Respiratory impairment in a mouse model of amyotrophic lateral sclerosis

Clarke G. Tankersley,1 Christine Haenggeli,2 and Jeffery D. Rothstein2,3

Departments of 1Environmental Health Sciences, 2Neurology, and 3Neuroscience, Bloomberg School of Public Health and School of Medicine, The Johns Hopkins University, Baltimore, Maryland

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Tankersley CG, Haenggeli C, Rothstein JD. Respiratory impairment in a mouse model of amyotrophic lateral sclerosis. J Appl Physiol 102: 926–932, 2007. First published November 16, 2006; doi:10.1152/japplphysiol.00193.2006.—Amyotrophic lateral sclerosis (ALS) is a progressive, lethal neuromuscular disease that is associated with the degeneration of cortical and spinal motoneurons, leading to atrophy of limb, axial, and respiratory muscles. Patients with ALS invariably develop respiratory muscle weakness and most die from pulmonary complications. Overexpression of superoxide dismutase 1 (SOD1) gene mutations in mice recapitulates several of the clinical and pathological characteristics of ALS and is therefore a valuable tool to study this disease. The present study is intended to evaluate an age-dependent progression of respiratory complications in SOD1G93A mutant mice. In each animal, baseline measurements of breathing pattern [i.e., breathing frequency and tidal volume (VT)], minute ventilation (VE), and metabolism (i.e., oxygen consumption and carbon dioxide production) were repeatedly sampled at variable time points between 10 and 20 wk of age with the use of whole-body plethysmographic chambers. To further characterize the neurodegeneration of breathing, VE was also measured during 5-min challenges of hypercapnia (5% CO2) and hypoxia (10% O2). At baseline, breathing characteristics and metabolism remained relatively unchanged from 10 to 14 wk of age. From 14 to 18 wk of age, there were significant (P < 0.05) increases in baseline VT, VE, and the ventilatory equivalent (VE/oxygen consumption). After 18 wk of age, there was a rapid decline in VE due to significant (P < 0.05) reductions in both breathing frequency and VT. Whereas little change in hypoxic VE responses occurred between 10 and 18 wk, hypercapnic VE responses were significantly (P < 0.05) elevated at 18 wk due to an augmented VT response. Like baseline breathing characteristics, hypercapnic VE responses also declined rapidly after 18 wk of age. The phenotypic profile of SOD1G93A mutant mice was apparently unique because similar changes in respiration and metabolism were not observed in SOD1 controls. The present results outline the magnitude and time course of respiratory complications in SOD1G93A mutant mice as the progression of disease occurs in this mouse model of ALS.

control of breathing; respiratory muscles; neurodegeneration

AMYOTROPHIC LATERAL SCLEROSIS (ALS) is a fatal motoneuron disease characterized by the selective and progressive loss of neurons in the motor cortex, brain stem, and spinal cord, leading to atrophy of target muscles. The total prevalence of ALS has been estimated to involve 3–6 persons in 100,000, and the disease is fatal in 1–5 yr after symptom onset, with respiratory paralysis being the primary cause of death (6, 11, 27). Severe diaphragmatic muscle weakness is the cause of respiratory problems in ALS patients and is also the principal cause of death (6). Therefore, recovery of respiratory neuromuscular function is critical to patient care. Consequently, therapies that specifically target respira-

ory motoneuron pools could potentially slow down, halt, or reverse respiratory dysfunction. Currently, preventative therapy in targeting respiratory motoneurons has shown limited success in ALS patients (2).

One significant limitation in human studies is the inability to predict the onset of disease before the degeneration of a large proportion of motoneurons. The disease is largely sporadic, but a familial form accounts for 1–2% of all cases based on mutations of the superoxide dismutase 1 (SOD1) gene. Overexpression of the SOD1 gene mutation in mice recapitulates the clinical and pathological characteristics of ALS (35). Therefore, these mice represent an important research tool in attempting to understand motoneuron biology and neurodegeneration. The SOD1G93A mutant mouse shows progressive loss of muscle function beginning with hindlimb dysfunction at ~90 days of age and ending in death between 120 and 140 days of age (24). Therefore, the purpose of this study is to noninvasively measure respiratory functional changes in SOD1G93A mutant mice as a predictive model of the familial form of ALS. Repeated measurements have allowed us to establish changes in the magnitude and pattern of breathing in mutant SOD1G93A mice at different stages of the disease (before disease onset, at early stages of the disease, and in advanced stages). In addition, mild chemical stimulation of breathing (i.e., hypercapnia and hypoxia) was performed to increase ventilation. The phenotypic profile established in the present study is the basis for evaluating benefits of therapeutic viral vector delivery of neuroprotective factors to respiratory motoneuron pools (13).

