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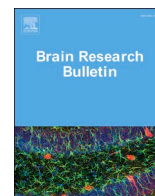


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Microtubule polarity flaws as a treatable driver of neurodegeneration

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ABSTRACT

Microtubule disruption is a common downstream mechanism leading to axonal degeneration in a number of neurological diseases. To date, most studies on this topic have focused on the loss of microtubule mass from the axon, as well as changes in the stability properties of the microtubules and/or their tubulin composition. Here we posit corruption of the normal pattern of microtubule polarity orientation as an underappreciated and yet treatable contributor to axonal degeneration. We include computational modeling to fortify the rigor of our considerations. Our simulations demonstrate that even a small deviation from the usual polarity pattern of axonal microtubules is detrimental to motor-based trafficking of organelles and other intracellular cargo. Additional modeling predicts that axons with such deviations will exhibit significantly reduced speed and reliability of organelle transport, and that localized clusters of wrongly oriented microtubules will result in traffic jams of accumulated organelles.

1. Introduction

Abundant evidence indicates that disruption of the microtubule array of the axon is at the heart of its degeneration during diseases of the nervous system and is likely a common downstream effector of many degenerative pathways (Matamoros and Baas, 2016; Falnikar and Baas, 2009; Huang et al., 2022). Various studies have suggested that these pathways result in a progressive loss of microtubule mass from the axon. In addition, changes occur in the stability properties of the microtubules as well as tubulin post-translational modifications that affect both the dynamics of the microtubules and their interaction with molecular motors and microtubule-severing proteins. As such, there is promise in potential therapies that restore these aspects of the microtubule array to a more normal status. Drugs that promote microtubule assembly and stabilization as well as drugs that affect tubulin acetylation are favored candidates (Soliman et al., 2022; Sferra et al., 2020; Brunden et al., 2017). While preclinical studies with such drugs have shown encouraging results, clinical trials have yet to yield similarly hopeful outcomes. Here we discuss an aspect of the axonal microtubule array that has received almost no attention in the neurodegeneration field that might be more consequential and treatable than those thus far considered. Specifically, we consider the polarity orientation of the microtubules.

Microtubules are intrinsically polar cytoskeletal polymers, with a

plus end favored for assembly and disassembly over a relatively quiescent minus end. The polarity of the microtubule arises from the fact that tubulin is a heterodimer, with beta tubulin exposed at the plus end of the microtubule and alpha tubulin exposed at the minus end. The polarity exists all along the length of the microtubule and manifests in the topography of the microtubule's surface, such that certain molecular motors move toward the plus end of the microtubule while other molecular motors move toward the minus end. In the axon, almost all microtubules have their plus end directed away from the cell body of the neuron, thus providing directionality to the anterograde and retrograde transport of various cargoes (Heidemann et al., 1981; Burton and Paige, 1981; Baas and Lin, 2011). There is tolerance for a small fraction of the microtubules to be of flipped orientation, but above that tolerance level, anterograde and retrograde transport become inefficient due to cargoes moving in the wrong direction. If patches of oppositely oriented microtubules arise in the axon, there can be organelle accumulations, called traffic jams, with escalating negative consequences for the axon (Baas and Mozgova, 2012; Shemesh et al., 2008; Shemesh and Spira, 2010).

At present, it remains unclear whether microtubule polarity flaws are a significant contributor to neurodegeneration. However, a plethora of studies in recent years aimed at ascertaining how axonal microtubules achieve their nearly uniform orientation have revealed that depletion or

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mutation of a growing list of different proteins results in microtubule polarity flaws in the axon (i.e. the appearance of abnormally high levels of flipped microtubules) (van Beuningen et al., 2015; Rao et al., 2017; Sánchez-Huertas et al., 2016; Klinman et al., 2017; Muralidharan et al., 2022; Ehaideb et al., 2014). Whether each of those proteins is critical for establishing the microtubule polarity pattern of the axon remains unclear, but what is clear is that the pattern, even once established, is highly vulnerable to corruption. That being the case, it seems reasonable to speculate that a wide variety of disease mechanisms could result in microtubule polarity flaws in the axon. Here we discuss this possibility, its potential contribution to neurodegeneration, and the potential benefits of therapeutics to correct microtubule polarity flaws that might occur.

