membrane potentials. Afterwards, the neurons were antidromically activated by stimulating their corresponding ventral roots in order to confirm their identity as MNs. The verified MNs with resting membrane potential more negative than –60 mV and overshooting APs were selected to study their synaptic properties. Spontaneous postsynaptic potentials (PSPs) were recorded for 5 – 30 minutes without any pharmacological or electrical intervention. To test the composition of spontaneous PSPs and possible reversal of inhibitory PSPs (IPSPs) due to the 3M KCl electrode, strychnine, an antagonist of glycine receptor, together with picrotoxin, an antagonist of GABA_A receptor, and 6,7-dinitroquinoxaline-2,3-dione (DNQX), an AMPA and kainate receptor antagonist, were applied to the ACSF in order to block the IPSPs and EPSPs. The ipsilateral dorsal root

was then stimulated to produce synaptic potentials by a five-pulse train at frequencies ranging from 1 – 25 Hz and intensities ranging from 1 – 5X of the threshold used for evoking oEPSPs. (2R)-amino-5-phosphonovaleric acid (APV), an NMDAR antagonist, and mephenesin, an inhibitor for poly-synaptic transmission (Lev-Tov and Pinco, 1992; Pinco and Lev-Tov, 1994), were separately applied to the ACSF to determine the cellular mechanisms underlying oEPSPs. Mephenesin was used as an inhibitor for polysynaptic transmission (Lev-Tov and Pinco, 1992; Pinco and Lev-Tov, 1994), and it is likely that this inhibition is due to mephenesin's blocking effects on persistent Na^+ or barium currents (Ducreux, 1978). Also, poly-synaptic transmission is more susceptible to mephenesin's effects than mono-synaptic transmission (Wright, 1954).

Data analysis

To analyze the data from root recordings, the amplitudes of evoked coAPs were calculated from their peak-to-peak values. As we have recently shown that the degree of system short-term depression (sSTD) in coAPs represents a form of excitability (Jiang et al., 2015), sSTD was quantified by converting the amplitudes of coAPs into percentage changes from the amplitudes of their respective, first-evoked coAPs of the individual. To analyze fast EPSPs, a 30-second time window of stable recording was performed in order to count the amplitudes of individual EPSPs and to calculate their excitabilities using the area under curve (AUC) method. To verify whether the reversed IPSPs exist, a 20-second time window was set to compare the amplitudes of spontaneous depolarizing PSPs, larger than 1 mV for reliable resolution, before and after blocking IPSPs. The spontaneous oEPSPs were analyzed by reviewing their occurrences and computing the AUC. Also, the intensity requirement for the evoked oEPSPs was statistically analyzed. The AUC for a time window (0.05 – 1 second after stimulation) was quantified for riluzole's effect on the evoked synaptic responses. For the effect of low Ca^{2+} ACSF, the AUC for a 1 second time window after stimulation, during which AP was truncated at -45 mV, was quantified. Statistical significance of these values between $\text{mSOD1}^{\text{G93A}}$ MNs and NT MNs were calculated using a two-way ANOVA and post-hoc t-test, a χ^2 test, a Wilcoxon signed-rank test, and a Mann-Whitney U test.

Results

Enhanced repetitive firings in S2 – 3 motor pools of $\text{mSOD1}^{\text{G93A}}$ mice

Synaptic and intrinsic properties in a neuron define its excitability. Since excitability exhibits use-dependent characteristics, we first compared overall excitability of the spinal motor system between $\text{mSOD1}^{\text{G93A}}$ mice (tested on 15 ventral roots from 8 mice) and their NT littermates (tested on 11 ventral roots from 6 mice) by measuring the evoked motor output (i.e. the evoked coAP) from the ventral roots of the S2 – 3 motor pools. Five electrical pulses were applied to each dorsal root at 25Hz and at three intensities (1.5X, 2X and 5X of root reflex threshold). The results showed that the largest response coAPs were evoked by the first of the five electrical pulses. Subsequent pulses (P2-5) elicited progressively smaller coAPs (Fig. 1), which is characteristic of sSTD (Jiang et al., 2015). The averaged amplitudes of the coAPs evoked by pulse 1 (P1) were similar between the two mouse groups (Fig. 1Ba). The significance of sSTDs in each group was determined by Wilcoxon signed-rand test (Table 1). The percentage changes of coAPs evoked by P2-5 quantified sSTD between

mSOD1^{G93A} and NT mice (Fig. 1Bb). A two-way ANOVA analysis, considering the factors of genotype and intensity, determined significantly less sSTD in mSOD1^{G93A} mice than in NT mice (for genotype: $p = 0.001$; for intensity: $p = 0.05$; post hoc t tests: indicated by the asterisks in Fig. 1Bb), which suggests higher motor excitability in mSOD1^{G93A} mice than in NT mice.

Spontaneous synaptic activities and enhanced oEPSPs in mSOD1^{G93A} MNs

Because synaptic excitability emerges from both excitatory and inhibitory synapses, the recordings on spontaneous synaptic activities were performed without blocking these two types of synapse. However, these spontaneous PSPs appear to be mostly excitatory, i.e. EPSPs, in both types of MNs in the mouse sacral cord. On the other hand, the spontaneous IPSPs were rare and small, and thus were not analyzed further. From 67 MNs (mSOD1^{G93A}: 39 cells from 18 mice, 2 of which were previously tested in the sSTD; NT: 28 cells from 18 mice), the spontaneous EPSPs were classified into two major groups based on their activity patterns and occurrences: individual EPSPs (i.e. fast EPSPs, Fig. 2A) and repetitive EPSPs (i.e. oEPSPs, Fig. 2B). Individual EPSPs occurred in isolation, were present in all MNs, and included both mono- and poly-EPSPs (identified by their rising phase; Mono-EPSPs: single rising phase; Poly-EPSPs: multiple rising phases; Fig. 2A). In each MN, the individual EPSPs varied widely in amplitude (range: 0.2 – 15 mV, limited by signal resolution, Fig. 2Aa – c) and in duration of a few hundred milliseconds. In general, poly-EPSPs (Fig. 2Ad) had larger amplitudes and longer durations than those of mono-EPSPs. To quantify excitability of individual EPSPs, we first averaged the maximum peaks of individual EPSPs in a 30 second recording period for each MN and compared these values between mSOD1^{G93A} MNs and NT MNs (Fig. 2Ca). The excitability of these individual EPSPs in the same time window was also analyzed by AUC, in which the baseline was adjusted manually when necessary. Both the averaged peaks and AUCs of individual EPSPs showed no statistical difference between the two types of MNs (Fig. 2Ca, b) and further suggest that electrical behaviors of individual mono-/poly-EPSPs in mSOD1^{G93A} MNs are normal in the P50 – 90 age range.