METHODS

Animals. In this study, transgenic mice overexpressing the human SOD1 gene with the G93A mutation [B6SJL-TgN(SOD1-G93A)1Gur] (n = 14 mice) were used, and transgenic mice carrying the normal allele of the human SOD1 gene were used as controls (n = 8 mice). All mice were purchased from Jackson Laboratory (Bar Harbor, ME), and the mice were studied between 10 and 20 wk of age. The animals were housed at the Johns Hopkins University Bloomberg School of Public Health, and water and mouse chow (Agway Pro-Lab RMH 1000) were provided ad libitum. All animal protocols were reviewed and approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutes.

Measurements of metabolic rate. Oxygen consumption (V˙O2) and carbon dioxide production (V˙CO2) were measured with a commercially available indirect open-circuit calorimetric system (Oxymax Deluxe; Columbus Instruments, Columbus, OH) in-line with 200-ml cylindrical Plexiglas chambers. Unhumidified compressed air was delivered through the chamber, under the control of a calibrated flow meter. The flow was adjusted to maintain a small difference between

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chamber inflow (21% O₂ in N₂) and outflow oxygen concentrations. The air flow out of the chamber was dried by a column of anhydrous CaSO₄ and was sampled for 30 s for fractional concentrations of O₂ and CO₂ using a limited-diffusion O₂ sensor and nondispersive infrared CO₂ sensor (Columbus Instruments). Sensor output was transmitted to a dedicated computer operated by data-acquisition software (Oxymax version 5.3; Columbus Instruments) for on-line computation and display of metabolic parameters. Gas-analyzer calibrations were conducted before each experiment using gas mixtures standardized by the manufacturer (Puritan Bennett, Linthicum Heights, MD). Reference air measurements were obtained intermittently to correct for sensor drift. Finally, VO₂ and VCO₂ data were normalized to standard temperature, pressure, and dry conditions (STPD) and standardized as a function of body weight.

*Total body plethysmography.* Whole-body plethysmography was used to repeatedly measure breathing frequency (f) and tidal volume (VT) in each animal. The theoretical basis for this method is based on the physical principle that, within a closed chamber, the volume of inspired air expands as the air is warmed and wetted and contracts on expiration as the air is cooled and dried (18). The animal was contained in the 200-ml cylindrical chamber using two rubber stoppers with openings allowing for a pressure transducer, a thermistor, and an outflow to allow air to pass through the chamber. Compressed air entered the top of the chamber through a port at a flow rate of 560 ml/min. As the animal became quiescent, the chamber was sealed with stopcocks that extended from the inflow and outflow ports to obtain measurements of f and VT. Cessation of air flow did not exceed 60 s.

*Exposure to hypoxic and hypercapnic challenge.* Hypoxic and hypercapnic challenges were used to chemically stimulate increases in ventilation and to determine changes in respiratory control mechanisms. After the magnitude and pattern of breathing were measured at baseline, hypercapnic ventilatory responses were evaluated after an acute (5 min) challenge to an inspired air mixture containing fractional CO₂ = 0.05 and fractional O₂ = 0.21 in N₂. The inspired gas mixture was returned to baseline, and ventilation measurements were repeated after a 20-min reacclimation period. A second inspirate challenge tested the response to hypoxia using fractional CO₂ = 0.00 and fractional O₂ = 0.10 in N₂. Each animal was returned to the cage after hypoxic exposure.

*Data analysis.* Because these experiments were noninvasive, we conducted repeated measurements in the same animals at variable time points with the progression of neurodegenerative disease. The age of symptom onset in SOD1G93A mutants occurs between 85 and 95 days of age, and mortality occurs between 120 and 140 days of age (13). In a majority of SOD1G93A transgenic mice used in the present study, breathing and metabolic measurements were sampled between 60 and 75 days of age and repeated during an age period of 120–130 days. In many of the mice, additional intermediate measurements were captured between these critical age periods. Sample sizes for each time point are reported in Table 1. Also, repeated measurements were performed 1–2 days before death in five animals. In control mice, repeated measurements were performed every 2 wk between 70–140 days of age.

