BDNF-dependent modulation of axonal transport is selectively impaired in ALS

Andrew P. Tosolini1,2*, James N. Sleigh1,2,3†, Sunaina Surana1,2,3†, Elena R. Rhymes1,2, Stephen D. Cahalan4 and Giampietro Schiavo1,2,3*

Abstract
Axonal transport ensures long-range delivery of essential cargoes between proximal and distal compartments, and is needed for neuronal development, function, and survival. Deficits in axonal transport have been detected at pre-symptomatic stages in the SOD1G93A and TDP-43M337V mouse models of amyotrophic lateral sclerosis (ALS), suggesting that impairments in this critical process are fundamental for disease pathogenesis. Strikingly, in ALS, fast motor neurons (FMNs) degenerate first whereas slow motor neurons (SMNs) are more resistant, and this is a currently unexplained phenomenon. The main aim of this investigation was to determine the effects of brain-derived neurotrophic factor (BDNF) on in vivo axonal transport in different α-motor neuron (MN) subtypes in wild-type (WT) and SOD1G93A mice. We report that despite displaying similar basal transport speeds, stimulation of wild-type MNs with BDNF enhances in vivo trafficking of signalling endosomes specifically in FMNs. This BDNF-mediated enhancement of transport was also observed in primary ventral horn neuronal cultures. However, FMNs display selective impairment of axonal transport in vivo in symptomatic SOD1G93A mice, and are refractory to BDNF stimulation, a phenotype that was also observed in primary embryonic SOD1G93A neurons. Furthermore, symptomatic SOD1G93A mice display upregulation of the classical non-pro-survival truncated TrkB and p75NTR receptors in muscles, sciatic nerves, and Schwann cells. Altogether, these data indicate that cell- and non-cell autonomous BDNF signalling is impaired in SOD1G93A MNs, thus identifying a new key deficit in ALS.

Keywords: Axonal transport, Amyotrophic lateral sclerosis, Motor neuron, BDNF, TrkB, p75NTR

Introduction
Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease primarily affecting motor neurons (MNs), leading to muscle atrophy, paralysis and ultimately death due to respiratory failure. Although only a small proportion of ALS-causing mutations are found in genes encoding components of the axonal transport machinery (e.g., KIF5A, DCTN1, ANXA11), altered axonal transport is a common pathological feature downstream of many ALS-causing mutations [1, 2]. Axonal transport maintains neuronal homeostasis by ensuring the long-range delivery of several cargoes, including cytoskeletal components, organelles, signalling molecules and RNA between proximal and distal neuronal compartments [3]. As a result, perturbations in axonal transport have severe consequences for neuronal homeostasis and function [4]. Indeed, we have previously demonstrated that deficits in in vivo axonal transport occur pre-symptomatically (i.e., before MN loss) across diverse ALS mice [5–9].

α-MNs are defined by the type of skeletal muscle fibre they innervate, and can be sub-classified according to their firing pattern into fast (FMNs) and slow (SMNs) MNs, each with distinct anatomical, metabolic, and...
functional properties [10–13], as well as diverse transcriptional profiles [14]. FMNs innervate type-IIB and -IIX fast-twitch fatigable and type IIA fast-twitch fatigue-resistant muscle fibres to execute fine motor control, whereas SMNs innervate type I slow-twitch fatigue-resistant muscle fibres to exert postural control [11]. Strikingly, FMNs are more susceptible to ALS pathology, whereas SMNs are predominantly resistant [13]. Preferential FMN vulnerability has been observed in SOD1 [15–17], TDP-43 [18], FUS [19] and C9ORF72 [20] mutant mice, with limb-onset ALS accounting for ~70% of human pathology [21], suggesting that preferential FMN vulnerability in ALS is conserved across species.

The exclusively FMN-innervated tibialis anterior (TA) muscle [22–24] undergoes pathological changes early in disease in ALS mice [15–17, 19], with neuromuscular junction (NMJ) denervation occurring before MN loss [25], and pathology in TA is observed in ALS patients [26]. In contrast, the predominantly SMN-innervated soleus muscle is more resistant to pathology [19, 23–25]. ALS induces a fast-to-slow muscle fibre type switch in the TA, with a significant reduction in type IIB fibres and a concomitant increase in type IIA/IIX fibres [23, 24, 27]. This precedes NMJ denervation and has, at least in part, attributed to a metabolic switch in fast-twitch glycolytic muscles [28, 29]. Intriguingly, this fast-to-slow muscle fibre switching is also observed in mice with muscle-specific ablation of brain-derived neurotrophic factor (BDNF), with phenotypes including reduced type IIB muscle fibres, motor endplate size, and expression of muscle-specific glycolytic genes, with concomitant increases in the amount of type IIX muscle fibres [22]. Furthermore, neurotrophic factors have been shown to regulate muscle and MN subtype identities [30]. Indeed, BDNF mediates fast glycolytic fibre types [22], neurutrin regulates slow-twitch motor unit development [31], whilst γ-MNs require muscle spindle-derived glial cell-derived neurotrophic factor (GDNF) for postnatal survival [32].

The neurotrophin BDNF controls the development and maintenance of neurons through binding to TrkB and p75NTR receptors. TrkB exists as three differentially spliced isoforms, namely the full-length TrkB receptor (TrkB.FL) and two shorter, kinase-deficient truncated isoforms, TrkB.T1 and TrkB.T2 [33]. The cytoplasmic tyrosine kinase domain present in TrkB.FL is fundamental for pro-survival signalling via ERK1/2, Akt and PLC-γ controlled pathways [34]. However, activation of these pathways is dampened by TrkB.T1 and TrkB.T2, which lack the essential kinase domain and sequester synaptic BDNF [35]. The physiological roles of p75NTR are equally complex [36], with higher affinity for pro-neurotrophins and a primary role in controlling neuronal apoptosis during development, whilst modulating neurotransmitter availability and NMJ organisation in the mature nervous system [37]. BDNF binding triggers TrkB.FL, TrkB.T1 and p75NTR homo- and/or hetero-dimerisation [36], and each complex elicits distinct signalling outputs (e.g., TrkB.FL-TrkB.T1 heterodimers inhibit TrkB.FL autophosphorylation) [35, 38]. Importantly, ALS patient spinal cords display abnormality in TrkB-mediated intracellular signalling [39], as well as increased p75NTR expression [40].

Despite in-depth knowledge of BDNF biology [34], the physiological landscape of BDNF signalling at the NMJ, as well as its possible perturbation in ALS, are currently less known. BDNF regulates both the pre- and post-synaptic components of the neuromuscular synapse [22], and is secreted by skeletal muscles during contraction [38]. Internalised BDNF-receptor complexes induce both local [34] and long-distance signalling [41]. The former controls local translation at nerve terminals [42], whilst the latter is driven by sorting of activated Trk receptors [43] to signalling endosomes, which undergo fast retrograde axonal transport to the soma [44], with signalling endosome flux dependent on TrkB activation [45]. Hence, understanding the regulation of BDNF-signalling in MN subtypes can provide novel clues regarding selective MN vulnerability in ALS.

Here, we assessed axonal transport dynamics of signalling endosomes in axons of different α-MN subtypes in wild-type (WT) and SOD1G93A mice in vivo. We find that BDNF stimulation promotes faster retrograde transport speeds of signalling endosomes in WT FMNs, but not in SMNs, as well as in embryonic primary ventral horn neurons. In SOD1G93A mice, transport is preferentially impaired in FMNs innervating TA, which become refractory to BDNF stimulation, a phenotype we also observed in cultured SOD1G93A embryonic primary ventral horn neurons. In addition, we show that truncated TrkB isoforms and p75NTR levels are upregulated in muscles, sciatic nerves and Schwann cells of SOD1G93A mice, thus identifying cell- and non-cell-autonomous dysregulation of BDNF signalling in ALS pathology.