In contrast to individual EPSPs, repetitive EPSPs consisted of poly-EPSPs and acted continuously with multiple peaks so that membrane potentials appeared oscillatory. Therefore, this distinctive behavior of poly-EPSPs was called oEPSPs. Typical oEPSPs started with small poly-EPSPs that repeated with increases in amplitude, oscillated above resting membrane potentials, and ended by progressively decreasing their amplitudes and returning to resting membrane potentials (Fig. 2B1, 2). In general, oEPSPs could last between 0.1 – 2 minutes and oscillate at 3 – 16 Hz with peaks between 10 – 15 mV. Out of 329 total periods of oEPSPs, only 17 oEPSPs generated APs at a rate of 0.05 AP/oEPSPs. The averaged AUC value of oEPSPs in each MN did not differ significantly between the two types of MNs (Fig. 2Cc). However, oEPSPs were recorded in more mSOD1^{G93A} MNs than NT MNs (mSOD1^{G93A} MNs: 34/39; NT MNs: 9/28; Chi² test, $p = 0.001$). Plotting the number of oEPSPs versus recording period also showed a higher oEPSP occurrence in mSOD1^{G93A} MNs than in NT MNs (NT: 0.30 ± 0.17 , $n = 9$; mSOD1^{G93A}: 0.85 ± 0.45 , $n = 34$; mean occurrence/min $\pm$ SD; $p = 0.01$, Mann-Whitney U test; Fig. 2Cd). Taken together,

these data suggest that the excitability is enhanced in the number of mSOD1^{G93A} MNs as manifested in a higher occurrence of oEPSPs.

After observing the spontaneous activities, we then tested their responses to pharmacological treatment and electrical stimulation in some MNs that were still in good condition. We first verified the involvement of the spinal network in oEPSP generation by bath application of mephenesin. As shown in Fig. 3A, 1mM mephenesin completely eliminated oEPSPs (mSOD1^{G93A}: n = 2; NT: n = 1) with partial blockage of individual PSPs. NMDARs are known to be involved in oscillatory activity (Bonnot et al., 2002) and rhythmic firing of the ventral roots of adult mSOD1^{G93A} mice (Jiang et al., 2009). We thus tested the involvement of NMDARs in generating the MN oEPSPs. Similar to mephenesin, 50 μ M APV by bath application completely blocked oEPSPs and reduced individual PSPs (mSOD1^{G93A}: n = 5; NT: n = 1; Fig. 3B). These results suggest that oEPSPs are composed of poly-PSPs from the spinal network and depend on NMDAR activity. In addition, some mSOD1^{G93A} MNs displayed slight hyperpolarization (< 1mV) during mephenesin or APV application (Fig. 3). This hyperpolarization might be the blocking effect of the drugs on either oEPSPs or individual EPSPs. It is also possible that the hyperpolarization was due to random variation in membrane potentials. Due to the limited amount of MNs, this hyperpolarization was not analyzed.

Lower threshold of oEPSPs in mSOD1^{G93A} MNs in response to electrical stimulation

Given the presence and the hyperexcitability of spontaneous oEPSPs in mSOD1^{G93A} MNs, we further tested whether sensory inputs can evoke oEPSPs in these MNs. In a total of 25 MNs, oEPSPs could be readily evoked in mSOD1^{G93A} MNs by a five-pulse train stimulation at 1 Hz and at an intensity as low as 1X threshold used for evoking the coAPs (Fig. 4A). A significantly higher stimulation intensity was required for NT MNs (Fig. 4B) than that for mSOD1^{G93A} MNs (stimulation intensity < 1.5X: n = 7 vs 1; stimulation intensity 1.5X and < 2X: n = 4 vs 6; stimulation intensity > 2X: n = 0 vs 7; mSOD1^{G93A} vs NT, Chi² test: p 0.01). These results suggest that oEPSPs can be induced by inputs from either the spinal network or peripheral afferents and could account for increased excitability in mSOD1^{G93A} MNs.

Contributions from the spinal network and NMDARs in evoked oEPSPs, similar to spontaneous oEPSPs, were confirmed by separately adding mephenesin or APV. In 5 MNs (4/5: mSOD1^{G93A} MNs; 1/5: NT MN), a short-train stimulation at an intensity of 1.5X threshold or stronger evoked oEPSPs, which was blocked by 1mM mephenesin. In one of these mSOD1^{G93A} MNs (Fig. 5A), an effort was made to avoid activating NMDARs by hyperpolarizing its resting membrane potential to -95 mV with a DC current. Under this condition, the 1.5X train stimulation evoked both mono-EPSPs, which produced a maximum depolarization of the membrane potential to -77 mV, and oEPSPs, which were blocked by 1 mM mephenesin. Because NMDARs are mostly closed at -70mV (Clarke et al., 2013), this result strongly suggests that oEPSPs originate from the spinal network. In addition, the effect of NMDARs on evoked oEPSPs was signified when evoked oEPSPs in two MNs were blocked by 50 μ M APV (Fig. 5B). Taken together, these results suggest that both spontaneous and evoked oEPSPs originate from spinal network and NMDAR activity.

