Reduced GABAergic Inhibition Explains Cortical Hyperexcitability in the Wobbler Mouse Model of ALS

Jose Luis Nieto-Gonzalez1, Jakob Moser2, Martin Lauritzen3,4, Thomas Schmitt-John2 and Kimmo Jensen1,5

1Synaptic Physiology Laboratory, Department of Physiology and Biophysics, 2Neurogenetics Group, Department of Molecular Biology, Aarhus University, DK-8000 Aarhus C, Denmark, 3Department of Neuroscience and Pharmacology and Center for Healthy Aging, University of Copenhagen, DK-2200 Copenhagen N, Denmark, 4Department of Clinical Neurophysiology, Glostrup Hospital, DK-2600 Glostrup, Denmark and 5Center for Psychiatric Research, Aarhus University Hospital, DK-8240 Risskov, Denmark

Address correspondence to Kimmo Jensen, Synaptic Physiology Laboratory, Department of Physiology and Biophysics, Building 1160, Aarhus University, DK-8000 Aarhus C, Denmark. Email: kimmo@fi.au.dk.

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of the central nervous system. Symptomatic and pre-symptomatic ALS patients demonstrate cortical hyperexcitability, which raises the possibility that alterations in inhibitory gamma-aminobutyric acid (GABA)ergic system could underlie this dysfunction. Here, we studied the GABAergic system in cortex using patch-clamp recordings in the wobbler mouse, a model of ALS. In layer 5 pyramidal neurons of motor cortex, the frequency of GABA$_A$ receptor-mediated spontaneous inhibitory postsynaptic currents was reduced by 72% in wobbler mice. Also, miniature inhibitory postsynaptic currents recorded under blockade of action potentials were decreased by 64%. Tonic inhibition mediated by extrasynaptic GABA$_A$ receptors was reduced by 87%. In agreement, we found a decreased density of parvalbumin- and somatostatin-positive inhibitory interneurons and reduced vesicular GABA transporter immunoreactivity in the neuropil. Finally, we observed an increased input resistance and excitability of wobbler excitatory neurons, which could be explained by lack of GABA$_A$ receptor-mediated influences. In conclusion, we demonstrate decreases in GABAergic inhibition, which might explain the cortical hyperexcitability in wobbler mice.

Keywords: GABA, layer 5, neocortex, patch-clamp, pyramidal cell

Introduction

In amyotrophic lateral sclerosis (ALS), neurons of the motor cortex, brain stem, and spinal cord are affected both in human patients and in animal models (Eisen and Weber 2001; Janette et al. 2002b; Boillée et al. 2006). The resulting upper motor neuron affection leads to spasticity and loss of motor control, whereas affection of lower motor neurons causes denervation of skeletal muscles, progressive muscular weakness, and eventually death. Despite intensive research, no effective cure is available, but there is progress in patient treatments, including respiratory support, multidisciplinary care, nutrition, and neuroprotection with riluzole. However, using animal models, some increased understanding has been gained regarding sporadic ALS pathophysiology. One such model is the wobbler mouse (Duchen and Strich 1968), and the recessive wobbler mouse (Duchen and Strich 1968), and the recessive gene leading to an amino acid replacement (Q967L) in the C-terminal domain of the corresponding Vps54 protein causing motor neuron degeneration. Vps54 protein is a vesicle-tethering factor known to be involved in the retrograde vesicle traffic from early and late endosomes to the trans Golgi network. Thus, the wobbler mouse associates ALS-like motor neuron degeneration with retrograde vesicle transport.

Diagnostic criteria for ALS require the simultaneous occurrence of both upper and lower motor neurons signs in the bulbar region and 2 spinal regions for clinical diagnosis (de Carvalho et al. 2008). Upper motor neuron signs in patients include spasticity and hyperreflexia, but the pathophysiology of upper motor neuron involvement is incompletely understood. However, ALS patients demonstrate cortical hyperexcitability (Caramia et al. 2000; Vucic et al. 2008), thought to be caused by changes in the intrinsic membrane properties of neurons (Pieri et al. 2009), or by an unfavorable tipping of the balance of neurotransmitter influences toward more excitation or less inhibition (Zieman et al. 1997; Zanette et al. 2002a). However, the latter possibility has not been investigated at the single-cell level.

