The Pathophysiology of Axonal Transport in Alzheimer's Disease

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Abstract

Neurons communicate in the nervous system by carrying out information along the length of their axons to finally transmit it at the synapse. Proper function of axons and axon terminals relies on the transport of proteins, organelles, vesicles and other elements from the site of synthesis in the cell body. Conversely, neurotrophins secreted from axonal targets and other components at nerve terminals need to travel towards the cell body for clearance. Molecular motors, namely kinesins and dyneins, are responsible for the movement of these elements along cytoskeletal tracks. Given the challenging structure of neurons, axonal transport machinery plays a crucial role in maintaining neuronal viability and function, allowing the proper neurotransmitter release at the presynaptic ending. On this basis, failure of axonal transport has been proposed as a key player in the development and/or progression of neurodegenerative disorders such as Alzheimer's disease (AD). Increasing evidence suggests that amyloid- β peptide, a hallmark of (AD), may disrupt axonal transport and in so doing, contribute to AD pathophysiology. Here we discuss the molecular mechanisms of axonal transport with specific emphasis on the possible relationship between defective axonal transport and AD.

Keywords: Axon; Alzheimer's disease; Amyloid- β peptide; Fast axonal transport; Molecular motors;

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1. INTRODUCTION

Alan Hodgkin and Andrew Huxley demonstrated in 1939, using the squid giant axon as an experimental model, that signal carried out along the axon is mediated by the active generation of an electrical potential, termed the action potential [1,2]. Later it was understood that action potentials trigger the release of neurotransmitters by depolarizing the axonal end, which allows the docking and subsequent fusion of the neurotransmitter vesicles. However, most of the proteins and cellular components that are essential for synaptic function are synthesized in the soma and transported long distances to reach the synaptic terminals. In this context, axonal transport is critical to maintain neuronal function and synaptic activity [3–5]. In this review, we aim to summarize the current knowledge on axonal transport and function as well as their pathological alterations in Alzheimer's disease (AD).

2. AXONAL TRANSPORT

The movement of cargoes inside cells is carried out by molecular motor proteins that move along cytoskeletal polymer tracks. Among the molecular motors that are involved in intracellular transport, three large superfamilies have been identified: kinesins, dyneins and myosins [3,5]. These motors differ in the tracks with which they engage, thus dynein and kinesin motors move along microtubules whereas myosins move along microfilaments [6,7]. Because of their length and organization, it is generally assumed that microtubules are the tracks for long-range axial movement in axons whereas microfilaments are the tracks for short-range movements [8]. In the present review we focus in those implicated in long-range movement as they might contribute higher to the maintenance of axon ending function.

Microtubules, the main component in the cytoskeleton, are tubular, dynamic and polarized structures composed of a long chain of α - and β -tubulin heterodimers undergoing continuous polymerization and depolymerization. The faster growing plus-end of

microtubules point towards the axon terminal, while the slower growing minus-end point towards the soma. This polarity orientation, which is not seen in dendrites, has important implications for the directionality of axonal transport. Thus, kinesins move towards the plus ends and participate in anterograde transport, selectively transporting molecules from the cell body to axons. Retrograde transport is carried out mainly by cytoplasmic dyneins, which are minus-end motors that move molecules from the axonal terminals to the soma [8,9].

Axonal transport is divided into fast and slow transport, differentiated by the movement rates and the cargoes involved. Slow anterograde transport is responsible for the movement of cytoskeletal elements and cytoplasm proteins at slow rates, and has been proposed to be involved in neuronal morphogenesis [10]. Fast axonal transport (FAT) is bidirectional: anterograde transport moves synaptic vesicles and organelles to the axon terminal whereas retrograde transport delivers trophic factors and returns old constituents to the cell body [8–10]. Since FAT is responsible for delivery of synaptic materials which ensures synaptic activity and neurotrophic factors that enhance neuronal survival; it is considered the key transport for neuronal survival and proper function.

While some cargoes have a fixed destination, others need to be distributed according to their own rules: mitochondria must be present along the axon but also concentrate in axon terminal and growth cones [14]. Consequently, many neuronal mitochondria are in motion, traveling in both anterograde and retrograde directions in order to be distributed along the full length of the axon [11–13].

Once a synapse is established, new components must continue to arrive to replace proteins and organelles targeted for degradation; the balance between the arrival and departure of components may determine whether a synapse is strengthened, weakened, or in a steady state [15]. Thus, axonal transport is more than just a monotonous transport down or back up the axon and is essential for the correct maintenance and survival of neurons.

2.1. Anterograde transport

2.1.1. Kinesins

Biomolecules and components are transported anterogradely from the cell body to the axon terminal by members of the kinesin superfamily (KIFs; [16,17]), which comprises more than 40 proteins (Fig. 1). The general structural features of KIFs are defined by the structure of conventional kinesin, kinesin-I. Kinesin-I consists of two kinesin heavy chain (KHC) subunits and two kinesin light chains (KLC). The KHC comprises a globular motor domain, an α -helical coiled-coil stalk domain and a carboxyl C-terminal tail domain. The globular motor domain shows a high degree of homology among kinesin family members and contains a microtubule-binding sequence and an ATP-binding sequence [5]. However, outside the motor domain, each KIF has a unique sequence. The range of diverse tail domains may confer unique cargo binding and regulatory specificity upon each kinesin protein [4,5].