Effects of riluzole and low Ca^{2+} ACSF on the hyperexcitability in mSOD1^{G93A} MNs

After identifying the synaptic hyperexcitability, we explored whether this hyperexcitability could be regulated by riluzole, the therapeutic drug for ALS. In this experiment, the EPSPs were evoked at 3X intensity of root reflex threshold in order to reliably produce APs. 10 minute application of 10 μM riluzole effectively reduced the evoked oEPSPs in all mSOD1^{G93A} MNs, but not fast EPSPs and APs (the ratio of oEPSP AUCs between riluzole and control: 58.4 ± 20.4 , mean $\% \pm \text{SD}$, $n = 6$ from 6 mSOD1^{G93A} mice; Fig. 6A). The results suggest that the therapeutic mechanism of riluzole is largely based on its inhibitory effect on the oEPSPs. In addition, the relationship between the synaptic hyperexcitability and the intracellular Ca^{2+} was examined in order to understand the pathophysiological function of Ca^{2+} in ALS and to benefit designing new therapeutic strategy. Using the same protocol as for riluzole test, we found that reducing the Ca^{2+} concentration in ACSF to 1 mM for 5 minutes dramatically decreased both evoked fast EPSPs and oEPSPs, and made the former unable to generate APs (ratio of whole EPSP AUCs between low Ca^{2+} test and control: 37.2 ± 17.9 , mean $\% \pm \text{SD}$, $n = 5$ from 3 mSOD1^{G93A} mice; Fig. 6B). The results suggest a positive correlation between the synaptic excitability and intracellular Ca^{2+} in mSOD1^{G93A} MNs.

Composition of spontaneous synaptic activities

The leak of chloride from the KCl electrode can change the intracellular chloride equilibrium potential and may reverse IPSPs. However, the chloride leak depends largely on electrode resistance, and MNs exhibit certain buffering capability (Coombs et al., 1955). To test whether such reversed IPSPs exist in our recordings, we compared the spontaneous EPSPs in five MNs from 4 NT mice before and after a 2-minute application of 5 μM strychnine and 10 μM picrotoxin. The results show that the treatment did not reduce but rather slightly enhanced the EPSPs, although it was not significant (before: $1.78 \pm 0.24\text{mV}$; after: $1.80 \pm 0.17\text{mV}$; $n = 5$, mean $\pm \text{SD}$, Wilcoxon signed rank test), as shown in Fig. 7Aa,b as an example. These data may imply a balance between chloride leak from the high resistance electrode and the ion buffering capacity of MNs. In four of these five MNs, the EPSPs were then largely blocked after a 2-minute application of 10 μM DNQX (Fig. 7Ac). More directly, we observed stable IPSPs with resting membrane potential at -70mV for up to 50 minutes in an unidentified ventral horn neuron (Fig. 7B). The same treatment clearly blocked both of its IPSPs and EPSPs in this neuron. Taken together, these data strongly suggest that in our study, the 3M KCl electrode has very limited effect, if any, on IPSPs, and confirms that the spontaneous EPSPs are mediated mostly through AMPARs.

Discussion

In the hypothesis of glutamate-induced excitotoxicity, the abnormal rise in extracellular glutamate and intracellular Ca^{2+} are two critical pathogenic steps leading to the degeneration of ALS MNs. Ca^{2+} entry is conducted by voltage-gated Ca^{2+} channels (VGCCs) and ionotropic glutamate receptors, including NMDARs and AMPARs. Studies using various methods, such as molecular biology and immunohistology, have shown abnormalities in the function and permeability of these receptors in ALS. Here, we investigated the functionality of adult mSOD1^{G93A} MNs using electrophysiology. Our results suggest that synaptic

hyperexcitability exists in adult mSOD1^{G93A} MNs and consists of several membrane mechanisms, which may all contribute to the transmembrane transmission of glutamate-induced excitotoxicity in this ALS mouse model.

The mechanisms underlying enhanced repetitive firings

The firing ability of a neuron is determined by intrinsic and synaptic properties and exhibits use-dependent characteristics. Not only do APs cause Na⁺ entry through Na⁺ channels, but they also trigger Ca²⁺ entry by activating VGCCs. In this study, we observed that the motor outputs at P1 and individual APs are comparable between mSOD1^{G93A} and NT mice. The enhanced repetitive firings evoked by a train stimulation suggest voltage-gated Na⁺ and Ca²⁺ channels as being the sources of excitotoxicity. Because the intrinsic excitability in mSOD1^{G93A} mice appears to decrease from hyperexcitability at early ages (Pieri et al., 2003; Kuo et al., 2004; Kuo et al., 2005) to normal or even hypoexcitability in adulthood (P40 – P50) (Delestree et al., 2014), it is less probable that the enhanced repetitive firings in our study arise from the channels' intrinsic properties. However, the intrinsic hyperexcitability might occur at the very late stage of the disease as the increased expression of beta3 subunit of voltage-gated Na⁺ channel was found at P120, but not at P75 in mSOD1^{G93A} MNs (Nutini et al., 2011). Thus, other cellular mechanisms might contribute to the enhanced repetitive firings in the age range we tested.

