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Acute glial activation by stab injuries does not lead to overt damage or motor neuron degeneration in the G93A mutant SOD1 rat model of Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease where motor neurons within the brain and spinal cord are lost, leading to paralysis and death. Recently, a correlation has been reported between head trauma and the incidence of ALS. Furthermore, new invasive neurosurgical studies are being planned which involve inserting needles directly to the spinal cord. We therefore tested whether acute trauma to the spinal cord via a knife wound injury would lead to accelerated disease progression in rodent models of ALS (SOD1^{G93A} rats). A longitudinal stab injury using a small knife was performed within the lumbar spinal cord region of presymptomatic SOD1^{G93A} rats. Host glial activation was detected in the lumbar area surrounding a micro-knife lesion at 2 weeks post-surgery in both wild type and SOD1^{G93A} animals. However, there was no sign of motor neuron loss in the injured spinal cord of any animal and normal motor function was maintained in the ipsilateral limb. These results indicate that motor neurons in pre-symptomatic G93A animals are not affected by an invasive puncture wound injury involving reactive astrocytes. Furthermore, acute trauma alone does not accelerate disease onset or progression in this ALS model which is important for future strategies of gene and cell therapies directly targeting the spinal cord of ALS patients.

Keywords

Amyotrophic lateral sclerosis (ALS); SOD1^{G93A} rats; acute trauma; stab injury; astrocyte activation; motor neuron; spinal cord

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Introduction

Amyotrophic Lateral Sclerosis (ALS) is typified by progressive loss of motor neurons in the brain and spinal cord, leading to paralysis and death within 3–4 years (Cleveland et. al., 2001; Boillee et. al., 2006). The mean age of onset for FALS is 60 years, and patients have no symptoms until shortly before the disease begins. While the majority of ALS cases are sporadic in nature, a small percentage (~10%) has a familial component. Although the cause of sporadic ALS remains unclear, a clear genetic link to point mutations in the cytosolic $\text{Cu}^{2+}/\text{Zn}^{2+}$ superoxide dismutase 1 (SOD1) has been shown in a small group of familial ALS (FALS) patients (Rosen et. al., 1993; Andersen et. al., 2003). This has led to the generation of transgenic mice and rats over-expressing multiple copies of mutant SOD1 (SOD1^{G93A}) that have many of the characteristics of both the familial and sporadic form of human disease (Gurney et. al., 1994; Hall et. al., 1998; Howland et. al., 2002). The exact etiology of ALS is still mystery, but recent observations suggest an involvement of acute trauma on the occurrence or disease progression of ALS (Chio et. al., 2005; Chen et. al., 2007).

Motor neuron death in ALS is a complex process, and may involve multiple pathways including formation of protein aggregates, axonal transport defects, oxidative damage, mitochondrial defects, alterations in calcium homeostasis (Julien et. al., 2001; Rowland et. al., 2001). While the primary trigger for ALS remains a mystery, there is also increasing evidence that excitotoxicity due to astrocyte dysfunction and inflammatory processes from microglia activation play a crucial role in disease progression (Hall et. al., 1998; Barbeito et. al., 2004; Boillee et. al., 2006). In rodent models of FALS expressing high levels of mutant SOD1, astrocytes are also reactive early in disease progression and are known to release factors that contribute to motor neuron death (Van Den et. al., 2008). Recent studies showed that in chimeric mice with both wild type and mutant SOD1 expressing cells there is a clear protective effect when wild type astrocytes are associated with mutant SOD1 expressing motor neurons (Clement et. al., 2003) suggesting a potential protective role under certain conditions. Most of the studies to date have focused on modulating the ratio of mutant SOD1 over expression and normal cells in transgenic mice and have shown that reducing the number of glia expressing the mutation can have a significant impact on motor neuron survival and disease progression (Clement et. al., 2003; Beers et. al., 2006).

Previous evidence points to a mechanism where the astrocytes become reactive, but lose regulation of their normal functions in ALS. However, it has not been previously determined whether spinal cord injuries, which initiate acute glial activation and inflammation, could affect motor neuron survival in presymptomatic SOD1^{G93A} rats. In this study we hypothesized that host glial activation by experimental stab injuries in the spinal cord of presymptomatic SOD1^{G93A} rats may trigger motor neuron degeneration through abnormal astrocyte responses. To address this issue, we used a model of experimental spinal cord injury at the lumbar spinal cord (L2–4) with a longitudinal stab injury using a micro knife. This injury model has been used to study the cell biology of penetrating spinal cord injury *in vivo* in a context that caused no grossly detectable behavioral impairments in animals unless the division of host astrocytes was inhibited (Faulkner et. al., 2004). Here we show that acute astrocyte activation by stab injury does not cause motor neuron degeneration in pre symptomatic rats carrying the ^{G93A} SOD1 mutation, a rat model of familial ALS.