Materials and methods

Animals

Mouse experiments were performed under license from the United Kingdom Home Office in accordance with the Animals (Scientific Procedures) Act (1986) and approved by the UCL Queen Square Institute of Neurology Ethics Committee. Mice were housed in individually ventilated cages in a controlled temperature/humidity environment and maintained on a 12 h light/dark cycle with ad libitum access to food and water. Transgenic mice carrying the mutant SOD1G93A transgene (TgN[SOD1-G93A]1Gur) were obtained from the Jackson Laboratory [46].
Colonies were maintained by breeding male heterozygous carriers with female (C57BL/6 × SJL) F1 hybrids. Mice were genotyped for the human SOD1 transgene using DNA extracted from ear notches and primers as previously described [5–7]. Female and male SOD1<sup>G93A</sup> mice display distinct patterns of disease, including differences in disease onset, progression and survival [47]. Therefore, only female hemizygous transgenic mice carrying the human SOD1<sup>G93A</sup> transgene (hereafter referred to as SOD1<sup>G93A</sup>) and WT littermates were used, allowing comparisons with our previous studies [5–8]. All experimental groups contained age-matched WT and SOD1<sup>G93A</sup> littermates to minimise the potential impact of differing oestrous cycles. For axonal transport, female WT mice had a mean age of 85.13 ± 14.97 days; we did not assess WT axonal transport separately at P73 and P94 as we have previously shown that there are no significant differences in transport between 1 and 18 months in WT mice [9, 48]. Female SOD1<sup>G93A</sup> postnatal day 73 (P73) mice had a mean age of 72.74 ± 0.67 and P94 mice had a mean age of 93.70 ± 0.46.

**In vivo axonal transport**

Signalling endosomes were visualised in vivo by injecting the fluorescent atoxic binding fragment of tetanus neurotoxin (H<sub>c</sub>T-555), as previously described [49, 50]. Briefly, H<sub>c</sub>T<sup>-</sup> (residues 875–1315) fused to an improved cysteine-rich region was expressed in bacteria as a glutathione-S-transferase fusion protein [51], cleaved and subsequently labelled with AlexaFluor555 C<sub>2</sub> maleimide (Thermo Fisher Scientific, A-20346). 5–7.5 µg of H<sub>c</sub>T-555 alone, or in combination with 25 ng of human recombinant BDNF (Peprotech, 450–02) or 25 ng of human recombinant GDNF (Peprotech, 450–10) (pre-mixed with phosphate buffered saline) were injected into single muscles. Briefly, after anaesthesia was initiated and maintained using isoflurane, the fur on the ventral and/or dorsal lower leg was shaved, and mice were placed on a heat-pad for the duration of the surgery. A small incision was made using iris spring scissors on the ventral surface below the patella for TA, or on the lateral aspect of the dorsal surface below the popliteal fossa for lateral head of gastrocnemius (LG). Injections were performed as a single injection targeting the motor end plate region [52] in a volume of ~3.5 µl using a 701 N Hamilton® syringe (Merck, 20,779) for TA and LG. For soleus injections, a vertical incision was made on the skin covering the lateral surface of lower hindlimb between the patella and tarsus to expose the underlying musculature. Subsequent vertical incisions were carefully made laterally along the connective tissue between LG and TA, and the deeper soleus muscle was exposed using forceps. 1 µl injections were performed into soleus using pulled graduated, glass micropipettes (Drummond Scientific, 5-000-1001-X10), as previously described [53]. The overlying skin was then sutured, and mice were monitored for up to 1 h. 4–8 h later, mice were re-anaesthetised with isoflurane, and the skin covering the entire lateral surface of the injected hindlimb was removed, along with the biceps femoris muscle to expose the underlying sciatic nerve. The connective tissue underneath the sciatic nerve was loosened using curved forceps to enable the placement of a small piece of parafilm aiding the subsequent imaging. The anaesthetised mouse was then transferred to an inverted LSM780 confocal microscope (Zeiss) enclosed within an environmental chamber maintained at 37 °C. Using a 40x, 1.3 NA DIC Plan-Apochromat oil- immersion objective (Zeiss), axons containing retrogradely mobile H<sub>c</sub>T-555-positive signalling endosomes were imaged every 0.3–0.4 s using an 80 × digital zoom (1024 × 1024, <1% laser power) (Fig. 1A, Additional file 1: Video S1); movies of three to five axons per animal were acquired. All imaging was concluded within 1 h of initiating anaesthesia.

**In vivo axonal transport analysis**

Confocal “.czi” images were opened in FIJI/ImageJ (http://rsb.info.nih.gov/ij/), converted to “.tif” and transport dynamics were then assessed semi-automatically (i.e., automated spot detection, followed by manual linking (see Additional file 1: Video S1)) using the TrackMate plugin [54]. Indeed, as determined by several parameters such as fluorescence intensity and diameter, the TrackMate automated spot detection method encloses all cargoes that fit the criteria within purple circles (Additional file 1: Video S1B). Endosomes selected for transport analysis were then manually connected across multiple adjacent frames (Additional file 1: Video S1C). This method provides single frame-to-frame velocities, which were then averaged across the entire run to give an average speed for each tracked endosome (as represented by an individual data point in Fig. 1C). Kymographs (Additional file 2: Fig. S2) were generated using FIJI/ImageJ to highlight axonal transport phenotypes, but were not used to assess axonal transport dynamics. Only thicker axons were selected for tracking [9]. Signalling endosomes with the following criteria were analysed: (1) organelles were tracked for a minimum of 10 and a maximum of 100 consecutive frames (i.e., ~3–40 s), including pauses. Terminal pausing carriers, which we defined by the absence of movement in ≥10 consecutive frames, were excluded; (2) for every individual axon, 15–40 signalling endosomes were tracked across at least 1000 frames (i.e., ~5–10 min); and (3) signalling endosome data representing an individual animal were comprised from at least three separate motor axons. The individual datapoints obtained from
each experimental group can be found in Additional file 2: Table S1. Relative frequency curves were generated to display the relative frame-to-frame movements of all signalling endosomes per animal (e.g., Fig. 1B). For all mice included in the analysis, the speeds of all individual endosomes were plotted (e.g., Fig. 1C), the mean speeds of all endosomes per individual axon were averaged (e.g., Fig. 1D), and finally, the mean speed of all endosomes per animal was also averaged (e.g., triangles in Fig. 1E). Importantly, the mean values across all analyses (e.g., Fig. 1C–E) were similar. For example, for WT soleus transport, the mean speed of all individual endosomes was 2.55 µm/s (Fig. 1C), the mean endosome speed per axon was 2.51 µm/s (Fig. 1D) and the mean endosome speed per animal was 2.54 µm/s (Fig. 1E). Owing to statistical overpowering of individual endosome speed data, statistical tests were only performed on the mean endosome speeds per axon (Fig. 1D) and per animal (Fig. 1E). The fastest individual endosome speed per animal was considered as the maximum speed (e.g., represented by circles in Fig. 1E). A pause was defined by an endosome that moved less than 0.1 µm between consecutive frames, and the time paused (%) is determined by the number of pauses divided by the total number of frame-to-frame movements assessed per animal (e.g., Fig. 1F).

In vitro axonal transport
Mixed ventral horn cultures were prepared as previously described [6–8]. Briefly, ventral horns from E11.5–13.5 WT and SOD1(G93A) mice were dissociated, centrifuged at 380 × g for 5 min, seeded into two-chambered microfluidic devices (Fig. 3A) [7], and maintained in motor neuron media (Neurobasal (Gibco) with 2% B27 (Gibco), 2% heat-inactivated horse serum, 1% Glutamax (Invitrogen), 24.8 µM β-mercaptoethanol, 10 ng/ml ciliary neurotrophic factor (Peprotech, 450–13), 0.1 ng/ml GDNF (Peprotech, 450–10), 1 ng/ml BDNF (Peprotech, 450–02) and 1 × penicillin streptomycin (Thermo Fisher; 15140122)) at 37 °C and 5% CO2. After 6 days in vitro (DIV6), 30 nM Hc-T-555 and ±50 ng/ml of BDNF was added to existing media for 45 min, then all media was replaced with fresh MN media containing 20 mM HEPES–NaOH (pH 7.4) ±50 ng/ml of BDNF for time-lapse microscopy. Live imaging was performed on an inverted LSM780 confocal microscope at 37 °C using a 40x, 1.3 NA DIC Plan-Apochromat oil-immersion objective.
In vitro TrkB and p75NTR western blot analysis

