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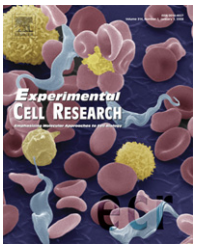

Review

What is slow axonal transport?☆

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ABSTRACT

While the phenomenon of slow axonal transport is widely agreed upon, its underlying mechanism has been controversial for decades. There is now persuasive evidence that several different mechanisms could contribute to slow axonal transport. Yet proponents of different theories have been hesitant to explicitly integrate what were, at least initially, opposing models. We suggest that slow transport is a multivariate phenomenon that arises through mechanisms that minimally include: molecular motor-based transport of polymers and soluble proteins as multi-protein complexes; diffusion; and en bloc transport of the axonal framework by low velocity transport and towed growth (due to increases in body size). In addition to integrating previously described mechanisms of transport, we further suggest that only a subset of transport modes operate in a given neuron depending on the region, length, species, cell type, and developmental stage. We believe that this multivariate approach to slow axonal transport better explains its complex phenomenology: including its bi-directionality; the differing velocities of transport depending on cargo, as well differing velocities due to anatomy, cell type and developmental stage.

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Contents

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Introduction

Neurons have a remarkable geometry, their axons can extend over the distance of meters and can contain a total volume that is hundreds of times greater than that of the cell body. Because the cell body contains most of the protein-synthesizing capacity, an enormous mass is transported into the axon to build and replace components required for neuronal function.

In the classic studies of axonal transport, a pulse of radio-labeled amino acid was applied to neuronal cell bodies that were then taken up and made into proteins over a time period of a few hours. Then at various intervals ranging from hours to several months the nerve was excised, divided into segments, and run out on protein gels, and the profile of radio-labeled proteins along the nerve was determined either by measuring the amount of radioactivity in each segment or by autoradiography (Fig. 1A). Transport was characterized by the movement of peaks of radio-labeled proteins (Fig. 1B) [1]. The original categorization of axonal transport into "fast" and "slow" components was based on the observation that different types of proteins moved at different velocities. The movement of membrane bound proteins at a rate roughly between 20 and 400 mm/day was called "Fast Axonal Transport". Where the movement of non-membrane bound proteins at a rate of 0.1–20 mm/day was called "Slow Axonal Transport". In contrast to the controversy surrounding slow transport, the mechanism of fast transport is now widely agreed to depend on the movement of membranous vesicles on microtubule 'tracks' powered by the motor proteins kinesin and dynein.

Classic examples of slow transport in the olfactory neurons of garfish and in the rat sciatic nerve are shown in Fig. 1 [2,3]. Slow axonal transport has three key features: (1) pulse labeled proteins move in the anterograde direction over the course of days, (2) the labeled proteins were seen to be transported as peaks or waves, and (3) different types of non-membrane bound proteins move at different velocities. The question we raise is "What is Slow Axonal Transport?" We make the

