Muscle fiber-type specific terminal Schwann cell pathology leads to sprouting deficits following partial denervation in SOD1<sup>G93A</sup> mice

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A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is an adult-onset disease characterized by the progressive death of motoneurons and denervation of muscle fibers. To restore motor function, surviving motoneurons in partially denervated muscles typically sprout axons to reinnervate denervated endplates. However, studies on the SOD1<sup>G93A</sup> rodent models of ALS indicate that sprouting is significantly limited in fast, but not slow, twitch muscles after disease onset. This limitation hastens the rate of muscle weakness and loss of motor function. The causes of this limitation are currently unknown. Sprouting could be limited because the SOD1<sup>G93A</sup> mutation weakens motoneurons making them incapable of expanding their field of innervation. Alternatively, motoneurons may be capable of sprouting, but unable to do so due to the loss of a permissive sprouting environment. To distinguish between the two possibilities, we compared the sprouting capacity of motoneuron subtypes by partially denervating the fast twitch plantaris (composed of type IIa/IIb muscle fibers) and slow twitch soleus muscles (type I/IIa fibers) prior to disease onset and weakening in SOD1<sup>G93A</sup> and WT mice. We found that only motoneurons innervating the SOD1<sup>G93A</sup> plantaris had a limited sprouting capacity. This was correlated with the selective loss of terminal Schwann cells (TSCs) at IIb fibers and an increase in macrophage infiltration. Treating SOD1<sup>G93A</sup> mice with the tyrosine kinase inhibitor, masitinib, significantly reduced infiltration, prevented TSC loss, and increased the sprouting capacity to near normal. These results suggest that TSCs at denervated type IIb muscle fibers are aberrantly targeted by infiltrating macrophages in SOD1<sup>G93A</sup> mice, and their loss accounts, at least in part, for the compromised sprouting capacity of the largest motoneurons during early stages of ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal, adult-onset, neurodegenerative disorder characterized, in part, by the progressive dysfunction and death of lower motor neurons innervating skeletal muscle fibers. While most cases of ALS are sporadic, approximately 10% have an inherited form, one class of which is caused by mutations in the superoxide dismutase (SOD1) gene (Ajroud-Driss and Siddique, 2015; Rosen et al., 1993). Transgenic mice over-expressing human mutant SOD1 with a glycine to alanine conversion (SOD1<sup>G93A</sup>) develop several pathologies typical of ALS, including progressive spinal motoneuron loss, denervation, and motor dysfunction (Chiu et al., 1995; Gurney et al., 1994).

To compensate for a loss of innervation, surviving motoneurons typically sprout terminal branches to reinnervate previously denervated endplates (Gordon et al., 2004; Son et al., 1996). Sprouting is mediated by terminal Schwann cells (TSCs), which are closely associated with neuromuscular junctions (NMJs) (Son and Thompson, 1995a). Following denervation, TSCs extend processes from denervated endplates to neighboring, innervated NMJs where they act as bridges to guide terminal sprouts from intact axons to the denervated endplates (Lee et al., 2017; Son and Thompson, 1995a). Functional compensation due to motoneuron loss can be quite remarkable since each motoneuron can expand its field of innervation by up to four times its normal size if more than 80% of its original innervation is lost (Brown and Ironton, 1978; Rafuse et al., 1992). Interestingly, several studies have shown that sprouting, by a subpopulation of motoneurons, is compromised in SOD1<sup>G93A</sup> mice and in patients with ALS (Dengler et al., 1990; Frey et al., 2000; Gordon et al., 2010, 2004; Hagedus et al., 2007; Pun et al., 2006; Schaefer et al., 2005; Sharma and Miller, 1996). More specifically, anatomical and electrophysiological studies have shown that a subpopulation of motoneurons innervating fast twitch muscles composed of type Ia and Ib fibers sprout poorly in SOD1<sup>G93A</sup> mice (Gordon et al., 2010, 2004; Hagedus et al., 2007; Pun et al., 2006; Schaefer et al., 2005; Sharma and Miller, 1996).
2005; Vallejo et al., 2013) while those innervating type I fibers in slow twitch muscles exhibit robust terminal sprouting (Pan et al., 2006). As a result, the contractile force of fast twitch muscles in SOD1\textsuperscript{G93A} mice declines rapidly as motoneurons die (Hegeduš et al., 2007) leading to an acceleration in motor dysfunction.

The cellular basis for the reduced sprouting capacity in SOD1\textsuperscript{G93A} mice is not well understood. The most vulnerable motoneurons in ALS, and first to die, are the largest and innervate type IIb fibers (Arbour et al., 2017; Murray et al., 2010). The SOD1\textsuperscript{G93A} mutation in these large motoneurons could compromise them and thus limit their capacity to expand their field of innervation (Frey et al., 2000; Pun et al., 2006). Alternatively, these motoneurons may be capable of sprouting, but unable to do so due to the loss of a permissive sprouting growth environment. Support for the latter comes from Pinter and colleagues (Carrasco et al., 2016b, 2016a) who reported a loss of TSCs at a subset of denervated endplates in SOD1\textsuperscript{G93A} fast twitch muscles (Gould et al., 2006) and postulated that the loss would result in compromised sprouting because the growth pathway provided by the TSCs was absent.

It is important to understand why sprouting is compromised because it can functionally compensate for the loss of many motoneurons after injury (Rafuse et al., 1992) and after a motor neuron disease such as polio myelitis (Trojan et al., 1991; Weichers, 1985). Finding ways to improve sprouting should, therefore, slow down the rate of muscle denervation and improve motor function during disease progression in ALS. In this study, we chose to examine why sprouting is compromised in SOD1\textsuperscript{G93A} mice by partially denervating the fast twitch plantar muscle, composed of type IIb and Ila fibers, and slow twitch soleus muscle (composed of type I and Ila fibers) prior to disease onset in SOD1\textsuperscript{G93A} and wild-type (WT) mice. We found that only motoneurons innervating the SOD1\textsuperscript{G93A} plantaris had limited sprouting capacities, and that this limitation was correlated with the selective loss of TSCs at Ila fibers. Furthermore, treating SOD1\textsuperscript{G93A} mice with masitinib, a receptor tyrosine kinase inhibitor, prevented the loss of TSCs at IIb fibers, and restored sprouting response to near WT levels. These results suggest that selective aberrant TSC behavior, not selective motoneuron vulnerability, accounts for the reduced sprouting capacity in fast twitch muscles in SOD1\textsuperscript{G93A} mice during early disease onset. Furthermore, our results suggest that pharmacological interventions maintaining TSCs at denervated endplates is a viable treatment strategy to attenuate the loss of motor function in ALS.

2. Materials and methods

2.1. Mice

The hemizygous B6.Cg-Tg(SOD1-G93A)1Gur/J (Jackson Laboratory; hereafter referred to as SOD1\textsuperscript{G93A}) mouse model of ALS was used in this study. These mice display extensive motoneuron cell death and progressive skeletal muscle weakening due to neuromuscular degeneration because they have a high copy number of the human SOD1 gene carrying a glycine to alanine transition at position 93 (Gurney et al., 1994). SOD1\textsuperscript{G93A} mice have a shortened life expectancy with mean survival at 161 ± 10 days (Heiman-Patterson et al., 2011). Onset of muscle-specific denervation begins at approximately P40 and continues throughout life with first visible motor deficits beginning at approximately P90 (Hegeduš et al., 2007). Hemizygous mice were genotyped at birth, so aged match littermates, not expressing the mutant gene, could be used as wild-type (WT) controls. All experiments were conducted in accordance to the guidelines of the Canadian Council on Animal Care and the policies of Dalhousie University.