In order to understand overall information processing in the spinal motor system, we recently studied short-term plasticity (STP) at a system level (Jiang et al., 2015), which is expressed as sSTD. Lasting from a few milliseconds to minutes, STP is believed to play an important role in determining motor input-output function under both normal and pathological conditions (Wolpaw and Tennissen, 2001). Compared to synaptic STP that focuses on mono-synaptic transmission (Zucker, 1989; Zucker and Regehr, 2002; Fioravante and Regehr, 2011), the sSTD is composed of more cellular mechanisms, including mono-synaptic, intrinsic, and network mechanisms (Jiang et al., 2015). In the mSOD1^{G93A} mice, immunostaining on glycinergic terminals (Chang and Martin, 2009) and Renshaw cells (Wootz et al., 2013) revealed degeneration of these inhibitory components to spinal MNs, and quantitative PCR on glycine receptor also found lower expression of the receptor's subunit mRNA (Chang and Martin, 2011). We have shown that decreases in inhibitory inputs enhance motor excitability by reducing sSTD (Jiang et al., 2015). In addition, NMDARs have been reported to enhance short-term synaptic transmission in the thalamic-anterior cingulate pathway (Shyu and Vogt, 2009). In mSOD1^{G93A} mice, the activity of NMDARs is likely enhanced due to increased levels of D-serine and glycine (Sasabe et al., 2007; Paul and de Belleruche, 2014, 2015), co-agonists of NMDARs, and a reduced zinc level, a potent inhibitor of NMDARs (Tokuda et al., 2007). The reduced sSTD might also result from the change in presynaptic release probability that is determined by Ca²⁺ level at synaptic terminals. In cortical mSOD1^{G93A} MNs, spontaneous excitatory postsynaptic currents were found to occur at higher frequency (Saba et al., 2016) due to over-expression of N-type VGCC (Pieri et al., 2013). Lautenschläger et al. (Lautenschläger et al., 2013) reported a decelerated cytosolic Ca²⁺ clearance due to disturbance of endoplasmic reticulum/mitochondria (ER/MT) in mSOD1^{G93A} MNs, which may enhance resting Ca²⁺. Thus, in mSOD1^{G93A} MNs, the terminal Ca²⁺ level is likely altered in a way to enhance

synaptic transmission, but its exact mechanism on the sSTD needs further investigation. Taken together, these data suggest that the synaptic mechanisms are more likely to be the causes for the reduced sSTD, i.e. the enhanced repetitive firings, in the spinal motor system of mSOD1^{G93A} mice.

The hyperexcitability of excitatory synaptic activity at subthreshold level

Our study shows that sub-threshold, fast EPSPs in spinal MNs occur much more frequently than APs. Most of these fast EPSPs are typical mono- and poly-EPSPs with no clear differences in averaged amplitudes and AUCs between mSOD1^{G93A} MNs and NT MNs. It has been demonstrated that fast EPSPs in spinal MNs are mediated mainly through AMPARs (Pinco and Lev-Tov, 1994), and the enhancement in Ca²⁺ permeability of AMPARs is detected at eight weeks after birth in mSOD1^{G93A} mice (Tortarolo et al., 2006). The higher Ca²⁺ permeability of AMPARs in mSOD1^{G93A} MNs implies greater Ca²⁺ entry, contributing to hyperexcitability in mSOD1^{G93A} MNs and thus, to ALS.

So far, electrophysiological studies on spinal MNs are mostly conducted using the preparation of either slice or single neuron. While these preparations have clear advantages, such as their feasibilities for patch clamp studies, one drawback is that most synaptic connections are lost. In ALS, available evidence has shown that a pathogenic mechanism may involve the increase of glutamate released from the pre-motor network (Milanese et al., 2011). In the current study, we therefore chose the whole cord preparation (Jiang and Heckman, 2006) in order to keep all network inputs intact and to trace this possible mechanism. To our knowledge, this is the first time that a spontaneous and poly-synaptic oEPSP was recorded in spinal MNs from both mSOD1^{G93A} mice and their NT littermates. Our data show the oEPSPs depend on pre-motor interneurons, NMDARs and intrinsic rhythmic firings. The hyperexcitability of oEPSPs in mSOD1^{G93A} MNs is likely due to the same pathological conditions in the NMDARs as for the STDs.

In the pathogenesis of ALS, ER stress and MT dysfunction are well demonstrated. Ca²⁺ entry may induce Ca²⁺ release from the ER and Ca²⁺ upload by MT, an intracellular process called Ca²⁺ induced Ca²⁺ release (CICR), which is characterized by oscillation of cytosolic Ca²⁺ level. Chronically enhanced CICR has been postulated as a cell death mechanism in ALS because it may cause ER Ca²⁺ depletion and MT Ca²⁺ overload leading to apoptosis or necrosis (Tadic et al., 2014). In MNs, CICR can be induced by the activation of NMDARs and the frequency of Ca²⁺ oscillation varies widely (Riout-Pedotti, 1997; Guertin and Hounsgaard, 1998). Identification of the oEPSP and its hyperexcitability in mSOD1^{G93A} MNs suggest that the internal CICR could be efficiently and maximally activated if resonance occurs between the external and the internal oscillations. Thus, the hyperexcitable oEPSPs might be particularly harmful to ER and MT in mSOD1^{G93A} MNs.

The effectiveness of transmitter clearance is another factor affecting a neuron's activity. The transmitter glutamate is quickly cleaned by several glutamate transporters, such as EAAT2 (Shigeri et al., 2004; Foran and Trotti, 2009). A reduced level of EAAT2 was reported in both familial and sporadic ALS patients (Rothstein et al., 1995; Jackson et al., 1999). In an mSOD1^{G93A} rat model, loss of EAAT2 was detected at the fourth week of birth and exacerbated severely at end stage (Howland et al., 2002). The loss of EAAT2 may underlie

the accumulation of glutamate in the extracellular space of neurons. This accumulation depolarizes neuronal membranes and gives rise to increased firings. Our data, however, did not show significant changes in resting membrane potentials or spontaneous firings and synaptic inputs, suggesting that the loss of EAAT2 is not a major component underlying synaptic hyperexcitability at the pre-symptom stage.