In this study, we examined gamma-aminobutyric acid (GABA)-mediated inhibition in the motor cortex of the wobbler mouse model of ALS. GABA is the major inhibitory neurotransmitter acting at GABA$_A$ receptors in 2 general fashions: 1) "phasic" inhibition mediated by rapid synaptic transmission and 2) a sustained type of "tonic" inhibition mediated by elevated ambient levels of extracellular GABA, activating extrasynaptic GABA$_A$ receptors (Chiu et al. 2005; Farrant and Nusser 2005). The GABAergic system not only orchestrates the brain rhythms in neuronal networks but also is thought to set the background excitability of single neurons (Walker and Semyanov 2008).

The aim of our study was to investigate whether the excitability is raised in the motor cortex of wobbler mice and whether reduced GABAergic activity could underlie hyperexcitability of pyramidal neurons. Using patch-clamp recordings, we analyzed both the functional and the structural changes of the GABAergic system and the consequence of these changes on the excitability of layer 5 pyramidal neurons. Our findings point to a reduced GABAergic inhibition of pyramidal neurons in wobbler mice, which could explain the increased excitability in cortex in this model of ALS.
Materials and Methods

**Preparation of Brain Slices**
Experiments were conducted according to institutional, national, and EU guidelines for the care and use of laboratory animals and approved by the Faculty of Health Sciences, Aarhus University. Wobbler mice were bred on a C57BL/6J background and kept with a 12:12 h light:dark cycle with unrestricted access to food and water. Offspring was genotyped around postnatal day (P) 8. P15-P25 wobbler (wr/wr; n = 9) and control mice (+/+, +/wr; n = 12) of both sexes were anesthetized with isoflurane. Mice were decapitated, and the brains were dissected out and transferred to ice-cold artificial cerebrospinal fluid (ACSF, in mM): 126 NaCl, 2.5 KCl, 2 CaCl2, 1 MgCl2, 25 NaHCO3, 10 NaH2PO4, and 10 Mg-glutamine (osmolality = 305–315 mosmol/kg), pH 7.4, when bubbled with carbogen (5% CO2-95% O2). Three hundred fifty micrometer coronal slices were cut on a Vibratome 3000 Plus (Vibratome) and rested for 1 h before recording. To improve slice quality, 2 mM l-kynurenine acid, 0.2 mM ascorbic acid, and 0.2 mM pyruvic acid were added during slicing and storage.

**Electrophysiological Recordings**
Slices were perfused with ACSF (35 ± 1 °C, 2.3 µL/min), and layer 5 pyramidal neurons of the motor cortex were visualized by a custom-built infrared microscope (Versascope; E. Marton Electronics), a +/− water immersion objective (Olympus), and a CCD100 camera (DAGE-MTI). Whole-cell patch-clamp recordings were carried out using a MultiClamp 700B Amplifier (Molecular Devices). For current-clamp recordings, the patch-pipettes contained (in mM): 140 CsCl, 0.05 EGTA, and 10 HEPES, adjusted to pH 7.2. During voltage-clamp recordings, the patch-pipettes contained (in mM): 140 CsCl, 0.05 EGTA, and 10 HEPES, adjusted to pH 7.2 with CsOH (280–290 mosmol/kg). Giga seals (>1 GΩ) were always obtained before break-in. Throughout voltage-clamp recordings, the whole-cell capacitance and series resistance were noted and resistances were compensated by 70% (lag 10 µs). Recordings were discontinued if series resistances increased by >50% or exceeded 20 MΩ.