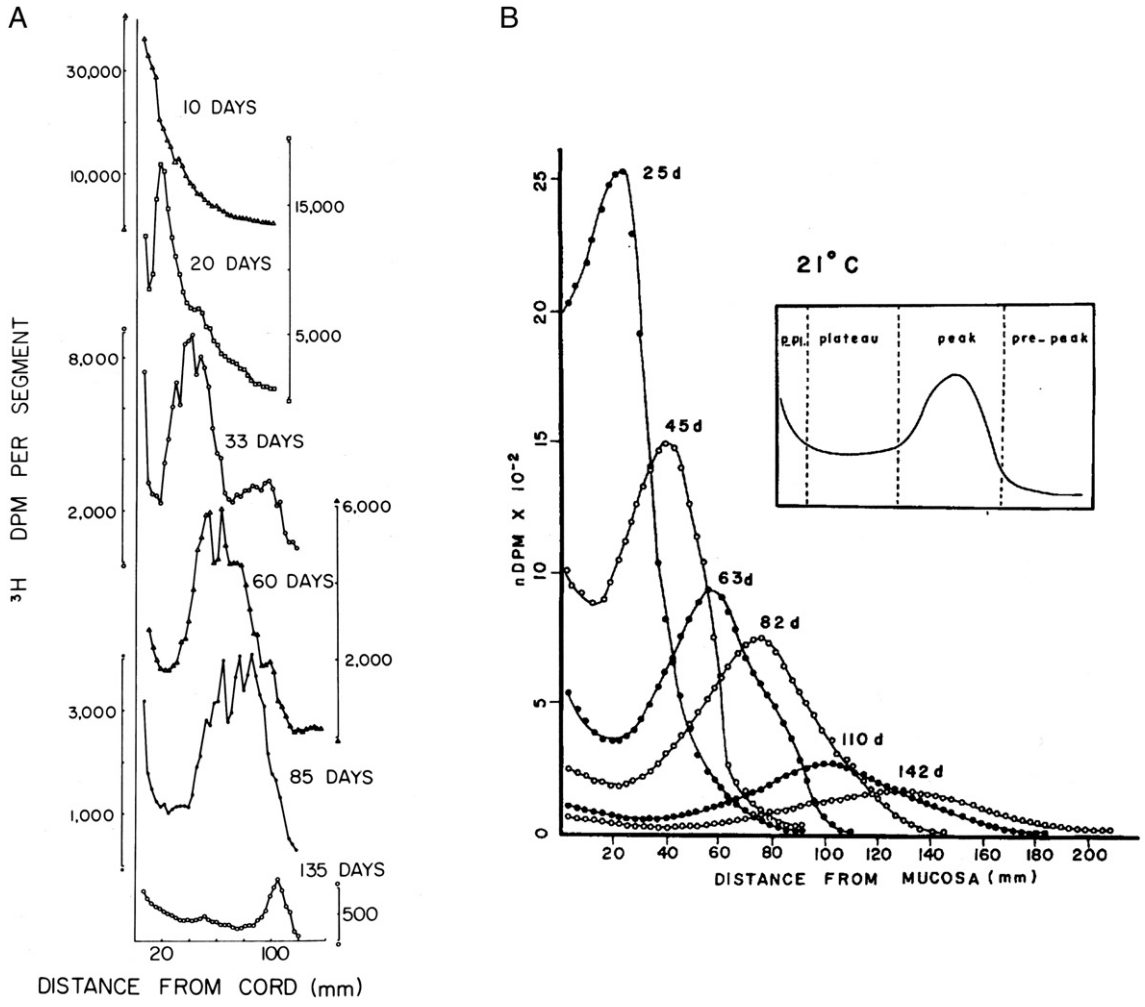

Fig. 1 – Classic examples of slow axonal transport. A. Titrated leucine was injected into the ventral horn of the spinal cord of young adult rats. At the times indicated, the sciatic nerve was excised, cut into segments, and the overall distribution of radio-labeled proteins was determined using a scintillation counter. Proteins moved down the axon as a coherent peak that slowed with time. B. Titrated leucine was injected into the olfactory cavities of 3 to 4 kg garfish (*Lepisosteus osseus*). At the times indicated, the distribution of radio-labeled proteins was determined as above. In these animals, which continue to grow during adulthood, the velocity of transport does not slow.

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argument that slow axonal transport cannot be explained by any single transport mechanism, but instead is a multivariate process.

Main text

Problems with the peaks

Peaks of labeled proteins (Fig. 1) are a defining feature of slow axonal transport. If the proteins were simply diffusing, this would produce an exponentially declining curve of labeled proteins along the axon [4]. The influential 'Structural Hypothesis' of Lasek explained the peak by proposing that the cytoskeleton formed a network structure that incorporated labeled proteins during assembly in the cell body and then moved coordinately to the axon tip [2]. This model is, indeed, the simplest explanation for the movement of the peak as is seen in Fig. 1A. It proposed that the entire axonal framework was in coherent motion thus 'overwhelming' diffusion [4]. An enormous amount of work went into testing, and ultimately falsifying this hypothesis [5–9].

