BDNF-regulation of in vivo axonal transport is selectively impaired in fast motor neurons in SOD1G93A mice.

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Abstract

Axonal transport ensures long-range, bidirectional delivery of essential cargos, such as mitochondria, autophagosomes and signalling endosomes, between proximal and distal compartments of neurons for neuronal homeostasis, function and survival (Sleigh et al, 2019; PMID: 31558780). SOD1G93A mice show in vivo deficits in axonal transport pre-symptomatically that suggest axonal transport impairment contributes to disease (Billett et al, 2010; PMID: 21059824). Brain-derived neurotrophic factor (BDNF) is critical for neurological development and synaptic growth; however, in adulthood its expression is reduced, and BDNF functions to mediate survival in a variety of neurons, and synaptic maintenance, including at the neuromuscular junction (NMJ). Motor neurons (MNs) are defined by the type of muscle fibre they innervate, α-MNs innervating tibialis anterior (TA) are comprised of fast-fatigable (FF) and fast-fatigue resistant (FFR) subtypes, and α-MNs innervating soleus (SOL) are comprised of slow-fatigue resistant (SFR) (Stijn, 2014; PMID: 25346699). In SOD1G93A mice, fast-fatigable motor neurons (e.g., innervating TA) are more vulnerable than slow-fatigue resistant motor neurons (e.g., innervating SOL) (Pun et al, 2006; PMID: 16473488). As the influence of both α-motor neuron subtype and BDNF regulation on axonal transport are currently unknown, determining the mechanisms that regulate BDNF-signalling in motor neuron subtypes, as well as in disease, will reveal novel clues about pathomechanisms influencing selective motor neuron vulnerability in ALS.

Methods

Axonal transport was visualised in vivo with intravitreal injections of fluorescently-labelled axotic fragment of tatuus neurotoxin (HT). HT was delivered into TA or soleus muscles of wild-type (WT) and SOD1G93A mice (at tine points that correspond with ~20%, 27% and -40%, p<0.05) of MNs. 4 hours later, sciatic nerves were exposed in live, anaesthetised animals, and imaged using a time course confocal microscopy at 37°C. Retrogradely transported, H-T1abeled signalling endosomes within single axons were tracked using TrackMate (Tinevez et al, 2006; PMID: 27713081). Muscle and sciatic nerve BDNF, receptors, and signalling pathways expression were assessed in SOD1G93A mice and age-matched, WT littermates by immunoblotting. TrkB and pTrkB levels were assessed via immunohistochemistry on NMJs from fast muscle fibres or sciatic nerve cryosections.

Results

Axonal transport is impaired in vivo in SOD1G93A mice, specifically motor neurons (α-MNs) and sensory neurons (β-MNs). BDNF-regulation of in vivo axonal transport is selectively impaired in fast motor neurons in SOD1G93A mice.

One-way ANOVA followed by a Bonferroni’s multiple comparison test, n=8-10.

Discussion

• Basally, FNH and SMN axons have similar axonal transport dynamics of signalling endosomes.
• BDNF enhances axonal transport specifically in fast motor neurons in wild-type mice.
• FNH axons display reduced axonal caliber in pathology whereas SMN axons remain unaltered.
• Axonal transport is selectively impaired in FNHs in SOD1G93A pathology.
• FNH axons become sensitive to BDNF stimulation in pathology.
• BDNF is expressed more in TA basal pathology and induces increases in TrkB T1 and pTrkB.
• Pathology does not alter pTrkB in SOD1G93A sciatic nerves.
• TrkB T1 and pTrkB receptor levels increase in SOD1G93A sciatic nerves, specifically in Schwann cells.

Conclusions

These data indicate that different MNimexcele subgroups have distinct axonal transport features, including sensitivity to BDNF stimulation, and that cell and non-cell autonomous BDNF signalling is impaired in SOD1G93A pathology.

Figure 7. Basal or pathological TrkB or pTrkB expression does not differ in tibialis anterior (TA) or soleus muscle (SOL). A) Representative immunoblots of TrkB expression in wildtype (WT) and SOD1G93A mice. B) WT (n=8), SOD1G93A (n=7) and their age-matched controls, were analyzed by one-way ANOVA followed by a Bonferroni’s multiple comparison test, n=8.

Figure 8. pTrkB expression is increased in sciatic nerves in disease. A) Representative immunoblots of pTrkB expression in wildtype (WT) and SOD1G93A mice. B) WT (n=8), SOD1G93A (n=7) and their age-matched controls, were analyzed by one-way ANOVA followed by a Bonferroni’s multiple comparison test, n=8.

Figure 9. TrkB T1 and pTrkB expression are increased in sciatic nerves in disease. A) Representative immunoblots of TrkB T1 and pTrkB expression in wildtype (WT) and SOD1G93A mice. B) WT (n=8), SOD1G93A (n=7) and their age-matched controls, were analyzed by one-way ANOVA followed by a Bonferroni’s multiple comparison test, n=8.