2. History

Prior to the discovery of cytoplasmic microtubule-based motors, the prevalent view was that microtubules of both orientations probably exist in the axon to accommodate both anterograde and retrograde organelle transport. This was before enzymes theorized to move organelles (now known as molecular motors) were known to preferentially move toward one end of the microtubule or the other. The first work suggesting a uniformly plus-end-out pattern of microtubules in the axon utilized a method developed by Heidemann and McIntosh called hooking (Heidemann and McIntosh, 1980; Heidemann and Euteneuer, 1982). These scientists discovered that if microtubule assembly is allowed to occur in the presence of a rather unique microtubule assembly buffer, the tubulin subunits preferentially add along the sides of existing microtubules rather than at their ends. The newly added tubulin forms a protofilament sheet that curves around to close on the existing microtubule. If the reaction is halted early enough, by introducing a fixative, the curved sheet stops short of closing, and appears, if viewed in cross-section, as a hooked appendage on the microtubule. Using electron microscopy, looking down on the microtubule, a clockwise hook corresponds to the plus end of the microtubule, while a counterclockwise hook corresponds to the minus end of the microtubule (Fig. 1A). The technique was believed to have a small amount of noise (inaccuracy), because microtubule arrays of known orientation (emanating from a centrosome or

basal body) produced common curvature of hooking anywhere from 90% to 100%. After the initial work documenting the axon's microtubule polarity pattern (Heidemann et al., 1981; Burton and Paige, 1981), decades of studies ensued on mechanistically how axonal microtubules achieve this level of organization (Yau et al., 2016; Thyagarajan et al., 2022) despite being free at both ends rather than being attached to a structure such as a centrosome or basal body.

With greater understanding of molecular motors, it became apparent that the older idea of mixed orientations of microtubules in the axon would not have served as well because it would have rendered much of the motion of organelles futile (“going in circles”), given that cargoes associate with motors that move toward one end of the microtubule or the other and transition from one microtubule in the array to another. The subsequent discovery of mixed microtubule orientation in vertebrate dendrites (Yau et al., 2016; Baas et al., 1988; Baas et al., 1989; Burton, 1988) re-introduced that conundrum, although clearly the shorter length of dendrites does not demand as efficient transport as in the axon. Even so, recent studies have shown that microtubules of each orientation are spatially segregated in dendrites, thus creating something akin to two lane traffic (Tas et al., 2017). Thus, polarity flaws in the microtubule arrays of either the axon or the dendrite could theoretically render their transport mechanisms less efficient.

A very different technique, sometimes referred to as comets, emerged around 15 years ago that made the significant advance of allowing microtubule orientation to be visualized in living cells (Baas and Lin, 2011). This technique was based on the discovery of microtubule-interacting proteins called +tips, which are proteins that associate mainly with the plus ends of microtubules as they assemble. As the microtubule assembles, the +tip molecules lose association with the region of the microtubule that was formerly at the plus end, while continuing to associate with the newly assembling plus end. When ectopically expressed, fluorescent fusions of +tips appear in cells as comet-shaped fluorescence at the plus end of rapidly assembling microtubules (Fig. 1B). The greatest intensity of fluorescence is toward the plus end of the microtubule, while the comet tail (representing the gradual loss of +tip association with the microtubule) is directed toward the minus end of the microtubule.