Mixed ventral horn cultures from E11.5–13.5 WT and SOD1<sup>G93A</sup> mouse spinal cords were prepared as above, and plated in MN media in a 12-well plate coated with poly-ornithine (1.5 mg/ml) and laminin (3 µg/ml). On DIV 6–7, each well was washed once in ice-cold PBS and lysates were prepared in RIPA buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 1 mM EGTA) with freshly added Halt<sup>™</sup> protease and phosphatase inhibitor cocktail (1:100, Thermo Fisher), and incubated on ice for 30 min. Lysates were spun at 14800 rpm at 4 °C for 15 min, the supernatant was then resuspended in 4 × Laemmli sample buffer (15% SDS, 312.5 mM Tris–HCl pH 6.8, 50% glycerol, 10% β-mercaptoethanol, 0.1% bromophenol blue) and loaded into 4–15% Mini-PROTEAN® TGX Stain-Free<sup>®</sup> protein gels (Bio-Rad). Western blotting was then performed using standard protocols. The primary antibodies used were TrkB (R&D Systems, AF1494, 1:500) and p75NTR (Biolegend, 839701, 1:500) (see Additional file 2: Table S3). Densitometry was performed on the bands at ~ 20 kDa for BDNF (Fig. 5A), ~ 140 kDa for TrkB.FL (Fig. 5B), ~ 75–100 kDa for truncated TrkB (Fig. 5C), ~ 75–85 kDa for p75NTR (Fig. 5C). As the steady-state levels of standard housekeeping proteins, such as GAPDH and β-actin, differ between muscle types, and can be affected by age, sex, and pathology [57, 58], post-immunoblotting Coomassie staining [55] between 10–25 kDa, 70–150 kDa and 60–100 kDa was used to assess relative levels of BDNF, TrkB (full-length and truncated isoforms), and p75<sup>NTR</sup>, respectively. The total protein load was used as an internal reference to accurately quantify relative protein levels, and data were then normalised using the sum of all data points per replicate [56].

Axon diameters

The axon diameters were measured following protocols established in ChAT.eGFP mice [9], using the same videos in the axonal transport analyses. Briefly, axon diameters were assessed by measuring the upper and lower positions of moving H<sub>2</sub>T-555 signalling endosomes from consecutive frames in unprocessed (i.e., not dissected, fixed, or sectioned), anatomically connected individual axons. A minimum of 10 positions were averaged for a single axon, and the mean axon diameters per animal were determined by averaging all axons from that animal (n ≥ 3 axons per animal). Similar to the in vivo transport experiments, this quantification is reliant upon intact NMJs, which can internalise H<sub>2</sub>T-555; hence, we cannot extrapolate diameters from denervated axons.

Muscle BDNF, TrkB and p75<sub>NTR</sub> western blot analysis

P73 (n = 5) and P94 (n = 5) WT and SOD1<sup>G93A</sup> mice were culled, and fresh TA and soleus muscles were immediately dissected, snap frozen in liquid nitrogen and stored at -80 °C. Protein extraction from frozen muscles was achieved by mechanically disrupting the tissue using a scalpel, followed by immersion in RIPA buffer containing freshly added Halt<sup>™</sup> protease and phosphatase inhibitor cocktail for 15 min on ice, and then homogenised on ice using an electrical homogeniser. Lysates were incubated at 4 °C with mild agitation for 2 h, after which they were centrifuged at 21000 g for 30 min at 4 °C. 20 µl of supernatant (~ 25–40 μg of protein) was treated with 6.5% trichloroacetic acid and the resulting pellet was washed with acetone. Proteins were resuspended in 1 × Laemmli buffer and loaded on 4–12% Bis–Tris polyacrylamide gels prior to western blotting. The primary antibodies used were against BDNF (Alomone ANT-010), TrkB (Millipore, 07–225) and p75<sup>NTR</sup> (Biolegend, 839701) (all 1:1000; see Additional file 2: Table S3). Densitometry was performed on the bands at ~ 20 kDa for BDNF (Fig 5A), ~ 140 kDa for TrkB.FL (Fig. 5B), ~ 75–100 kDa for truncated TrkB (Fig. 5B) and ~ 70–85 kDa for p75<sup>NTR</sup> (Fig. 5C). As the steady-state levels of standard housekeeping proteins, such as GAPDH and β-actin, differ between muscle types, and can be affected by age, sex, and pathology [57, 58], post-immunoblotting Coomassie staining [55] between 10–25 kDa, 70–150 kDa and 60–100 kDa was used to assess relative levels of BDNF, TrkB (full-length and truncated isoforms), and p75<sup>NTR</sup>, respectively. The total protein load was used as an internal reference to accurately quantify relative protein levels, and data were then normalised using the sum of all data points in a replicate [56]. P73 and P94 WT and SOD1<sup>G93A</sup> data points were combined as there were no timepoint-specific differences (data not shown).

Muscle immunohistochemistry (IHC)

P73 (n = 3) and P94 (n = 3) WT and SOD1<sup>G93A</sup> mice were culled, and TA and soleus muscles were immediately dissected and post-fixed in 4% paraformaldehyde (PFA) for 15–60 min. Muscle fibres were teased apart in bundles of 1–10 fibres and stained with α-bungarotoxin (BTX; Thermo Fisher Scientific, B13423, 1:500) for 1 h. Fibres were then permeabilized with 2% Triton X-100 in PBS for 90 min, then immersed in a blocking solution containing 4% bovine serum albumin and 1% Triton X-100 in PBS for 30 min at room temperature. Primary antibodies (see Additional file 2: Table S3) against TUJ1 (Synaptic Systems, 302306, 1:50), synaptophysin (Syn; Synaptic Systems, 302306, 1:50) and β-III-tubulin (BIII-T; Millipore, 13-0010) were used at 1:500, followed by Alexa Fluor 594- or 488-conjugated secondary antibodies (Thermo Fisher Scientific, A11001, 1:1000). Sections were then coverslipped with Fluoromount (Southern Biotech Assay, 01-510-12). Muscle fibres were teased apart in bundles of 1–10 fibres, stained with α-bungarotoxin (BTX; Thermo Fisher Scientific, B13423, 1:500) for 1 h. Fibres were then permeabilized with 2% Triton X-100 in PBS for 90 min, then immersed in a blocking solution containing 4% bovine serum albumin and 1% Triton X-100 in PBS for 30 min at room temperature. Primary antibodies (see Additional file 2: Table S3) against TUJ1 (Synaptic Systems, 302306, 1:50), synaptophysin (Syn; Synaptic Systems, 302306, 1:50) and β-III-tubulin (BIII-T; Millipore, 13-0010) were used at 1:500, followed by Alexa Fluor 594- or 488-conjugated secondary antibodies (Thermo Fisher Scientific, A11001, 1:1000). Sections were then coverslipped with Fluoromount (Southern Biotech Assay, 01-510-12).
**Sciatic nerve western blot analysis**

P73 (n = 5) and P94 (n = 5) WT and SOD1<sup>G93A</sup> mice were culled, and sciatic nerves were immediately dissected, snap frozen in liquid nitrogen and stored at − 80 °C. Thawed sciatic nerves were then immersed in NP-40 lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris–HCl, pH 8.0) with freshly added Halt™ protease and phosphatase inhibitor cocktail (10% weight/volume) (100x, Fisher, 78442). Lysates underwent mechanical disruption using a plastic pestle before being left on ice for 0.5 h and then centrifuged for 20 min at 10,000 g. Proteins were re-suspended in 4 × Laemmli buffer, and 40 µg/sample were loaded on 4–12% Bis–Tris polyacrylamide gels prior to western blotting. Primary antibodies (see Additional file 2: Table S3) against TrkB (Millipore, 07–225, 1:1000), p75<sup>NTR</sup> (Biolegend, 839701, 1:2000), ERK1/2 (CST, 9102, 1:1000), p-ERK1/2 (CST, 9101, 1:1000), AKT (CST, 9272, 1:1000), p-AKT (CST, 9275, 1:1000) and Cofilin (Cytoskeleton, ACF1L02, 1:500) were used to quantify protein levels. N.B. The expression of TrkB, AKT and p-AKT were below detection levels. All bands were first standardised to cofilin, and then normalised by the sum of all data points in a replicate [56]. P73 and P94 WT and SOD1<sup>G93A</sup> data points were combined as there were no timepoint specific differences (data not shown).