Because variation in VT might be attributable to the animal’s body temperature, we examined temperature differences as a function of time in separate groups in SOD1 mutant (n = 4) and control (n = 3) mice. Temperature transponders (IPTT-300, Bio Medic Data Systems) were implanted subcutaneously in a tissue space above the shoulders. A temperature probe (IPTT-6007) was used to measure each animal’s temperature noninvasively under identical experimental conditions as described above. Between 10 and 20 wk of age, weekly temperature measurements were repeated in each animal at 15-min intervals during a 90-min experiment, and the average of the last five consecutive measurements was reported.

The values reported are means ± SE. To evaluate statistically significant changes in breathing, two-way ANOVAs were performed with group (mutant vs. control) and time (16 or 18 wk vs. 10 wk) as

### Table 1. Breathing characteristics during hypoxic and hypercapnic challenge

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hypoxic (10% O₂)</th>
<th>Hypercapnic (5% CO₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, wk</td>
<td>f (breaths/min)</td>
<td>VT (l)</td>
</tr>
<tr>
<td>10</td>
<td>224.4 ± 0.2</td>
<td>207.5 ± 1.5</td>
</tr>
<tr>
<td>12</td>
<td>229.5 ± 1.3</td>
<td>220.5 ± 1.4</td>
</tr>
<tr>
<td>14</td>
<td>254.1 ± 3.2</td>
<td>265.1 ± 1.7</td>
</tr>
<tr>
<td>16</td>
<td>231.5 ± 4.3</td>
<td>247.1 ± 1.4</td>
</tr>
<tr>
<td>18</td>
<td>235.9 ± 5.1</td>
<td>257.9 ± 1.7</td>
</tr>
<tr>
<td>20</td>
<td>243.1 ± 6.2</td>
<td>265.1 ± 1.7</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>SOD1 mutant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breathing frequency,</td>
<td>256.7 ± 0.3</td>
<td>310.5 ± 1.3</td>
</tr>
<tr>
<td>Total volume, l</td>
<td>259.5 ± 1.5</td>
<td>332.5 ± 1.5</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. SOD1, superoxide dismutase 1; P < 0.05 vs. 10 wk of age.
main effects. Post hoc mean comparisons between data obtained within groups at 16 or 18 wk were compared with data obtained at 10 wk of age using paired r-tests. Comparisons between mutant and control groups at specific time points were performed with unpaired t-tests. Lastly, paired t-tests were used to compare acute changes occurring 1–2 days before death. Statistical significance was established at an α-level of 0.05.

RESULTS

In Fig. 1, top, the age-dependent changes in body weight and minute ventilation (VE) are depicted in SOD1 mutant and control mice. A significant (P < 0.05) decrease in body weight was evident between weeks 16 and 18 in the mutants. This rapid decline in body weight for the mutant SOD1G93A mice was in contrast to a predictable increase (P < 0.05) in body weight consistent with normal growth and maturation in control mice. There was also a significant (P < 0.05) increase in VE that occurred between 14 and 16 wk of age in SOD1G93A mutant mice. The increased VE response in mutant mice was further magnified at 18 wk of age. This same VE response was significantly (P < 0.05) lower in control mice than in mutants at 10, 16, and 18 wk of age, and there was no apparent age-dependent change in VE as seen in mutant mice.

In Fig. 1, bottom, age-dependent changes in breathing pattern are illustrated for mutant and control groups of mice. Although there were no consistent age-dependent changes in f responses for either SOD1G93A mutant or control mice through 18–20 wk of age, there were significant (P < 0.05) increases in VT after 14 wk in both groups. The SOD1G93A mutant mice demonstrated a significantly (P < 0.05) greater VT response relative to control at 10 and 12 wk and again at 18 wk of age.

This latter increase in VT led to the elevated VE response seen in SOD1G93A mice (Fig. 1, top). In SOD1 control mice, the significant age-dependent changes in VT were generally offset by modest reductions in f, leaving baseline VE relatively stable.

The average body temperature between the SOD1 mutant and control groups of mice was significantly different (36.3 ± 0.1°C and 35.6 ± 0.1°C; P < 0.05) at 10 wk of age. However, there was no detectable difference (35.9 ± 0.1°C and 36.0 ± 0.2°C; P > 0.05) between the groups at 18 wk of age. In addition, there were no differences (P > 0.05) in body temperature as a function of time between 10 and 18 wk of age in either group.