Excitotoxicity and therapy

Presently, ALS is still an incurable MN disease, and riluzole is the only therapy targeting excitotoxicity and produces limited benefit. At clinical doses, the major effect of riluzole is to reduce intrinsic excitability (Bellingham, 2011). The facts that riluzole inhibits the oEPSPs as we showed here and intrinsic repetitive firings (Kuo et al., 2006) further suggest that the drug's therapeutic targets include both MNs and interneurons. On the other hand, this study and the recent findings in adult mSOD1^{G93A} mice (Jiang et al., 2009; Delestree et al., 2014) suggest that hyperexcitability mainly exists in synaptic transmission in this ALS model. Externally, enhanced excitatory pressures to ALS MNs come from at least three sources including enhanced excitatory synaptic inputs as shown in this study, decreases in inhibitory synaptic inputs (Chang and Martin, 2009; Wootz et al., 2013), and loss of EAAT2 (Howland et al., 2002). In ALS MNs, Ca²⁺-permeable AMPARs are enhanced due to inefficient RNA editing (Tortarolo et al., 2006; Kwak et al., 2010), NMDARs become hyperexcitable due to abnormal metabolisms of their co-agonists (Paul and de Belleruche, 2014, 2015), and disturbed Ca²⁺ homeostasis eventually triggers the cell death (Tadic et al., 2014; Leal and Gomes, 2015). Thus, a combinatory therapy designed with the understandings of these detailed hyperexcitabilities and their relationship could be expected to be more potent than a single therapy with riluzole. In fact, individual survival tests that targeted the NMDARs (Wang and Zhang, 2005; Gerber et al., 2013) and AMPARs (Van Damme et al., 2003; Tortarolo et al., 2006) in mSOD1^{G93A} mice were all reported to produce some benefits. Therefore, a synergistic effect of such combinatory therapy is possible and is worthy of testing.

Conclusion

In adult mSOD1^{G93A} mice, we observed several forms of hyperexcitability that are expressed in the electrical activities of spinal MNs. The degree of sSTD in the motor output decreases and results in enhanced repetitive firings. The excitability of NMDARs in spinal networks increases and results in hyperactive oEPSPs. As we have shown that the function of AMPARs is unchanged, AMPAR contribution to the excitotoxicity is likely manifested by enhanced permeability to Ca²⁺. Although the sample size in this study is relatively low due to the demanding nature of the technique, the data are important as they likely reveal cellular mechanisms underlying the transmembrane transmission of glutamate-induced excitotoxicity in a special form of ALS.

Acknowledgments

This work was supported by grants from National Institute of Health (NIH NINDS NS077863) and from the ALS Association (277).

Abbreviations

ACSF	artificial cerebrospinal fluid
ALS	amyotrophic lateral sclerosis
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AP	action potential
APV	(2 <i>R</i>)-amino-5-phosphonovaleric acid
Ca²⁺	calcium
CICR	calcium-induced calcium release
coAP	compound action potential
DNQX	6,7-Dinitroquinoxaline-2,3-dione
ER	endoplasmic reticulum
EPSP	excitatory postsynaptic potential
I – V	Current – voltage
mACSF	modified artificial cerebrospinal fluid
MT	mitochondria
MN	motoneuron
mSOD1^{G93A}	G93A mutated SOD1 protein
NT	non-transgenic
IPSP	inhibitory postsynaptic potential
NMDAR	N-methyl-D-aspartate receptors
oEPSP	oscillating excitatory postsynaptic potential
Na⁺	sodium
sSTD	system short-term depression
STP	short-term plasticity
VGCC	voltage-gated calcium channel

References

- Amendola J, Gueritaud JP, Lamotte d'Incamps B, Bories C, Liabeuf S, Allene C, Pambo-Pambo A, Durand J. Postnatal electrical and morphological abnormalities in lumbar motoneurons from transgenic mouse models of amyotrophic lateral sclerosis. *Arch Ital Biol.* 2007; 145:311–323. [PubMed: 18075124]

- Bellingham MC. A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: what have we learned in the last decade? *CNS Neurosci Ther.* 2011; 17:4–31. [PubMed: 20236142]
- Bendotti C, Tortarolo M, Suchak SK, Calvaresi N, Carvelli L, Bastone A, Rizzi M, Rattray M, Mennini T. Transgenic SOD1 G93A mice develop reduced GLT-1 in spinal cord without alterations in cerebrospinal fluid glutamate levels. *J Neurochem.* 2001; 79:737–746. [PubMed: 11723166]
- Bonnot A, Whelan PJ, Mentis GZ, O'Donovan MJ. Locomotor-like activity generated by the neonatal mouse spinal cord. *Brain Res Brain Res Rev.* 2002; 40:141–151. [PubMed: 12589913]
- Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, Sisodia SS, Rothstein JD, Borchelt DR, Price DL, Cleveland DW. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron.* 1997; 18:327–338. [PubMed: 9052802]
- Chang Q, Martin LJ. Glycinergic innervation of motoneurons is deficient in amyotrophic lateral sclerosis mice: a quantitative confocal analysis. *Am J Pathol.* 2009; 174:574–585. [PubMed: 19116365]
- Chang Q, Martin LJ. Glycine receptor channels in spinal motoneurons are abnormal in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci.* 2011; 31:2815–2827. [PubMed: 21414903]
- Clarke RJ, Glasgow NG, Johnson JW. Mechanistic and structural determinants of NMDA receptor voltage-dependent gating and slow Mg²⁺ unblock. *J Neurosci.* 2013; 33:4140–4150. [PubMed: 23447622]
- Coombs JS, Eccles JC, Fatt P. The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *The Journal of physiology.* 1955; 130:326–374. [PubMed: 13278905]
- Delestree N, Manuel M, Iglesias C, Elbasiouny SM, Heckman CJ, Zytnicki D. Adult spinal motoneurons are not hyperexcitable in a mouse model of inherited amyotrophic lateral sclerosis. *The Journal of physiology.* 2014; 592:1687–1703. [PubMed: 24445319]
- Ducreux C. Mephensin blocks persistent Na⁺ or Ba²⁺ currents underlying paroxysmal depolarizations in PTZ- or Ba-treated molluscan neurones. *Comp Biochem Physiol C.* 1978; 59:51–57. [PubMed: 24522]
- Ferraiuolo L, Kirby J, Grierson AJ, Sendtner M, Shaw PJ. Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. *Nat Rev Neurol.* 2011; 7:616–630. [PubMed: 22051914]
- Fioravante D, Regehr WG. Short-term forms of presynaptic plasticity. *Curr Opin Neurobiol.* 2011; 21:269–274. [PubMed: 21353526]
- Foran E, Trotti D. Glutamate transporters and the excitotoxic path to motor neuron degeneration in amyotrophic lateral sclerosis. *Antioxid Redox Signal.* 2009; 11:1587–1602. [PubMed: 19413484]
- Gerber YN, Privat A, Perrin FE. Gacyclidine improves the survival and reduces motor deficits in a mouse model of amyotrophic lateral sclerosis. *Front Cell Neurosci.* 2013; 7:280. [PubMed: 24409117]
- Guertin PA, Hounsgaard J. NMDA-Induced intrinsic voltage oscillations depend on L-type calcium channels in spinal motoneurons of adult turtles. *Journal of neurophysiology.* 1998; 80:3380–3382. [PubMed: 9862937]
- Hamson DK, Hu JH, Krieger C, Watson NV. Lumbar motoneuron fate in a mouse model of amyotrophic lateral sclerosis. *Neuroreport.* 2002; 13:2291–2294. [PubMed: 12488813]
- Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, Erickson J, Kulik J, DeVito L, Psaltis G, DeGennaro LJ, Cleveland DW, Rothstein JD. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proceedings of the National Academy of Sciences of the United States of America.* 2002; 99:1604–1609. [PubMed: 11818550]
- Jackson M, Steers G, Leigh PN, Morrison KE. Polymorphisms in the glutamate transporter gene EAAT2 in European ALS patients. *Journal of neurology.* 1999; 246:1140–1144. [PubMed: 10653305]