2.2. Surgeries

All surgeries were performed under aseptic conditions on P30 mice. Animals were anesthetized with a mixture of isoflurane (Baxter) and oxygen throughout surgery. For partial denervation of plantaris and soleus muscles, a small (~1.5 cm) incision was made in the skin, left of the dorsal midline at the level of, and rostral to, the iliac crest. Another incision was then made along the border between the spinalis muscles and fat layer parallel to the spine to expose the L4-S1 spinous processes. The L5 spinous process was removed and a 10–0 suture was used to ligate and gently lift the L5 root to cut the nerve distal to the suture. The ligation ensured regeneration was prevented. Mice were allowed 1, 2, 3, 14, or 30 days of recovery, after which they were killed by isoflurane inhalation and cervical dislocation.

In some experiments, the plantaris and soleus muscles were completely denervated by cutting the tibial nerve. Briefly, under isoflurane anesthesia, a small incision (~1 cm) was made along the rostral/caudal axis of the lower hindlimb behind the knee. The underlying layer of fat was carefully separated to expose the branching point of the tibial nerve. A suture (7–0 to 10–0) was used to ligate the tibial nerve prior to its branching point to the triceps surae muscles. The nerve was then cut distal to the ligation. Mice recovered for 3 days and then were killed by isoflurane inhalation and cervical dislocation.

2.3. Preparation and administration of masitinib mesylate

A 100 mg/ml stock solution of masitinib mesylate (AdooQ Bioscience) was prepared by dissolving 50 mg masitinib mesylate powder in 500 μl distilled water (dH2O). This solution was aliquoted and stored at −20 °C. The amount of masitinib stock was calculated per mouse for the desired 30 mg/kg dose and diluted to 150 μl in 10% dextrose solution. The masitinib and dextrose mixture was fed to mice using a syringe with a blunted needle. Mice were administered masitinib 3 days prior to the partial denervation surgery and daily after surgery until the final acute experiment.

2.4. Ex vivo isometric tension recordings

Mice were anesthetized with a mixture of isoflurane and oxygen. The plantaris or soleus muscle, along with its supplying nerve, were rapidly dissected free and placed into carbogenated (95% O2, and 5% CO2) Tyrode’s solution containing the following: 125 mM NaCl, 24 mM NaHCO3, 5.37 mM KCl, 1 mM MgCl2, 1.8 mM CaCl2, and 27.75 mM dextrose. The proximal muscle tendon (soleus) or hindlimb bones (plantaris) were carefully pinned in a Sylgard (Dow Corning)-coated recording chamber that was continuously perfused with carbogenated Tyrode’s. Thread was tied to the distal end of each muscle and connected to a force transducer (FT 03; Grass Technologies). A fine-tipped, fire polished glass stimulated suction electrode (World Precision Instruments, Inc.) was used to deliver electrical current (0.05–0.1 ms duration) to the innervating nerve via an S88 stimulator (Grass Technologies) that was isolated from ground using a stimulus isolation unit (PSIU6; Grass Technologies). Muscle length was adjusted to maximal isometric contraction. Tetanic forces were recorded at 50 Hz (1 s duration) using a Digidata 1322A analog-to-digital board and AxoScope version 10.2 software (Molecular Devices).

2.5. Immunofluorescence and imaging

Soleus and plantaris muscles from partially denervated, completely denervated, and contralateral unoperated limbs were dissected from the lower hindlimb immediately after euthanasia and transferred to phosphate buffered saline (PBS). Muscles were then pinned at physiological length and fixed in 4% paraformaldehyde (PFA) for 15–20 min. Next, they were washed in PBS, permeabilized in −20 °C methanol for 6 min, washed again in PBS, and then securely pinned in a Sylgard dish immersed in PBS under a dissecting microscope. One tendon was removed so the muscle fibers could be carefully teased into bundles using forceps.

To visualize pre-synaptic structures at the NMJs, muscle fiber...
bundles were incubated overnight in 0.3% Triton-PBS containing 10% normal goat serum and a cocktail of primary antibodies, described in Table 1. The next day, muscles were washed in PBS and then incubated for 1 h in 0.3% Triton/PBS with the appropriate secondary antibody solutions, including goat anti-mouse Alexa Fluor 488/Cy2 (1:500; Invitrogen/Jackson Laboratories), goat anti-rabbit Alexa Fluor 647 (1:500; Invitrogen), and goat anti-rat Cy3 (1:500; Jackson Laboratories). To visualize post-synaptic acetylcholine receptors (AChRs), all fibers were labeled with either rhodamine (1:500) or Alexa Fluor 647-conjugated α-bungarotoxin (BTX; 1:250; Invitrogen) in 0.3% Triton in PBS. Muscles were finally rinsed in PBS and mounted on slides using 50% glycerol/PBS and 0.03 mg/ml p-phenylenediamine to prevent fading.

Teased muscles immunostained for muscle fiber-types were pre-blocked for 30 min in 0.1% Triton-PBS containing 10% normal goat serum. Muscle fibers were then incubated overnight at room temperature in 0.01% Triton-PBS containing primary antibodies against different myosin isoforms (Table 1), washed in PBS, and finally incubated for 2 h in 0.01% Triton-PBS containing BTX (1:250 or 1:500, depending on the fluorophor) and the appropriate secondary antibodies including goat anti-mouse Cy3 IgG (1:500; Jackson ImmunoResearch), Alexa Fluor 594 IgM (1:500; Invitrogen) and goat anti-rabbit Alexa Fluor 488 (1:500; Invitrogen). Muscles were mounted on slides as described above.

Z-stacks were acquired with a laser-scanning confocal microscope (Zeiss Microimaging) utilizing Zeiss Zen 2009 software (Zeiss Microimaging). Three-dimensional rendering was achieved on a subset of z-stacks using the surfaces function in Imaris software (Oxford Instruments).

2.6. Classification of innervation, terminal sprouts, terminal Schwann cell presence, and macrophage density

For quantification of endplate innervation, BTX-positive zones of AChRs were located and visualized by focusing up and down using an upright fluorescence microscope (Leica Instruments). For any given BTX-positive zone, it was determined whether it was innervated, partially innervated, or denervated by the presence, or lack thereof, of presynaptic structures imaged on a different fluorescence channel. If presynaptic structures (either SV2 alone, or SV2 and TUJ1 together) were present in the same configuration as the postsynaptic structure (BTX), it was termed innervated. Endplates with partial colocalization of presynaptic structures were classified as partially innervated, while endplates without any discernible presynaptic structures were classified as denervated. TSCs were characterized as S100β+ cells overlying endplates without any discernible presynaptic structures were classified as partially innervated, while endplates with partial colocalization of presynaptic structures (either SV2 alone, or SV2 and TUJ1 together) were present in the same configuration as the postsynaptic structure (BTX). If BTX-positive zone, it was determined whether it was innervated, partially innervated, or denervated by the presence, or lack thereof, of presynaptic structures. The presence of presynaptic innervation and TSC presence at the NMJ.

Whole mounted muscles stained for BTX and immunolabeled for TuJ1 were used to quantify terminal sprouts. Endplate-rich regions were chosen at random and both the total number of terminal sprouts and number of sprouts extending from an innervated endplate to previously denervated endplate (termed successful sprouts), were quantified. Terminal sprouts were clearly identifiable as small caliber axons extending from terminals innervated by a larger caliber axon (Chipman et al., 2014; Schaefer et al., 2005). A minimum of 250 endplates were counted per muscle, and the proportions of total and successful sprouts were represented as a percentage of the endplates counted.

Whole mounted muscles immunostained for CD11b, S100β and labeled with BTX were used to determine macrophage density (cells/mm²) at 6 endplate-rich regions/muscle. Four muscles were analyzed per group.

2.7. Experimental design and statistical analysis

Two-way ANOVAs were performed to examine the differences between group over time and Mann–Whitney tests were used to compare between groups. Data were analyzed using GraphPad Prism 5 software and were considered statistically significant at $p < 0.05$.