In retrospect, it is now clear that the peak is not the result of a pulse of radio-labeled protein being incorporated into a moving network, or any other form of coordinated cargo movement. Fast transport also produced waves or peaks in these pulse labeling experiments [10], but there is nothing coordinated about the movement of cargo by fast transport, which is bi-directional, intermittent, and independent for each membranous structure [11]. It now seems generally accepted that the peak is indicative of active transport, but provides little other information about the mechanisms of transport or the properties of the transported cargo.

From the earliest studies it was clear that slow axonal transport occurs at two broad rates: slow component-a (SCa) at 0.1–1 mm/day and slow component-b (SCb) at 2–10 mm/day [12]. SCa is associated with the movement of proteins that form the cytoskeletal polymers from soluble subunits: microtubules from tubulin and neurofilaments from neurofilament proteins. The transport of these proteins has dominated the discussion of and experiments on slow transport for decades [13,14]. The transport of a diverse array of hundreds of different 'soluble proteins,' including cytoskeletal-associated proteins, actin, glycolytic enzymes and synaptic proteins, is associated with the 5–10× faster SCb [15]. A substantial problem that must be addressed is why different proteins move at different rates. In the past, there have been several attempts to create a unifying theory that explains both SCa and SCb [11,16,17]. While we agree that some rate differences arise because of differing amounts of time coupled to a single transport mechanism (e.g. kinesin), we think an alternative is that SCa and SCb vary in rates because the cargo varies in the extent to which it is transported by *different* mechanisms of transport.

While slow transport has a net anterograde motion, there is significant retrograde transport that has been rarely discussed. The classic studies of Fink and Gainer showed that when proteins were labeled in the middle of the axon 'slow transport' occurred in both directions [18,19]. Similarly, retrograde transport, at SCb rates, of soluble protein tracers are routinely used in neuroanatomical studies [20–23]. In addition, dynein has been implicated in the retrograde transport of neurofilament polymers [24,25]. The observation that non-membrane bound proteins (i.e. not moved by fast transport) are transported in both the anterograde and retrograde directions clearly suggests that slow axonal transport arises through the action of multiple transport modes.

Because most macromolecular synthesis occurs in the cell body [26], large amounts of protein must be delivered to the distal axon, both for maintenance and for growth at the growth cone. However, the impossibility of maintaining long axons with protein delivered at the rate of slow transport was persuasively argued years ago, but largely ignored until recently [27–29]. That is, the rate of slow axonal transport appears to be too slow to adequately supply the axon with proteins. For example it would take almost 27 years for neurofilament proteins moving at a rate of 0.1 mm/day to make it to the of end of the 1 m long human sciatic nerve [26].

In brief, we suggest that a model for slow axonal transport must be able to explain the following: why different non-membrane bound proteins are transported at different velocities; the observations of retrograde transport; how long axons (i.e. >1 m) are supplied with proteins; and must explain variation in transport rates that occur through development and along the length of the axon. Below, we will suggest a model that, in essence, proposes that the axon is analogous to a chromatography column that lengthens by stretching and is supplied with proteins through diffusion and microtubule motor-based transport.

Current proposals for slow transport: "back to the future"

The current explicit proposals for slow transport share two mechanistic similarities. The first is that movement of proteins, both as cytoskeletal polymers and as protein complexes, is driven by kinesin and dynein motors moving along microtubules, the same basic mechanism that is now well-established for fast axonal transport [5,7,30]. The second is that the slow rate of transport is the result of an essentially chromatographic mechanism: the transported proteins cycle between binding to, transport along, and unbinding from the matrix that makes up the axonal framework.

Gross and Weiss were the first to explicitly propose a chromatography type model in their 'Microstream Hypothesis' [31,32]. In this model, the axoplasm surrounding microtubules form moving streams, a result of 'force generating enzymes' at the microtubule surface, that move proteins within the 'microstream.' It is now thought that molecular motors, instead of streams of fluid, are responsible for transport along microtubules. A similar theory was proposed by Ochs called the Unitary Hypothesis. It posited that 'motorized' movement along microtubules non-selectively dragged axonal components down the axon [16]. As our discussion of the potential contributors to slow axonal transport will now show, important current thinking on slow transport has returned to ideas of low-efficiency coupling to the same 'microtubule railroad' that underlies fast transport. In addition, we discuss the contribution of en bloc transport of the cytoskeleton ('low velocity transport') and diffusion or dispersion mechanisms. The combination of these transport mechanisms address yet

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another issue prominent in early, but not current, studies of slow transport: changes in its rate with developmental status and neuronal type [32].