- Jiang M, Schuster JE, Fu R, Siddique T, Heckman CJ. Progressive changes in synaptic inputs to motoneurons in adult sacral spinal cord of a mouse model of amyotrophic lateral sclerosis. *J Neurosci*. 2009; 29:15031–15038. [PubMed: 19955354]
- Jiang MC, Heckman CJ. In vitro sacral cord preparation and motoneuron recording from adult mice. *J Neurosci Methods*. 2006; 156:31–36. [PubMed: 16574242]
- Jiang MC, Elbasiouny SM, Collins WF 3rd, Heckman CJ. The transformation of synaptic to system plasticity in motor output from the sacral cord of the adult mouse. *J Neurophysiol*. 2015; 114:1987–2004. [PubMed: 26203107]
- Kuo JJ, Siddique T, Fu R, Heckman CJ. Increased persistent Na(+) current and its effect on excitability in motoneurons cultured from mutant SOD1 mice. *The Journal of physiology*. 2005; 563:843–854. [PubMed: 15649979]
- 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; 574:819–834. [PubMed: 16728453]
- Kuo JJ, Schonewille M, Siddique T, Schults AN, Fu R, Bar PR, Anelli R, Heckman CJ, Kroese AB. Hyperexcitability of cultured spinal motoneurons from presymptomatic ALS mice. *Journal of neurophysiology*. 2004; 91:571–575. [PubMed: 14523070]
- Kwak S, Hideyama T, Yamashita T, Aizawa H. AMPA receptor-mediated neuronal death in sporadic ALS. *Neuropathology*. 2010; 30:182–188. [PubMed: 20102521]
- Lautenschlager J, Prell T, Ruhmer J, Weidemann L, Witte OW, Grosskreutz J. Overexpression of human mutated G93A SOD1 changes dynamics of the ER mitochondria calcium cycle specifically in mouse embryonic motor neurons. *Exp Neurol*. 2013; 247:91–100. [PubMed: 23578819]
- Leal SS, Gomes CM. Calcium dysregulation links ALS defective proteins and motor neuron selective vulnerability. *Front Cell Neurosci*. 2015; 9:225. [PubMed: 26136661]
- Lev-Tov A, Pinco M. In vitro studies of prolonged synaptic depression in the neonatal rat spinal cord. *The Journal of physiology*. 1992; 447:149–169. [PubMed: 1593445]
- 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; 54:116–126. [PubMed: 23466698]
- McGown A, McDermid JR, Panagiotaki N, Tong H, Al Mashhadi S, Redhead N, Lyon AN, Beattie CE, Shaw PJ, Ramesh TM. Early interneuron dysfunction in ALS: insights from a mutant sod1 zebrafish model. *Ann Neurol*. 2013; 73:246–258. [PubMed: 23281025]
- Milanese M, Zappettini S, Onofri F, Musazzi L, Tardito D, Bonifacino T, Messa M, Racagni G, Usai C, Benfenati F, Popoli M, Bonanno G. Abnormal exocytotic release of glutamate in a mouse model of amyotrophic lateral sclerosis. *Journal of neurochemistry*. 2011; 116:1028–1042. [PubMed: 21175617]
- Nutini M, Spalloni A, Florenzano F, Westenbroek RE, Marini C, Catterall WA, Bernardi G, Longone P. Increased expression of the beta3 subunit of voltage-gated Na⁺ channels in the spinal cord of the SOD1G93A mouse. *Mol Cell Neurosci*. 2011; 47:108–118. [PubMed: 21458573]
- Paul P, de Belleruche J. The role of D-serine and glycine as co-agonists of NMDA receptors in motor neuron degeneration and amyotrophic lateral sclerosis (ALS). *Front Synaptic Neurosci*. 2014; 6:10. [PubMed: 24795623]
- Paul P, de Belleruche J. Experimental approaches for elucidating co-agonist regulation of NMDA receptor in motor neurons: Therapeutic implications for amyotrophic lateral sclerosis (ALS). *J Pharm Biomed Anal*. 2015; 116:2–6. [PubMed: 25604957]
- Pieri M, Caioli S, Canu N, Mercuri NB, Guatteo E, Zona C. Over-expression of N-type calcium channels in cortical neurons from a mouse model of Amyotrophic Lateral Sclerosis. *Exp Neurol*. 2013; 247:349–358. [PubMed: 23142186]
- Pieri M, Albo F, Gaetti C, Spalloni A, Bengtson CP, Longone P, Cavalcanti S, Zona C. Altered excitability of motor neurons in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Neurosci Lett*. 2003; 351:153–156. [PubMed: 14623129]
- Pinco M, Lev-Tov A. Synaptic transmission between ventrolateral funiculus axons and lumbar motoneurons in the isolated spinal cord of the neonatal rat. *J Neurophysiol*. 1994; 72:2406–2419. [PubMed: 7884467]