In live cell imaging, the shape of the comet is sometimes ambiguous,

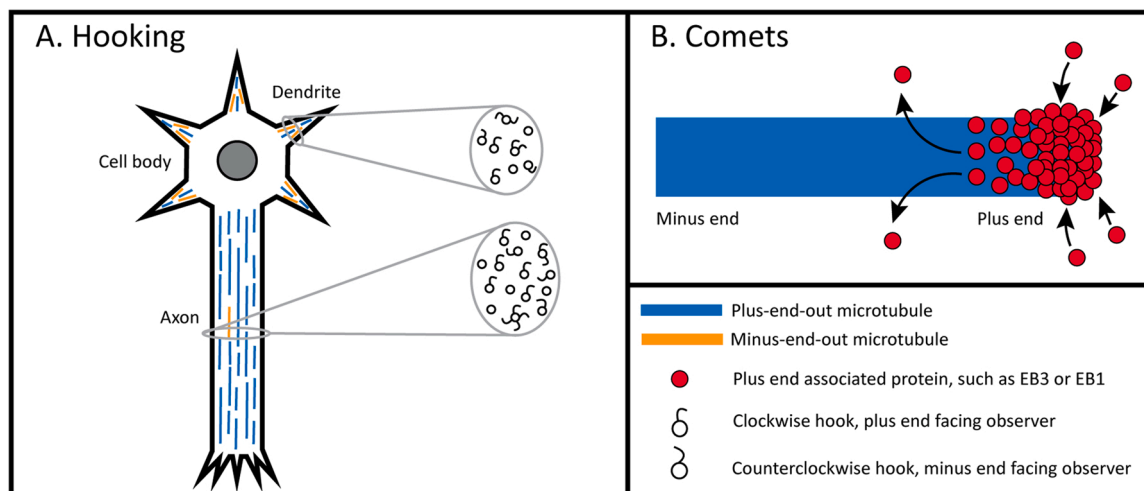


Fig. 1. Methods for determining microtubule polarity orientation in axons and dendrites. (A) Schematic of microtubule hooking technique. After extracting holes in the membrane to allow exogenous tubulin to enter, a special microtubule assembly buffer promotes the formation of lateral protofilament sheets on existing microtubules that appear as hooks when viewed in cross-section with electron microscopy. Clockwise hooks facing the observer indicate the plus end of the microtubule, while counterclockwise hooks indicate the minus end of the microtubule. Axonal microtubules display almost all clockwise hooks while dendritic microtubules (in vertebrate neurons) display roughly equal numbers of clockwise and counterclockwise hooks. (B) Schematic of EB comet technique. Microtubule plus-end-tracking proteins (+tips), such as end-binding protein 3 (EB3) or end-binding protein 1 (EB1) associate with the plus end of the microtubule during bouts of assembly. Ectopic expression in cells of an EB fused to a fluorescent protein results in a comet-like appearance of fluorescence at the plus end of the microtubule, and this reveals the orientation of the microtubule.

but is usually not needed to reveal microtubule polarity orientation, because the direction of the assembly is sufficient to reveal which end is the plus end. The theoretical exception would be a microtubule moving with minus-end-leading while assembling from the plus end, but such a scenario is likely rare and would require the movement to be notably faster than the assembly to give a deceptive appearance regarding microtubule polarity orientation. A more significant limitation of this technique is that it only reveals the polarity orientation of microtubules that are in the process of undergoing rapid bouts of assembly, because there would be no comets at the tips of microtubules undergoing disassembly or remaining the same length during the time period of observation. A great advantage of this technique is that the same sample can be analyzed at different stages of development, age, or disease progression.

Data from the comet and hooking methods generally align well. Interestingly, the comet method unequivocally revealed a very small fraction of minus-end-out microtubules in axons, thus demonstrating that the small number of counterclockwise hooks observed in the hooking studies on the axon were probably not noise in the technique after all. Additional mechanistic studies suggested that flipped microtubules arise in the axon for various reasons, for example during branch formation and other plastic events in which a great deal of microtubule severing occurs, but also that mechanisms exist in the axon to clear out the flipped microtubules (Qiang et al., 2010). In particular, a process called microtubule polarity sorting appears to be the principal means for keeping down the numbers of flipped microtubules in the axon (Rao and Baas, 2018; Craig et al., 2017; Rao et al., 2017; Del Castillo et al., 2020). In this motor-driven process, microtubules are transported within the axon with their plus end leading, such that plus-end-out microtubules move anterogradely to populate the axon while minus-end-out microtubules move retrogradely back into the cell body. While other mechanisms exist to ensure that new microtubules in the axon are plus-end-out, the polarity-sorting mechanism is the only mechanism thus far identified that can account for removing minus-end-out microtubules from the axon.