Slow transport of polymers

We begin our discussion of specific modes of transport with two recent models. Both the "Stop-and-Go" hypothesis and the "Cut-and-Run" hypothesis postulate the slow movement of cytoskeletal polymers, as opposed to soluble proteins, is based on inefficient coupling to the microtubule motor 'rail-road' underlying fast transport. The "Stop-and-Go" hypothesis is not just a general assumption that slow axonal transport is powered by molecular motors, but a specific hypothesis that polymers are actively transported. This hypothesis is based on time-lapse observations of the movement of fluorescently labeled cytoskeletal polymers following photobleaching or photoactivation. These experiments have revealed intermittent bi-directional movement of individual polymers at rates consistent with kinesin and dynein mediated transport (~0.4–0.6 um/s) [7,11,33,34]. While some cytoskeletal polymers move at a high velocity, it is widely agreed that the majority remain embedded in a stationary axonal framework [9,35–37]. The "Stop-and-Go" hypothesis proposes that slow axonal transport arises in vivo because cytoskeletal polymers move rapidly but very rarely, e.g. neurofilaments are postulated to spend 8% of the time on the 'tracks' and even then are paused 97% of the time. Overall, this transport has been suggested to average to the rate of slow axonal transport [8].

There is clear evidence that the rate of slow axonal transport decreases during development [38]. There is also indirect evidence that "Stop-and-Go" transport may decrease during axonal elongation based on observations of the lengths of transported cytoskeletal polymers and the lengths of these polymers seen in neurons of various ages. That is, the average length of transported neurofilaments is in the range of 4 to 10 μm and for microtubules in the range of 2 to 5 μm [39,40]. While microtubules have an average length of ~4 μm in early embryonic neurons [41], in more mature neurons polymer length is on average 100 μm for both neurofilaments and microtubules [42–44]. If short polymers are less frequent in long axons, it suggests that "Stop-and-Go" transport may make its most significant contribution early in development. This may be particularly important for providing seeds for polymerization.

The "Cut-and-Run" hypothesis has been developed in acknowledgement that most polymers are long in mature axons. It proposes that molecular motors are constantly attempting to transport all microtubules, but only short microtubules that are not cross-linked within the axonal framework [37] undergo fast movement [7]. This model presumes that polymerization occurs in one part of the cell to make long polymers, those are severed into short polymers, which are then transported down the axon. Those short polymers then either depolymerize to provide subunits for existing microtubules or act as nucleation seeds for the creation of new long microtubules. The strength of this variation of the "Stop-and-Go" model is that it incorporates data that suggest that short (<10 μm), but not long microtubules appear to be transported by microtubule based motors in the axon.

One proposal to explain soluble transport in terms of these models is that slow axonal transport of non-polymeric proteins are carried indirectly (ride 'piggy back') on moving cytoskeletal polymers [11]. We agree that this will make a contribution to slow axonal transport, but suggest that additional modes of transport are required, particularly to explain those soluble proteins in SCb that move faster than any of the cytoskeletal polymers (Table 1). As a further example, measuring transport of tubulin and various microtubule-associated proteins with a method similar to the original radiolabeling approach (Fig. 1), tubulin was shown to move significantly more slowly than the microtubule-associated proteins [45]. Based on indirect, 'piggy back' movement of cargo, it is difficult to see how 'soluble' cargoes could move faster than their polymeric carriers [46]. We propose that the majority of slow transport of soluble proteins occurs by direct association of soluble proteins with motors based on clear new evidence [15] and older work on viral transport, as we discuss below.