**Statistical analyses**

GraphPad Prism 9 (GraphPad Software) was used for statistical analyses. Normal distribution was first ascertained by the D’Agostino and Pearson omnibus normality test, and parametric data were statistically assessed using unpaired, two-tail t-tests, one-way or two-way analyses of variance (ANOVA) with Holm-Sidaks multiple comparisons tests. Non-normally distributed data were analysed by a two-tailed Mann–Whitney U test or Kruskal–Wallis test with Dunn’s multiple comparisons test.

**Results**

In vivo axonal transport is differentially regulated in MN subtypes by BDNF

To investigate the influence of α-MN and skeletal muscle subtypes [10–12] on axonal transport dynamics in vivo, we labelled neurotrophin-containing signalling endosomes with a fluorescent atoxic tetanus neurotoxin binding fragment (H<sub>c</sub>T) [59, 60] to assess axonal transport dynamics in sciatic nerves of live mice [49, 50]. H<sub>c</sub>T is internalised into MNs upon binding to nidogens and polysialogangliosides at distal terminals [61], and
Fig. 2  BDNF stimulation differentially impacts axonal transport of signalling endosomes in motor neurons innervating fast and slow muscles in wild-type mice. A Average and maximum endosome speeds upon intramuscular BDNF stimulation in motor axons innervating tibialis anterior (average: *p = 0.029; maximum: p = 0.281), B lateral gastrocnemius (average: *p = 0.017; maximum: p = 0.176), and C soleus (average: p = 0.485; maximum: p = 0.937). D Time signalling endosomes paused upon BDNF stimulation in motor axons innervating tibialis anterior (*p = 0.014), E lateral gastrocnemius (*p = 0.009) and F soleus (p = 0.559). Data were assessed by Mann–Whitney U tests (n = 5–8). Means ± SEM are plotted for all graphs. The colour coding of individual datapoints is consistent within the muscle and treatment type and reflects the same animal. *p < 0.05, **p < 0.01. See also Additional file 2: Figs. S1 and Table S1.

Fig. 3  BDNF stimulation enhances axonal transport of signalling endosomes in primary embryonic ventral horn neurons from WT, but not SOD1<sup>G93A</sup> mice. A Schematic of primary embryonic ventral horn cultures plated in microfluidic chambers (MFCs) with or without 50 ng/µl of BDNF added to both somatic and axonal compartments. B Speed distribution curves of signalling endosome transport in WT and SOD1<sup>G93A</sup> primary ventral horn neurons in MFCs. C Axonal transport dynamics of the same cultures upon addition of 50 ng/ml of BDNF, with the mean endosome speeds shown in D (WT vs. SOD1<sup>G93A</sup>: p = 0.068; WT −/− BDNF: p = 0.041; SOD1<sup>G93A</sup> −/− + BDNF: p = 0.735; WT vs. SOD1<sup>G93A</sup> + BDNF: *p = 0.037). E Mean speeds in individual motor neuron axons (WT vs. SOD1<sup>G93A</sup>: p = 0.7308; WT −/− BDNF: ***p < 0.001; SOD1<sup>G93A</sup> −/− + BDNF: p = 0.576; WT vs. SOD1<sup>G93A</sup> + BDNF: ***p < 0.001). F Speed of individual H<sub>2</sub>T-555-positive signalling endosomes (white circles; black lines represent the median and the dashed lines represent the upper and lower quartiles). G Percentage of signalling endosomes pausing (WT vs. SOD1<sup>G93A</sup>: p = 0.122; WT −/− BDNF: p = 0.745; SOD1<sup>G93A</sup> −/− + BDNF: p = 0.154; WT vs. SOD1<sup>G93A</sup> + BDNF: p = 0.791). Statistical analyses were performed using unpaired, two-tailed t-tests (n = 3 biological replicates). See also Additional file 2: Table S2.
is retrogradely transported in Rab7-positive signalling endosomes [44]. Using H$_2$T-555, we separately targeted the FMN-innervated TA, the FMN- and SMN-innervated (i.e., mixed) LG and the predominantly SMN-innervated soleus muscles in WT mice, and after 4–8 h, we performed time-lapse intravital microscopy (Fig. 1A; Additional file 1: Video S1). Speed distribution curves (Fig. 1B), as well as the average and maximum velocities (Figs. 1C–E) indicate that endosome transport dynamics in axons innervating TA, LG and soleus are similar, albeit with less pausing in TA axons (Fig. 1F).

We next assessed whether peripheral stimulation with BDNF impacts signalling endosome transport dynamics, given the influence of this neurotrophin on endocytosis, endosomal flux and pro-survival signalling events [41, 45]. Co-injection of H$_2$T-555 with 25 ng of recombinant BDNF increased the mean speeds of signalling endosomes in motor axons innervating the TA (Fig. 2A; Additional file 2: Fig. S1A) and LG (Fig. 2B; Additional file 2: Fig. S1B), whilst concurrently reducing their pausing (Figs. 2D–E). However, BDNF stimulation had no influence on transport in soleus motor axons (Fig. 2C, F; Additional file 2: Fig. S1C). We then tested if this response was specific for BDNF or a general feature of neurotrophic factors, by stimulating FMN axons with GDNF, which is known to activate distinct signalling cascades via RET and GFRα receptors [8, 12]. In contrast to BDNF, application of 25 ng of recombinant GDNF did not influence transport of H$_2$T-555-positive signalling endosomes (Additional file 2: Fig. S1D–E). Altogether, these data indicate that FMNs and SMNs have similar axonal transport speeds under basal conditions, and that BDNF stimulation enhances axonal transport dynamics specifically in FMNs.

**Axonal transport is selectively impaired in TA-innervating axons of SOD1$^{G93A}$ mice**

SOD1$^{G93A}$ mice display early and persistent axonal transport deficits [5], but the precise contributions of fast and slow MNs, as well as BDNF stimulation, are currently unresolved. To fill this gap, we first assessed the effect of BDNF on in vitro axonal transport in embryonic primary ventral horn neurons in microfluidic chambers (Fig. 3A). Under basal conditions, we observed similar transport speeds between WT and SOD1$^{G93A}$ neurons (Fig. 3B, D–F), thus supporting a neurodegenerative, rather than neurodevelopmental, transport phenotype in SOD1$^{G93A}$ mice [5]. Moreover, TrkB.FL, truncated TrkB and p75NTR levels do not differ between WT and SOD1$^{G93A}$ cultures (Additional file 2: Fig. S2). However, application of 50 ng/ml of recombinant BDNF increased WT endosome retrograde transport speeds (without altering pausing), but had no effect in SOD1$^{G93A}$ primary ventral horn neurons, suggestive of dysregulated BDNF signalling in SOD1$^{G93A}$ MNs (Fig. 3C–G).

Next, we assessed in vivo axonal transport dynamics at postnatal day 73 (P73) and 94 (P94), which correspond to SOD1$^{G93A}$ disease timepoints with ~20% and ~40% loss of lumbar MNs, respectively [5]. Axonal transport of signalling endosomes was impaired at both timepoints in SOD1$^{G93A}$ FMNs innervating the TA (Fig. 4A, B; Additional file 2: Fig. S3), and with significant alterations in pausing (Fig. 4C). Contrastingly, axonal transport was unaffected in the predominantly SMNs innervating the soleus (Fig. 4D–F; Additional file 2: Fig. S3) and the mixed population of FMNs and SMNs innervating the LG (Additional file 2: Fig. S4A–C) at both disease timepoints in SOD1$^{G93A}$ mice.

We then assessed diameters of the axons in which endosomes were tracked to determine if there were any changes that might contribute to the transport phenotypes. Using our established methods [9], we show that the mean diameter of motor axons innervating the TA, LG and soleus are similar in WT mice (Additional file 2: Fig. S5A, B). In agreement with previous reports that TA motor units are preferentially vulnerable in ALS mice [13], we found a reduction in the mean diameters of TA
motor axons at P73 (Additional file 2: Fig. S5C) that persisted and plateaued by P94 (Additional file 2: Fig. S5D). Suggestive of delayed pathology, LG motor axons displayed diameter reductions at P94 only (Additional file 2: Fig. S5D). Consistent with our in vivo axonal transport data, soleus motor axon diameters remained unaltered at both timepoints (Additional file 2: Fig. S5C, D).
We then assessed the impact of BDNF stimulation on axonal transport in SOD1<sup>G93A</sup> mice (see Additional file 2: Table S1). In contrast to WT mice (e.g., Fig. 2), BDNF failed to enhance transport in SOD1<sup>G93A</sup> motor axons innervating the TA (Fig. 4G–I) and LG (Additional file 2: Table S1). In contrast to WT mice (e.g., Fig. 2), motor axons remained unresponsive (Additional file 2: Table S1). In contrast to WT mice (e.g., Fig. 2), motor axons remained unresponsive (Additional file 2: Table S1).