**Data Acquisition and Analysis**
Currents were low-pass filtered (8-pole Bessel) at 3 kHz, digitized at 20 kHz, and acquired using a BNC-2110 A/D converter and a PCl-6014 board (National Instruments) and custom-written LabVIEW 6.1-based software (EVAN v. 1.4, courtesy of I. Mody), which was also used to detect and analyze spontaneous inhibitory postsynaptic currents (sIPSCs) with amplitude detection thresholds of −7 −8 pA. All events were inspected before an average of 50–100 events was made. Amplitude, 10–90% rise time, and frequency were measured, whereas (in mM) 126 NaCl, 2.5 KCl, 2 CaCl2, 1 MgCl2, 25 NaHCO3, 10 NaH2PO4, and 10 Mg-glutamine (osmolality = 305–315 mosmol/kg), pH 7.4, when bubbled with carbogen (5% CO2-95% O2). Three hundred fifty micrometer coronal slices were cut on a Vibratome 3000 Plus (Vibratome) and rested for 1 h before recording. To improve slice quality, 2 mM l-kynurenine acid, 0.2 mM ascorbic acid, and 0.2 mM pyruvic acid were added during slicing and storage.

**Immunohistochemical Procedures**
Mice (P16–P20, control n = 9, wobbler n = 9) were perfused with phosphate-buffered saline (PBS) followed by 4% PFA in PBS and postfixed in the same solution for 2–3 h. After overnight incubation in 30% sucrose in PBS, 40-µm sections were cut according to stereotaxical coordinates between bregma 0 and +1 mm (Paxinos and Franklin 2001) on a Leica CM1900 cryostat (Leica Microsystems A/S). Parvalbumin- and somatostatin-positive neurons were revealed by bright field and vesicular GABA transporter (VGAT) by confocal laser microscopy. Free floating sections were used with antibody incubation times of 18–24 h. Sections were washed in PBS, and endogenous peroxidase activity was blocked with ethanol and incubated for 1 h at room temperature in 3% normal donkey serum and 0.3% Triton X-100 in PBS and then in primary antibody (1:8000 goat anti-parvalbumin, SWANT, 1:8000 mouse monoclonal anti-somatostatin, CURE, UCLA) at 4 °C. The sections were incubated with biotinylated donkey anti-rabbit IgG (1:2000, Jackson) at 4 °C and using the ABC kit for 1 h at room temperature. Peroxidase activity was revealed by 0.02% diaminobenzidine with 0.01% H2O2 and enhanced with 0.04% nickel ammonium sulfate (Nieto-Gonzalez et al. 2009).

Sections processed for VGAT immunohistochemistry were preincubated free floating in blocking solution (3% normal donkey serum with 0.3% Triton X-100 in PBS) at room temperature for 1 h before incubation with the primary antibodies in blocking solution at 4 °C (mouse monoclonal anti-VGAT, 1:500; Synaptic Systems). Sections were incubated with secondary antibody (donkey anti-mouse Alexa568, 1:500; Molecular Probes) at 4 °C and mounted with fluorescence mounting medium (Dako).

**Image Acquisition and Analysis**
Bright field images were captured on a Leica AF6000 LX workstation. To quantify the number of parvalbumin- and somatostatin-positive neurons, we measured neuronal density instead total number because wobbler mice show a thinner cortex than wild type (WT) (Supplementary Figure 1). For VGAT quantification, image stacks were obtained using a confocal microscope (Zeiss LSM 510 META, ×40). ImageJ (National Institutes of Health) was used to calculate mean gray values after background subtraction. All the images were sampled through the neuropil using square boxes of 25 µm (110 pixels) (Davis-Lopez de Carrizosa et al. 2009).

**Solutions and Drugs**
All drugs were from Sigma except pyruvic acid (MP Biomedicals) and tetrodotoxin (TTX) (Alomone). Stock solutions of SR95531 (6 mM) were prepared in ACSF containing 50% dimethyl sulfoxide.