Soluble transport

The assertion that soluble proteins are transported by slow axonal transport has never been particularly controversial; glycolytic [47] and cytoskeletal-associated proteins [45] that do not form polymers move by slow axonal transport. What has been debated is how soluble transport occurs. First, we think it likely that 'soluble protein' in the context of axonal transport refers to protein complexes [5,48], which are empirically defined as those that remain in the S3 fraction (i.e. the supernate after centrifugation at 100,000 *g* for 1 h) during a biochemical purification [49]. It is questionable if any protein exists in isolation in a cell: immuno-precipitation of any single soluble protein invariably pulls down a complex of interacting proteins [50]. Glycolytic enzymes have long been known to form complexes with the cytoskeleton [51]. Similarly, dynein and kinesin were originally isolated by 'soluble protein' methodology but are now well known to function as multi-protein complexes [52]. Here again, the older idea that slow transport involves movement of protein complexes [53] has seen a resurgence in popularity [15,54].

Especially illuminating evidence that multi-protein cargoes can bind directly to kinesin and dynein and 'ride' along microtubule tracks within neural axons comes from the axonal transport of neurotropic viruses such as Herpes simplex virus (HSV) [55]. Although rarely discussed in studies of axonal transport we think viral transport, despite occurring at fast transport rates, provides important insights into the likely mechanism(s) for slow transport of soluble protein. These include precedents that help answer some of the questions on slow transport raised earlier, such as bi-directionality and

Table 1 – Solubility and velocity of transport

Protein	Percent soluble	Velocity (mm/day)
Neurofilament	>10	0.1
Tubulin	30	1
Actin	50	2
SCb	100	10

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differential rates of transport. Indeed, the life cycle of such viruses normally utilizes both retrograde and anterograde 'motorized' transport within axons, thus directly confirming a 'round trip ticket' for soluble protein complexes.

Essentially, the initial infection occurs in the periphery and the viral capsid is retrogradely transported along microtubules by dynein to the cell body. Infectious progeny are assembled in the cytoplasm and then transported anterogradely along axonal microtubules via kinesin for release into the extracellular space. Although these viruses are enveloped, transport does not require an outer membrane for transport in either direction: the transported structure is the naked viral particle [56,57]. Accordingly, various viral proteins have been shown to bind directly to the motor complexes [58,59] with a subset of these required for transport [56,60]. Thus, the protein composition of the complex regulates directionality of movement [61,62]. We postulate an entirely similar scenario as the mechanism underlying motorized slow transport of 'soluble' proteins. Strong support for this idea comes from an innovative paper on the transport of Slow Component-b proteins [15]. Using red and green tags to label different types of non-membrane bound proteins, strong evidence was found that soluble proteins are transported bi-directionally by kinesin and dynein as multi-protein complexes.

We do not see the differences in the rates of viral transport and slow transport to be problematic. As with polymer transport, we think the slow rates of transport of soluble protein complexes simply represents inefficient and variable coupling to the microtubule system. Conversely, there are reports of non-polymer protein transport occurring at rates typical of *fast* transport, including neurofilament proteins [63], small amounts of tubulin [64], and actin associated proteins [65]. In addition, a wide array of rates for slow transport of tubulin, actin, and alpha-synuclein have been reported [30,66]. Thus, we see a continuum in the rates at which 'soluble' proteins can be transported by motors; not only do different proteins move at different rates (and direction), but the same protein can also move at different rates. We suggest that motors will transport anything they bind, either directly or indirectly, that is not tightly associated with the cytoskeletal framework. This includes proteins often regarded as soluble and short cytoskeletal polymers. In our eyes, there is no special distinction between soluble and polymer transport: what can be moved by motor transport is moved.

However, this raises the issue of how soluble protein complexes interact with motors. In the case of viruses, it appears that motor-binding functions have been strongly selected and are specific [67], as might be expected in view of the crucial role of microtubule transport in their life cycle. This seems unlikely for more general transport of soluble proteins. It would be surprising if the wide array of proteins transported in the axon all had specific motor-binding functions. Rather, we think the mechanism is probably non-specific for most proteins for the following reason. Kinesin binds and transports negatively charged beads [68] and negatively charged fluorescently labeled dextrans [69]. As most proteins have a net negative charge these may be transported non-specifically. This is not to discount that there are specific mechanisms where by motors binds to other proteins [49]. There is clear evidence that scaffolding proteins such as Liprin-α, GRIP1, and JIP bind to kinesin [70] to offer the ability to selectively target proteins to specific regions of the cell.