Methods

Animals

Breeder SOD1^{G93A} rats, which had been originally generated by Howland *et al.* (Howland et. al., 2002), were obtained from Taconic (Hudson, NY), and colonies were developed by crossing male founders with female Sprague Dawley rats (Suzuki et. al., 2007b). Heterozygous

SOD1^{G93A} progeny were identified with polymerase chain reaction (PCR) of tail DNA with primers specific for human SOD1. They were maintained in a room with controlled illumination (lights on 0600-1800 h) and temperature (23 ± 1 °C), and given free access to laboratory chow and tap water. All the animal experiments in the present study were carried out in accordance with the guidelines for University of Wisconsin-Madison and National Institutes of Health standards of animal care.

Surgery procedures and behavioral tests

Presymptomatic SOD1^{G93A} male rats with slow disease progression (Suzuki et. al., 2007b) were used for this study. All surgery procedures were performed under full general anesthesia with isoflurane in oxygen-enriched air. Unilateral longitudinal stab injuries were made in 115-day-old SOD1^{G93A} males as previously described (Faulkner et. al., 2004). The stab injuries ran parallel to the long ascending and descending tracts and passed primarily through gray matter at the level of L2-4. All surgeries were performed using an operating microscope (Zeiss, Oberkochen, Germany) and a rodent stereotaxic apparatus (David Kopf, Tujunga, CA). The spinal cord was exposed by laminectomy of a single vertebra. The longitudinal stab injuries were 2 mm in length and 1 mm depth and were made with a retractable wire knife (David Kopf) without rupturing the dura.

To analyze motor function in SOD1^{G93A} rats, open field locomotor scores were evaluated in a small enclosure using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale to assess hind limb function on days 0, 1, 2, 3, 4, 8, 10 and 14 after the surgery. The BBB locomotor rating scale was obtained as previously described (Klein et. al., 2005; Suzuki et. al., 2007b). Briefly, each rat was allowed to walk around in a wading pool with a textured floor while we observed hind and fore limb movements for approximately 3 to 5 minutes. Each hind limb score was based on the 21 point scoring scale from no movement (0) to normal locomotion (21). Scoring takes into account paw rotation, toe clearance, weight support, the frequency of each, and the amount of movement occurring from each joint.

Immunohistochemistry

The animals were transcardially perfused with chilled 0.9% saline and 4% paraformaldehyde-phosphate buffered saline. The lumbar spinal cords were collected and sectioned in 35 μ m increments using a cryostat. One in six sections were processed for Nissl staining or immunostained for glial fibrillary acidic protein (GFAP, rabbit polyclonal 1:2000, Dako, Glostrup, Denmark), nestin (mouse monoclonal, 1:200, Millipore, Billerica, MA), choline acetyl transferase (ChAT, goat polyclonal, 1:200, Millipore), CD11b (OX-42, 1:500, MorphoSys, Planegg, Germany), or Ki67 (mouse monoclonal, 1:500, BD Biosciences, San Jose, CA). After incubation with the primary antibodies, the cultures were rinsed in PBS, and incubated for 30 min with secondary antibody conjugated to Cy3 (goat anti-IgG, 1:1000, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) or Alexa 488 (goat anti-IgG, 1:1000, Invitrogen, Carlsbad, CA, USA). All images were optimized by using a fluorescence microscope with a digital camera (SPOT II) or a laser scanning confocal microscope (Nikon, Tokyo, Japan).

Lumbar motor neuron count was performed as described previously (Suzuki et. al., 2007a). In brief, spinal cord sections (adjacent 5 sections to the lesion sites) were processed for fluorescent Nissl staining. Fluorescence microscopy images of the lumbar spinal cord were captured bilaterally with a spot camera. A circular area-of interest was positioned over the ventral horn on both sides of the spinal cord. Area of interest, size, and position were standardized across sections by choosing a circular circumference that matched the curvature of the ventral horn. Each Nissl-stained neuron in the ventral horn with a distinct nucleolus and a darkly stained cytoplasm was manually traced using Metamorph Imaging software (Molecular devices,

Downingtown, PA). Alpha-motor neurons were defined as having cross-sectional area $\geq 700 \mu\text{m}^2$. All alpha-motor neurons were counted in the selected area of the ventral horn.