**Results**

**Recordings from Layer 5 Pyramidal Neurons in the Motor Cortex**
To validate the neuronal type identified by infrared illumination, a subset of putative layer 5 pyramidal neurons (n = 17), placed in

**Nonparametric test for 2 independent samples was used to compare means with a 5% significance level. Data are presented as means ± standard error of the mean, with n indicating the number of neurons.**

**Morphological Identification**
A set of pyramidal neurons (n = 19) was intracellularly labeled by including 0.5% biocytin in the internal pipette solution facilitated by depolarizing current steps (0.2–1 nA) of 500 ms at 1 Hz for 10–20 min after recording. Only 2 of the injected neurons were not pyramidal. Slices were transfected to a solution of 4% paraformaldehyde (PFA) at 4 °C overnight and then to 30% sucrose in phosphate buffer at 4 °C overnight. Thereafter, biocytin was revealed by an ABC kit (Vector Laboratories) or with fluorescent biotinylate conjugated avidin–biotin (2 µL/mL; Vector Laboratories). Following a wash, slices were embedded with fluorescent mounting medium (Dako) and visualized on a confocal microscope (Zeiss LSM 510 META) or alternatively dehydrated by serial alcohol, cleared with xylene, and mounted in gelatin-coated slides with a mixture of diastrene, plasticizer and xylene (Fluka).
the motor cortex (M1) according to the stereotaxical atlas (bregma 0 to +1 mm), were patch-clamped and filled with biocytin to reveal their electrophysiological and morphological features (Cho et al. 2004; Zhang 2004; Molnar and Cheung 2006). In current-clamp, neurons with a membrane potential more negative than -55 mV were included and showed a nonbursting or bursting firing pattern (Fig. 1A). The labeled neurons were localized within layer 5 (Fig. 1A) with a typical pyramidal shaped soma (Fig. 1A) and characterized by either thick tufted apical dendrites or slender terminal tufts. This indicates that the 2 major classes of layer 5 pyramidal neurons (Molnar and Cheung 2006) were present in this study. Figure 1B, C shows fluorescent examples of a control and a wobbler layer 5 pyramidal neuron.

**Downregulation of the Frequency of sIPSCs in Wobbler Mice**

In order to test if the spontaneous GABAAergic synaptic inputs in motor cortex are affected in the wobbler mouse, we carried out whole-cell voltage-clamp recordings from layer 5 pyramidal neurons. Using CsCl-filled patch-pipettes, recordings were made at a $V_{\text{hold}}$ of -70 mV in the presence of kynurenic acid (2 mM) to block ionotropic glutamate receptors. Figure 2A, B illustrate sweeps of sIPSCs from a pyramidal neuron from a control and a wobbler mice. Individual sIPSCs in wobbler mice tended to display smaller amplitudes; however, the mean sIPSC amplitude was not statistically different (Fig. 2C) (52.0 ± 8.1 pA in control, $n = 7$, vs. 42.7 ± 8.1 pA in wobbler, $n = 7$, $P > 0.05$). In contrast, the sIPSC frequency was significantly lower in wobbler mice (Fig. 2D, 37.8 ± 8.1 Hz in control, $n = 7$, vs. 10.5 ± 4.3 Hz in wobbler, $n = 7$, $P < 0.01$), that is, a reduction to 28% of the control level.

**Reduced Frequency of Miniature Inhibitory Postsynaptic Currents in Wobbler Mice**

We then performed whole-cell recordings of miniature inhibitory postsynaptic currents (mIPSCs) in layer 5 pyramidal neurons in the presence of TTX (1 μM) in order to block action potentials (Fig. 3A). To analyze the waveform of mIPSCs, average mIPSCs in each cell were made (Fig. 3B). No differences were found in mIPSC amplitude or rise time (10–90%) (Fig. 3C, D) between control (27.1 ± 1.1 pA, 325 ± 19 μs, $n = 10$) and wobbler mice (25.5 ± 1.9 pA, 322 ± 23 μs, $n = 10$). However, a significant increase was found in the weighted decay time constant (Fig. 3E) (3.9 ± 0.23 ms for control, $n = 10$, vs. 5.8 ± 0.45 ms for wobbler, $n = 10$, $P < 0.001$). In addition, the mIPSC frequency (Fig. 3F) was decreased from 16.2 ± 1.0 Hz for