Collectively, these data demonstrate MN subtype-specific alterations in transport of signalling endosomes in SOD1<sup>G93A</sup> mice and a preferential reduction of FMN axon diameters in pathology. Furthermore, diseased FMNs innervating TA become insensitive to BDNF stimulation at early symptomatic stages of ALS progression in SOD1<sup>G93A</sup> mice.

Truncated TrkB and p75<sup>NTR</sup> are increased in SOD1<sup>G93A</sup> muscles, but not at NMJs

For subsequent experiments, we focused solely on TA and soleus muscles, because of the clear differences in axonal transport phenotypes in motor axons innervating these muscles (i.e., Fig. 4; Additional file 2: Fig. S4A–C), and their distinct fibre type compositions (i.e., TA = fast muscle; soleus = slow muscle) [11]. We first determined the levels of BDNF and its receptors in TA and soleus muscles using western blot (Fig. 5A–C). We found higher basal BDNF levels in TA compared to soleus, without significant changes in disease (Fig. 5D). Between TA and soleus muscles, there were no discernible differences in the levels of BDNF and its receptors in TA and soleus muscles (Fig. 5A–C).

To determine if the observed changes in neurotrophin receptors were confined to the synapse, we evaluated the synaptic expression of total TrkB and p75<sup>NTR</sup> at the NMJ by immunostaining. Pre-synaptic axon terminals were identified by combined synaptophysin (Syn) and βIII-tubulin (TUJ1) staining, the post-synaptic region was labelled with α-bungarotoxin (α-BTX), whereas terminal Schwann cells were stained with an S100 antibody. TrkB (Fig. 6A) and p75<sup>NTR</sup> (Fig. 6B) staining was observed at the pre-synaptic axon terminals (i.e., Syn/TUJ1 regions), post-synaptic NMJ compartment (i.e., α-BTX regions), and peri-synaptically in terminal Schwann cells (i.e., S100 regions). By applying a Syn/TUJ1-α-BTX mask to the TrkB or p75<sup>NTR</sup> immunolabelled regions in partially or fully innervated, but not vacant, NMJs, we found that the mean fluorescence...
of TrkB (Fig. 6C; Additional file 2: Fig. S6A–C) and p75NTR (Fig. 6D; Additional file 2: Fig. S6D-F) did not significantly differ between TA or soleus NMJs in WT or SOD1GA mice.

Altogether, these data reveal that, whereas truncated TrkB and p75NTR are increased in SOD1GA whole muscles, this increase is not reflected at the NMJ. TrkB.T1 and p75NTR are elevated in SOD1GA sciatic nerves

We then performed western blot analyses on whole sciatic nerves, probing for TrkB and p75NTR, as well as the phosphorylation of key downstream signalling molecules, ERK1/2 (Fig. 7A). Consistent with our muscle data (Fig. 5F–G), there is more TrkB.T1 (Fig. 7B) and p75NTR (Fig. 7C) in SOD1GA sciatic nerves. However, we did not find alterations in phosphorylated ERK1/2 (Fig. 7D), confirming previous reports [7]. However, we were unable to detect TrkB.FL in our experimental conditions.

To pinpoint the cellular source of increased TrkB.T1 and p75NTR, we immunostained sciatic nerve sections for TrkB (Fig. 7E) and p75NTR (Fig. 7F), using TUJ1 and S100 as markers of axons and Schwann cells, respectively. We then applied TUJ1 and S100 masks to TrkB (Fig. 7E) and p75NTR (Fig. 7F) immunolabelled regions in WT and SOD1GA sciatic nerves. These analyses revealed no differences in TrkB content in axons or Schwann cells in WT and SOD1GA sciatic nerves (Fig. 7G). In contrast, we observed increased p75NTR mean fluorescence, specifically in Schwann cells (Fig. 7H).

Collectively, these experiments further confirm that non-cell autonomous dysregulation of TrkB.T1 and
p75NTR signalling in peripheral sciatic nerves might contribute to SOD1<sup>G93A</sup> pathology.

**Discussion**

As summarised in Fig. 8, in this study we show that FMN and SMN axons have similar transport kinetics of signalling endosomes under basal conditions in vivo, with peripheral BDNF able to boost axonal transport speeds exclusively in FMNs. Furthermore, BDNF stimulation increases endosome speeds in WT, but not SOD1<sup>G93A</sup> primary embryonic ventral horn neuronal cultures. In early symptomatic SOD1<sup>G93A</sup> mice, axonal transport is selectively impaired in FMNs innervating the TA, which also become refractory to BDNF stimulation. Moreover, pathology increases truncated TrkB and p75<sup>NTR</sup> in both muscle and sciatic nerve, including in myelinating Schwann cells, but not at the NMJ. Altogether, these data suggest that cell- and non-cell autonomous BDNF signalling is impaired in an α-MN subtype-specific manner in SOD1<sup>G93A</sup> pathology.

**α-MN subtypes display distinct axonal transport dynamics in WT and SOD1<sup>G93A</sup> mice**

This is the first study to dissect axonal transport dynamics in different α-MN subtypes in vivo. In WT mice, we found no difference in the mean or maximum endosome speeds suggesting that motor unit type does not influence basal transport dynamics. However, we observed that signalling endosomes paused less frequently in TA-innervating axons. Stationary organelles may sterically hinder axonal transport [62], forcing approaching cargoes to switch to a different microtubule track [63] to overcome obstacles on the original microtubule [64].

In basal conditions, WT and SOD1<sup>G93A</sup> primary ventral horn cultures had similar axonal transport dynamics. However, application of BDNF increased axonal transport speeds in WT, but not SOD1<sup>G93A</sup> primary neurons. This was not due to overt differences in TrkB, truncated TrkB or p75<sup>NTR</sup> receptors. Moreover, the levels of two downstream effectors of BDNF-TrkB signalling, ERK1/2 and AKT, are unchanged between WT and SOD1<sup>G93A</sup> cultures [8]. However, an important caveat of these analyses is that these mixed cultures contain several neuronal (e.g., α- and γ-MN, as well as cholinergic glutamatergic, glycnergic and GABA-ergic interneurons) and non-neuronal (e.g., different glia and fibroblasts) subtypes. Furthermore, the inability of SOD1<sup>G93A</sup> motor neurons to respond to BDNF may be due to multiple mechanisms, including differential recruitment of dynein adapters (e.g., snapin [65]), as well as their altered local translation [3, 42, 66]. In this regard, we have previously reported that pharmacological inhibition of IGF1R specifically increases the levels of the dynein adaptor BICD1 by promoting its axonal translation [7], thus restoring physiological transport in SOD1<sup>G93A</sup> MNs in vivo.
BDNF stimulation specifically enhances axonal transport in WT FMNs, an effect not observed with GDNF. This was surprising because GDNF is important for MN survival and development [12, 30], is added to primary MN media [7–9], and enhances axonal transport of signalling endosomes in primary WT ventral horn neurons [8]. However, our observation that GDNF does not modulate axonal transport of signalling endosomes in vivo in WT FMNs, supports previous findings that specific neurotrophic factors elicit discrete signalling in different MN subtypes [22, 30, 31]. In this regard, γ-MNs require muscle spindle-derived GDNF for postnatal survival [32]. Moreover, we have recently reported that RET inhibition rescues in vivo deficits in axonal transport of signalling endosome in SOD1G93A mice [8]. Altogether, this evidence indicates that neurotrophic factors elicit distinct effects on axonal transport in vivo.