3. Results

3.1. LS spinal nerve transection partially denervates plantaris and soleus muscles in WT and SOD1G93A mice to the same extent

To examine why sprouting is compromised in a subset of motoneurons in SOD1G93A mice prior to disease onset (defined here as prior to the onset of motoneuron death), we partially denervated the fast twitch plantaris (composed of type IIB and IIA fibers) and slow twitch soleus muscle (composed of type I and Ila fibers) at P30 (Hegedus et al., 2007) by cutting and ligating the L5 ventral root (Fig. 1A). We chose to partially denervate muscles at P30 because we wished to characterize terminal sprouting prior to any loss in motoneuron plasticity due to disease progression and cell death. The plantaris and soleus were chosen because they contain different motor unit subtypes (based on their muscle fiber composition) that are differentially vulnerable in ALS (Frey et al., 2000; Hegedus et al., 2008, 2007). More specifically, the plantaris muscle contains 50% type IIB, 25% Ila fibers, and 0% type I fibers while the soleus contains 50% type Ila, 31% type I and only 3% IIB fibers (Bloomberg and Quadrilatero, 2012). In addition, both muscles are innervated by the same spinal nerves.

Before examining whether motoneuron subtypes have differential capacities to sprout after partial denervation, we first needed to ascertain whether the relative distribution of plantaris and soleus motoneurons in the L4 and L5 spinal nerves differed between WT and SOD1G93A mice. To determine this, we partially denervated both muscles in WT and SOD1G93A mice by ligating and cutting the L5 spinal nerve at P30 and then quantified the degree of partial denervation 3 days later using ex vivo tetanic force recordings upon tibial nerve stimulation. Contralateral muscles in each mouse served as unoperated controls. As shown in the tetanic force recordings (Fig. 1B,C), cutting the L5 spinal nerve caused an approximate 40% reduction in plantaris muscle contractile force in both the WT and SOD1G93A mice (Fig. 1B,D).

In the soleus, transecting the L5 spinal nerve caused a 50% loss of force compared to the contralateral control in both the WT and SOD1G93A mice (Fig. 1C,D).

To determine whether the extent of NMJ denervation in plantaris and soleus muscles was comparable to the loss in contractile forces, we characterized the degree and pattern of endplate innervation using BTX
staining and SV2 immunohistochemistry to identify post-synaptic AChRs and synaptic vesicles, respectively. As shown in Fig. 1E, the pattern of innervation, when present, occasionally differed. Consequently, we sub-characterized endplate innervation based on the extent of co-labeling between pre- and post-synaptic markers. NMJs exhibiting ~100% co-localization were considered fully innervated, while BTX⁻ endplates with limited contact with pre-synaptic markers were identified as partially innervated (Fig. 1E). Endplates with a complete absence of pre-synaptic markers were considered denervated (Fig. 1E).

Using this classification scheme, we found that both the plantaris (Fig. 1F) and soleus muscles (Fig. 1G) were equally denervated in the WT and SOD1<sup>G93A</sup> mice after transection of the L5 spinal nerve, and the extent of denervation was comparable to the loss of force. These results indicate that the degrees of partial denervation caused by cutting the L5 spinal nerve were similar between the two muscles, and that the SOD1<sup>G93A</sup> mutation itself does not alter the relative distribution of

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**Fig. 1.** Quantification of endplate denervation after spinal nerve section. A, Schematic illustration showing site of spinal nerve transection (red x) used to partially denervate the plantaris and soleus muscles in WT and SOD1<sup>G93A</sup> mice. B,C Representative tetanic recordings (mN) from partially denervated (PD) and unoperated (unop) plantaris (B) and soleus muscles (C) in WT and SOD1<sup>G93A</sup> mice 3 days post-surgery. D, Mean (± SD) maximum tetanic force of partially denervated WT and SOD1<sup>G93A</sup> plantaris and soleus muscles shown as a percentage of force generated from the unoperated muscles in the contralateral limb (p = 0.500 for plantaris and 0.223 for soleus, Mann-Whitney test). E, Representative confocal images from a PD plantaris muscle, stained for synaptic vesicle protein 2 (SV2) and BTX, show different NMJ innervation patterns. F,G Percent endplate innervation (mean ± SD) in unoperated and partially denervated WT and SOD1<sup>G93A</sup> plantaris (F) and soleus (G) 3 days post-sural nerve transection (p = 0.057 and p = 0.200 for plantaris and soleus muscles, respectively; Mann-Whitney test). ns, not significant; unop, unoperated; PD, partially denervated; SOL, soleus; PLA, plantaris. Scale bar = 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
soleus and plantaris motoneurons in the L4 and L5 spinal nerves prior to P30.

3.2. Reinnervation via sprouting is limited following partial denervation of SOD1G93A plantaris muscles

To ascertain whether motoneurons innervating plantaris and soleus muscles have the same capacity to sprout and innervate denervated fibers prior to disease onset, we transected the L5 spinal nerve at P30 and examined endplate innervation 14 days later to allow time for reinnervation to occur (Son and Thompson, 1995a). As before, unoperated, contralateral muscles were used as internal controls. The examples in Fig. 2A show that some endplates in partially denervated soleus and plantaris muscles in WT and SOD1G93A mice remained denervated, or partially innervated, 14 days after spinal nerve transection (Fig. 2A; arrowheads and asterisks, respectively). When quantified, we observed that the number of innervated endplates was higher 14 days after spinal nerve transection, compared to 3 days after (yellow lines in Figs. 2B,C indicate degree of innervation after 3 days), in partially denervated soleus and plantaris muscles in WT mice. However, while the degree of innervation was the same in partially denervated SOD1G93A and WT soleus muscles (Fig. 2C), partially denervated SOD1G93A plantaris muscles contained significantly fewer innervated endplates compared to partially denervated WT plantaris muscles (Fig. 2B, p = 0.0079). These results suggest that sprouting occurs normally in partially denervated soleus muscles in SOD1G93A mice but is significantly compromised in SOD1G93A plantaris muscles prior to disease onset (i.e. death of motoneurons).

To examine whether sprouting is compromised in SOD1G93A plantaris muscles beyond 14-days post injury, we examined endplate innervation and sprouting in partially denervated WT and SOD1G93A soleus and plantaris muscles 30 days after spinal nerve transection. Fig. 3A shows examples where dual immunolabeling with Tuj1 and SV2 revealed several phenotypes including fully denervated endplates (arrowheads), partially innervated endplates (arrows), terminal sprouts that successfully contacted another endplate (asterisks) and sprouts that did not contact an endplate (+). As observed 14 days after spinal nerve transection, the percentage of innervated endplates in partially denervated WT and SOD1G93A soleus was the same (Fig. 3C; yellow lines indicate degree of innervation at 3 days). In contrast, endplate innervation remained significantly lower in partially denervated SOD1G93A plantaris muscles compared to WT plantaris (Fig. 3B), indicating that reinnervation had not progressed. Interestingly, the degree of endplate denervation was the same in the unoperated and partially denervated SOD1G93A plantaris muscles 30 days after spinal nerve transection. This was not due to an increase in innervation in the partially denervated muscles because the percentage of innervation remained unchanged from the mean values taken after 3 days (Fig. 3B; yellow lines indicate 3-day values). Rather, the observed endplate denervation in the unoperated plantaris likely reflects the progressive denervation that is known to occur in SOD1G93A fast twitch muscles between P30 and P60 (Hagedus et al., 2007; see Discussion).