A motor-based soluble transport model offers a natural explanation for the observed variation in rates of slow axonal transport. There is a clear correlation between the solubility of a protein and its rate of transport. The greater solubility, the higher the rate of transport (Table 1) [71,72]. For example, proteins that are transported in SCb are 100% soluble and move at the rate of 10 mm/day. This high rate of transport approaches the transport rate of neurotropic virus. We propose that because soluble proteins spend more time unbound to a stationary matrix (in the proximal axon of elongating neurons and throughout the axon in mature axons), they are more often free to diffuse and to interact with a motor-based active transport mechanism. With regards to the effects of both diffusion and active transport, either could have the effect of increasing the rate of slow axonal transport.

Axonal stretching

In the course of studies testing the Structural Hypothesis, several groups noted that axonal stretching occurred in rapidly growing *Xenopus* neurons cultured on laminin [73–75]. Nonetheless, because of the many reports that there is a stationary microtubule cytoskeleton in growing axons [35,36,76,77] axonal stretching has not been viewed as a slow axonal transport mechanism.

Our own skepticism over whether the axonal framework is stationary began with studies in which axonal elongation was induced experimentally by applied mechanical tension [78]. In the course of these experiments, we marked the membrane surface of axons with highly adhesive vinyl beads. In contrast to the observations of Bray [79], we found that in our experimentally elongated neurites the surface marker beads moved substantially with elongation and that the distance between markers increased. We suggested intercalated addition of membrane surface area [78] and agree with the interpretation of Chang [80] who suggested that the movements of the beads reflect stretching of the underlying cytoskeletal framework. Further, and also in contrast to the observations of Bray [79], we have seen on many occasions that branch points moved in a manner indicating axonal stretching (Fig. 2). That is, the distance between the cell body and branch point increases when the branch point moves, indicating either stretch or intercalated addition between the branch point and cell body.

In addition, recent evidence suggests that axonal stretching may occur in vivo and in rats [81]. Using standard orthopedic leg-lengthening procedures ('traction'), elongation of the adult rat femur by 1 mm/day for a distance of 70 mm (doubling of original bone length) caused a similar 70 mm lengthening of the (myelinated) sciatic nerve. Examination of the sciatic nerve showed a highly significant increase (doubling) in internodal length throughout the sciatic nerve accompanied by minimal nerve degeneration and without significant reduction of axon caliber [81]. This increase in internodal length suggests axons grow by stretching in vivo.

Further indirect evidence for axonal stretching comes from experiments that show that experimentally applied tension can lead to lengthening and growth of the axon on a much greater scale than demonstrated by earlier work of Bray [82]

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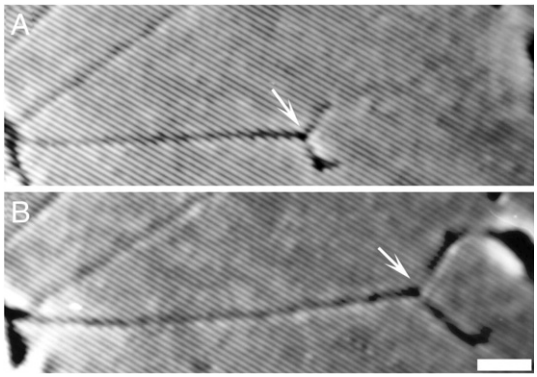

Fig. 2 – Branch points move during axonal elongation. A. Phase image of a branch point of a chick dorsal root ganglion neuron grown on plastic. B. The same axon 55 m later. Arrows point to a branch point that is seen to advance.

and Heidemann [83,84]. Embryonic rat sensory neurons were elongated using a specialized culture chamber in which two initially contiguous platforms are pulled apart by a stepper motor [85,86]. Dorsal root ganglia explants plated onto these platforms are initially connected by axons of about 100 μm but can be elongated to lengths of 10 cm at an astonishing sustained rate of 8 mm/day (330 μm/h). This is approximately ten times faster than the growth cone mediated rate and could be continued for many days. These axons are ultrastructurally normal, conduct action potentials normally and, more surprisingly, show a higher density of Na+ and K+ channels than spontaneously elongated axons [85]. Thus, the enormous scale of the axonal elongation and the normal structure and function of the elongated axons emphasize the role of tension in true growth of axons. But, as Pfister et al. discuss, their results also suggest that stretching of the axons occurs during axonal elongation.