Through the efforts of various laboratories using different experimental models, it has now been demonstrated that disruption of cytoplasmic dynein, various dynein-related proteins, KIFC1, TRIM46, augmin or prickle1 result in microtubule polarity flaws in the axon (van Beuningen et al., 2015; Rao et al., 2017; Sánchez-Huertas et al., 2016; Klinman et al., 2017; Muralidharan et al., 2022; Ehaideb et al., 2014).

The list is growing and probably also includes a variety of static cross-linkers of microtubules that tamp down aberrant movements of microtubules that would otherwise disrupt the normal polarity-sorting process. The important message from this work is that preserving the nearly uniform microtubule polarity pattern of the axon is ongoing work for the neuron, throughout its life, and is surprisingly vulnerable to corruption. The same is probably true of dendrites, given that their microtubules are not randomly organized but have a high degree of spatial organization within their polarity pattern.

3. Implications of pathological corruption of the axonal microtubule polarity pattern

The nearly uniform microtubule polarity pattern of the axon is important for many axonal properties and duties, but chief among them is the regulation of axonal transport (Fig. 2). There is ongoing transport of different classes of cargoes, mainly membrane-bound organelles but other cargoes as well, some of which move anterogradely down the axon and others of which move retrogradely. Retrograde transport, for example, is critical for autophagy (Stavoe and Holzbaur, 2019). Fig. 2A shows orderly transport of anterograde and retrograde cargoes in a healthy axon, with that orderliness corrupted in a diseased axon, shown in Fig. 2B, containing abnormally high levels of flipped microtubules. We posit that this can lead to the axon pulling away from its synapse and retracting in a common neuropathological phenomenon known as “dying back” of the axon (Fig. 2B) and the loss of synapses (Fig. 2C and D).

The efficiency of axonal transport diminishes with aging but so too with various disease mechanisms. For example, when tau forms pathological filaments, the “PAD” domain is constitutively revealed, resulting in the aberrant phosphorylation-based inhibition of molecular motors crucial for axonal transport (Combs and Kanaan, 2017). This occurs via increased activity of the kinase GSK3Beta. In *SPG4* Hereditary Spastic Paraplegia, mutant forms of spastin can elicit similar effects by aberrantly activating casein-kinase-2 (Leo et al., 2017). In addition, microtubules become less acetylated in various neurodegenerative disorders, in part because of increased HDAC6 activity, and this would make the microtubules less effective at interacting with certain motors including Kinesin-1, the principal motor for anterograde organelle transport (Yates et al., 2021; Rao et al., 2017; Benoy et al., 2017; Kumar et al., 2022; Reed et al., 2006). But what if there is an “elephant in the room”