KIFs use the chemical energy of ATP to drive conformational changes that generate the motile force to transport cargoes along microtubules. Kinesins couple the binding, hydrolysis and release of ATP within their motor domains to a cycle of allosteric conformational changes that result in motion along the wall of the microtubule [8].

KIFs are classified into 14 kinesin families [18] that can be broadly grouped into three types, depending on the position of the motor domain in the molecule: N-kinesins have the motor domain in the amino-terminal region, M-kinesins have the motor domain in the middle and C-kinesins have the motor domain in the carboxy-terminal region. The position of the motor domain determines the directionality of the kinesin; accordingly, N-kinesins are directed towards the plus-end and C-kinesins towards the minus-end of microtubules. Most of the 14 families of kinesins are N-kinesins, the ones responsible for the anterograde transport [6].

2.1.2. Fast and slow axonal transport functions

FAT, which occurs at rates of approximately 200-400 mm/day [11,19], moves membrane bounded organelles (such as lysosomes or Golgi-derived vesicles), synaptic vesicle precursors (SVPs), active zone components in piccolo-bassoon transported vesicles (PTVs) [6,12] ion channels [20,21], molecules that are abundant in growth cones [22], neurotransmitters, neuropeptides [12], membrane lipids and mitochondria [12,23,24] down the axon.

The diversity of the cargo-binding domains among kinesins explains how KIFs can transport numerous different cargoes [5]. However, cytoplasmic proteins and neurofilament proteins are transported by slow axonal transport [12].

Slow axonal transport comprises two kinetic components: slow component A which travels at a rate of 0.2-1 mm/day and carries neurofilament and microtubules protein such as tubulin, and slow component B, which travels twice as fast as slow component A (2-8 mm/day) and moves proteins such as clathrin, actin, actin-binding proteins and cytosolic enzymes and proteins [11].

Recent evidences have shown that the so called “slow transport” is not really slow; indeed, its components are transported by fast motors perhaps similar or identical to the motors that move membranous organelles. But despite moving at fast rates, the average rate of movement is slow due to prolonged pauses between movements [8,25–27].

2.2. Retrograde transport

2.2.1. Dyneins

Dyneins are responsible for the minus end-directed retrograde transport that drives elements from the axon terminal to the cell body [28] (Fig. 2). Dyneins are a multi-subunit complex that consists of two catalytic dynein heavy chains (DHC) and several dynein light (DLC) and dynein intermediate chains (DIC) [9]. The DHC harbor the ATPase activity and

bind microtubules, whereas the other chains are involved in cargo binding and binding to dynactin [29]. Indeed, dynein interacts with its cargoes via an adapter complex called dynactin, which consists of the protein p150^{glued} (a subunit of the dynactin complex 1, DCTN1) and the p150 dynamitin (known as dynactin complex 2, DCTN2) along with many other subunits [9].

The composition of retrograde traffic, which moves at a rate of 150-300mm/day, includes mitochondria, some synaptic vesicle components and endosomal recycling vesicles (containing neurotrophic factors) [11]. Viruses (herpes simplex, rabies and polio) [30] and toxins (tetanus toxin) [31] also appear to take advantage of retrograde transport to move from distal sites to neuronal cell bodies and infect the central nervous system (CNS).

Whereas the cargo specificity of kinesin is thought to depend on tail diversity, dynein cargo interactions are thought to be determined by the diversity of dynein-associated polypeptides [28]. However, C- type kinesins, from the kinesin-14 family, have the same directionality as dyneins and thus could potentially also serve as retrograde motors in axons [8].

2.2.2. Retrograde transport functions

Axonal retrograde signaling has conventionally been thought to rely on retrograde transport of endosomes containing endocytosed neurotrophin-receptor complexes to neuronal cell bodies [32–34]. These endosomes are then responsible for evoking the changes in gene expression and metabolic activity required for survival and differentiation. This hypothesis, known as the “signaling endosome hypothesis” was first proposed by Mobley and colleagues [35] and has been further supported by mounting evidence indicating that both tyrosine receptor kinase (Trk) and their neurotrophin ligands undergo endocytosis and retrograde transport [36–39].

On the other hand, the retrograde axonal transport of proteins also allows the neurons to receive information about the external environment surrounding the nerve terminal. In many regions of the nervous system, signals produced by target cells and surrounding glia or in response to injury are received at axon terminals and then retrogradely propagated to the cell body where they regulate gene transcription and other cellular processes required for development and adult function [34]. This information allows neurons to respond to a number of extracellular signals in different ways, for instance, by either surviving or undergoing apoptosis, destabilizing or strengthening synaptic connections with targets, or inducing changes in neuronal phenotype. Other functions of retrograde axonal transport may include the removal of unwanted proteins from the nerve terminal region, and the retrieval of material which would otherwise be removed from the neuron [32].

3. AXONAL TRANSPORT DEFECTS IN AD

Axonal transport is essential for the proper function of neurons and thus, the dysfunction of any of the components involved, from the molecular motors themselves to a lack of energy for the ATP-dependent motors or alterations in the connection between motors and their cargos, has been implicated in various neurologic diseases. The identification of primary mutations in the axonal transport machinery has provided convincing evidence for the alteration of FAT as the cause for the pathology in several diseases including hereditary spastic paraplegia (HSP), Charcot-Marie-Tooth disease (CMT), spinal and bulbar muscular atrophy (SBMA; also known as Kennedy's disease) and Perry syndrome.