The VO₂ and VCO₂ results and the respective measurements of ventilatory equivalent (VEQ) are shown in Fig. 2. Within each group of mice, VO₂ and VCO₂ remained unchanged as a function of age in both mutant and control mice. However, both VO₂ and VCO₂ were significantly (P < 0.05) greater in the SOD1G93A mutant than in control mice between 10 and 12 wk of age, indicating a higher metabolic rate in SOD1G93A mice. With the use of either VO₂ or VCO₂ as a reference, the average VEQ results in mutant mice showing significant (P < 0.05) increases after 16 wk of age, suggesting that the increased VE observed in SOD1G93A mutant mice was not attributable to a greater metabolic demand.

The chemical control of breathing in SOD1G93A mutant and control groups of mice is depicted in Fig. 3. In both groups, the VE response to hypercapnia was elevated above the same response to hypoxia. The VE responses to chemical stimulation in control mice were relatively stable as a function of time. To
the contrary, there were modest but significant \( P < 0.05 \) increases in hypoxic and hypercapnic \( V_E \) responses in the SOD1<sup>G93A</sup> mice at 16 and 18 wk of age, respectively. At both time periods, the increase in \( V_E \) to chemical stimulation occurred as a result of a significant \( P < 0.05 \) increase in \( V_T \) (Table 1).

As shown in Fig. 4, after 18 wk of age, there is an abrupt fall in baseline and hypercapnic breathing that was associated with imminent death in SOD1<sup>G93A</sup> mutant mice. Although the sudden depression in ventilation is accompanied by an average fall in body weight of 2.7 g, the change in body weight does not account for the rapid disintegration in ventilation.

**DISCUSSION**

The findings of the present study suggest that SOD1<sup>G93A</sup> mutant mice demonstrate three phases of a respiratory disease phenotype beginning with relatively stable breathing characteristics from 10 to 14 wk of age. This first phase is associated with significantly elevated \( V_E \) and \( V_O_2 \) in mutant compared with control mice at 10 wk. A second phase occurs at 16 wk, in which there is a progressive increase in \( V_T \) and \( V_E \) that is accentuated through 18 wk of age. This increase in the magnitude of ventilation occurs as metabolism remains relatively unchanged, suggesting that the breathing pattern, especially
Vt is no longer optimized and the respiratory apparatus is becoming less efficient. This change in baseline Vt and Ve occurs in parallel with modest but significant increases in the same responses to hypoxia (at 16 wk) and hypercapnia (Fig. 3). Although the onset of hindlimb paralysis occurs as early as 10 wk of age (25), changes in ventilation increase (not decrease) between 14 and 18 wk of age. Furthermore, the age-dependent increase in Vt and Ve for SOD1G93A mutant mice is not attributable to variation in body temperature from 10 to 18 wk. It is also important to note that the chemical sensory and transducing mechanisms appear to be intact through 18 wk of age in the SOD1G93A mutant mice.

After 18 wk of age, a third phase of the disease phenotype in the SOD1G93A mutant mice is characterized by a rapid decline in ventilatory function. During a 2- to 5-day period, both baseline and hypercapnic Ve responses precipitously drop with imminent death in these mice. Whereas f/ remains stable and Vt increases up to 18 wk of age, both breathing parameters decay rapidly to undetectable levels shortly after 18 wk. The rapid decay in breathing pattern suggests that the progressive loss of phrenic motoneuron function, which occurs before 18 wk, becomes a significant fraction after 18 wk of age and leads to acute respiratory failure (17). During this rapid decay period, the ventilatory response to chemical stimulation is also compromised. Given that there are a proportion of functional neurons driving respiratory muscle activity with disease progression, hypercapnic or hypoxic stimulation presumably increases efferent nerve activity. However, the increase in hypercapnic ventilation above baseline responses during the end stage of disease is modest compared with the ventilatory differences observed before 18 wk of age (Figs. 3 and 4). Furthermore, hypoxic ventilatory responses were undetectable in our plethysmographic apparatus after 18 wk of age.