To characterize the various sprouting phenotypes between different muscle groups, we quantified the percentage of endplates with terminal sprouts (Fig. 3D) and the percentage of terminal sprouts that successfully contacted a denervated endplate (Fig. 3E). We found that the percentage of endplates with terminal sprouts was the same in partially denervated soleus muscles in SOD1G93A and WT mice. In contrast, this percentage was significantly lower in partially denervated SOD1G93A plantaris muscles compared to their WT counterparts (Fig. 3D). Furthermore, the percentage of sprouts that successfully reinnervated an endplate was the same in partially denervated WT and SOD1G93A soleus muscles. However, the number of successful sprouts was significantly lower (p = 0.006) in partially denervated plantaris muscles in SOD1G93A mice compared to the same muscle in WT mice (Fig. 3E). Together with the innervation results, this data suggests that the impairment in endplate reinnervation following partial denervation of the SOD1G93A plantaris muscle, but not SOD1G93A soleus, is likely due to a reduction in sprouting competence.

3.3. TSCs at denervated endplates in SOD1G93A plantaris muscles decline over time following partial denervation

It is well established that TSCs are essential for initiating and facilitating collateral reinnervation by neighboring motoneurons following partial denervation (Son and Thompson, 1995a, 1995b). Therefore, we next sought to investigate if there were differences in TSC morphology between muscle groups that could explain our findings of impaired reinnervation. TSCs and endplates were visualized in whole mounted muscle fibers labeled with BTX and immunostained for presynaptic markers Tuj1/SV2, and S100β to visualize all Schwann cells, including TSCs. We chose to examine muscles 1, 2, and 3 days after partial denervation, as this corresponds to the time that TSCs extend from denervated endplates (Son and Thompson, 1995a). Fig. 4A shows representative labeling in the four muscle groups 3 days after partial denervation. Denervated endplates associated with TSCs are indicated by arrows while denervated endplates not associated with a TSC are demarcated by an arrowhead (Fig. 4). When quantified, > 80% of the denervated endplates in WT plantaris and soleus muscles were associated with TSCs 1 to 3 days after partial denervation (Fig. 4B). In contrast, while > 90% of denervated endplates in partially denervated SOD1G93A soleus muscles were associated with TSCs 1 to 3 days after spinal nerve transection, there was a significant decline in the percentage of denervated endplates in SOD1G93A plantaris muscles associated with TSCs over the same time-period (Fig. 4C). By 3 days, the number of denervated endplates associated with TSCs was significantly less in the SOD1G93A plantaris muscles (40.9 ± 26.2%) compared to the soleus in the same SOD1G93A mice (94.1 ± 6.97%).

3.4. A subset of TSCs become disassociated from endplates in completely denervated SOD1G93A plantaris muscles

To investigate whether all denervated endplates in SOD1G93A plantaris muscles lose TSCs, we completely denervated the soleus and plantaris muscles by cutting and ligating the tibial nerve (Fig. 5A). The presence of TSCs at endplates was then quantified 3 days later. As shown in Fig. 5B, most endplates (> 80%) in denervated WT soleus and plantaris muscles, as well as denervated SOD1G93A soleus muscles, were associated with TSCs. In contrast, significantly fewer endplates (52.2 ± 11.7%) in completely denervated SOD1G93A plantaris muscles were associated with TSCs 3 days after denervation. These findings indicate that only a subset of plantaris endplates lose TSCs 3 days following injury induced denervation.

To rule out the possibility that TSCs are only lost at denervated plantaris endplates after a nerve injury, we quantified the presence of TSCs at denervated endplates in unoperated SOD1G93A mice at a time
When disease-related denervation is well underway in fast twitch muscles (Fig. 3B; see also Pun et al., 2006; Hegedus et al., 2007). We found that only 18.5 ± 7.58% of the denervated endplates in SOD1G93A plantaris muscles were associated with TSCs at P90. This indicates that TSCs are not only lost at endplates denervated by nerve injury, but are also lost at endplates denervated by normal disease progression in SOD1G93A mice (see also Carrasco et al., 2016a).

3.5. TSCs loss is muscle fiber-type specific

The observation that approximately 50% of the endplates in completely denervated SOD1G93A plantaris muscles is intriguing considering the mouse plantaris is composed of approximately 50% type IIb fibers (Bloemberg and Quadrilatero, 2012). Based on this correlation, we decided to examine whether the aberrant loss of TSCs was preferentially associated with IIb muscle fibers in SOD1G93A plantaris muscles following partial denervation. To do so, we partially denervated plantaris muscles in WT and SOD1G93A mice at P30 and labeled endplates on teased muscle fibers 3 days later with BTX. Fibers were also immunostained with antibodies against S100β and myosin heavy chain isoforms IIa or IIb. Fig. 6A and C show representative images of endplates in partially denervated SOD1G93A plantaris muscles immunostained for myosin IIa and IIb, respectively (for WT comparison images, see Fig. 4A). Fig. 6A shows S100β+ TSCs associated with two endplates on type IIa fibers while the endplate on the IIb fiber in Fig. 6C (arrowhead) was not associated with a TSC. Interestingly, the two
endplates on the adjacent negatively stained, and presumably type IIa fibers, were associated with TSCs (Fig. 6C). When quantified, we found that virtually none of the endplates on type IIa muscle fibers in WT and SOD1<sup>G93A</sup> plantaris muscles lacked TSCs (Fig. 6B). Likewise, none of the endplates associated with type IIb fibers in WT plantaris muscles lacked TSCs (Fig. 6D). In contrast, only 64.7 ± 5.56% of endplates on type IIb muscle fibers in SOD1<sup>G93A</sup> plantaris muscles had TSCs (Fig. 6D). Due to antibody constraints, we could not determine the innervation status of the endplates. However, because 100 ± 0.0% and 99.7 ± 0.526% of innervated endplates retained TSCs 3 days after partial denervation in WT and SOD1<sup>G93A</sup> mice, respectively, it is highly likely that endplates lacking TSCs were denervated. Taken together, our results reveal that a population of TSCs associated with type IIb muscle fibers in SOD1<sup>G93A</sup> plantaris muscles are lost 3 days post partial denervation. As a result,
these endplates are unable to participate in the reinnervation process because TSCs are necessary to guide terminal sprouts to denervated endplates.

3.6. Macrophages infiltrate partially denervated muscles and interact with TSCs in SOD1G93A plantaris muscles

Macrophages are known to infiltrate and populate denervated muscles in order to clear cellular debris and contribute to inflammation and recovery (Sakaguchi et al., 2014). Macrophage infiltration also occurs in humans with ALS and in SOD1G93A rodents during disease progression (Trias et al., 2018, 2017) and after injury (Carrasco et al., 2016a). Macrophages and microglia are also known to phagocytose myelin debris after peripheral and central nerve injuries (Gaudet et al., 2011). Based on these observations, we reasoned that macrophages, recruited into partially denervated muscles, could interact with TSCs at type IIb muscle fibers and contribute to their morphological changes and ultimate removal from these endplates.

Fig. 7A shows representative examples of intramuscular CD11b+ macrophages in WT and SOD1G93A plantaris and soleus muscles 3 days after partial denervation. Quantification of macrophage density near endplate-rich regions showed that SOD1G93A plantaris and soleus muscles were infiltrated by significantly more macrophages compared to their WT counterparts, although neither WT nor SOD1G93A plantaris contained as many macrophages as partially denervated soleus muscles (Fig. 7B). Given that macrophage infiltration to the soleus was greater than in the plantaris, we decided to examine macrophage/endplate interactions. When the macrophages around the denervated endplates in SOD1G93A plantaris and soleus muscles were examined closely, we noticed clear differences in patterning. As shown in the computer rendering (Fig. 7C), macrophages were often located very near endplates and in direct contact with TSCs in SOD1G93A plantaris muscles while they were diffusely distributed around the endplates in SOD1G93A soleus muscles. When quantified, we found that the number of macrophages at denervated endplates was significantly higher in partially denervated SOD1G93A plantaris muscle compared to the soleus (Fig. 7D), even though substantially more macrophages infiltrated the soleus muscle in SOD1G93A mice (Fig. 7B). Therefore, it appears that macrophage infiltration does not necessarily correlate with TSC loss per se, but rather there may be pathological interactions with a subset of TSCs within the fast twitch muscles, specifically, that contributes to their removal from denervated endplates.