**Figure 1.** Morphological identification of layer 5 pyramidal neurons in mouse motor cortex. (A) Photomicrograph showing the localization and higher magnification details of a layer 5 pyramidal neuron labeled with biocytin. The inset depicts the firing pattern of the injected layer 5 pyramidal neuron. (B and C) Morphology of a control and wobbler neuron injected with biocytin revealed by avidin–fluorescein isothiocyanate.
control (n = 10) to 5.8 ± 0.7 Hz for wobbler (n = 10, P < 0.001), that is, a reduction to 36% of control. TTX produced a stronger reduction of sIPSCs frequency in control than in wobbler mice. TTX reduced the sIPSC frequency by 57.1% in control (or by 21.6 Hz) but only by 44.8% in wobbler (by 4.7 Hz).

Wobbler Mice Show Reduced Numbers of Parvalbumin- and Somatostatin-Positive Neurons and Decreased VGAT Immunoreactivity

Previously, a decrease in the number of parvalbumin-positive neurons in the cortex of ALS patients has been reported (Nihei et al. 1993). To test if this was also the case in this mouse model of ALS, we carried out immunohistochemistry against parvalbumin, somatostatin, and the VGAT, a marker of GABAergic terminals, assessed in 3 animals in each group including 15 alternative slices. These stainings could quantitatively indicate whether our electrophysiological results were associated with structural changes in the GABAergic system. In regions of interest of 200 × 500 μm placed in layer 5 of primary motor cortex, wobbler mice showed a decrease in the density of parvalbumin-positive neurons (control 43.73 ± 2.05 cells/0.1 mm² and wobbler 31.93 ± 1.34 cells/0.1 mm², P < 0.05; Fig. 4A,B,G) and somatostatin-positive neurons (control 18.05 ± 2.1 cells/0.1 mm² and wobbler 11.35 ± 1.93 cells/0.1 mm², P < 0.05; Fig. 4C,D,G). We also observed a decrement in the optical density of GABAergic synaptic boutons, as evaluated by the VGAT staining (control 23.86 ± 1.53 counts per pixel (cpp) and wobbler 15.45 ± 0.8 cpp, P < 0.05; Fig. 4E-G). These findings may be associated with the reduction in synaptic GABA release found above.

Wobbler Mice Show a Reduction in Tonic GABA<sub>A</sub> Receptor-Mediated Currents

To examine the contribution of extrasynaptic GABA<sub>A</sub> receptors to the inhibition of layer 5 pyramidal neurons, we measured tonic GABA<sub>A</sub> receptor-mediated currents in presence of kynurenic acid (2 mM) in control and wobbler mice (Fig. 5). Injection of the GABA<sub>A</sub> antagonist SR95531 (>100 μM) into the slice chamber blocked the sIPSCs and revealed an outward shift in the holding
The shift in the baseline noise is illustrated by the Gaussian fit to an all-points histogram (middle panels) obtained from the recording shown to the left. Right panels illustrate the time course of the change in the holding current in response to SR95531. For quantification, we normalized the SR95531-sensitive current to the cell capacitance (Fig. 5C, significantly smaller in wobbler mice, 42.76 ± 1.04 pF and 37.26 ± 0.78 pF, respectively, P < 0.05). This resulted in average tonic current densities (pA/pF), which were 2.77 ± 0.96 pA/pF (n = 8) for control and 0.99 ± 0.30 pA/pF (n = 8) for wobbler mice. The significant differences were seen in decay time constant (increased by 47%) and mIPSC frequency (decreased by 64%) for control and wobbler mice (P < 0.05).
0.35 ± 0.17 pA/pF (n = 6) for wobbler mice (Fig. 5D), that is, a reduction by 87% (P < 0.05).

