Older but not slower: ageing does not alter axonal transport dynamics of signalling endosomes in vivo

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Efficient bi-directional axonal transport is critical for the function and survival of neurons. Defects in this process have been identified in early stages of several late-onset neurological disease models. Axonal transport is also thought to naturally decline with age, which could exacerbate pathological deficiencies and may alter disease onset and/or progression. Here, by using the atoxic binding fragment of tetanus neurotoxin (HcT), we monitored the transport kinetics of axonal signalling endosomes, which are intracellular compartments essential for neuronal differentiation and homeostasis. HcT can be injected into muscles, where it is taken up by nerve termini and hijacks the retrograde delivery of signalling endosomes. Assessing the dynamic properties of signalling endosomes in live, female, wild-type mice aged from one to over 13 months, we saw no significant alterations in transport speeds or pausing. Our work indicates that decline in signalling endosome kinetics does not occur before one year in vivo, suggesting that its deterioration during normal ageing is unlikely to be affecting previously reported disease-associated endosome transport deficits.

Objective

Using real-time, intravital imaging, our principal aim was to determine whether wild-type animals show an aging-related decline in axonal transport of signalling endosomes in vivo over an extended period.

Introduction

Neurons possess long, thin processes called axons, which require specialised transport mechanisms for the anterograde (cell soma to axon terminal) and retrograde (terminal to soma) delivery of different cargoes, such as organelles and proteins, to support neuronal function and survival [Maday 2014[1]]. Defective axonal transport is thought to underlie several neurological conditions, including amyotrophic lateral sclerosis and Alzheimer’s disease, as its impairment is amongst the earliest indicators of pathology in disease models [Millecamps 2013[2]]. Axonal transport has also been reported to decline with age in a number of different experimental settings including mouse sciatic nerve and hippocampal explants [Gilley 2012[3]] [Milde 2015[4]] Drosophila melanogaster wing preparations [Vagnoni 2015[5]] and non-invasive, tracer experiments analysing rodent cargo delivery en masse [Kim 2011[6]] [Li 2003[7]] [McQuarrie 1989[8]] [Minoshima 2008[9]] however, there is little evidence on how ageing affects the real-time transport of individual cargoes in living mammals. We therefore analysed signalling endosome kinetics in peripheral axons of wild-type mice aged one month to over a year by injecting HcT into the muscles of the lower leg with subsequent exposure and imaging of the sciatic nerve (Figure 1A and Supplementary Video 1).

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Figure 1. In vivo signalling endosome axonal transport dynamics are unchanged up to over a year in wild-type mice. (A) Representative series of images (i to iv) acquired by time-lapse confocal microscopy of HcT-555 (pseudocoloured cyan) being retrogradely transported (left to right) in sciatic nerve axons from a live wild-type mouse (postnatal day 408). Four individual endosomes are tracked by coloured triangles (iv), the displacement profiles of which have been plotted (v). Scale bars = 10 μm. (B) The speed distribution curves of signalling endosome are similar for 1-month and ≥13-month-old wild-type animals. The same holds true for the three and eight month data, which are not plotted. (C) Speed distribution curve produced by combining data from four different time points (1, 3, 8 and ≥13 months; 19 mice). A smoothened curve is fitted to the data, which shows that approximately 50% of individual endosome steps are faster than 1.88 μm/s. A value of 1.89 μm/s was calculated from the raw data. (D) The mean average speed of individual signalling endosomes across the four ages are plotted. Each data point represents an individual endosome. (E) There is no significant difference between ages in the mean average (×, P = 0.851, one-way ANOVA) and the maximum (○, P = 0.757, one-way ANOVA) signalling endosome speeds calculated individually for each mouse. (F-G) No difference is also detected between ages in the percentage of endosomes pausing for at least one frame (F, P = 0.876, one-way ANOVA) or the percentage of time paused (G, P = 0.221, one-way ANOVA). Pairwise statistical comparisons of the data from different ages in panels E-G were also not significant (P > 0.05, Tukey’s multiple comparisons test). NS, not significant. Means ± standard error of the mean (S.E.M.) are plotted for all graphs. n = 3 (one month), 3 (three months), 4 (8 months), and 9 (≥13 month).

Supplementary Video 1. In vivo imaging of axonal transport. Representative video constructed from images acquired by time-lapse confocal microscopy of HcT-555 (pseudocoloured cyan) being retrogradely transported (left to right) in exposed sciatic nerve axons of a live, anaesthetised, wild-type mouse (P408). HcT was injected into the gastrocnemius and tibialis anterior muscles of the right leg, and is being transported towards the spinal cord. The video consists of 199 frames, covering 546 s, and is being played at a rate of 5 frames/s. The video is 66.4 mm x 18.6 mm.
Results and discussion
HcT was injected into the gastrocnemius and tibialis anterior muscles of the right hind limb of live, anaesthetised, wild-type, female mice at four different ages of 1, 3, 8, and ≥13 months. 4–6 h post-injection, the right sciatic nerve was exposed and imaged using time-lapse confocal microscopy in order to identify and track individual HcT-containing signalling endosomes being retrogradely transported towards the spinal cord (Figure 1A and Supplementary Video 1). The speed of all individual endosome steps between two consecutive frames was calculated and relative frequencies plotted (Figure 1B-C). The speed distribution curves of all four time points were very similar (Figure 1B, only one and ≥13 month data are shown). We therefore combined the data from all ages into one curve in order to produce a reference speed profile for wild-type mice (Figure 1C). The average speed of single endosomes across at least five consecutive frames was also plotted for the four time points (Figure 1E). When these values were averaged to produce mean and maximum endosome speeds for each animal, we saw no significant differences between any of the time points (Figure 1E, average speed, $P = 0.851$, one-way ANOVA; maximum speed, $P = 0.757$, one-way ANOVA; pairwise comparisons, $P > 0.05$, Tukey’s multiple comparisons test). Moreover, there was no difference in either the percentage of endosomes pausing for at least one frame or the percentage of time spent paused (Figure 1F-G, $P = 0.876$, one-way ANOVA; maximum speed, $P = 0.221$, one-way ANOVA; pairwise comparisons, $P > 0.05$, Tukey’s multiple comparisons test).