HSP comprises a heterogeneous inherited group of lower motor neuron diseases, characterized by progressive limb spasticity and weakness. Several forms of HSP are caused by mutations in genes related to intracellular trafficking. In particular, a missense mutation in the KIF5A gene, encoding for an anterograde-specific molecular motor, kinesin 5, was found

in a family with HSP type 10 [40]. Moreover, the pathogenic mutants of KIF5A produced dysfunction of anterograde transport *in vitro* [41]. Mutations in another kinesin, KIF1B, were identified as responsible for CMT disease type 2A, a common inherited peripheral neuropathy, characterized by motor and sensory deficits. Also, knock-out mice models of KIF1B developed progressive muscle weakness and symptoms similar to CMT type 2 [42]. One of the proteins involved in retrograde transport is mutated in SBMA, a lower motor neuron disease caused by a polyQ expansion in the androgen receptor (AR). The mutation was found in the p150 subunit of DCTN1 which is essential for the binding of dynein to cargo [43]. Other DCTN1 mutants cause a neurodegenerative disorder with parkinsonism and dementia, known as Perry syndrome [44] (Table 1).

In all the aforementioned neurodegenerative disorders, there are also other mutations and proteins that cause secondary dysfunction of FAT. For instance, the most common mutations involved in about 40% of the autosomal dominant forms of HSP are in the gene SPG4, which encodes spastin, an ATPase that regulates microtubule dynamics [45]. Knock-out mice for this gene show accumulation of organelles and cytoskeleton protein and progressive swelling of the axon, indicating an impairment in axonal transport [46]. Moreover, mutant spastin is able to disrupt FAT in the squid axoplasm [47]. Similarly, mutations in MFN2 and RAB7, which code for mitofusin-2 and Rab-7 protein respectively, have been identified in cases of CMT type 2. Mitofusin-2 is a mitochondrial GTPase involved in the transport of mitochondria along the axon. MFN2 mutants disrupt both anterograde and retrograde mitochondrial transport [48,49]. Rab-7 regulates endosomal and lysosomal retrograde transport [50,51]. Another example is SBMA, in which the polyQ AR expression causes the phosphorylation of KHCs by c-Jun NH2-terminal kinase (JNK) and prevents kinesin-I binding to microtubules [52].

When looking at the latter cases, the mechanisms by which FAT defects cause the development or progression of the disease are not well understood and the causative role of FAT is as yet inconclusive. However, in these pathologies, the presence of primary mutations in axonal transport elements has provided direct evidence that FAT defects are a key player in the development of the diseases. This has allowed for the extrapolation of this hypothesis to other neurodegenerative disorders in which primary mutations associated with axonal transport machinery have not been described and the role for FAT defects is not as clear. This is the case for such common neurodegenerative diseases as Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) or AD, in which the known proteins causing the disorder, huntingtin [53,54], α -synuclein [55], superoxide dismutase 1 (SOD1) [56] and A β [57] or tau [58] respectively, have been shown to affect in one way or another the axonal transport machinery [59,60]. For example, polyglutamine huntingtin causes tubulin deacetylation which in turn prevents the binding of the motor proteins to the microtubules [53,54], and SOD1 activates p38 and the subsequent phosphorylation of KLC inhibits its binding to the cargo [56] (Table 2).

We are particularly interested in elucidating the role of FAT dysfunction in the pathogenesis of AD. Thus this review will try to compile all the evidence so far in relation to this issue.

3.1 AD

AD is a neurodegenerative process that affects firstly the hippocampus and temporal cortex and then the whole brain as the disease progresses. It is characterized by the presence of two histopathological hallmarks: senile plaques and neurofibrillary tangles (NFT). Senile plaques are formed by insoluble aggregates of amyloid- β peptide (A β) [61,62], a peptide released by the cleavage of the amyloid precursor protein (A β PP) through the sequential

action of the β -secretase protein (beta-site A β PP cleaving enzyme 1; BACE1) and γ -secretase, a multi-protein complex in which presenilins (PS) are the catalytic subunits [63–65]. These aggregates are surrounded by dystrophic neurites bearing NFT, which consists of intracellular aggregates of the tau microtubule-associated protein in a hyperphosphorylated state [66].

Several lines of evidence suggest that disruption of axonal transport is a hallmark of many neurodegenerative diseases such as AD. A β PP, BACE1 and PS1, the major proteins involved in AD etiology and progression, undergo axonal transport, therefore they might be affected. Furthermore A β and tau seem to play an essential role in transport along the axons. Other data demonstrates that AD is also associated with unbalanced distribution and dysregulation of neurotrophins due to impaired axonal transport.