Statistical Analysis

Prizm software (Graphpad software Inc., La Jolla, CA) was used for all statistical analyses. All cell-counting data was analyzed by one-way functional ANOVA using Newman-Keuls *post hoc* test. Differences in BBB score were calculated using two-way ANOVA with Bonferonni *post hoc* test. Differences were considered significant when $P < 0.05$.

Results

Spinal cord injury by a micro-knife does not change motor function in ipsilateral limb in SOD1^{G93A} rats

To establish the lesion model, a wire micro-knife lesion parallel to the rostral-caudal axis was unilaterally performed at L2-4 in wild type rats (Fig. 1A). At 2 weeks post-surgery, the animals were perfused and the level of the knife injury in the spinal cord was observed by Nissl staining (Fig. 1B). A longitudinal stab injury did not cause severe trauma to the motor neuron pools within the ventral horn beneath the lesion (Fig. 1B) as shown in previous studies (Faulkner et al., 2004).

A unilateral knife lesion was then made at L2-4 in 115-day-old SOD1^{G93A} or wild type rats ($n=5$ for each). We next used a cohort of presymptomatic SOD1^{G93A} male rats with slow disease progression (Suzuki et al., 2007b) that showed normal motor function prior to the surgery (Day 0 in Fig. 2). Our recent data from the latest SOD1^{G93A} rat colony indicated that the median to reach disease onset and endpoint were 146 and 184 days. To establish whether a stab injury could result in any positive or negative effects on limb function in SOD1^{G93A} rats, we assessed hind limb function using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale in each limb of all animals up to 2 weeks following injury. While reduced hind limb changes were noticed at an early time point after a micro-knife injury in all animals, there was no significant difference in hind limb function between the injured-side and the non-injured side in any animal over time (Fig. 2).

A stab injury leads to host glial activation

We next determined the degree of reactive astrocytes in the lumbar spinal cord of WT and SOD1^{G93A} rats. At 2 weeks post surgery, all animals were perfused and the spinal cords sectioned and immunostained for glial fibrillary acidic protein (GFAP) and nestin which can detect host astrocyte and progenitor activation (Fig. 3A, B). Significant astrocyte activation, labeled with anti-GFAP and nestin antibodies, was detected in the injured core of the spinal cord (Fig. 3C–E). Furthermore, double staining with GFAP and motor neuron marker choline acetyltransferase (ChAT) revealed that astrocytes surrounding large motor neurons had increased astrocyte activation in the ventral horn of the spinal cord of injured SOD1^{G93A} rats (Fig. 3F,G).

Previous studies have shown that in normal mice, it is possible to produce a small stab wound injury to the dorsal spinal cord which induces local reactive astrocyte division but does not result in motor neuron death (Faulkner et al., 2004). However, if the dividing astrocytes are ablated in the same injury model by using a mouse model where thymidine kinase is expressed from the GFAP promoter, motor neurons will not survive (Faulkner et al., 2004). Therefore dividing astrocytes may be important to maintain motor neurons under these conditions. To address this issue, we performed double staining with proliferation marker Ki67 and GFAP and analyzed levels of astrocyte proliferation using the lumbar spinal cord sections of SOD1^{G93A} and WT rats 2 weeks after injury (Fig. 3H–J). In the injured core, we found a few

double positive cells in both of SOD1^{G93A} and WT rats (Fig. 3I). However, we could not find Ki67-positive astrocytes within the ventral horn where motor neurons appear in these sections (Fig. 3J).

We determined the levels of GFAP expression in the ventral horn of the spinal cord within the core of a stab injury (Fig. 4A–D, I). In both WT and SOD1^{G93A} rats, a stab injury tended to increase GFAP expression, although the difference did not reach statistical significance (Fig. 4I). Furthermore, SOD1^{G93A} rats showed a significant increase of GFAP expression in both non-injured and injured sides compared to the level of non-injured ventral horn in WT rats ($P < 0.05$; Fig. 4I). We also determined the levels of microglial activation by using immunostaining for microglial marker CD11b (OX-42). Microglial activation was observed in the area of micro knife injury in all animals (Fig. 4E–H, J). Particularly, the level of microglial activation was significantly increased by up to 3 times compared to the non-injured side of the ventral horn in SOD1^{G93A} ($P < 0.05$; Fig. 4J). However, there was no difference in OX-42 expression between WT and SOD1^{G93A} rats. Together, these results suggest that glial activation may be undergoing in the spinal cord of SOD1^{G93A} rats, although those animals did not show significant changes in motor function.