Layer 5 Pyramidal Neurons of Wobbler Mice Show Increased Excitability

As presented above, wobbler mice show a downregulation in both the phasic and the tonic GABA<sub>A</sub> receptor–mediated inhibition in the neocortex. We speculated that this might lead to an increased excitability of the pyramidal neurons (Vucic et al. 2008). Therefore, we measured the input resistance in current-clamp recordings from layer 5 pyramidal neurons in control and wobbler mice (Fig. 6A,B). The neurons showed a resting membrane potential of −64.7 ± 1.2 mV for control (n = 9) and −68.2 ± 2.6 mV for wobbler (n = 5, P > 0.05). Injection of current pulses evoked membrane potential deflections that differed between control and wobbler mice. Figure 6A,B shows the response of 2 layer 5 pyramidal neurons

![Image of Layer 5 Pyramidal Neurons](https://example.com/image.png)

**Figure 4.** Reduced immunoreactivity for parvalbumin and somatostatin neurons and VGAT in wobbler mice. (A–D) Immunohistochemistry for parvalbumin and somatostatin neurons indicating a decreased number in layer 5 of wobbler mouse neocortex. (E and F) VGAT immunohistochemistry indicating a reduced number of synaptic GABAergic boutons in the neuropil of wobbler mouse. (G) Histograms depicting the quantitative changes in the number of parvalbumin- and somatostatin-positive neurons and optical density of VGAT in the neuropil.
to pulses of similar amplitude. The input resistance was larger in the wobbler mouse neurons (117.4 MΩ) compared with the exemplar control neuron (77.7 MΩ). In addition, the current threshold, that is, the minimal injected current to evoke an action potential, was significantly lower in wobbler mice (120.0 ± 21.9 pA, n = 5) compared with controls (204.4 ± 10.4 pA, n = 8, P < 0.05), whereas no change was found in the depolarization voltage between control (27.16 ± 1.53 mV, n = 5) and wobbler (27.45 ± 2.06 mV, n = 8, P > 0.05), indicating that the layer 5 pyramidal neurons in wobbler mice are more excitable due to an increased input resistance. To examine whether this increase in excitability could be related to the defective GABA<sub>A</sub> function, we measured the input resistance in presence of the GABA<sub>A</sub> antagonist picrotoxin (50 μM). The

**Figure 5.** Reduced tonic GABA<sub>A</sub>-mediated inhibition in wobbler mouse. (A and B) Whole-cell recordings of tonic GABA<sub>A</sub> receptor-mediated current (left panel) in presence of kynurenic acid. Injection of the GABA<sub>A</sub> receptor antagonist SR95531 (>100 μM) into the recording chamber blocked the sIPSCs and revealed a tonic current in control and wobbler mice. The middle panel illustrates all-points histogram of the traces in the left panel. The right panel illustrates the time course of the blockade of the tonic current by SR95531 in control and wobbler neurons. (C) Scatter plot depicting the cell capacitances in control and wobbler mice. The average cell capacitance was significantly different (P < 0.05). (D) Histogram showing the significant decrease in the wobbler mouse tonic current when normalized to cell capacitance to calculate the current density (P < 0.05).
resting membrane potential of the neurons in presence of picrotoxin was $-67.8 \pm 1.9$ mV for control ($n = 5$) and $-65.1 \pm 1.2$ mV for wobbler ($n = 9$). In agreement with GABA$_A$-mediated inhibition having important effects on excitability, picrotoxin converted the input resistance of control neurons into that of wobbler neurons, whereas picrotoxin had no significant effect on wobbler neurons per se. Figure 6F illustrates the average input resistance for the control without picrotoxin ($76.9 \pm 4.0$ MΩ, $n = 8$) and with picrotoxin ($142.9 \pm 18.9$ MΩ, $n = 5$, $P < 0.01$) and the wobbler mouse without picrotoxin ($132.1 \pm 18.1$ MΩ, $n = 5$) and with picrotoxin ($145.4 \pm 13.5$ MΩ, $n = 9$, $P > 0.05$). This indicates that GABA$_A$ receptor-mediated influences decrease the input resistance of layer 5 pyramidal neurons in control but not in wobbler neurons.