- 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; 9:408–419. [PubMed: 16474388]
- 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; 589:2245–2260. [PubMed: 21486770]
- Rioult-Pedotti MS. Intrinsic NMDA-induced oscillations in motoneurons of an adult vertebrate spinal cord are masked by inhibition. *Journal of neurophysiology.* 1997; 77:717–730. [PubMed: 9065844]
- Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncel RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol.* 1995; 38:73–84. [PubMed: 7611729]
- Saba L, Viscomi MT, Caioli S, Pignataro A, Bisicchia E, Pieri M, Molinari M, Ammassari-Teule M, Zona C. Altered Functionality, Morphology, and Vesicular Glutamate Transporter Expression of Cortical Motor Neurons from a Presymptomatic Mouse Model of Amyotrophic Lateral Sclerosis. *Cerebral cortex.* 2015
- Saba L, Viscomi MT, Caioli S, Pignataro A, Bisicchia E, Pieri M, Molinari M, Ammassari-Teule M, Zona C. Altered Functionality, Morphology, and Vesicular Glutamate Transporter Expression of Cortical Motor Neurons from a Presymptomatic Mouse Model of Amyotrophic Lateral Sclerosis. *Cereb Cortex.* 2016; 26:1512–1528. [PubMed: 25596588]
- Sasabe J, Chiba T, Yamada M, Okamoto K, Nishimoto I, Matsuoka M, Aiso S. D-serine is a key determinant of glutamate toxicity in amyotrophic lateral sclerosis. *EMBO J.* 2007; 26:4149–4159. [PubMed: 17762863]
- Shigeri Y, Seal RP, Shimamoto K. Molecular pharmacology of glutamate transporters, EAATs and VGLUTs. *Brain Res Brain Res Rev.* 2004; 45:250–265. [PubMed: 15210307]
- Shyu BC, Vogt BA. Short-term synaptic plasticity in the nociceptive thalamic-anterior cingulate pathway. *Molecular pain.* 2009; 5:51. [PubMed: 19732417]
- Tadic V, Prell T, Lautenschlaeger J, Grosskreutz J. The ER mitochondria calcium cycle and ER stress response as therapeutic targets in amyotrophic lateral sclerosis. *Front Cell Neurosci.* 2014; 8:147. [PubMed: 24910594]
- Tokuda E, Ono S, Ishige K, Naganuma A, Ito Y, Suzuki T. Metallothionein proteins expression, copper and zinc concentrations, and lipid peroxidation level in a rodent model for amyotrophic lateral sclerosis. *Toxicology.* 2007; 229:33–41. [PubMed: 17097207]
- Tortarolo M, Grignaschi G, Calvaresi N, Zennaro E, Spaltro G, Colovic M, Fracasso C, Guiso G, Elger B, Schneider H, Seilheimer B, Caccia S, Bendotti C. Glutamate AMPA receptors change in motor neurons of SOD1G93A transgenic mice and their inhibition by a noncompetitive antagonist ameliorates the progression of amyotrophic lateral sclerosis-like disease. *J Neurosci Res.* 2006; 83:134–146. [PubMed: 16323214]
- Van Damme P, Leyssen M, Callewaert G, Robberecht W, Van Den Bosch L. The AMPA receptor antagonist NBQX prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis. *Neurosci Lett.* 2003; 343:81–84. [PubMed: 12759169]
- Van Den Bosch L, Van Damme P, Bogaert E, Robberecht W. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica et biophysica acta.* 2006; 1762:1068–1082. [PubMed: 16806844]
- van Zundert B, Peuscher MH, Hynynen M, Chen A, Neve RL, Brown RH Jr, Constantine-Paton M, Bellingham MC. Neonatal neuronal circuitry shows hyperexcitable disturbance in a mouse model of the adult-onset neurodegenerative disease amyotrophic lateral sclerosis. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2008; 28:10864–10874. [PubMed: 18945894]
- Wang R, Zhang D. Memantine prolongs survival in an amyotrophic lateral sclerosis mouse model. *Eur J Neurosci.* 2005; 22:2376–2380. [PubMed: 16262676]
- Wolpaw JR, Tennissen AM. Activity-dependent spinal cord plasticity in health and disease. *Annual review of neuroscience.* 2001; 24:807–843.

- Wootz H, Fitzsimons-Kantamneni E, Larhammar M, Rotterman TM, Enjin A, Patra K, Andre E, Van Zundert B, Kullander K, Alvarez FJ. Alterations in the motor neuron-renshaw cell circuit in the Sod1(G93A) mouse model. *J Comp Neurol*. 2013; 521:1449–1469.
- Wright EB. Effect of mephenesin and other depressants on spinal cord transmission in frog and cat. *Am J Physiol*. 1954; 179:390–401. [PubMed: 13218179]
- Zucker RS. Short-term synaptic plasticity. *Annual review of neuroscience*. 1989; 12:13–31.
- Zucker RS, Regehr WG. Short-term synaptic plasticity. *Annual review of physiology*. 2002; 64:355–405.

Highlights

1. Hyperexcitability was observed in motor outputs due to diminished sSTD in the spinal motor system of mSOD1^{G93A} mice
2. Spinal network- and NMDAR-dependent oEPSPs were first observed and were hyperactive in mSOD1^{G93A} MNs
3. Fast EPSPs were found to be similar between mSOD1^{G93A} MNs and NT MNs at P50 - 90.

Figure 1.