Reactive astrogliosis by a stab injury is not accompanied by motor neuron loss

In order to establish whether astrocyte activation followed by a micro-knife injury could affect motor neuron survival, we next counted Nissl-stained cells within the lumbar spinal cord region adjacent to the damage. The spinal cord sections were prepared and stained with fluorescent Nissl. Images of the lumbar spinal cord (L2–4) were captured with a CCD camera, and the number of large motor neurons was determined by measuring soma size of Nissl-stained cells in the ventral horn. There was no obvious difference in the number of large motor neurons in the non-injured side of the spinal cord in both WT and SOD1^{G93A} rats (Fig. 5A), and the ratio of motor neurons was not different between the injured and non-injured sides in both WT and SOD1^{G93A} rats (Fig. 5B).

Furthermore, we double labeled sections throughout each animal for both ChAT and ubiquitin, one marker of degenerating motor neurons in this model (Clement et. al., 2003; Suzuki et. al., 2007a). Most of the large surviving ChAT⁺ motor neurons within chimeric regions of the transplanted spinal cord did not express ubiquitin. A few ChAT⁺/ubiquitin⁺ neurons, which represent early degenerating motor neurons, were found in the lumbar spinal cord of SOD1^{G93A} rats (Fig. 6 and Table 1). In contrast, there were no ubiquitin positive cells in the spinal cord of WT rats at 2 weeks post injury (Table 1). Although spinal cord injury did not change motor function in presymptomatic SOD1^{G93A} rats, there may be undergoing degeneration based on ubiquitin staining. Together, we conclude that while a micro-knife injury induced significant host astrogliosis, it was not accompanied by either limb dysfunction or motor neuron degeneration in the presymptomatic SOD1^{G93A} rats.

Discussion

Here we hypothesized that glial activation by a stab injury may lead to increased motor neuron loss and early onset of disease symptoms in presymptomatic SOD1^{G93A} rats. We found that while a micro-knife injury leads to significant host astrogliosis in the transgenic animals, it was not accompanied by either limb dysfunction or motor neuron degeneration. This suggests that acute trauma or insertion of a needle to inject cells into the spinal cord of pre symptomatic animals has no acute effect on motor neuron health.

Astrocyte activation is one of the key components of the cellular response in spinal cord injuries and ALS, and the passage from the quiescent to the reactive state is associated with a strong upregulation of GFAP (McGeer et. al., 1991; Hall et. al., 1998). Astrocytes also play a crucial

role in the function and survival of motor neurons and may be involved in the pathogenesis of ALS (Clement et. al., 2003; Yamanaka et. al., 2008). The initial onset of motor neuron damage could trigger secondary abnormal responses in astrocytes carrying the SOD1 mutation, leading to further propagation of the disease. In an excellent study using chimeric animals with both wild type and mutant SOD1 expressing cells, neighboring cells that did not express mutant SOD1 delayed degeneration and significantly extended survival of mutant SOD1 expressing motor neurons (Clement et. al., 2003). Moreover, reduction of mutant SOD1 expression in astrocytes by selective silencing, which was demonstrated by crossbreeding transgenic mice containing floxed mutant SOD1 with mice expressing Cre driven by GFAP promoter, also affected disease progression (Yamanaka et. al., 2008). Using co-culture with motor neurons and astrocytes, other recent studies showed that a non-cell-autonomous interaction between both cell types was responsible for motor neuron degeneration in ALS (Nagai et. al., 2007; Di Giorgio et. al., 2007; Di Giorgio et. al., 2008; Marchetto et. al., 2008).