The selective dying of motoneurons in ALS patients is inherited as a dominant phenotype in 5–10% of all cases of the disease (referred to as familial ALS). In the familial subgroups of patients, mutations in the SOD1 gene account for 15–20% of the cases. There are presently more than 100 human mutations that are known to be associated with clinical outcomes of ALS (1, 9). Transgenic mice overexpressing either the wild-type form of the human SOD1 gene or a specific amino acid substitution, generally leading to altered protein structure, have been studied in numerous ways to investigate disease mechanisms of ALS (28). The mechanisms by which the mutant protein leads to cell death are not yet fully understood, but multiple processes have been implicated, including mitochondrial dysfunction, excitotoxicity, oxidative stress, protein aggregation, and altered cellular transport (5). The toxicity does not appear to be due to any reduced function of the antioxidant activity of the SOD1 protein, as many of the mutant forms of the protein have normal dismutase activity. Moreover, mice null for the SOD1 gene do not develop motoneuron disease and neither do transgenic mice overexpressing normal SOD1 protein (4, 37). Several transgenic mice models encoding different mutations in the human SOD1 gene have been characterized by a unique disease time course and specific survivorship curve; the SOD1G93A mutant mice show a relatively early disease progression (10).

The ventilatory control characteristics of human ALS patients have led some investigators to conclude that nocturnal oxygen desaturation followed by chronic CO2 retention occurs as a disease outcome of respiratory muscle weakness (19). A truncated Vt has been suggested as an alteration in ventilatory control that ultimately leads to respiratory muscle weakness (23). In another study (36), longitudinal data on breathing pattern and respiratory mechanics in ALS patients showed evidence consistent with a restrictive ventilatory pattern derived from progressive weakness of respiratory muscle function. Patients compensated for a decline in respiratory muscle function by demonstrating a rapid shallow breathing pattern (i.e., increasing f and decreasing Vt). This change in breathing pattern occurred with only modest changes in arterial blood gases. Finally, by assessing inspiratory occlusion pressure within 0.1 s as an index of respiratory drive, the authors suggested that the chemosensory and central integration mechanisms remained relatively stable. Patients with progressive respiratory muscle weakness demonstrated little change in inspiratory occlusion pressure within 0.1 s but showed a dramatic fall in their maximally generated pleural pressures. Similar results demonstrating a preservation of hypercapnic and hypoxic ventilatory chemosensitivities have been observed in another study of nonrespirator-dependent ALS patients (12).

On the basis of past models of respiratory control involving neuromuscular dysfunction (e.g., 34), aspects of the ventilatory profile in the SOD1G93A mice in this study were unexpected, particularly related to the increased VeQ for O2 and CO2. These results suggested that the increased Ve response between 16 and 18 wk were out of proportion with the level of metabolism. The respiratory system is generally coupled to metabolic regulation and adjusts blood gases and pH accordingly. In addition, the ventilatory apparatus is regulated to work efficiently, and adjustments in breathing pattern can minimize work expenditure by the respiratory system. These data, however, suggest that the SOD1G93A mice demonstrate a phase in the disease process before end stage in which the Ve response is increased for a given level of metabolic demand. The significant increases in Vt during the same age range suggest that a greater amount of work is required for a deeper breathing pattern. There is little evidence in the present study to suggest that the greater Ve response likely occurs as a result of higher
levels of CO₂ retention. Furthermore, the chemosensitivity to hypoxia or hypercapnia does not appear to be negatively affected; in fact, there may be evidence to support an increased ventilatory chemosensitivity. How the SOD1(G93A) mutation may be involved in these respiratory complications requires future studies. One possible direction is to explore the mutational role of aberrant SOD1 in promoting greater VT responses just before acute respiratory failure.

Changes in VEQ for O₂ and CO₂ have been measured during breathing at rest, and a rise in VE in the face of an unchanged VO₂ and VCO₂ has been used to indicate an increased ratio between dead space ventilation and VT (14). It is possible that there was an elevation of dead space ventilation-to-VT ratio in the SOD1(G93A) mutants from 16 to 18 wk of age, just before respiratory failure, but there are no lung functional or structural data in SOD1 mutant mice to directly address this possible mechanism. Another novel observation is suggested by the differences in VO₂ and VCO₂ between the SOD1(G93A) mutant and control groups at 10–12 wk of age. The present data indicate that there was a twofold greater VO₂ in the mutant mice than in control mice (Fig. 2). The increased metabolic rate appears to be associated with a greater body temperature at 10 wk in SOD1(G93A) mutant vs. that shown in control mice. These observations appear to be consistent with the hypothesis that a variety of SOD1 mutations may result from a specific mitochondrial dysfunction, which modulates an imbalance in energy homeostasis. Dupuis et al. (8) described several aspects of the metabolic deficiencies in SOD1 transgenic mouse models that led to higher energy expenditures. Specifically, the investigators observed greater oxygen consumption rates in SOD1 mutants (i.e., G86R and G93A mutations) than in control mice. The greater energy expenditure was associated with greater food intakes in both of the mutations at 75 days of age. Although the investigators eliminated several possibilities, such as thermogenesis and elevated body temperature, there was not a clear explanation of what mechanisms are involved in the higher metabolic rate in SOD1 mutants.