We have previously demonstrated that axonal transport is impaired in pre-symptomatic SOD1G93A [5–7] and TDP-43M337V mice [9]. Importantly, compromised axonal transport is not a general disease by-product as heterozygous mutant FUS [9] and Kennedy’s disease [67] mice do not display in vivo transport deficits, despite displaying MN loss. However, in these studies axonal transport was assessed upon injection of both the TA and LG with BDNF. In this work, we found that only TA axons display transport deficits in SOD1G93A mice and without changes in pausing, suggesting that this is not due to a general impairment in the retrograde transport machinery. Interestingly, the transport deficits observed in TA did not worsen during disease, indicative of a pathological plateau, which was also observed in TDP-43M337V mice [9]. However, we are unable to account for transport dynamics in denervated MNs, as only axons with internalised H_{C/T} can be assessed by intravital imaging.
The precise mechanism by which FMNs selectively display impairments in retrograde axonal transport remains elusive. Mutant, but not WT, SOD1 (i.e., SOD1G93A and SOD1G85R) interacts with the dynein motor complex [68], suggesting that vulnerable FMNs may accumulate more of this pathological protein, thus impinging upon retrograde transport regulation. Mutant SOD1 also aberrantly interacts with the stress granule protein G3BP1 [69], thus potentially disturbing processes involved in axonal maintenance (e.g., stress granule dynamics, RNA localisation) [3]. Axonal transport deficits also impact local translation, as Rab7-containing organelles, which include signalling endosomes, are sites for mitochondrial-associated local mRNA translation [66]. Whether these pathological phenomena occur specifically in vulnerable FMNs, but not in resistant SMNs, or whether other organelles also display transport deficits specifically in vulnerable FMNs, remains to be determined.

Dysregulated truncated TrkB and p75NTR in ALS mice
Dynamic NMJ remodelling precedes motor unit loss in ALS mice [17, 70], however it is currently not known whether neuromuscular BDNF signalling in fast versus slow muscles is altered in disease. Here, we report that BDNF signalling in SOD1G93A mice is dysregulated in embryonic ventral horn neurons and that adult MNs are refractory to BDNF stimulation, with TA axons displaying ~38% reduction in transport speeds in early symptomatic SOD1G93A mice (P73). As physiological BDNF and TrkB levels fluctuate (e.g., upon exercise [38]), persistent BDNF insensitivity can have severe consequences for MN homeostasis, impacting translation and signalling events in axon terminals, along the axon and within MN soma [3, 22, 38, 41, 42].

We initially hypothesised that this BDNF insensitivity might be due to: (1) reduced muscle BDNF; (2) altered TrkB and p75NTR relative levels; (3) imbalanced TrkB.FL and truncated TrkB ratios; or (4) a combination of the above. In our study, we observed an increase of truncated TrkB and p75NTR levels in TA and soleus muscles and sciatic nerves, suggestive of a role for these receptors in SOD1G93A pathology. Remarkably, TA, which is refractory to exogenous BDNF application in SOD1G93A mice and displays differential vulnerability in ALS, selectively expresses more truncated TrkB, but not p75NTR. The increased concentration of these receptors on the plasma membrane may reduce the availability of BDNF to bind TrkB.FL. Hence, an imbalanced ratio of TrkB.FL, truncated TrkB and p75NTR could, in principle, diminish the pro-survival signalling of TrkB.FL [38], thus contributing to the vulnerability of the TA motor unit. However, the distribution of these receptors and BDNF in skeletal muscle are dynamic, as synaptic or muscular activity increases the bioavailability of BDNF and phosphorylated TrkB.FL, whilst decreasing TrkB.T1 [38]. Furthermore, symptomatic SOD1G93A mice upregulate p75NTR along with apoptotic markers in α-MNs [71], while deleting TrkB.T1 ubiquitously or specifically in astrocytes delays MN death in SOD1G93A mice [72, 73]. Conversely, viral overexpression of TrkB.T1 induces MN degeneration [74]. These studies suggest that BDNF-mediated signalling pathways are altered in ALS and that their modulation might have therapeutic benefits [35]. Indeed, harnessing the pro-survival activity of p75NTR prevents MN death and extends the lifespan of SOD1G93A mice, in part, by rescuing p-TrkB, p-Akt, p-ERK and p-CREB levels in SOD1G93A spinal cords [75].

However, a caveat to our TrkB immunostaining approach is that the commercially available TrkB antibodies bind to the extracellular domain of this receptor, and thus cannot distinguish TrkB.FL from TrkB.T1. A limitation of our experimental approach is that while western blotting allows us to evaluate the TrkB isoforms, it lacks MN subtype specificity; conversely, our immunostaining experiments enable MN subtype detection, but lack TrkB isoform differentiation. Hence, dissecting the endogenous levels of TrkB isoforms in FMNs and SMNs is currently not possible. In addition, we observed tissue-specific differences in the molecular weights of full-length and truncated TrkB, as well as p75NTR receptors. In skeletal muscle and embryonic ventral horn cultures, TrkB.FL migrated at ~140 kDa (Fig. 5B and Additional file 2: Fig. S2ai), whereas in sciatic nerve and brain, we observed TrkB.FL at ~120 kDa (Fig. 5B and data not shown). In skeletal muscle, TrkB.T1 and TrkB.T2 were identified at ~80–95 kDa, whereas in sciatic nerve, the TrkB.T1 isoform was observed as a single band at ~95 kDa. Such differences may be due to tissue-specific post-translational modifications [76]. For example, there are ten N-terminal glycosylation sites in TrkB [77], and its phosphorylated form has been observed at both ~120 kDa and ~140 kDa [75] in WT and SOD1G93A spinal cords. Total and phosphorylated TrkB.FL have also been shown to migrate at ~140 kDa in skeletal muscle [37]. p75NTR was detected as two distinct bands at ~75 kDa and ~85 kDa in skeletal muscle (Fig. 5C), likely due to N- and O-linked glycosylation [78], with the upper band (i.e., ~85 kDa) representing the fully glycosylated form and the lower band (i.e., ~75 kDa) the non-glycosylated form [79].
Collectively, our data indicate that the BDNF signalling axis is essential for maintenance and homeostatic regulation of FMNs, which is selectively impaired in SOD1<sup>G93A</sup> pathology in a cell- and non-cell autonomous manner.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40478-022-01418-4.

**Additional file 1. Video S1.** Intravital time-lapse microscopy of HCT-555-positive signalling endosomes (white) in at least three somatic nerve axons of a live, anaesthetised mouse.

**Additional file 2. Figure S1.** Retrograde transport dynamics of signalling endosomes in wild-type mice. **Figure S2.** TrkB<sub>FL</sub>, truncated TrkB and p75<sup>NTR</sup><sub>ER</sub> levels do not differ between wild-type (WT) and SOD1<sup>G93A</sup> primary embryonic ventral horn neurons in mass culture. **Figure S3.** Kymographs of in vivo retrograde transport of HCT-555-positive signalling endosomes from live, anaesthetised mice; **Figure S4.** Retrograde transport dynamics of signalling endosomes in axons innervating lateral gastrocnemius (LG) and soleus muscles in WT and SOD1<sup>G93A</sup> mice; **Figure S5.** Fast motor axon diameters decrease with progression of SOD1<sup>G93A</sup> pathology; **Figure S6.** TrkB and p75<sup>NTR</sup><sub>ER</sub> expression at the neuromuscular junction (NMJ) in WT and SOD1<sup>G93A</sup> tibialis anterior and soleus muscles. **Table S1.** Number of animals, axons, cargoes and frame-to-frame movements assessed for each in vivo axonal transport experimental group. **Table S2.** Number of animals, axons, cargoes and frame-to-frame movements assessed for each primary ventral horn culture used for in vitro axonal transport experiments; **Table S3.** Primary antibodies used in this study; **Table S4.** Secondary antibodies used in this study.

## Acknowledgements

This work is dedicated to the memory of Paul Victor Tosolini. We thank James Dick and the personnel of the Denny Brown Laboratories for assistance in maintaining the mouse colonies (Queen Square Institute of Neurology, University College London), and Nicol Birs, Jose Norberto Sagullo Vargas and David Villarroel-Campos (Queen Square Institute of Neurology, University College London) for critical reading of the manuscript. This work was supported by a Junior Non-Clinical Fellowship from the Motor Neuron Disease Association (Tosolini/Oct20/973-799 (APT)), the Medical Research Council Career Development Award (MR/S006990/1) (JNS); the Sir Henry Wellcome fellowship 103191/A/13/Z (JNS); Human Frontier Science Program long-term fellowship LT00220/2017-L (SS), Medical Research Council Studentship (ERR), Horserace Betting Levy Board and the Mellow foundation provided salary support (SOB); Wellcome Senior Investigator Awards (107116/Z/15/Z and 223822/Z/21/Z) (GG), and a UK Dementia Research Institute Foundation award (UKDRI-1002) (GS).