3.7. The selective tyrosine kinase inhibitor, masitinib, reduces macrophage infiltration, TSC loss and improves reinnervation in partially denervated SOD1G93A plantaris muscles

The close association between macrophages and endplates, along with TCSs, in the SOD1G93A plantaris muscles is intriguing because it suggests that they could contribute to TSC removal. To investigate this possibility, we administered masitinib mesylate to WT and SOD1G93A mice 3 days before partially denervating the plantaris muscle, and then daily thereafter. Masitinib mesylate was chosen because it is a selective receptor tyrosine kinase inhibitor (Hahn et al., 2008) that prevents proliferation and migration of hematopoietic cells such as macrophages, and has been shown to decrease macrophage infiltration in SOD1G93A rat extensor digitalis longus (EDL) muscles after the onset of paralysis (Trias et al., 2017).

As occurred in SOD1G93A rats (Trias et al., 2017), masitinib significantly decreased the number of macrophages near endplate rich regions of SOD1G93A plantaris muscles 3 days after partial denervation compared to untreated animals (Fig. 8A). Masitinib treatment has little effect on macrophage infiltration in WT mice (Fig. 8A). Furthermore, there were significantly fewer CD11b+ macrophages per denervated endplate in WT and SOD1G93A plantaris muscles after masitinib treatment, compared to untreated SOD1G93A mice, 3 days after partial denervation (Fig. 8B). To determine whether this decrease in macrophage number affected TSC patterning, we quantified the percentage of denervated endplates with TSCs in partially denervated plantaris muscles from masitinib treated and untreated WT and SOD1G93A mice. Fig. 8C shows that approximately half of the denervated endplates in untreated animals lacked TSCs 3 days after partial denervation while virtually all denervated endplates in masitinib treated SOD1G93A mice were associated with TSCs at the same time point. In fact, the number of plantaris endplates with TSCs was the same (> 95%) in masitinib treated WT and SOD1G93A mice (Fig. 8C).

Finally, to determine whether the prevention of TSC loss enhances terminal sprouting and successful reinnervation in plantaris muscles, we quantified endplate innervation in masitinib treated and untreated mice 14 days after partial denervation. Fig. 8D shows that the number of reinnervated endplates in plantaris muscles was significantly higher in masitinib treated SOD1G93A mice compared to untreated mice, although the percentage was not as high as masitinib treated WT mice. Taken together, this data strongly suggest that macrophage infiltration contributes to the loss of type IIb TSCs in SOD1G93A plantaris muscles, and that this loss attenuates terminal sprouting and reinnervation of denervated endplates after partial denervation in muscles containing type IIb fibers. Furthermore, these results indicate that the impaired sprouting response is not solely an anomaly of SOD1G93A motoneurons, but rather it is due, in part, to TSC pathology.

4. Discussion

In this study, we demonstrate that TSCs associated with type IIb fibers in plantaris muscles abnormally vacate denervated endplates when muscles are partially denervated by spinal nerve transection prior to disease onset in SOD1G93A mice. The same was not true for type Ia or type I fibers in the plantaris and soleus muscles. This pathology was
correlated with reduced terminal sprouting from neighboring motorneurons and reinnervation of denervated type IIb muscle fibers. In addition, infiltration of macrophages around denervated endplates was significantly higher in SOD1\textsuperscript{G93A} plantaris muscles after partial denervation compared to soleus muscles in the same animals. Administration of the tyrosine kinase inhibitor, masitinib, reduced macrophage infiltration in partially denervated SOD1\textsuperscript{G93A} plantaris muscles and prevented the loss of TSCs at denervated endplates on type IIb fibers. Most importantly, pharmacological prevention of TSC loss at denervated endplates restored terminal sprouting to near WT values in partially denervated plantaris muscles in SOD1\textsuperscript{G93A} mice. Together, these findings support the hypothesis that a subpopulation of motorneurons innervating fast twitch muscles fail to sprout in SOD1\textsuperscript{G93A} mice, not because the mutation adversely affects the sprouting capacity of the motorneurons themselves, but because TSCs associated with them behave aberrantly (Carrasco et al., 2016a). Furthermore, our results suggest that pharmacological interventions preventing the loss of TSCs at denervated endplates may be useful for slowing the development of

![Muscle fiber-type specific absence of TCSs at endplates in SOD1\textsuperscript{G93A} plantaris muscles. A,C Confocal images of endplates on plantaris muscle fibers in SOD1\textsuperscript{G93A} mice labeled with BTX and immunostained for S100\textbeta and myosin Ila (A) or I Ib (C). B,D Quantification of TSC presence (mean ± SD) at endplates on type Ila (B) and I Ib (D) plantaris muscle fibers in WT and SOD1\textsuperscript{G93A} mice. p = 0.3223 and p = 0.0133 for Ila and I Ib, respectively (Mann-Whitney test). Scale bar = 50 μm.](image)
muscle weakness and improving overall motor function in ALS.

4.1. Sprouting in SOD1G93A mice

Our observation that sprouting is compromised in SOD1G93A plantaris, but not soleus muscles, is consistent with previous studies examining sprouting in fast and slow twitch muscles in SOD1G93A rodent models of ALS (Frey et al., 2000; Hegedus et al., 2007; Pun et al., 2006; Schaefer et al., 2005). More specifically, electrophysiological studies on fast twitch medial gastrocnemius (MG), tibialis anterior (TA) and extensor digitalis longus (EDL) muscles in SOD1G93A mice showed a near parallel decline in motor unit numbers and force between P40 to P80, while neither declined over the same time period in the soleus (Hegedus et al., 2007, 2008). These results suggest that little sprouting had occurred by P80 even though 65% of the motoneurons innervating fast twitch muscles had died (Hegedus et al., 2008; Pun et al., 2006). Using anatomical techniques, Caroni and colleagues (Pun et al., 2006) reported a complete loss of motoneurons innervating type IIb fibers (i.e. fast fatigue resistant units, FF) in the fast twitch lateral gastrocnemius muscle by P55 in SOD1G93A mice. This loss was accompanied by robust and sustained sprouting by motoneurons innervating type I fibers (i.e. S motoneurons) with only modest and transient sprouting from motoneurons innervating fast twitch muscles containing type Ia and IIb fibers (Guido et al., 2010). One population capable of sprouting while the other was not.