Discussion

In an animal model of ALS, the wobbler mouse, we have reported that neocortical layer 5 pyramidal neurons show increased excitability. Also, we found a downregulation in both action potential-dependent and -independent release of GABA onto the pyramidal neurons. In addition, tonic inhibition mediated by presumed extrasynaptic GABA$_A$ receptors was decreased. Measurements of current threshold for action potentials and input resistance revealed that the increased excitability in layer 5 pyramidal neurons could be explained by the decrease in GABAergic inhibition. In agreement with these findings, immunoreactivity for parvalbumin, somatostatin, and VGAT was reduced in the neocortex. Thus, in this first report of cortical single-cell electrophysiology in a mouse model of ALS, the GABAergic system is downregulated, which could explain the hyperexcitability in presymptomatic ALS patients.

The Wobbler Mouse: A Model of ALS

The wobbler mouse closely resembles sporadic ALS in many respects and thus is a valuable ALS model. For example, it resembles ALS with regard to tumor necrosis factor involvement (Bigini et al. 2008), mitochondrial dysfunction (Santoro et al. 2004), progesterone-induced preserved Na,K-ATPase (Gonzalez Deniselle et al. 2002), various ALS-specific inclusions (van Welsel et al. 2002), and ubiquitinylated aggregates (Ishigaki et al. 2004). The recent observation in wobbler mice of TDP-43 and ubiquitin changes characteristic of sporadic ALS (Dennis and Citron 2009), which are not observed in superoxide dismutase (SOD)1 model mice, further argue that wobbler is a valid ALS animal model. In addition, our finding of cortical hyperexcitability demonstrates a further similarity to human ALS. Although we would argue that the wobbler mouse is a very useful ALS model, until now, no mutation in Vps54 in human patients has been found, and therefore, the wobbler's relevance for genetic or familial ALS should be interpreted with great care.

Phasic Inhibition Is Reduced in Wobbler Mice

In a recent study, Vucic and coworkers (2008) showed that cortical hyperexcitability develops prior to clinical symptoms in ALS. Although there are several possible explanations for this finding (Kierman 2009), the cortical hyperexcitability could be caused by a defective GABAergic system. In the wobbler mice homozygous for the mutation, the first motor defects appear at the juvenile stages (3–4 weeks old) and stabilize in older animals (3–5 months old) (Boillée et al. 2003). When we examined the GABAergic system in layer 5 pyramidal neurons in 2- to 3-week-old mice, we found a decrease in the frequency of spontaneous GABA release, and the fraction of TTX-sensitive (action potential-driven) IPSCs appeared to be small in wobbler mice (4.7 vs. 21.6 Hz in WT). Whereas our data point to a reduced GABAergic activity in wobbler mice, this could be explained by a reduction in the activity in individual interneurons or a decreased number of interneurons and their synaptic boutons or a combination. The decrease in the frequency of mIPSCs, and the reduction in parvalbumin and VGAT immunoreactivity, points to a significant loss of GABAergic interneurons and their synaptic boutons in wobbler mice. Interestingly, ALS patients also show a decreased number of parvalbumin-positive interneurons in the cortex (Nihei et al. 1993) similar to wobbler mice (current data).

The significant prolongation of the mIPSC decay might represent a compensatory mechanism to regain the inhibitory synaptic strength following the reduction in both phasic and tonic inhibition. Compensatory upregulation of one type of inhibition, due to a loss of another, has been reported previously (Ortinski et al. 2006). The precise mechanism underlying the slower IPSC kinetics is currently not clear, but possible explanations include an altered receptor subunit composition in the postsynaptic membrane in wobbler mouse neurons or differences in the location of interneuron–pyramidal cell synapses and the size of the synaptic cleft (Evers et al. 1989). Change in GABA uptake (by GABA transporter 1) is less likely to modify the decay kinetics of small quantal GABA responses (Jensen et al. 2003).

Interestingly, changes in GABA$_A$ receptor subunit composition have been described in lower motor neurons in another model of ALS, the transgenic SOD-G93A mouse (Carunchio et al. 2008). SOD-G93A neurons show an increase in the expression of α1 subunits of the GABA$_A$ receptor, and an increased affinity for GABA, leading to a higher Cl$^-$ influx into the neurons. The authors argued that this Cl$^-$ influx could cause cell swelling and potentially be harmful to the spinal motor neurons in ALS conditions (Carunchio et al. 2008).