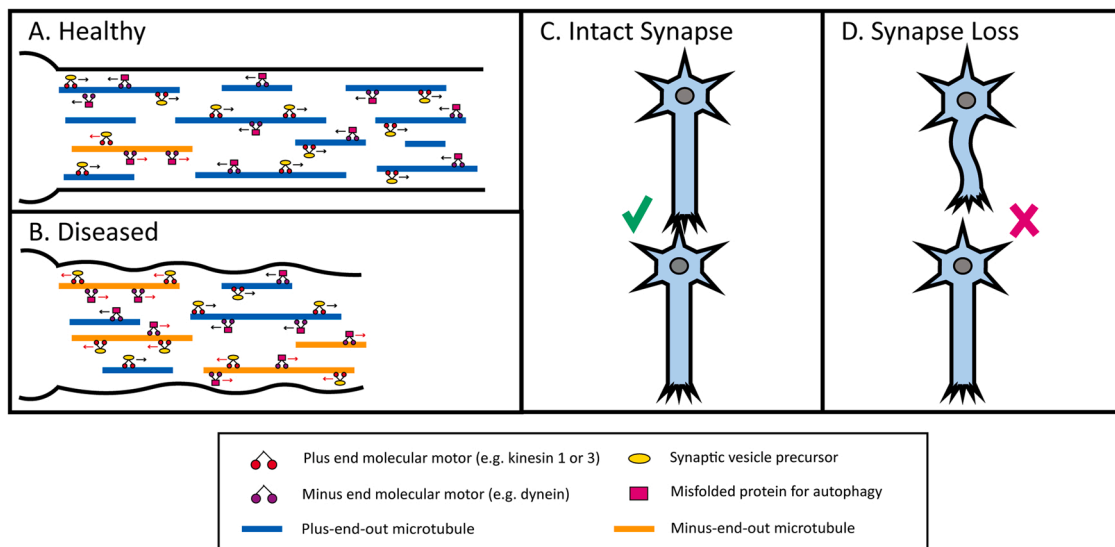


Fig. 2. High levels of flipped microtubules (i.e. incorrectly oriented microtubules, referred to here as polarity flaws) in diseased axons can account for aberrant axonal transport of various cargoes, leading to dieback of the axon and synapse loss. (A) Schematic of anterograde and retrograde traffic in the axon of a healthy neuron. (B) Schematic of aberrant anterograde and retrograde traffic in a diseased axon. (C) Intact synapse. (D) Synapse loss.

that has escaped notice, even after decades of study on neurodegenerative diseases? What if neurodegenerative mechanisms induce microtubule polarity flaws in axons (and perhaps dendrites) that significantly impair their normal functionality?

It is already known from studies such as the ones just mentioned that molecular motors are affected by disease mechanisms, and it is also known that molecular motors fuel the microtubule polarity-sorting process that rids the axon of wrongly oriented microtubules (Rao and Baas, 2018; Craig et al., 2017; Rao et al., 2017; Del Castillo et al., 2020). In addition though, researchers have almost certainly only scratched the surface of uncovering myriad proteins and pathways whose dysregulation can cause microtubule polarity flaws.

3.1. Computational modeling reveals potential repercussions of microtubule polarity flaws

To investigate the impact of microtubule polarity flaws on organelle trafficking, we turned to mathematical modeling. First, we considered an idealized model system in which molecular motors such as Kinesin-1 move toward the plus-ends of microtubules at an average velocity, v_0 . If some microtubules are oriented with minus-end-out, then for every microtubule that has this flipped orientation, not only does the system lose one of its railways for correctly directed axonal transport, but it also gains a railway sending cargo in the wrong direction. Expressing this idea mathematically, if a fraction p_+ of microtubules is oriented with plus-end-out, and molecular motors switch frequently between microtubules, the average velocity is given by a weighted average:

$$v = p_+v_0 - (1 - p_+)v_0 = (2p_+ - 1)v_0 \quad (1)$$

This means, for instance, that 10% of microtubules flipped would produce a 20% reduction in average cargo transport speed relative to a uniform plus-end-out polarity pattern, 20% of microtubules flipped yields a 40% reduction in average cargo transport speed, and so on.

In addition to reducing the average speed of cargo transport, mixed microtubule polarity patterns introduce randomness to cargo transport that significantly reduces the likelihood for motor-based transport to deliver cargo to a destination in a predictable time frame. To illustrate this point, we extended our theoretical considerations to develop the following stochastic model for one-dimensional motor-based cargo transport in an axon of length L_{axon} : We assumed that a fraction, p_+ , of axonal microtubules have a plus-end-out polarity orientation, and that this polarity distribution is uniform along the length of the axon. We considered an ensemble of molecular motors, each initialized at the proximal end of an axon with an initial probability (p_+) of being in contact with a plus-end-out microtubule and probability ($1 - p_+$) of being in contact with a minus-end-out microtubule. To simulate the subsequent motion of molecular motors, we assumed that each molecular motor travels toward the plus end of a microtubule with velocity v_0 and may dissociate from a microtubule at an average rate, k_{off} , which encompasses detachment due to mistiming of its processive stepping cycle or detachment arising because the motor reached the end of a microtubule. After a motor dissociation event, we assumed that the motor reattaches to another nearby microtubule in the array, with a probability, p_+ , to attach to a plus-end-out microtubule and resume walking in the anterograde direction, and a probability, $1 - p_+$, to attach to a minus-end-out microtubule and begin traveling toward the neuronal cell body. Note that we approximate the reattachment process as instantaneous, motivated by the high density of microtubules in the axon. If a simulated molecular motor moves along a minus-end-out microtubule to the proximal end of the axon ($x = 0$, in our coordinate system), the molecular motor remains at this position until a stochastic event in which it reattaches to a plus-end-out microtubule and begins moving in the opposite direction. If a molecular motor reaches the distal end of the axon ($x = L_{axon}$), the simulation is stopped for this molecular motor, because our goal is to simulate the time scale associated with