Difference in the sSTD between mSOD1^{G93A} and NT mice. A. Two mouse examples, one from a NT and another from an mSOD1^{G93A} mouse, show the responsive patterns of coAPs evoked by five pulse stimuli at 25 Hz and at three stimulating intensities as indicated. Note: the original data are truncated and expanded to enhance the resolution of coAPs. B. Histograms display the sSTDs in the two types of mice. The averaged amplitudes of coAPs evoked by P1 are plotted in a as mean mV $\pm$ SEM. The degrees of sSTD and their comparisons between two groups of mice are plotted in b as mean % $\pm$ SEM. The significant differences in sSTD between the mSOD1^{G93A} mice and their NT littermates are shown by the asterisks (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$; post hoc t-test).

Figure 2.

Spontaneous synaptic activities in MNs between mSOD1^{G93A} and NT mice. A. Examples show individual mono-EPSPs selected at different amplitudes (a: minimum; b: mean; c: maximum) and poly-EPSPs (d) observed in mSOD1^{G93A} and NT MNs. Note: the mono-EPSPs in a are averages of five EPSPs for better resolution. B. Two examples show the spontaneous oEPSPs recorded in an mSOD1^{G93A} MN and a NT MN. The expanses indicated by numbers exhibit the oscillation. The start and end of the oEPSPs are indicated by arrows. C. The histograms display averaged amplitudes (a) and AUC (b) of individual

EPSPs and averaged AUCs of oEPSPs (c) recorded in mSOD1^{G93A} and NT MNs. The data are presented as mean $\pm$ SEM. The scattered plot in d illustrates the occurrence of oEPSPs during the recording in each MN (p : calculated by Mann-Whitney U test).

Figure 3.

Mechanisms under the spontaneous oEPSPs. A. An example shows the inhibitory effect of mephenesin on the spontaneous oEPSPs in an mSOD1^{G93A} MN. The inhibitory effect of mephenesin is shown in the time course (top rows), of which oEPSPs and poly-EPSPs, indicated by inset numbers, are sampled and detailed in the bottom row for better time resolution. B. An example recording displays the effect of APV on blocking the spontaneous oEPSPs in another mSOD1^{G93A} MN. The data are plotted in the same way as in A to show the inhibitory effect of APV on sampled oEPSPs and EPSPs.

Figure 4.

Comparison of the evoked oEPSPs between mSOD1^{G93A} and NT MNs. A. Sample recordings display the simultaneously evoked coAPs on a ventral root and oEPSPs in an mSOD1^{G93A} MN. In a, the top rows, under each listed intensity, show continuous root recordings with the triangles indicating the stimuli while the bottom rows display the evoked coAPs indicated by the triangles. In b, the top rows are the intracellular recordings from the same mSOD1^{G93A} mice, in which the arrows indicate the stimuli. The bottom rows display the selected oEPSPs in the shadow boxes of the top rows. Note: the bottom plots in a and b

are expanded in time and amplitude for better resolution. B. Sample recordings display the same tests as shown in A for a NT mouse.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 5.

Mechanisms under the evoked oEPSPs. A. An example shows the inhibitory effect of mephenesin on the evoked oEPSPs in an mSOD1^{G93A} MN. In a, evoked oEPSPs in the control were mostly blocked during the application of 1 mM mephenesin with partial recovery during wash. Selected EPSPs, indicated by arrows, and oEPSPs indicated by shadow boxes, are expanded in b. Note: 1. The two selected EPSPs are plotted at the same voltage scale; 2. The resting membrane potential was hyperpolarized by a DC current injection. B. An example shows the inhibitory effect of APV on the evoked oEPSPs in another mSOD1^{G93A} MN. Note: The APs in this MN are truncated and no DC current was injected.

Figure 6.

The inhibitory effects of riluzole and low Ca^{2+} ACSF on the evoked synaptic responses. A. Sample responses of the evoked EPSPs to riluzole treatment in an mSOD1^{G93A} MN. The APs are truncated on the top row, the evoked oEPSPs marked by the boxes are expanded and overlapped on the right. The evoked APs and fast EPSPs in the shadows are expanded and overlapped on bottom. B. Sample responses of the evoked EPSPs to low Ca^{2+} ACSF in another mSOD1^{G93A} MN. The APs on the top row are truncated. The detailed EPSPs and APs are expanded on the bottom row.

Figure 7.

Composition of spontaneous EPSPs. A. Responses of spontaneous EPSPs in a MN to antagonists strychnine, picrotoxin, and DNQX. The top rows plot EPSP amplitudes that are larger than 1 mV and collected in 20 seconds. The curves on the bottom rows are sample EPSPs. B. Responses of IPSPs and EPSPs in an unidentified ventral horn neuron to the same drug treatment. Note: stry. = strychnine, pic.= picrotoxin.

Table 1

Statistics of the coAPs in each group evoked at different intensities.

NT	P1	P2	P3	P4	P5
1.5X	21.29 ± 3.67	9.53 ± 2.11	4.39 ± 1.01	2.77 ± 0.65	1.46 ± 0.36
2X	24.73 ± 4.24	12.19 ± 2.74	9.06 ± 2.36	5.07 ± 1.13	3.96 ± 0.87
5X	25.46 ± 4.26	14.28 ± 3.48	10.21 ± 2.65	7.70 ± 2.20	4.48 ± 1.14
mSOD1 ^{G93A}	P1	P2	P3	P4	P5
1.5X	19.37 ± 4.13	15.18 ± 3.30	8.00 ± 1.87	4.54 ± 1.29	3.38 ± 1.43
2X	19.25 ± 4.63	14.74 ± 3.38	9.24 ± 2.16	7.58 ± 1.97	4.57 ± 1.24
5X	21.60 ± 4.31	15.96 ± 3.03	11.19 ± 2.29	8.25 ± 2.07	5.99 ± 1.53

Note:

¹The coAP values were expressed as mean mV ± SEM.

²Comparisons were made in coAPs between P1 and P2 – 5.

³P 0.01 for all comparisons calculated by Wilcoxon signed-rand test.