3.1.1 Axonal transport impairment and AD: primary dysfunction

Unlike other neurodegenerative diseases, there is no known specific mutation in the molecular machinery of axonal transport that results in AD. However, some evidence suggests that axonal transport defects might be the primary mechanism underlying the disease. Axonal transport defects may represent an early stage in AD pathogenesis as axonal swelling and reduced transport are observed before other AD hallmarks appear in human brains and in transgenic mouse models of AD [67]. Indeed, transgenic mice overexpressing AD-related proteins such as wild-type or mutant A β PP [54,55] or mutant PS1 [67,68] display axonal transport dysfunction. Familial AD (FAD) mutations in PS1 might contribute to neuronal degeneration by altering kinesin-based transport mechanisms and impairing FAT of axonal cargoes [69,70]. These axonal transport impairments are also observed in transgenic mice expressing human ApoE4, a gene associated with increased risk of AD [71]. Furthermore, a polymorphism in the KLC1 subunit of kinesin-1 was found to be significantly

associated with AD, though a larger scale study should be carried out to further confirm this [72].

On the other hand, since AD-related proteins, such as A Disintegrin And Metalloproteinase domain-containing protein 10 (ADAM10), A β PP, BACE1, PS1 and Tau, are transported down the axon by axonal transport machinery, failure of these proteins to reach their destination or their accumulation due to impaired transport could also lead to the development of the disease.

A β PP undergoes fast anterograde axonal transport [73] by an indirect interaction via JNK interacting protein 1 (JIP1) [74–76]. JIP1 binds to a conserved 11-aa motif located at the C-terminus of KLC [77]. Additionally, recent evidence shows that the scaffolding protein JIP1 is also responsible for coordinating A β PP bidirectional transport by switching between anterograde and retrograde motile complexes as knocking out its expression in neurons produces deficits in the anterograde and retrograde transport of A β PP. JIP 1 interacts with both KLC and p150 and its phosphorylation by JNK determines the directionality of A β PP transport [78]. Also, A β PP functions as a kinesin-I membrane receptor, mediating the axonal transport of BACE1 and PS1 [79]. In fact, A β PP, BACE1 and PS1 are thought to travel anterogradely within the same vesicle-like membrane compartment and processing of A β PP to amyloid- β by secretases can occur in this compartment transported by kinesin-I [79,80], though there is some controversy regarding this model [81,82]. A recent study showed actually that it is upon neuronal activity induction that BACE1 and A β PP vesicles merge and thus facilitate A β PP cleavage [83].

Tau protein also undergoes axonal transport, though it is thought to be much slower. In fact, the rate of anterograde transport of tau was in accordance with the reported rate for the slow component of axonal transport [84,85]. Nonetheless, other studies have shown that tau

move at rates consistent with fast transport, albeit infrequently [86]. This agrees with recent findings reporting that cytoskeletal proteins such as neurofilaments, vimentin or tubulin, which were previously thought to move in the slow component of axonal transport, are probably rapidly transported by fast motors including kinesin and dynein molecular motors [87–90]. The rapid movement coupled with frequent pauses combine for an overall slow rate of transport [8,26,27].

3.1.2 Secondary dysfunction of axonal transport in AD

3.1.2.1 A β oligomers inhibit anterograde axonal transport

Both monomeric and fibrillar A β [91,92] have been shown to disrupt axonal transport. However, since mounting evidence suggested soluble oligomers of A β (A β Os) to be the most neurotoxic species responsible for synapse failure and early AD pathogenesis, the role of A β Os in the disruption of FAT was assessed. A β Os inhibited FAT when applied to squid [57] or to murine hippocampal neurons [93]. Though evidence shows that A β might disrupt both anterograde and retrograde transports. However, most reports have shown that A β has a greater effect on the anterograde transport [92,94–96]. In this regard, it is plausible that a defect in anterograde-moving mitochondria or synaptic vesicles could lead to dysfunction of synaptic activity and neuronal degeneration, features of AD pathology. While it is clear that A β impairs FAT, the mechanisms by which A β affects transport remain elusive. Some studies suggested that A β exerts its inhibitory effects via actin polymerization and aggregation [91], but it has been further demonstrated that A β did not cause major alterations to the cytoskeleton nor to the microtubule network which remained intact [92,93]. Thus, a dysregulation of intracellular signaling cascades, instead of disintegration of cytoskeleton, might be the cause behind FAT disruption in AD.

It is important to consider that most reports have used micromolar concentrations of A β to show dramatic reductions of axonal transport. Thus, the use of high levels of A β could lead to non-specific toxicity that secondarily impairs axonal transport. However, it has been also possible to observe changes in axonal transport induced by low concentration of A β [91]. Tang et al. used small quantities of A β and still showed a small decrease in the velocity of some cargoes, suggesting that effects on axonal transport are not dependent on generic A β -related toxicity. Also, and unlike previous reports [91], they demonstrated that transport defects induced by A β are reversible upon washout of the oligomers [97].

3.1.2.1.1 Downstream effects of A β : kinases activity

Many pieces of evidence indicate that FAT disruption by A β is dependent on both N-methyl D-aspartate receptor (NMDAR) and glycogen synthase kinase-3 β (GSK3 β) since NMDAR antagonists and inhibition of GSK3 β prevented A β -mediated FAT disruption [92,93,97] (Fig. 3). Both NMDAR and GSK3 β are functionally related since during long-term potentiation (LTP) the activation of NMDA and PI3K/Akt phosphorylates and inactivates GSK3 β , thus preventing the induction of long-term depression (LTD). In turn, activation of the phosphatase PP1 during LTD causes dephosphorylation and subsequent activation of GSK3 β and inhibition of Akt [98]. A β Os have been shown to affect NMDAR function, inducing abnormal calcium influx, neuronal oxidative stress [99] and aberrant activation of kinases, including GSK3 β [100–102]. Although both drugs are thought to affect multiple pathways in AD, it is worth noting here that the NMDAR antagonist memantine is one of the few treatments approved for AD [103,104] and GSK3 β inhibitors are being studied as a possible therapeutic agent too [105–107].