Fig. 7. Macrophages infiltrate partially denervated muscles and interact with TSCs more frequently and denervated endplates in plantaris compared to soleus muscles in SOD1G93A mice. A, Representative confocal images of partially denervated plantaris and soleus muscles in WT and SOD1G93A mice 3 days after surgery. Muscles were labeled with BTX and immunostained for CD11b. B, Macrophage density (mean ± SD) around endplates on plantaris and soleus muscles in WT and SOD1G93A mice (*p = 0.0143; Mann-Whitney test). C, Imaris 3-dimensional renderings of confocal images taken of endplates on plantaris and soleus muscle fibers in SOD1G93A mice 2 days after surgery showing S100 (green), macrophages (CD11b – red), and BTX (white). D, Macrophages per denervated endplates was significantly higher in SOD1G93A PLA compared to SOL. scale bars = 5 μm; SOD1G93A SOL left scale = 20 μm, right scale = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Fig. 8. Decreasing intramuscular macrophage density increases TSC presence and sprouting in partially denervated SOD1<sup>G93A</sup> plantaris muscles. A, Mean (± SD) macrophage density at endplates in partially denervated WT and SOD1<sup>G93A</sup> plantaris muscles in masitinib treated and untreated mice, 3 days after partial denervation. (*p = 0.0143). B, Mean (± SD) macrophages per denervated endplate in partially denervated WT and SOD1<sup>G93A</sup> plantaris muscles in masitinib treated and untreated mice. *p = 0.0147 WT untreated vs. SOD1<sup>G93A</sup> untreated, *p = 0.0147 WT treated vs. SOD1 treated, and *p = 0.0143 SOD1<sup>G93A</sup> untreated vs. SOD1<sup>G93A</sup> treated, Mann-Whitney tests. C, Mean (± SD) percentage of denervated endplates with TSCs in partially denervated plantaris muscles from WT and SOD1<sup>G93A</sup> mice after 3 days of recovery that were treated or not treated with masitinib. (*p = 0.0278, WT untreated vs SOD1<sup>G93A</sup> untreated; *p = 0.0159, WT untreated vs WT treated; *p = 0.0079, SOD1<sup>G93A</sup> untreated vs SOD1<sup>G93A</sup> treated; and **p = 0.0079, SOD1<sup>G93A</sup> untreated vs WT treated). D, Mean (± SD) percent innervation of endplates in partially denervated WT and SOD1<sup>G93A</sup> plantaris muscles 14 days of surgery. Mice were treated or untreated with masitinib 3 days prior to the partial denervation. (*p = 0.0143, SOD1<sup>G93A</sup> untreated vs SOD1<sup>G93A</sup> treated; *p = 0.0286, SOD1<sup>G93A</sup> treated vs WT treated; **p = 0.0079, SOD1<sup>G93A</sup> untreated vs WT untreated; ns p = 0.0556, WT untreated vs SOD1<sup>G93A</sup> treated (Mann-Whitney test used throughout).
Although the identities of the motoneuron subtypes were not identified in the study, it is tempting to speculate from our results that the population of motoneurons with the low sprouting capacity were innervating type IIb fibers.

In a more recent study, Martineau et al. (2018) used repetitive in vivo imaging of identified NMJs from single motor units in the EDL over three months to show that, as axons withdraw from NMJs, new axons sprout to nearby endplates on type IIb fibers in SOD1<sup>G93A</sup> mice. At present, it is not entirely clear why these results differ from the above studies. One possibility is that the previous studies used the fast-progressing SOD1<sup>G93A</sup> mouse model rather than the slower progressing SOD1<sup>G93B</sup> model. One might expect macrophage infiltration would be greater, and occur more rapidly, in SOD1<sup>G93A</sup> mice because motoneurons die faster. This synchronous infiltration could then lead to the rapid loss of TSCs and related attenuation in sprouting response. It is interesting to note that few motor units reinnervated their own vacated NMJs during disease progression in the SOD1<sup>G93B</sup> mouse model (Martineau et al., 2018). Whether this is due to macrophage infiltration and the loss of TSCs is not known and thus requires further investigation.

Interestingly, we did not observe further denervation in partially denervated SOD1<sup>G93A</sup> plantaris muscles between P30 and P60 (Fig. 3B) even though additional motoneurons would have died during this time period (Hegedus et al., 2007). Although the reasons for this observation is not known, these results support previous studies showing that the rate of denervation in SOD1<sup>G93A</sup> mice is attenuated if the muscles are partially denervated and functionally overloaded at P40 (Gordon et al., 2010), as well as following sciatic nerve crush (Sharp et al., 2018), peripheral axotomy (Kong and Xu, 1999), and tibial nerve crush in SOD1<sup>G93A</sup> rats (Franz et al., 2009). Functional overload could enhance neuromuscular stability in SOD1<sup>G93A</sup> mice because it converts type IIb fibers to become Ila fibers, resulting in less forceful motor units and preserved innervation (Gordon et al., 2010). However, other studies have reported that damage to peripheral nerves accelerates motor axon degeneration in SOD1<sup>Wt</sup> mice (Schram et al., 2019; Sharp et al., 2005). It is possible that factors such as injury severity, location, and disease stage may contribute to these conflicting results, and further studies exploring how axonal injury influences MN survival versus degeneration are warranted.

4.2. Loss of TSCs at denervated endplates in SOD1<sup>G93A</sup> mice

Our results showing TSC loss at denervated endplates on type IIb fibers are consistent with previous publications documenting irregularities in TSC morphology and activity during disease progression in SOD1<sup>G93A</sup> mice (Carrasco et al., 2016b, 2016a). For example, TSCs were found to be unusually disorganized at endplates in the fast twitch medial gastrocnemius muscle prior to denervation in SOD1<sup>G93A</sup> and SOD1<sup>G85R</sup> mice, and later, as the disease progressed, became absent from denervated NMJs (Carrasco et al., 2016b). Electron microscopy later confirmed the absence of TSCs at endplates 7 days after denervation leading Pinter and colleagues (Carrasco et al., 2016a) to suggest that this abnormality could hinder reinnervation from neighboring motoneurons.

Although ALS is not initiated by immune alterations, disease progression is amplified by activated microglia and inflammatory responses in the central nervous system (CNS) (Beers and Appel, 2019; Thonhoff et al., 2018). Inflammatory responses, including intramuscular infiltration of skeletal muscles with macrophages, have also been documented outside the CNS in animal models of ALS and in patients with the disease (Beers and Appel, 2019; Martinez-Muriana et al., 2016; Trias et al., 2017; Van Dyke et al., 2016; Wang et al., 2017). Furthermore, interventions reducing intramuscular macrophage infiltration attenuate the rate of endplate degeneration (Martinez-Muriana et al., 2016; Trias et al., 2018, 2017; Van Dyke et al., 2016; Wang et al., 2017) and improve the overall sprouting capacity caused by the loss of TSCs at type IIb fibers (present study).

While little is known about macrophage-SC interactions in ALS, a great deal is known about the role of macrophages in Wallerian degeneration. Following a peripheral injury, SCs produce monocyte chemotactic proteins, which contribute to the recruitment of macrophages to the injury site, where they work to phagocytose axonal and myelin debris produced by the degenerating distal nerve segment (Caillaud et al., 2019; Stoll et al., 1989; Toews et al., 1998). Indeed, macrophage-SC dynamics are essential for normal axonal regeneration and remyelination after a peripheral nerve injury (Barrette et al., 2008; Stratton et al., 2018). Therefore, it is not intuitive why intramuscular macrophages would change from a cell required for axonal repair to one detrimental to it in ALS. Macrophage infiltration alone likely does not result in TSC loss given that the WT and SOD1<sup>G93A</sup> soleus muscles both had higher numbers of macrophages than the plantaris (Fig. 7B). One possible explanation for this is that the number of terminal sprouts generated in the soleus are also higher (Fig. 3D). Perhaps more macrophages are required to clear debris to accommodate this regenerative response in the soleus.

Gene expression is known to be altered as early as P40 in the spinal cord and muscle of SOD1<sup>G93A</sup> mice (de Oliveira et al., 2013; Saris et al., 2013). It is, therefore, conceivable that altered gene expression in type IIb TSCs could make them more susceptible to phagocytosis. Interestingly, studies by Rotshenker and colleagues suggest that myelin in the CNS and peripheral nervous system normally down-regulate their own phagocytosis by sending a “do not eat me” message to macrophages by expressing CD47 on their cell surface (Gitik et al., 2014, 2011). It is possible that the SOD1<sup>G93A</sup> mutation alters CD47 expression and/or its function in TSCs in SOD1<sup>G93A</sup> mice causing the cells to be targeted and degraded by infiltrating macrophages. However, if true, it still does not alone explain why TSCs associated with denervated type IIb endplates are more likely to be selectively targeted.