Similarly, coherent movement and a velocity gradient for mitochondria 'docked' to the cytoplasmic matrix were recently reported by one of us [87]. In the proximal axon, we found mitochondria attached to the cytoskeletal framework were stationary relative to the substrate and fast axonal transport fully accounted for mitochondrial transport. In the distal axon, we found both fast mitochondrial transport and a coherent slow transport of the mitochondria docked to the axonal framework (low velocity transport — LVT). LVT was distinct from previously described transport processes: it was coupled with stretching of the axonal framework and, surprisingly, was independent of growth cone advance. Fast mitochondrial transport decreased and LVT increased in a proximo-distal gradient along the axon. These findings suggest that viscoelastic stretching of the axoplasm due to tension exerted by the growth cone, with or without advance, is seen as LVT.

Axonal stretching seems an important mechanism for simultaneously lengthening the axon and moving material away from the cell body. Given the observations that suggest bulk transport occurs in some cases, it seems worthwhile to further investigate axonal stretching as a possible mechanism that may contribute to the phenomenon of slow axonal transport during development. In mature axons that are not elongating, there is only a minor need for low velocity transport or stretching of the axon in order to preserve axonal tension. We suggest that after axons stop lengthening, the contribution of stretching to transport may be small. As the rate of axonal lengthening slows in adults, a decrease in axonal stretching could explain observations of age related slowing in the rate of axonal transport [8,38,88] (Fig. 1).

Diffusion and dispersion

We argue that any transport mechanism that can mediate the transport of non-membrane bound proteins in the axon should be considered as a contributor to slow axonal transport. Given our earlier discussion of the transport of 'soluble' protein, and the uncertainty of the size of the transported cargo, diffusion will make varying contributions to transport. Whatever the size, given the cell body as a source, because that is the site of protein synthesis, and the axon as a sink, because that is where protein degradation occurs, "Fick's Law" predicts that diffusion will lead to net transport. Further, we think it likely that the 'motorized' movement in the axoplasm aids true diffusion (due only to thermal energy) by 'dispersion.' As an analogy, auto traffic in a tunnel would dramatically increase the rate of transport of smoke from one end to the other compared to true diffusion because it would be dispersed. In a sense, dispersion is related to the 'Microstream Hypothesis" mentioned earlier. The movement of material on the 'microtubule railroad' will passively entrain material in the adjacent cytoplasm and aid its movement. A dichotomy in thought now exists with regard to the importance of diffusion. On one hand, Brown's group has devised a model for neurofilament transport that ignores diffusion [8]. On the other hand, Van Veen and Van Pelt [89] have proposed that 'diffusion' may be more effective than anticipated [90]. Studies on the passive movement of truly soluble markers in neurons, e.g. dextrans, have shown surprisingly rapid labeling by the markers and, in view of the tightly packed, cross-linked axoplasm, relatively low values for the viscosity of axoplasm [91]. Similarly, there are several more recent mathematical models that suggest that diffusion makes an important contribution to slow axonal transport [92]. We note that, once again, both may be correct depending on the cargo. We suggest diffusion/disperson could be an important mode of slow axonal transport that deserves further study.

Less is more: why decreasing the amount of transport along the axon is the solution to ensuring an adequate supply of axonal proteins

In the classic model of slow axonal transport, the rate of transport was assumed to be constant along the axon. One of the primary objections with this model is that the rate of slow axonal transport appears to be too slow to adequately supply long axons with proteins. This is serious problem that has never been adequately addressed. As noted earlier, neurofilament proteins would require 27 years moving at 0.1 mm/day to reach the end of the human sciatic nerve. Essentially no proteins would reach the end of the axon. Alvarez [28] correctly argued this point very strongly and proposed protein synthesis along the axon accounts for the predicted missing protein. While we agree that Alvarez's skepticism is justified, based on estimates that less than 1% of total protein is synthesized in the axon [26] and that protein translation is not required in the distal axon for

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elongation, it would seem that axonal protein synthesis cannot fully account for the predicted mass deficit [93].