A β Os appear to interfere with NMDAR and cause LTD-like stimuli, activating GSK3 β and thus disrupting axonal transport [93,98,108]. PS1 mutations also impair axonal transport

by increasing GSK3 β activity [69,70]. Presumably, according to the model that has been proposed, GSK3 β inhibits kinesin-I by phosphorylating KLC and causing cargo to dissociate from the motor complex [109]. Most studies are in agreement with this model and suggest that GSK3 β acts as a negative regulator of kinesin-I. For instance, an excess of GSK3 β reduces anterograde mitochondrial movement in neural cells [110], whereas inhibiting GSK3 β enhances anterograde mitochondrial transport in hippocampal neurons [111]. Furthermore, reducing GSK3 β levels enhances anterograde and retrograde transport of A β PP in *Drosophila melanogaster* [112].

Impairment of long-term potentiation by A β Os is also prevented by inhibitors of casein kinase-2 (CK2), suggesting a possible role of CK2 in axonal transport [57]. In fact, A β Os were able to stimulate CK2 kinase activity *in vitro* [113]. It has been demonstrated that the mode of FAT inhibition of both GSK3 β and CK2 is similar, involving the phosphorylation of KLC which promotes detachment of conventional kinesin from its transported cargoes, leading to a reduction of FAT [57,58,109]. However, unlike GSK3 β , which only affects anterograde FAT, overexpression of CK2 and A β Os inhibit both directions, suggesting that CK2 can affect dynein as well [57]. Another study demonstrated that the inhibition of FAT by polyQ-AR was dependent on the activation of JNK. Active JNK was able to phosphorylate KHC and inhibited kinesin-1 microtubule binding activity [114]. However, the mechanism of A β Os-mediated activation of JNK or whether A β Os can even directly activate the JNK pathway is unknown.

The alterations in the signaling pathways presented above fit with the fact that alterations or dysregulation in the activity of protein kinases and abnormal phosphorylation are key features of AD [109,115,116]. Indeed, GSK3 β can be activated by fibrillar forms of A β [117] and active GSK3 β is found in association with NFT in AD brains and is the main kinase thought to be involved in the hyperphosphorylation of tau [114]. Also, the kinase

activities of CK2 and JNK are elevated in AD [115,116]. Therefore, CK2, GSK3 β and JNK might contribute to the axonal transport failure observed in AD by phosphorylating kinesin and thus promoting a premature uploading of the cargoes at an incorrect segment of the axon.

3.1.2.1.2 Downstream effects of A β : mitochondrial trafficking

There is also controversy regarding the effect of A β on mitochondrial trafficking. Most studies indicate that A β severely disrupt mitochondrial transport [92,93,95,96], whereas others showed no effects on mitochondrial motility [97]. However, A β has been reported to impair mitochondrial morphology [119] and function, including the disruption of membrane properties, calcium homeostasis, oxidative phosphorylation and ROS production among others [120]. In particular, ROS produced in the mitochondria are thought to activate GSK3 β since the activity of this enzyme is inhibited by antioxidant treatment with melatonin [121]. Accordingly, reducing the production of mitochondrial superoxide by overexpression of superoxide dismutase 2 (SOD2) rescued the axonal transport deficits typically shown in Tg2576 mice [122]. On this basis, A β -induced mitochondrial dysfunction has been suggested to decrease axonal transport in neurons [123]. This is in accordance with the finding that the deficiency of mitochondrial ATPase paraplegin disrupts both anterograde and retrograde transport in a mouse model of HSP [124].

3.1.2.1.3 Role of increased A β production in axonal transport

There are some studies suggesting a causal relationship between failed axonal transport and A β generation and its aberrant accumulation. For instance, some studies have shown that A β PP processing might occur during its axonal transport and that the production of A β is stimulated by inhibition of axonal transport [79]. Accordingly, it has been shown that reduction of kinesin-1 promotes axonal defects, increases A β generation and enhances amyloid deposition in *Drosophila* and mice [67,125]. However, this view has been refuted by

some researchers who showed that A β PP and BACE1 move in different vesicles and so A β PP cleavage might not occur within moving vesicles [126]. Another recent study showed that A β PP and BACE1 vesicles merged upon neuronal stimulation suggesting that overproduction of A β may be dependent on increased neuronal activity, although this does not preclude an additional contribution by axonal transport deficits [83].

On the other hand, A β *per se* might directly induce impairments in axonal transport [91–95,97]. Whether aberrant A β production is the primary cause underlying axonal transport deficits or whether this aberrant A β generation is the result of impaired axonal transport is unclear. Moreover, subtle changes in axonal transport functionality due to old age could enhance A β generation and this in turn would induce further deterioration of axonal transport [67,127]. However, the etiological cause of this vicious cycle is as yet unclear.