## Author contributions

Conceptualisation: APT and GS. Investigation: APT, JNS, SS, ERR and SC. Writing and figure production: APT and GS, with input from all authors. Funding acquisition: APT and GS. All authors read and approved the final manuscript.

## Availability of data and materials

All data generated or analysed during this study are included in this published article [and associated supplementary information].

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

## References

1. De Vos KI, Hafezparast M (2017) Neurobiology of axonal transport defects in motor neuron diseases: opportunities for translational research? Neurol Biol Dis 105:283–299. https://doi.org/10.1016/j.nbd.2017.02.004
2. Brown RH, Al-Chalabi A (2017) Amyotrophic lateral sclerosis. N Engl J Med 377:162–172. https://doi.org/10.1056/NEJMra1603471
3. Vargas JNS, Sleigh JN, Schiavo G (2022) Coupling axonal mRNA transport and local translation to organelle maintenance and function. Curr Opin Cell Biol 74:97–103. https://doi.org/10.1016/jceb.2022.01.008
4. Sleigh JN, Rossor AM, Fellows AD et al (2019) Axonal transport and neurodegenerative disease. Nat Rev Neurosci 15:691–703. https://doi.org/10.1038/s41582-019-0257-2
5. Blilud SN, Sahai E, Kelly G et al (2010) Deficits in axonal transport precede ALS symptoms in vivo. Proc Natl Acad Sci USA 107:20523–20528. https://doi.org/10.1073/pnas.1008691107
6. Gibbs KL, Kalmar B, Ryhmers ER et al (2018) Inhibiting p38 MAPK alpha rescues axonal retrograde transport defects in a mouse model of ALS. Cell Death Dis 9:596. https://doi.org/10.1038/s41419-018-0624-8
7. Fellows AD, Ryhmers ER, Gibbs KL et al (2020) IGF1R regulates retrograde axonal transport of signalling endosomes in motor neurons. EMBO Rep 21:e49129. https://doi.org/10.15252/embr.201949129
8. Ryhmers ER, Tosolini AP, Fellows AD et al (2022) Bimodal regulation of axonal transport by the GDNF-RET signalling axis in healthy and diseased motor neurons. Cell Death Dis 13:589. https://doi.org/10.1038/s41419-022-05031-0
9. Sleigh JN, Tosolini AP, Gordon D et al (2020) Mice carrying ALS mutant TDP-43, but not mutant FUS, display in vivo defects in axonal transport of signalling endosomes. Cell Rep 30:3655–3662.e2. https://doi.org/10.1016/j.celrep.2020.02.078
10. Ragagnin AMG, Shadrav S, Vidal M et al (2019) Motor neuron susceptibility in ALS/FTD. Front Neurosci 13:532. https://doi.org/10.3389/fnins.2019.00532
11. Stifani N (2014) Motor neurons and the generation of spinal motor neuron diversity. Front Cell Neurosci 8:293. https://doi.org/10.3389/fncel.2014.00293
12. Kanning KC, Kaplar A, Henderson CE (2010) Motor neuron diversity in development and disease. Annu Rev Neurosci 33:409–440. https://doi.org/10.1146/annurev.neuro.051508.135722
13. Nijsen J, Comley LH, Hedlund E (2017) Motor neuron vulnerability and resistance in amyotrophic lateral sclerosis. Acta Neuropathol 133:863–885. https://doi.org/10.1007/s00401-017-1708-8
14. Blum JA, Klemm S, Shadrach JL et al (2021) Single-cell transcriptomic analysis of the adult mouse spinal cord reveals molecular diversity of autonomic and skeletal motor neurons. Nat Neurosci 24:572–583. https://doi.org/10.1038/s41593-020-00795-0
15. Frey D, Schneider C, Xu L et al (2000) Early and selective loss of motoneuron axons in motoneuron disease alleviated by CNTF. Nat Neurosci 3:408–419. https://doi.org/10.1038/nneurosci.2020.07-16.0217
16. Pun S, Santos AF, Saxena S et al (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/nneurosci.2020.07-16.0217
17. Tremblay E, Martineau É, Robitaille R (2017) Opposite synaptic alterations at the neuromuscular junction in an ALS mouse model: when motor units matter. J Neurosci 37:8901–8918. https://doi.org/10.1523/JNEUROSCI.3090-16.2017
35. Tessarollo L, Yanpallewar S (2022) TrkB truncated isoform receptors.

31. Correia JC, Kelahmetoglu Y, Jannig PR et al (2021) Muscle-secreted neurotrophin receptor p75NTR. Acta Neuropathologica Communications 2022:10:121.

29. Dobrowolny G, Lepore E, Martini M et al (2018) Metabolic changes associated with muscle expression of SOD1G93A. Front Neurol 9:251–334. https://doi.org/10.3389/fneur.2018.00321.

28. Palamiuc L, Schlagowski A, Ngo ST et al (2015) A metabolic switch toward lipid use in glycolytic muscle. Proc Natl Acad Sci USA 112:16111–16120. https://doi.org/10.1073/pnas.1500541116.

27. Hegedus J, Putman CT, Tyreman N, Gordon T (2008) Preferential motor neuron survival is promoted by specific exercise in a mouse model of amyotrophic lateral sclerosis. J Physiol 587:3561–3572. https://doi.org/10.1113/jphysiol.2009.169748.

26. Jenkins TM, Alix JJP, Fingret J et al (2020) Longitudinal multi-modal assessment of muscle damage in murine muscle wasting. J Neurol Sci 415:114608. https://doi.org/10.1016/j.jns.2020.114608.

25. Alhindi A, Boehm I, Chaytow H (2021) Small junction, big problems: Neurovascular junction pathology in mouse models of amyotrophic lateral sclerosis. Neuromuscular Disorders 31:1–7. https://doi.org/10.1016/j.nmd.2021.01.008.

24. Peggion C, Massimino ML, Biancotto G et al (2017) Absolute quantification of cPKCα and cPKCβI. Front Mol Neurosci 10:147. https://doi.org/10.3389/fnmol.2017.00132.

23. Deforges S, Branchu J, Biondi O et al (2009) Motoneuron survival is promoted by specific exercise in a mouse model of amyotrophic lateral sclerosis. J Physiol 586:3337–3351. https://doi.org/10.1113/jphysiol.2007.149726.

22. Delezie J, Weihrach M, Maier G et al (2019) BDNF is a mediator of myofiber type specification in mouse skeletal muscle. Proc Natl Acad Sci USA 116:11161–11162. https://doi.org/10.1073/pnas.1900541116.

21. Kiernan MC, Vucic S, Cheah BC et al (2011) Amyotrophic lateral sclerosis. Lancet 377:942–955. https://doi.org/10.1016/S0140-6736(11)61156-7.

20. Liu Y, Pattamatta A, Zu T et al (2016) C7orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD. Neuroreport 90:521–534. https://doi.org/10.1016/j.neuro.2016.04.005.

19. Korobeynikov VA, Lyashchenko AK, Blanco-Redondo B et al (2022) cPKCα and cPKCβI. Front Mol Neurosci 10:147. https://doi.org/10.3389/fnmol.2020.00132.

18. Ebstein SY, Yagudayeva I, Schneider NA (2019) Mutant TDP-43 causes early-stage dose-dependent motor neuron degeneration in a TARDBP knockin mouse model of ALS. Cell Rep 26:350–367.e4. https://doi.org/10.1016/j.celrep.2018.12.045.

17. Hurtado E, Cilleros V, Nadal L et al (2017) Muscle contraction regulates BDNF/TrkB signaling to modulate synaptic function through presynaptic cPKCa and cPKCB1. Front Mol Neurosci 10:147. https://doi.org/10.3389/fnmol.2017.00147.

16. Mutoh T, Sobue G, Hamano T et al (2000) Decreased phosphorylation of p75NTR in spinal cords from patients with amyotrophic lateral sclerosis. Neurochem Res 25:239–245. https://doi.org/10.1023/a:1007575504321.

15. Lowry KS, Murray SS, McLean CA et al (2001) A potential role for the p75 low-affinity neurotrophin receptor in spinal motor neuron degeneration in murine and human amyotrophic lateral sclerosis. Amyotroph Lateral Sclerosis 2:127–134. https://doi.org/10.1080/14680260110035032.