There is precedence for the targeting and degradation of specific spinal neuronal subtypes by glia in ALS (Di Giorgio et al., 2008; Nagai et al., 2007) indicating that distinct cell recognition and subsequent degradation can take place. In addition, it is well recognized that distinct subclasses of motoneurons with differing activation patterns selectively innervate discrete subtypes of muscle fibers. Because Ca<sup>2+</sup> responses in TSCs discriminate between patterns of activity and regulate presynaptic plasticity (Todd et al., 2010), it seems plausible that different subclasses of TSCs exists to match motoneuron activation patterns and muscle fiber contractile properties. Evidence for different TSC subtypes comes from the study by De Winter et al., (2006) where they showed that semaphorin 3A (Sema3A), an inhibitor of axonal regeneration, is selectively upregulated in TSCs associated with type Iib muscle fibers in SOD1<sup>G93A</sup> mice (De Winter et al., 2006), which could contribute to the limited reinnervation of these endplate types. Indeed, prolonged Sema3A exposure has been shown to induce neural progenitor cell apoptosis in vitro (Bagnard et al., 2001), which could explain why TSCs at these endplates are lost. Furthermore, the axon-refractory activity of Sema3A has also been shown to be tyrosine kinase activity-dependent (Bagnard et al., 2001), providing a possible mechanism for how masitinib (a tyrosine kinase inhibitor) prevents the selective loss of TSCs from denervated NMJs associated with type Iib muscle fibers. Whether other proteins such as CD47 are selectively expressed or repressed in TSCs at denervated type Iib fibers in SOD1<sup>G93A</sup> mice remains to be determined.
Evidence to support this notion comes from a recent randomized clinical trial where mitoxantrone, combined with riluzole, slowed Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) decline in patients with ALS to a clinically meaningful degree (Mora et al., 2019). Furthermore, clinical improvement was greater when the mitoxantrone/riluzole treatment was initiated at a less severe stage of the disease (Mora et al., 2019). These clinical findings are consistent with our study because we would predict better outcomes if mitoxantrone was administered early in disease progression when the larger motoneurons innervating type IIb fibers are still functioning and capable of sprouting. Improved sprouting capacity would maintain motor function longer and slow the ALSFRS-R decline. Finally, while more research is clearly warranted, this study highlights the NMJ as early therapeutic target to improve motoneuron function and enhance quality of life for patients with ALS.

Author Statement

Julia Harrison: Methodology, Data analysis, Writing- Original draft.
Vctor Rafuse: Conceived the project, acquired funding for the project, participated in data analysis, and reviewed and edited the final version of the manuscript.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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References

Ajroud-Driss, S., Siddique, T., 2015. Sporadic and hereditary amyotrophic lateral sclerosis (ALS). Biochem. Biophys. Acta 1852, 679–684. 10.1016/j.bbadis.2014.08.010.
Arbour, D., Velec, C. Vande, Robitaille, R., 2017. New perspectives on amyotrophic lateral sclerosis. Mol. Cell. Neurosci. 6, 349–362. 10.1016/j.mcn.2016.09.022.
de Oliveira, G.P., Alves, C.J., Chiu, A., 2013. Early gene expression changes in spinal cord from SOD1(G93A) amyotrophic lateral sclerosis animal model. Front. Cell. Neurosci. 7, 216. 10.3389/fncel.2013.00216.
de Winter, F., Vo, T., Stum, F.J., Wisman, L.A.B., Bo, P.R., Niclou, S.P., van Muijswinkel, F.L., Verhaagen, J., 2006. The expression of the chemorepellent Semaphorin IA is selectively induced in terminal Schwann cells of a subset of neuromuscular synapses that display limited anatomical plasticity and enhanced vulnerability in motor neuron disease. Mol. Cell. Neurosci. 32, 102–117. 10.1016/j.mcn.2006.09.002.
Dengler, R., Konstanzer, A., Kutherford, G., Hesse, S., Wolf, W., Struppler, A., 1990. Amyotrophic lateral sclerosis: macro-EMG and twitch forces of single motor units. Muscle Nerve 13, 545–550. 10.1002/mus.880130612.
Di Giorgio, F.P., Boulting, G.L., Bobrowicz, S., Egan, K.C., 2008. Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. Cell Stem Cell 3, 637–648. 10.1016/j.stem.2008.01.017.
Franz, C.K., Quach, E.T., Kruy, C.A., Federici, T., Kliem, M.A., Snyder, B.R., Ropper, A., Boullis, N.M., 2009. A conditioning lesion provides selective protection in a rat model of amyotrophic lateral sclerosis. PLoS One 4, e7357. 10.1371/journal.pone.0007357.
Frey, D., Schneider, C., Xu, L., Borg, J., Spooren, W., Caroni, P., 2000. Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron disease. J. Neurosci. 20, 2534–2542.
Gauthier, R., Popovich, P.G., Riesz, R.M.S., 2021. Wallerian degeneration: Gaining perspective on inflammatory events after peripheral nerve injury. J. Neuroinflammation 8. 10.1186/s12974-020-01224.
Glik, M., Liras-Zalzams, S., Oldenberg, P.-A., Reichert, F., Rothshenker, S., 2011. Myelin down-regulates myelin phagocytosis by microglia and macrophages through interactions between CD47 on myelin and SIRPalpha (signal regulatory protein-alpha) on phagocytes. J. Neuroinflammation 8, 24. 10.1186/1742-2094-8-24.
Glik, M., Kleinhaus, R., Hadas, S., Reichert, F., Rothshenker, S., 2014. Phagocytic receptor activation and immune inhibitory receptor SIRPalpha inhibits phagocytosis through pallin and colluin. Front. Cell. Neurosci. 8, 104. 10.3389/fncel.2014.00104.
Gordon, T., Hegedus, J., Tam, S.L., 2004. Adaptive and maladaptive motor axonal sprouting in aging and motoneuron disease. Neurobiol. Res. 26, 174–185. 10.1002/nrb.1442.
Guido, T.W., Buss, R.R., Vinsant, S., Prevette, D., Sun, W., Knudson, C.M., Milligan, C.E., Oppenheimer, R.W., 2006. Complete dissociation of motor neuron death from motor dysfunction by axon deletion in a mouse model of ALS. J. Neurosci. 26, 8774–8786. 10.1523/JNEUROSCI.2153-06.2006.
Guido, A.N., Campos, G.E.R., Neto, H.S., Marques, M.J., Minatel, E., 2010. Fiber type composition of the sternomastoid and diaphragm muscles of dystrophin-deficient mdx mice. Anat. Rec. (Hoboken). 293, 1722–1728. 10.1002/ar.21124.
Gurney, M.E., Pu, H., Chiu, A.Y., Dal Canto, M.C., Polchow, C.Y., Alexander, D.D., Caliendo, J., Hentati, A., Kwon, Y.W., Gurney, M.E., Pu, H., Chiu, A.Y., Canto, M.C.D., Polchow, C.Y., Alexander, D.D., Caliendo, J., Hentati, A., Kwon, Y.W., Deng, H.X., Chen, W., Zhai, P., Sufit, R.L., Siddique, T., 1994. Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science 80 (264), 1772–1775.
Hahn, K.A., Ogilvie, G., Rask, T., Devachelle, P., Leblanc, A., Legendre, A., Powers, B., Leventhal, P.S., Kinet, J.-P., Palmerini, F., Dubreuil, P., Moussey, A., Hermine, O., 2008. Mnsatinb is safe and effective for the treatment of canine mast cell tumors. J. Vet. Intern. Med. 22, 1301–1309. 10.1111/j.1939-1676.2008.04746.x.
Hegedus, J., Putman, C.T., Gordon, T., 2007. Time course of preferential motor unit loss in the SOD1 G93A mouse model of amyotrophic lateral sclerosis. Neurobiol. Dis. 28, 154–164. 10.1016/j.nbd.2007.07.003.
Hegedus, J., Putnam, C.T., Tyreman, N., Gordon, T., 2008. Preferential motor unit loss in the SOD1 G93A transgenic model of amyotrophic lateral sclerosis. J. Physiol. 586, 3337–3351. 10.1111/j.1469-7711.2007.05896.x.
Heiman-Patterson, T.D., Sher, R.B., Blankenhorn, E.A., Alexander, G., Deitch, J.S., Kunst, C.B., Cox, G., 2011. Effect of genetic background on phenotype variability in transgenic mouse models of amyotrophic lateral sclerosis: A window of opportunity in the search for genetic modifiers. Amyotrophic Lateral Scler. 12, 79–86. 10.3109/17482636.2010.575216.
Kong, J., Xu, Z., 1999. Peripheral axotomy slows motoneuron degeneration in a transgenic mouse line expressing mutant SOD1 G93A. J. Comp. Neurol. 412, 373–380. 10.1002/(sici)1096-9861(19990920)412:2<373::aid-cne13>3.0. CO;2-v.
Lee, Y.B., Thompson, W.J., Harlow, M.L., 2017. Schwann cells participate in synapse elimination at the developing neuromuscular junction. Curr. Opin. Neurobiol. 40, 176–181. 10.1016/j.conb.2017.01.010.
Martineau, É., Di Polo, A., Vande, C., Robitaille, R., 2018. Dynamic neuromuscular
remodeling precedes motor-unit loss in a mouse model of ALS. Elife, e41973. https://doi.org/10.7554/elif.e41973.