3.1.2.2 Role of tau in anterograde axonal transport

Tau is a microtubule-associated protein involved in microtubule dynamics and maintenance [128]. Since axonal transport is highly dependent on microtubule tracks and tau aggregates play a key role in AD pathology, the effects of tau on microtubule-dependent FAT have been explored. Unlike with A β , the effect of tau on axonal transport is still inconclusive. Several studies have reported that tau perfusion in the squid axoplasm [58] or tau overexpression in cortical neurons, retinal ganglion cells, neuroblastoma cells and CHO cells [129,130] inhibited axonal transport. The observed impairment preferentially affected anterograde kinesin-dependent transport, whereas dynein-mediated movements were less affected [58,129–131]. As a result, mitochondria and other vesicles accumulated within the cell body and disappeared from the neurites [130]. Similarly, transgenic mice overexpressing the shortest human tau isoform in CNS neurons showed filamentous tau inclusions and

insoluble hyperphosphorylated tau, typical features of AD, as well as impaired FAT. Interestingly, treatment with microtubule-stabilizing drugs restored FAT [132].

A mechanism proposed for tau regulation of kinesin-based transport proposes a competition between tau and kinesin-1 for the kinesin-1 binding site on microtubules [129,130]. Indeed, tau constructs lacking the microtubule-binding repeats (MTBRs) did not show this effect [130]. In addition, tau could regulate fast axonal transport by alternative mechanisms. For example, tau filaments directly activate the phosphatase PP1, causing the dephosphorylation and activation of axonal GSK3 β . Activated GSK3 β phosphorylates KLCs, prompting dissociation of kinesin-1 and cargo [58,109]. Accordingly, GSK3 β has been reported to phosphorylate tau and subsequently increase its binding to kinesin-1 [133]. So, it is also possible that when tau is overexpressed it could sequester kinesin, preventing the molecular motor from binding to other cargoes and thus allowing the accumulation of neuronal components in the cell body. Indeed tau overexpression impairs mitochondrial transport but does not inhibit its own transport along axons [85,129].

On the other hand, Yuan et al. reported that the overexpression of the wild-type form of tau did not affect rates of transport along axons *in vivo* [134], suggesting that only overexpression at high levels or mutations that affect its phosphorylation state may play a role in axonal transport. This is consistent with another study in which high levels of monomeric tau did not affect either *in vitro* axonal transport or in squid axoplasm [114]. Whether tau affects axonal transport is as yet controversial, but recent studies showed that it somehow might play a role. Indeed, tau reduction has been reported to prevent A β -induced defects in axonal transport in transgenic mice [135,136] suggesting a connection between tau and A β effects.

3.1.2.3 Defects in retrograde neurotrophins signaling

Neurotrophins are a family of homodimeric and secreted proteins that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and neurotrophin 4/5 (NT4/5). Neurotrophins bind to two types of receptors – a high affinity Trk and a low-affinity pan-neurotrophin receptor (p75). Each Trk is preferentially activated by one or more NTs — TrkA by NGF, TrkB by BDNF and NT-4/5, and TrkC by NT-3 — and is responsible for mediating a wide range of cellular responses including within neurons cell survival, proliferation, fate of neural precursors, axon and dendrite growth and patterning. Trk receptors also regulate synaptic strength and plasticity [137–139]. Neurodegenerative diseases such as AD are linked to imbalanced distribution and dysregulation of neurotrophins [138,140,141]. Moreover BDNF has been demonstrated to protect neurons against A β neurotoxicity [142].

BDNF is considered a major regulator of synaptic plasticity and plays an essential role in learning and memory, neuronal survival and development [143] and has neuroprotective effects in rodent and primate models of AD [144]. It has been shown that mRNA and protein levels of BDNF are reduced in the hippocampus and neocortex of AD brains as well as the levels of its full-length receptor TrkB [141,145–147]. Moreover, polymorphisms in the BDNF gene have been associated with higher risk of AD, though there is still some controversy [148–152]. BDNF-TrkB signaling is based on axonal transport. BDNF activates TrkB in axon terminals and the formation of the ligand-receptor complex initiates their internalization into endosomes, which requires Ca²⁺ influx [153], and their retrograde transport along microtubules [123,154]. The movement of the BDNF-TrkB signaling endosome depends on TrkB activation since a kinase-dead receptor variant is neither phosphorylated in response to BDNF nor transported retrogradely. Also this retrograde transport depends on clathrin-mediated endocytosis, since its inhibition prevents internalization and retrograde transport of TrkB after BDNF treatment. Finally, disruption of dynein function reduces Trk trafficking

and thus abolishes the ability of BDNF to support survival of neurons, suggesting this transport is also dynein-dependent [155]. Impairment of anterograde transport is thought to be caused by A β -induced activation of GSK3 β which causes phosphorylation of kinesin and subsequent release of the cargoes [109]. Unlike with anterograde transport, it is not known the mechanism by which A β interacts with dynein.