Similarly, mathematical models of axonal elongation based on the need for distal transport invariably point out that growth or remodeling is strongly constrained by transport [28,94–96]). Yet experiments in culture involving either growth cone-mediated growth [97] or experimentally induced growth [86] clearly indicate that axonal elongation is not constrained to the expected degree.

Conversely, relatively short axons produce a different problem at a theoretical level. If proteins were transported down the axon at a constant rate with little or no degradation, when they reach the synapse they would need to all be degraded [98]. Yet there has been little experimental support for this. Instead, recent work using conditional expression of NF-L suggests that cytoskeletal proteins degrade along the length of axons and not just at the synapse [99].

We are suggesting that in normal axons the flux due to slow axonal transport decreases along the axon in a manner that allows axonal diameter to be constant along the axon and that minimizes unneeded protein degradation [88]. Specifically, the excess protein that would have been delivered to the synapse is instead deposited along the axon. Support for our model has come from the experimental observations of Xu et al. [38] and the idea of a decrease in slow axonal transport along the axon has recently been incorporated into a model proposed by Brown's group [8]. In addition, we point out that the transport profiles of slow axonal transport during and after axonal elongation are likely to be different. We agree that in cases where slow axonal transport declines more steeply than normally, thinning of the distal region of the axon and distal degeneration are expected to occur. Such effects are well documented both during aging [100] and in many neurodegenerative diseases.

How long axons, for example in giraffes, whales, and humans, are adequately supplied with proteins by slow axonal transport is unknown. While the rates of slow axonal transport are often quoted at between 0.1 and 1 mm/day for the transport of cytoskeletal polymers, a plausible suggestion is that in long axons the rates of transport could be much higher. In flatfish, which are asymmetrical, the rate of slow axonal transport is faster on the long side [101]. The same holds true in the two branches of the nerve projecting from the dorsal root ganglion [102]. Slow axonal transport in the peripheral branch is faster than in the central branch, which only extends a short distance to the spinal cord. In addition, a survey of the rates of axonal transport in the model organisms reveals that the rate of slow axonal transport scales with axonal length [88]. Nonetheless, increases in transport may not be sufficient. Alternatives such as increasing the half-life of proteins [88], import of proteins from non-neuronal cells, and local translation [28] are important alternatives that need to be further examined. Lively debate surrounded these topics decades ago. Given the technologies now available in live imaging, they should be revisited more systematically.

Conclusions

Our core argument is that slow axonal transport cannot be explained by a single mechanism. Through this paper we have cited examples in the context of the arguments posed above, but to give it more force we are restating it here as concisely as possible. Our argument can be broken into three parts: different modes of transport have been observed, the reported transport phenomena are too complex to be explained by a single model, and the existing models explain only limited aspects of slow axonal transport.

In brief, there is direct evidence for multiple types of transport: polymers are actively transported, soluble proteins are actively transported, all proteins undergo diffusion, and axons stretch.

Transport is too complex to be explained by a single mechanism for example: soluble proteins (SCb) move at a faster rate than proteins that form polymers (SCa), the velocity of slow axonal transport slows down during development, and retrograde transport is seen in some cases.

There are outstanding questions that can't be answered with existing models: how are very long axons supplied with proteins; how are hundreds of different types of proteins all actively transported; how is axonal diameter controlled, how is the rate of protein synthesis for hundreds of different proteins controlled, and where does protein degradation occur along the axon?

We suggest that a comprehensive model of slow axonal transport should include the known modes of transport, be able to explain complex transport phenomena, and should be able to provide compelling answers to the questions we have assembled from numerous groups.

We suggest that a multivariate model of slow axonal transport is required to explain this complex phenomenon: "Stop-and-Go" to explain how polymeric seeds are delivered to the axon; microtubule motor-based soluble transport to explain why soluble proteins move by slow axonal transport; diffusion because it transports soluble proteins; and LVT and towed growth to explain how axonal lengthening occurs.

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