Martinez-Muriana, A., Mancuso, R., Francos-Quijorna, I., Olmos-Alonso, A., Osta, R., Perry, V.H., Navarro, X., Gomez-Nicola, D., Lopez-Vales, R., 2016. CSFIR blockade slows the progression of amyotrophic lateral sclerosis by reducing microgliosis and invasion of macrophages into peripheral nerves. Sci. Rep. 6, 25663. https://doi.org/10.1038/srep25663.

Mora, J.S., Genge, A., Chio, A., Estol, C.J., Chaverri, D., Hernández, M., Marín, S., Masciás, J., Rodríguez, G.E., Paipa, A., Dominguez, R., Gamez, J., Salvador, M., Lunetta, C., Ballario, C., Riva, N., Mandrioli, J., Mounsy, A., Kinet, J., Auclair, C., Dubreuil, P., Arnold, V., Mansfield, C.D., Hermine, O., 2019. Mastitis as an add-on therapy to riluzole in patients with amyotrophic lateral sclerosis: A randomized clinical trial. Amyotroph. Lateral Scler. Front. Degener. 1–10. https://doi.org/10.1080/21678421.2019.1632346.

Murray, L.M., Talbot, K., Gillingwater, T.H., 2010. Review: neuromuscular synaptic dysfunction in motor neuron disease alleviated by CNTF. Nat. Rev. Neurol. 6, 1135–2990.2010.01061.x.

Nagai, M., Re, D.B., Nagata, T., Chalazonitis, A., Jessell, T.M., Wichterle, H., Przedborski, M., Murray, L.M., Talbot, K., Gillingwater, T.H., 2010. Review: neuromuscular synaptic dysfunction in motor neuron disease alleviated by CNTF. Nat. Rev. Neurol. 6, 1135–2990.2010.01061.x.

Nagai, M., Re, D.B., Nagata, T., Chalazonitis, A., Jessell, T.M., Wichterle, H., Przedborski, M., Murray, L.M., Talbot, K., Gillingwater, T.H., 2010. Review: neuromuscular synaptic dysfunction in motor neuron disease alleviated by CNTF. Nat. Rev. Neurol. 6, 1135–2990.2010.01061.x.

Nagai, M., Re, D.B., Nagata, T., Chalazonitis, A., Jessell, T.M., Wichterle, H., Przedborski, S., 2007. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat. Neurosci. 10, 615–622. https://doi.org/10.1038/nn1876.

Pun, S., Santos, A.F., Saxena, S., Xu, L., Caroni, P., 2006. Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. Nat. Neurosci. 9, 408–419. https://doi.org/10.1038/nn1653.

Rafuse, V.F., Gordon, T., Oronco, R., 1992. Proportional enlargement of motor units after partial denervation of cat tibialis surae muscles. J. Neurophysiol. 68, 1261–1276. https://doi.org/10.1152/jn.1992.68.4.1261.

Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O’Regan, J.P., Deng, H.K., 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362, 59–62. https://doi.org/10.1038/36259a0.

Sakaguchi, S., Gershon, M., Suzuki, T., Sawano, S., Anderson, J.E., Do, M.Q., Ohtsubo, H., Mizuno, W., Sato, Y., Nakamura, M., Furuse, M., Yamada, K., Ikeuchi, Y., Tatsumi, R., 2014. Implication of anti-inflammatory macrophages in regenerative motor-neuromuscular reinnervation: Promotion of myoblast migration and neural chemorepellent semaphorin 3A expression in injured muscle. J. Int. Biochem. Cell Biol. 54, 272–285. https://doi.org/10.1016/j.bjolcel.2014.05.032.

Saris, C.G.J., Groen, E.J.M., van Voight, P.W.J., van Es, M.A., Blauw, H.M., Veldink, J.H., van den Berg, L.H., 2013. Gene expression profile of SOD1(G93A) mouse spinal cord, blood and muscle. Amyotroph. Lateral Scler. Frontotemporal Degener. 14, 190–198. https://doi.org/10.3109/21678421.2012.749914.

Schlaeter, A.M., Sanes, J.R., Lichtman, J.W., 2005. A compensatory subpopulation of motor neurons in a mouse model of amyotrophic lateral sclerosis. J. Comp. Neurol. 490, 209–219. https://doi.org/10.1002/cne.20620.

Schram, S., Tsang, S.C., Schmidt, G., Piponov, H., Heldor, C., Kems, J., Gonzalez, M., Song, F., Loeb, J.A., 2019. Mutant SOD1 prevents normal functional recovery through enhanced glia activation and loss of motor neuron innervation after peripheral nerve injury. Neurobiol. Dis. 124, 469–478. https://doi.org/10.1016/j.nbd.2018.12.020.

Sharma, K.R., Miller, R.G., 1996. Electrical and mechanical properties of skeletal muscle underlying increased fatigue in patients with amyotrophic lateral sclerosis. Muscle Nerve 19, 1391–1400. https://doi.org/10.1002/(SICI)1097-4598(19961119)11:1<1391:AID-MUS3>3.0.CO;2-T.

Sharp, P.S., Dick, J.R.T., Greensmith, L., 2005. The effect of peripheral nerve injury on disease progression in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Neuroscience 130, 897–910. https://doi.org/10.1016/j.neuroscience.2004.09.069.

Sharma, K.R., Miller, R.G., 1996. Electrical and mechanical properties of skeletal muscle underlying increased fatigue in patients with amyotrophic lateral sclerosis. Muscle Nerve 19, 1391–1400. https://doi.org/10.1002/(SICI)1097-4598(19961119)11:1<1391:AID-MUS3>3.0.CO;2-T.

Wang, H.A., Lee, J.D., Lee, K.M., Woodruff, T.M., Noakes, P.G., 2017. Complement C3a-C5aR1 signaling drives skeletal muscle macrophage recruitment in the hSOD1(G93A) mouse model of amyotrophic lateral sclerosis. Skelet. Muscle 7, 10. https://doi.org/10.1186/s13395-017-0128-8.

Wiechers, D.O., 1985. Acute and latent effect of poliomyelitis on the motor unit as revealed by electromyography. Orthopedics 8, 870–872.

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