However, what is most interesting is the reported ability of A β to impair this BDNF retrograde trafficking. In fact, it has been found that the retrograde transport rate of BDNF-GFP vesicles and the somatic accumulation of BDNF-GFP are significantly reduced in AD transgenic mouse neurons [156]. This finding is in agreement with a study in which A β PP overexpression disrupted NGF retrograde transport with a concomitant cholinergic neurodegeneration [68]. However, Cotman and colleagues also found that the retrograde transport deficit was reversed using γ -secretase inhibitors, suggesting that increased A β levels and not total A β PP was responsible for these transport deficits. Also A β Os alone were capable of impairing BDNF trafficking in wild-type neurons [156]. The differences between these results can be attributed to the use of different neurotrophins (BDNF vs. NGF), different neuronal types (cortical vs. basal forebrain cholinergic neurons) and/or different models (tg2576 neurons vs. Ts65Dn mice). In fact, Ts65Dn is a Down syndrome (DS) mouse model that carries an extra chromosome with triplicated copies of many other genes in addition to A β PP [157].

On the other hand, mitochondria are important for axonal transport of neurotrophic factors [158]. Since A β is known to impair mitochondrial function and trafficking, BDNF-TrkB signaling could be affected by a lack of energy supply for the retrograde axonal transport [123]. Another interesting link between neurotrophins and AD is the fact that the progression of tau pathology in AD follows the neuroanatomical track of the BDNF

retrograde transport pathway. In the adult brain, BDNF is mainly produced in the entorhinal cortex and traffics to the subiculum and CA1 fields and then to the basal forebrain and amygdale, which is the neuroanatomical track for the progression of neurofibrillary tangles in AD [159].

3.1.3 Synaptic dysfunction in AD as a consequence of FAT disruption

Synaptic loss, which occurs early in AD development, is considered the best pathological correlate of cognitive deficits [160,161]. While it is clear that A β can directly disrupt synapses, there are other indirect mechanisms through which A β could cause synapse degeneration. It is likely that synaptic dysfunction in AD may be caused by axonal transport defects. Indeed, axonal transport is essential to transport many synaptic elements needed for the proper functioning of the synapse. For instance, synapses are sites of high-energy demand and in need of a great number of mitochondria. Electron microscopy and immunohistochemistry showed mitochondria located near the presynaptic membrane and tethered to vesicle release sites [162]. Synaptic transmission requires mitochondrial ATP generation and control of Ca²⁺ for neurotransmitter exocytosis, vesicle recruitment and synaptic plasticity, among others. Mitochondria have been demonstrated to act as the main Ca²⁺ buffers at synapses, uptaking and releasing Ca²⁺ to regulate the cytosolic concentrations needed for normal neurotransmission [24,163–166]. Therefore, the impairment of mitochondrial supply to the synapses could lead to synaptic dysfunction and degeneration.

In line with this hypothesis, mutations of Dynamin-related protein 1 (Drp-1) in *Drosophila* that affect mitochondrial transport and distribution also resulted in defective synaptic transmission. These studies demonstrated synaptic depression and asynchronous release of neurotransmitters but only showed modest effects on Ca²⁺ levels, suggesting that calcium buffering in the absence of mitochondria is not the main cause of the physiological

defects in these mutants [166–169]. Thus, the other major defect in synapses lacking mitochondria might be related to ATP production. In accordance with this, Verstreken et al. found that giving ATP to neurons could partially rescue synaptic depression of drp1 mutants [170]. Moreover, Calkins et al. reported decreased mitochondrial numbers and motility in A β -treated mouse hippocampal neurons and suggested this impairment in axonal transport as a likely cause for the decrease in synaptophysin staining [95].

Besides mitochondria, SVPs and PTVs are crucial to the nerve terminal and its proper function. PTVs are the core components of the presynaptic cytomatrix at the active zone of neurotransmitter release, tethering proteins that mediate efficient synaptic vesicle clustering. Both PTVs and SVPs are transported anterogradely along axons and their arrival at and departure from an established synapse may determine whether this synapse is strengthened or weakened [171,172]. So, impaired axonal transport and the inability of those components to reach the synapse could lead to inappropriate synaptic function and ultimately, its degeneration [57,70]. Additionally, as discussed earlier, appropriate neurotrophic support depends on axonal transport and its disruption may cause defective neurotrophic function and ultimately lead to neurodegeneration [70].

In support of this hypothesis relating synapse degeneration and axonal transport, mutations in conventional kinesin and dynein that impair anterograde and retrograde transport respectively, showed degeneration of sensory and motor neurons [40,173,174]. Altogether, all the aforementioned data indicate that deficits in FAT could be underlying synapse loss seen in various neurodegenerative diseases.

4. CONCLUSION

Axonal transport is of the utmost importance for the proper maintenance and function of neurons. Its disruption has been proposed as a hallmark of several neurodegenerative

diseases. In particular, many pieces of experimental evidence are consistent with the fact that axonal transport defects could be the cause behind AD pathology. Key proteins to AD pathology such as A β and tau have been shown to disrupt transport machinery and thus deprive axonal terminals of the necessary elements for their function. This could subsequently lead to synaptic dysfunction and degeneration, both shown in AD. However, the precise role of such axonal transport defects in the onset of the disease remains to be elucidated. Also, whether the defective axonal transport is the primary cause leading to the disease or if it is just a consequence of AD pathology is still inconclusive. These questions will be the subject of future studies in the field.