14. Moyna-Alvarado G, Guerra MV, Wu C et al (2020) BDNF/TrkB signaling endosomes in axons coordinate CREB/mTOR activation and protein synthesis in the cell body to induce dendritic growth in corticobasal neurons. BioRev. https://doi.org/10.1101/2020.08.22.262929.

13. Santos AR, Compido D, Duarte CB (2010) Regulation of local translation at the synapse by BDNF. Prog Neurobiol 92:505–516. https://doi.org/10.1016/j.pneurobio.2010.08.004.

12. Lazo OM, Schiavo G (2021) Rab10 regulates the sorting of internalised TrkB to retrograde axonal transport. BioRev. https://doi.org/10.1101/2021.04.07.438771.

11. Deinhardt K, Salinas S, Verastegui C et al (2006) Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway. Neuron 52:293–305. https://doi.org/10.1016/j.neuron.2006.08.018.

10. Wang T, Martin S, Nguyen TH et al (2016) Flux of signalling endosomes undergoing axonal retrograde transport is encoded by presynaptic activity and TrkB. Nat Commun 7:12976. https://doi.org/10.1038/ncomms12976.

9. Gurney ME, Pu H, Chiu Ay et al (1994) Motor neuron degeneration in mice that express a human CuZn superoxide dismutase mutation. Science 264:1772–1775.

8. Heiman-Patterson TD, Deitch JS, Blankenhorn EP et al (2005) Background and gender effects on survival in the TgN(SOD1-G93A)1Gur mouse model of ALS. J Neurosci 25:1–7. https://doi.org/10.1523/JNEUROSCI.201605-2005.2016.

7. Sleigh JN, Tosolini AP, Schiavo G (2020) In vivo imaging of anterograde and retrograde axonal transport in rodent peripheral nerves. Methods Mol Biol 2143:271–292. https://doi.org/10.1007/978-1-0716-0585-1_20.

6. Tosolini AP, Villanoe-Campos D, Schiavo G, Sleigh JN (2021) Expanding the toolkit for in vivo imaging of axonal transport. J Vis Exp. https://doi.org/10.3791/63471.

5. Restani L, Giribaldi F, Manich M et al (2012) Botulinum neurotoxin A and E undergo retrograde axonal transport in primary motor neurons. PLoS Pathog 8:e1003087. https://doi.org/10.1371/journal.ppat.1003087.

4. Mohan R, Tosolini AP, Morris R (2014) Targeting the motor end plates in the mouse hindlimb gives access to a greater number of spinal cord motor neurons: an approach to maximize retrograde transport. Neuron Science 274:318–330. https://doi.org/10.1016/j.neuroscience.2014.05.045.

3. Mohan R, Tosolini AP, Morris R (2015) Intramuscular injections along the motor end plates: a minimally invasive approach to shuttle tracers directly into motor neurons. J Vis Exp. https://doi.org/10.3791/52846.

2. Tinevez J-Y, Perry N, Schindelin J et al (2017) TrackMate: an open and flexible toolkit for in vivo imaging of axonal transport. J Vis Exp. https://doi.org/10.3791/201605000018.

1. De Meyer F, Perry N, Zitser I et al (2017) TrackMate: an open and extensible platform for single-particle tracking. Methods 115:80–90. https://doi.org/10.1016/j.ymeth.2016.09.016.

Restani L, Giribaldi F, Manich M et al (2012) Botulinum neurotoxin A and E undergo retrograde axonal transport in primary motor neurons. PLoS Pathog 8:e1003087. https://doi.org/10.1371/journal.ppat.1003087.

Mohan R, Tosolini AP, Morris R (2014) Targeting the motor end plates in the mouse hindlimb gives access to a greater number of spinal cord motor neurons: an approach to maximize retrograde transport. Neuron Science 274:318–330. https://doi.org/10.1016/j.neuroscience.2014.05.045.
57. Wyckelsma VL, McKenna MJ, Levinger I et al (2016) Cell specific differences in the protein abundances of GAPDH and Na(+)-K(+)-ATPase in skeletal muscle from aged individuals. Exp Gerontol 75:8–13. https://doi.org/10.1016/j.exger.2015.12.010

58. Vigelis A, Dybboe R, Hansen CN et al (2015) GAPDH and β-actin protein decreases with aging, making Stain-Free technology a superior loading control in Western blotting of human skeletal muscle. J Appl Physiol 118:386–394. https://doi.org/10.1152/japplphysiol.00840.2014

59. Surana S, Tosolini AP, Meyer IFG et al (2018) The travel diaries of tetanus and botulinum neurotoxins. Toxicon 147:58–67. https://doi.org/10.1016/j.toxicon.2017.10.008

60. Surana S, Villarroel-Campos D, Lazo OM et al (2020) The evolution of the axonal transport toolkit. Traffic 21:13–33. https://doi.org/10.1111/tra.12710

61. Bercsenyi K, Schmieg N, Bryson JB et al (2014) Tetanus toxin entry. Acta Neuropathologica Communications          (2022) 10:121

62. Che DL, Chowdary PD, Cui B (2016) A close look at axonal transport: cargos slow down when crossing stationary organelles. Neurosci Lett 610:110–116. https://doi.org/10.1016/j.neulet.2015.10.066

63. Gu Y, Sun W, Wang G et al (2012) Rotational dynamics of cargos at pauses during axonal transport. Nat Commun 3:1030. https://doi.org/10.1038/ncomms2037

64. Ross JL, Ali MY, Warshaw DM (2008) Cargo transport: molecular motors navigate a complex cytoskeleton. Curr Opin Cell Biol 20:41–47. https://doi.org/10.1016/jceb.2007.11.006

65. Zhou B, Cai Q, Xie Y, Sheng Z-H (2012) Snapin recruits dynein to mitochondria in axons. Cell 150:1776–1786. https://doi.org/10.1016/j.celrep.2012.06.010

66. Cioni J-M, Lin JQ, Holtermann AV et al (2019) Late endosomes act as a reserve pool of molecules for axonal transport. eLife 8:e43200. https://doi.org/10.7554/eLife.41973

67. Smith KS, Rush RA, Rogers M-L (2015) Characterization and changes in neurotrophin receptor p75-expressing motor neurons in SOD1(G93A) G1H mice [corrected]. J Comp Neurol 523:1664–1682. https://doi.org/10.1002/cne.23763

68. Yanpallewar SU, Barrick CA, Buckley H et al (2012) Deletion of the BDNF truncated receptor TrkB.T1 delays disease onset in a mouse model of amyotrophic lateral sclerosis. PLoS ONE 7:e39946. https://doi.org/10.1371/journal.pone.0039946

69. Yanpallewar S, Fulgenzi G, Tomassoni-Ardori F et al (2021) Delayed onset of inherited ALS by deletion of the BDNF receptor TrkB.T1 is non-cell autonomous. Exp Neurol 337:113576. https://doi.org/10.1016/j.expne urol.2020.113576

70. De Wit J, Eggers R, Evers R et al (2006) Long-term adeno-associated viral vector-mediated expression of truncated TrkB in the adult rat facial nucleus results in motor neuron degeneration. J Neurosci 26:1516–1530. https://doi.org/10.1523/JNEUROSCI.4543-05.2006

71. Matusica D, Alfonsi F, Turner BJ et al (2018) Inhibition of motor neuron death in vitro and in vivo by a p75 neurotrophin receptor intracellular domain fragment. J Cell Sci 129:517–530. https://doi.org/10.1242/jcs.173864

72. Yanpallewar S, Lage K, Weinert BT et al (2012) Proteomic analysis of lysine acetylation sites in rat tissues reveals organ specificity and subcellular patterns. Cell Rep 2:419–431. https://doi.org/10.1016/j.celrep.2012.07.006

73. Hanin M, Talvenheim J, Le J et al (1995) Extracellular domain of neurotrophin receptor trkB: disulfide structure, N-glycosylation sites, and ligand binding. Arch Biochem Biophys 322:256–264. https://doi.org/10.1006/abbi.1995.1460

74. Baldwin AN, Shooter EM (1995) Zone mapping of the binding domain of the rat low affinity nerve growth factor receptor by the introduction of novel N-glycosylation sites. J Biol Chem 270:4594–4602. https://doi.org/10.1074/jbc.270.9.4594

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.