Tonic Inhibition Is Reduced in Wobbler Mice

Tonic GABA$_A$ receptor–mediated inhibition can be enhanced by elevating the extracellular GABA levels, which is believed to activate extrasynaptic GABA$_A$ receptors (Farrant and Nusser 2005). Accordingly, in neocortex, tonic inhibition can be enhanced by blocking GABA uptake via GABA transporters (Keros and Hablitz 2005) or knocking out GAT1 (GABA transporter 1) (Bragina et al. 2008), leading to elevated ambient cortical GABA levels. Neocortical pyramidal neurons have been shown to express typical extrasynaptic GABA$_A$ receptors containing γ2 subunit (Drasbek and Jensen 2006) and γ2 subunit combinations (Scimemi et al. 2006). It is also clear that the tonic inhibition in neocortex is cell type specific (Vardya et al. 2008).

In the present study, we found that layer 5 pyramidal neurons of the neocortex of young wobbler mice showed a strong decrease in tonic inhibition. This decrease might be explained by a loss of extrasynaptic GABA$_A$ receptors or a significant reduction in GABA release. It is known from other brain regions that the synaptic GABA release can control the magnitude of tonic inhibition and is correlated to the activity of GABAergic interneurons. Thus, in the hippocampus, the sIPSC frequency is
correlated to the magnitude of tonic inhibition (Glykys and Mody 2007), and in the thalamus, the action potential blocker TTX reduces tonic GABA inhibition (Bright et al. 2007). Thus, the most obvious explanation for our results is that the decrease in synaptic GABA release in wobbler mice might lead to lower ambient GABA levels and to a reduced tonic inhibition.

Figure 6. Pyramidal neurons in wobbler mice show an increased input resistance and loss of GABA, receptor–mediated influence. (A–D) Voltage membrane response to negative and positive current pulses (increments 40 pA) for control and wobbler mice in the absence and presence of picrotoxin (50 μM). As shown, current pulses of the same amplitudes evoked larger voltage deflections in the wobbler neurons than in control in absence of picrotoxin. (E) Plots illustrating the current/voltage relationship to determine the input resistance for the 2 layer 5 pyramidal neurons illustrated in [A] and [B]. (F) Histogram summarizing the differences between control and wobbler mice in absence and presence of picrotoxin.
Layer 5 Pyramidal Neurons Are More Excitable in Wobbler Mice

ALS patients show a hyperexcitability in the cortex (Eisen and Weber 2001; Vucic et al. 2008). In mouse models of ALS, hyperexcitability in the spinal cord and in cultured cortical neurons has been shown to arise from an increase in persistent Na\(^+\) currents (Pier et al. 2009). On the other hand, in our study, we found that a decreased GABAergic inhibition could account for the hyperexcitability of the layer 5 pyramidal neurons. Indeed, when we measured excitability parameters, an increased excitability in wobbler mice was seen without change in depolarization voltage and picrotoxin changed the electrophysiological characteristics of control mouse neurons to resemble wobbler neurons. This indicates that changes in activity of the GABA\(_A\) receptor system can modify intrinsic parameters of excitability in the cells. We conclude that decreased GABAergic inhibition can explain the cortical hyperexcitability in wobbler mice.

Functional Consequences

Since cortical hyperexcitability appears to be a common and early feature in ALS (Vucic and Kiernan 2006; Vucic et al. 2009), this finding has supported the idea that the upper motor neuron dysfunction leads to anterograde (dying-forward) transneuronal degeneration of the anterior horn cells due to excitotoxicity (reviewed by Eisen and Weber 2001 and Kiernan 2009). It is attractive to speculate on a possible link between the motoneuronal degeneration and the loss of inhibitory activity (present data; Nihei et al. 1993). If the decrease in inhibition could drive the pyramidal neurons to release glutamate into spinal regions, perhaps in conjunction with a dysfunction of the glutamate uptake by astrocytes (Shaw 2005), it could provide support for the dying-forward hypothesis in ALS. However, future studies are necessary to demonstrate this.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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Conflict Of Interest: None declared.

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