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ABBREVIATIONS: AD, Alzheimer's disease; A β , amyloid- β peptide; A β Os, soluble oligomers of A β ; A β PP, amyloid β precursor protein; AR, androgen receptor; AT, axonal transport; BACE1, beta-site A β PP cleaving enzyme 1; BDNF, brain-derived neurotrophic factor; CK2, casein kinase 2; CMT, Charcot-Marie-Tooth disease; CNS, central nervous system; DCTN1, dynactin complex 1; DHC, dynein heavy chain; DIC, dynein intermediate

chain; DLC, dynein light chain; FAT, fast axonal transport; GSK3 β , glycogen synthase kinase 3 β ; HSP, hereditary spastic paraplegia; JIP1, JNK interacting protein 1; JNK, c-Jun NH2-terminal kinase; KHC, kinesin heavy chain; KIF, kinesin superfamily; KLC, kinesin light chain; LTD, long-term depression; LTP, long-term potentiation; NFT, neurofibrillary tangles; NMDA, N-methyl-D-aspartate; NMDAR, NMDA receptor; PS1, presenilin 1; PTV, piccolo-bassoon transported vehicle; SBMA, spinal and bulbar muscular atrophy; SVP, synaptic vesicle precursor; Trk, tyrosin receptor kinase

Table 1. Primary mutations of axonal transport elements

AT dysfunction	Disease	Mutated gene	Protein implicated	References
Anterograde axonal transport	HSP-10	KIF5A	Kinesin 5	40,41
	CMT2A1	KIF1BB	Kinesin 1	42
Retrograde axonal transport	SBMA	DCTN1	Dynactin	43
	Perry syndrome	DCTN1	Dynactin	44

Abbreviations. CMT: Charcot-Marie-Tooth disease, HSP: hereditary spastic paraplegia 10, SBMA: spinal and bulbar muscular atrophy

Table 2. Secondary dysfunction of axonal transport

AT dysfunction	Disease	Mutated gene	Protein implicated	References
Anterograde axonal transport	HSP4	SPG4	Spastin	45,46,47
	CMT2A2	MFN2	Mitofusin-2	48,49
	SBMA	AR	PolyQ AR	52
	Huntington's disease	-	PolyQ huntingtin	53,54
	Parkinson's disease	-	α -synuclein	55
	ALS	SOD1	Cu/Zn superoxide dismutase-1	56
	AD	-	A β , Tau	57,58,92-94,129,130
Retrograde axonal transport	HSP4	SPG4	Spastin	45,46,47
	CMT2B	RAB7	Rab-7	50,51
	Huntington's disease	-	PolyQ huntingtin	51,52
	ALS	SOD1	Cu/Zn superoxide dismutase-1	56
	AD	-	A β , Tau	57,58,92-94,129,130

Abbreviations. ALS: amyotrophic lateral sclerosis, HSP: hereditary spastic paraplegia 10, CMT: Charcot-Marie-Tooth disease, SBMA: spinal and bulbar muscular atrophy

FIGURES LEGENDS

Fig. 1. Anterograde axonal transport by the kinesin motor. Kinesin is composed of two kinesin heavy chain (KHC) (orange) and two kinesin light chain (KLC) (green). The KHC contains the motor domains (heads), the central coiled-coiled stalk and the tails that interact with the KLC subunits. The motor domain binds to microtubules and hydrolyzes ATP to propel cargoes along the microtubules. Cargoes are attached to the kinesin by the KLC (A). Microtubules in the axon have the plus-ends pointing towards the synaptic terminals, and provide the tracks for the kinesin. Kinesins transport mitochondria, vesicles containing A β PP, synaptic vesicle precursors (SVPs) and piccolo-bassoon transport vesicles (PTVs) towards the plus-ending of microtubules, that is, towards the nerve terminal (B).

Fig. 2. Retrograde axonal transport by the dynein motor. Dynein is a large protein complex composed of two dynein heavy chain (DHC) (orange) with the microtubule binding and ATPase domains, two dynein intermediate chain (DIC) (blue), two DLIC (yellow), several DLC subunits (Roadblock, LC8 and Tctex1) and the dynactin complex. Dynactin complex links the dynein motor to cargoes, and the largest dynactin subunit, p150Glued (purple), forms an elongated dimer that interacts with the DIC and binds to microtubules (A). Microtubules in the axon have the plus-ends pointing towards the synaptic terminals, and provide the tracks for the dynein. Dynein transports mitochondria and neurotrophins towards the minus-ends of microtubules, that is, towards the cell body (B).

Fig. 3. Signaling pathways involved in A β -dependent FAT disruption. A β Os are suggested to interfere with NMDA-R and cause LTD-like stimuli. In LTD, calcium enters through NMDA-R and activates PP2B, which dephosphorylates PP1 and activates it. PP1 activation leads to

the dephosphorylation and subsequent activation of GSK3 β . GSK3 β can then phosphorylate kinesin at the level of KLC and disrupt it from the cargoes. CK2 is also thought to phosphorylate KLC and disrupt axonal transport, but the mechanism through which A β OS cause CK2 activation are still unknown. Similarly, it is also unknown how A β Os produce the JNK activation that leads to phosphorylation of the KHC and disruption from microtubule binding.

FIGURE 1

FIGURE 2

FIGURE 3