Several ventilatory characteristics of SOD1(G93A) mutants are similar to the ventilatory changes associated with chemically induced hypermetabolism using well-studied mitochondrial uncouplers, such as 2,4-dinitrophenol (16, 22, 30). Several investigators (e.g., Ref. 16) have shown that a rise in VE is adjusted proportional to the elevation in VO₂, when mitochondrial uncoupling is chemically induced. Others (22, 30) have suggested that mitochondrial uncouplers stimulate VE by other mechanisms that may or may not include the effect of increased VO₂ and VCO₂. There is considerable debate, however, surrounding the specific effects of the SOD1 mutation on mitochondrial function. Mattiazzi et al. (20) suggested that mitochondrial respiration and ATP production are significantly impaired in brain and spinal cord from SOD1(G93A) mutants. However, these changes were observed only at 17 wk and not at an early age of 13 wk. Although the SOD1(G93A) mutant protein may lead to mitochondrial dysfunction, it remains speculative to suggest that the SOD1(G93A) mutation “uncouples” mitochondrial oxidative phosphorylation (3).

Transgenic mice overexpressing mutant SOD1 genes have been successfully used to study many aspects of ALS as a model of neuromuscular degeneration. Like human prognostic studies (15, 19, 29, 31), the results from the present study suggest that age-dependent changes in the ventilatory profile (i.e., the phase of hyperventilation between 16 and 18 wk) can be used as a predictor of a final phase of respiratory failure and imminent death. Future studies are needed to test age-dependent changes in ventilatory function in response to specific interventions aimed at delaying the onset or rate of respiratory failure. For example, Kaspar et al. (13) demonstrated that the viral vector-mediated delivery of IGF-1 significantly delayed the progression of disease in SOD1(G93A) mice. Adeno-associated virus (AAV) encoding the IGF-1 gene was injected in the hindlimbs and intercostal muscles of presymptomatic or symptomatic SOD1(G93A) mice. In both treatment paradigms, the retrograde transport on AAV-IGF-1 to the spinal cord motoneurons led to clear behavioral and neuropathological improvements. Continuous intrathecal infusion of recombinant human IGF-1 before the onset of disease symptoms also significantly improved survival and locomotor function in transgenic SOD1(G93A) mice (26). Furthermore, reducing mutant SOD1 in motoneurons using small interfering RNA constructs delivered by viral vector also showed significant improvements in motor function (21, 32, 33). The use of stem cells is another promising therapeutic approach, in which the transplantation of embryonic stem cell-derived motoneurons into the spinal cord was shown to restore function to animals with spinal cord injury or motoneuron function (7). It is unclear whether therapeutic interventions, such as viral vector delivery, can alter respiratory control mechanisms and improve ventilatory function. The goal of future studies is to determine whether neuromuscular function in the diaphragm of SOD1(G93A) mutant mice can be improved by targeting phrenic motoneuron pools using retrograde delivery of AAV-IGF-1.

In summary, by characterizing the respiratory impairment in SOD1(G93A) transgenic mice, the results of the present study demonstrate a unique age-dependent ventilatory profile that was not apparent in the control mice. At 16–18 wk of age, the SOD1(G93A) mutant mice demonstrated elevated ventilation at baseline and during chemical stimulation, including acute hypoxic and hypercapnic challenges. The increase in ventilation occurred in the absence of an increased metabolic rate, which indicates decay in the ventilatory efficiency before the onset of respiratory failure. From these findings, the therapeutic potential of AAV-mediated delivery of neurotrophic factors to improve respiratory function can be assessed at different phases of the respiratory disease phenotype to model intervention in ALS patients.

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