Several studies have provided indirect evidence that axonal transport declines with age by using non-invasive approaches. For example, radiolabelled tracers have been used to show that slow axonal transport is compromised by 24 months in rat ventral motor axons and optic nerves [McQuarrie 1989[6]] while α-synuclein transport velocity is reduced by 11 months in mouse sciatic nerves [Li 2003[7]]. Similarly, magnetic resonance imaging has shown that axonal transport rates are diminished by 13 months in rat brains, and are in decline in the mouse olfactory bulb by 15 months [Kim 2011[8]]. Nevertheless, these studies may be affected by axon loss, and do not directly assess axonal transport of individual cargoes in identifiable axons in live animals. Only a single previous report has used intravital imaging to assess the transport of individual cargoes in living, anaesthetised mice. Takihara et al. reported that the duration, distance and speed of mitochondrial transport in retinal ganglion neurons increased from two to four months of age, but then declined by 12-13 months, getting worse by 23-25 months [Takihara 2015[9]]. Our data presented here contrast with these findings up to 13 months and indicate that axonal transport of signalling endosomes remains unchanged in vivo in wild-type mice aged over a year. This discrepancy may be caused by differences in myelination, which is known to affect the stability of the microtubules used by molecular motors for axonal transport [Kirkpatrick & Brady 1994[10]]. Alternatively, it may reflect the type of cargo being transported [Bilsland 2010[11]] or innate properties of the central and peripheral nervous systems [Milde 2015[12]].

Conclusions
Wild-type, female mice show no difference in the axonal transport dynamics of signalling endosomes in the peripheral nervous system between four time points spanning one month to over a year. This work is the first to show that real-time axonal transport speeds and pausing are unaffected in live, anaesthetised mice aged over a year. These data indicate that previously reported defects in endosome dynamics in mouse models of late-onset nervous system disease using fluorescently labelled HcT [Bilsland 2010[12]] are unlikely to be compounded by natural, age-related decline in the transport process, thus also facilitating the future analysis of slower progressing disease models.

Limitations
The presented analyses only include data from female mice, which may not be reflective of both sexes. We have generated data from males at one and three months, and there does not appear to be a difference between sexes ($n = 3$; average speed, $P = 0.972$; maximum speed, $P = 0.801$; % pausing endosomes, $P = 0.550$; % time paused, $P = 0.304$; two-way ANOVA, data not shown). Moreover, males and females have previously been reported to show no distinctions in vesicular and mitochondrial transport dynamics in nerve explant preparations across multiple ages [Milde 2015[12]] however, although unlikely, this may not be true in vivo for signalling endosomes at the later time points of 8 and ≥13 months. Moreover, we only analysed a single type of cargo, which move exclusively in the retrograde direction. In addition, transport kinetics are not evaluated beyond 13 months of age, and hence we cannot comment on what happens towards the end of the natural lifespan of laboratory mice. When injected into muscle, HcT is taken up at both neuromuscular junctions (NMJs, motor termini) and muscle spindles ( proprioceptive sensory termini). The sciatic nerve consists of both motor and sensory axons, so we cannot be certain about the type of neuron analysed for each animal using this modality of injection. Nevertheless, given that muscles possess considerably more NMJs than spindles (for example, approximately 20-fold more in the cleidomastoid muscle [Tapia 2012[32]]; [Bricha 1987[33]] and approximately 80% of HcT-containing sciatic axons stain for the motor marker ChAT [Bilsland 2010[12]] it is likely that we
were predominantly analysing motor nerves. Finally, due to variability in the amount of HcT uptake between different nerves, we cannot reliably measure the frequency of cargoes being transported as has been done previously \cite{Gilley2012,Milde2015}.

**Conjectures**

It would be important to grow mice to two years and see whether the previously reported transport deficits in neuronal explants at this age \cite{Milde2015} are observed in an *in vivo* setting. Furthermore, intravital imaging of other axonal, bi-directionally transported cargoes such as mitochondria would allow us to determine whether anterograde transport is disturbed and whether these findings are cargo specific, central or peripheral nervous system dependent, or generally applicable.
Additional Information

Methods and supplementary material
Please see https://sciencematters.io/articles/201605000018.

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Ethics statement
All mouse handling and experiments were performed under license from the United Kingdom Home Office in accordance with the Animals (Scientific Procedures) Act (1986) and approved by the University College London – Institute of Neurology Ethics Committee.

Citations


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