The immunostaining results using anti-ubiquitin antibodies indicate that a degenerating process asymptotically occurred at this time point in the spinal cord of SOD1^{G93A} rats. Many different studies indicate changes at the cellular and molecular level prior to overt behavioral changes in the animals during the presymptomatic change. A quantitative analysis previously showed that in SOD1^{G93A} mice there were significant pathological changes at much earlier time points before recognizing onset of disease symptoms (Fischer et. al., 2004). Denervation at neuromuscular junctions occurred by day 47 followed by loss of several motor axons from the ventral root between days 47 and 80, loss of large motor neuron cell bodies in the lumbar spinal cord after day 80, and recognized signs of clinical disease at about 80 days (Fischer et. al., 2004). A recent observation using GFAP-luciferase/SOD1^{G93A} transgenic mice also revealed that the first induction of GFAP bioluminescence signals, which was associated with the significant upregulation of GFAP immunoreactivity, was asymptotically detected in the lumbar spinal cord at 25–30 days of age (Keller et. al., 2008). As in these SOD1^{G93A} mouse studies, we are currently attempting to address how signs of clinical disease are related to pathological changes during the time course of disease progression in a rat model of ALS. In context of therapeutic approaches for human patients, the present study would ideally be performed using animals after disease onset based on BBB scores declining. Unfortunately the decline is so steep in this model that it does not give time to see if this would make the progression worse.

In rodent models of brain and spinal cord injury, reactive astrocytes are essential for wound healing, restrict inflammation, and preserve survival and function of neurons after the injury. Astrocytes respond to traumatic injury in the brain and spinal cord by altered gene expression, hypertrophy and proliferation that occur in a graded fashion in relation to the severity of the injury (Eddleston et. al., 1993; Eng et. al., 1994). Excellent studies using a mouse model where thymidine kinase demonstrated that dividing astrocytes may be involved in survival of motor neurons in a stab injury model (Faulkner et. al., 2004). It has been well established that the peak time of astrocyte cell division is 2 to 5 days after such stab injuries (Hatten et. al., 1991; Norton et. al., 1992). We could not confirm dividing astrocytes within the ventral horn in both SOD1^{G93A} and WT rats 2 weeks after injury (Fig. 3J). However, our results do not contradict these previous studies, because a Ki67 can only be detected in the cells that are actively dividing.

ALS seems to be more prevalent in a large cohort of professional soccer players in Italy (Chio et. al., 2005). It has also been suggested that head injury was related to a high risk of ALS in an American population (Chen et. al., 2007). This suggests that trauma in general may trigger ALS in human patients who are susceptible to the disease. Our data in a rat model of ALS suggest that knife wound trauma in animals that are going to get the disease later in life does not lead to accelerated disease onset or increase pathology. This argues that the astrocyte

response to injury remains functional in pre symptomatic animals. In the course of trauma-induced and genetically-induced spinal cord degeneration, spinal cord vulnerability may be linked to disease modifiers that are activated by selective disease process and possibly depends on a common ground of molecular factors that can be switched on by different pathological triggers. Although it is still a mystery how biomedical stress initiates the cascade of molecular events leading to the irreversible neurodegeneration in ALS, our present results support the idea that a pathological process may not be perfectly overlapped in injury-induced and ALS spinal cord degeneration. Further investigations may be interesting to compare the levels of inflammatory cytokines between ALS and spinal cord injury models, which plays a key role in the amplification and possibly initiation of inflammation in both diseases (Moisse et. al., 2006; Alexander et. al., 2009).

While gene and cell therapies directly to the motor neurons within the spinal cord may be able to help in the battle against ALS (Azzouz et. al., 2006; Hedlund et. al., 2007; Suzuki et. al., 2008), the present observations suggest that motor neurons survive exogenous physical stimuli, which is important for future strategies of these therapies. As disturbances of the normal astrocytic function could have a direct or indirect effect on the survival of motor neurons, astrocytes may be an interesting therapeutic target. A recent study successfully demonstrated that transplanted glial restricted progenitor cells into the cervical spinal cord could differentiate into mature astrocytes and ameliorate disease progression and prolong survival period in SOD1^{G93A} rats (Lepore et. al., 2008). As an alternative approach, stem cell-based transplantation could be used as these cells migrate and integrate into the spinal cord. For both drug delivery and astrocyte replacement, stem cells isolated and expanded in culture can be modified to release growth factors and glial cells following transplantation into the spinal cord. Our recent study has shown that human neural progenitor cells (hNPC) can be modified to make growth factors such as GDNF, which are normally hard to deliver into the brain and spinal cord (Klein et. al., 2005; Suzuki et. al., 2007a). When these cells are transplanted into rat models of ALS, they differentiate into cells with astrocyte like properties, continue to release GDNF and protect the dying motor neurons. Furthermore, a reported clinical trial was recently carried out in Italy, with autologous mesenchymal stem cells transplanted into the thoracic spinal cord in ALS patients, although there were no adverse effects or significant improvements (Mazzini et. al., 2008). These approaches were performed by injections with needles directly into spinal cords. The present results, indicating no adverse effect on disease progression in the presymptomatic stage of ALS, may bring us important information, as we should safely evaluate efficiency of these therapies in ALS patients without any side effect.

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Figure 1. A stab injury model using a micro wire-knife in a rat spinal cord

(A) Schematic illustrations of stab injury model in the lumbar region of rat spinal cords. The stab injuries ran parallel to the long ascending and descending tracts and passed primarily through gray matter at the level of L2-4. The longitudinal stab injuries were 2 mm in length and 1 mm depth and were made with a retractable wire knife. (B) Representative photomicrograph showing tissue adjacent to the stab injury. A micro wire was inserted on the dorsal side of the spinal cord (arrow) and affected gray matter (asterisk). Scale bar: 200 μ m.

Figure 2. Ipsilateral limb function is not affected by stab injuries in SOD1^{G93A} rats
 Basso-Beattie-Bresnahan (BBB) locomotor rating scales in the WT (**A**) or SOD1^{G93A} rats (**B**). A significant difference was not observed between the injured side and non injured side in any group.

Figure 3. Host glial activation following stab injuries in SOD1^{G93A} rats

Immunostaining with anti-GFAP and nestin in the lumbar spinal cord of WT (A) and SOD1^{G93A} rats (B). Significant astrocyte activation in the injured side of the spinal cord, while there were few positive signals in the non-injured side. Particularly, high expression of GFAP (C) and nestin (D) was observed in the injured core. (F) Double labeling with GFAP and ChAT revealed astrocyte activation within regions of motor neurons. (G) A photomicrograph using higher magnification in the area represented by a broken-lined box in F. Double staining with GFAP and proliferation marker Ki67 in the injured core (H, I) and the ventral horn (J). While a few GFAP⁺/Ki67⁺ cells (designated by arrow heads) were found in the injured core, there is

no double positive cell in the ventral horn. Scale bars: 200 μm in A, B; 100 μm in C-E; 50 μm in F; 20 μm in H-J.

Figure 4. Analysis of glial activation following a stab injury in the spinal cord of SOD1^{G93A} rats Photomicrographs of the non-injured (**A**) and injured (**B**) WT rats and SOD1^{G93A} rats (**C**, **D**) indicate astroglial activation in the ventral horn of lumbar spinal cord. Microglial activation was compared between non-injured and injured spinal cord by immunostaining for microglial marker CD11b (OX-42) in WT (**E**, **F**) or SOD1^{G93A} animals (**G**, **H**). Densitometrical results of the relative intensity of GFAP (**I**) or OX-42 (**J**) –labeling. Scale bars: 100 μ m in A-H. *: $P < 0.05$ vs. non-injured WT; **: $P < 0.05$ vs. non-injured WT and SOD1^{G93A}.

Figure 5. Motor neurons are maintained following a stab injury in the lumbar spinal cord of SOD1^{G93A} rats

(A) Motor neuron counts showed that a significant difference was not observed in the number of lumbar motor neurons (L2-4) between the WT and SOD1^{G93A} animals. (B) The ratio of motor neurons compared to the non-injured side. There was no obvious difference in the number of large motor neurons in the non-injured side of the spinal cord in both WT and SOD1^{G93A} rats.

Figure 6. Ubiquitin expression in motor neurons of SOD1^{G93A} rats

Even though spinal cord injury did not change motor function of SOD1^{G93A} rats at 129 days of age, double labeled sections for both ChAT (**A**) and ubiquitin (**B**) revealed that there is undergoing degeneration in the lumbar spinal cord. Most of the large surviving ChAT⁺ motor neurons within chimeric regions of the transplanted spinal cord did not express ubiquitin (designated by arrows). A few ChAT⁺/ubiquitin⁺ neurons (designated by an arrow head), which represent early degenerating motor neurons, were found in the lumbar spinal cord of SOD1^{G93A} rats but not in WT rats. Scale bar: 50 μ m.

Table 1

Number of ubiquitin and ChAT-positive neurons following a stab injury.

		No. of ubiquitin ⁺ neurons	No. of ChAT ⁺ neurons
WT	Non-injured	0	6.7 ± 1.9
	Injured	0	7.8 ± 0.6
SOD1 ^{G93A}	Non-injured	0.2 ± 0.1	5.1 ± 1.3
	Injured	0.2 ± 0.2	5.2 ± 1.7