molecular motors reaching the distal end of the axon.

This coarse-grained description of molecular motor movement within a microtubule array is a type of biased random walk model (Berg, 1934), designed to illustrate how the microtubule polarity pattern governs the speed and reliability of organelle delivery. Sample simulated trajectories (Fig. 3A) show individual molecular motors switching directions stochastically, corresponding to events in which a motor dissociates from its microtubule “track” and attaches to a different microtubule. The model has four tunable parameters: the average molecular motor velocity, v_0 , the axon length, L_{axon} , the fraction of microtubules with plus-end-out, p_+ , and the rate at which molecular motors dissociate from a microtubule and “switch tracks”, k_{off} . In this study, our objective is to investigate the effect of polarity flaws on normalized time scales and velocities for cargo transport, relative to a characteristic time scale $\tau = \frac{L_{axon}}{v_0}$ and a characteristic velocity v_0 , allowing us to focus our investigation on two functionally significant tunable parameters: p_+ and k_{off} .

To investigate the impact of microtubule polarity flaws on cargo trafficking, we focused on the following quantitative outputs of the model: (1) Mean first passage time (MFPT): In biological processes, a mean first passage time is defined as an average timescale for a stochastic event to first occur (Polizzi et al., 2016). Here, we were interested in the MFPT for molecular motors to arrive at the distal end of the axon ($x = L_{axon}$). By simulating the movement of an ensemble of motors, recording the arrival time for each one and calculating the mean of these arrival times, we determined the MFPT for the ensemble. The dimensionless quantity, $\frac{MFPT}{\tau}$, provides a measure of the impact of microtubule polarity pattern flaws on organelle transport time. (2) Average velocity: The inverse of the normalized mean first passage time yields a normalized average velocity for molecular motors traversing the axon: $(\frac{MFPT}{\tau})^{-1} = (\frac{L_{axon}}{MFPT}) \cdot \frac{1}{v_0} = \frac{v}{v_0}$. (3) Fraction of molecular motors traversing axon in $\Delta t < 2\tau$. As a measure of the reliability of motor-based transport in delivering organelles to the axonal terminal, we choose a time frame, 2τ , which is twice the expected time for cargo delivery in a microtubule array with uniform plus-end-out polarity. To determine whether organelle transport can reliably occur within a flawed polarity pattern on a comparable time frame as expected for uniform polarity, we record what fraction of molecular motors reach the axonal terminal in $\Delta t < 2\tau$.

Simulations of $MFPT/\tau$ as a function of microtubule plus-end-out probability, p_+ , demonstrate that flaws in the axon’s uniform microtubule polarity pattern substantially increase the time required for a molecular motor to travel the length of the axon (Fig. 3B). When more than ~20% of microtubules are oriented with minus-end-out, the predicted time frame for cargo transport is also sensitive to the motor dissociation constant k_{off} . We varied k_{off} over several orders of magnitude, relative to a characteristic frequency, $\tau^{-1} = \frac{v_0}{L_{axon}}$. Motors with high processivity (small $\frac{k_{off}}{\tau^{-1}}$) are likely to traverse much of the axon before experiencing a dissociation event, which allows motors initialized on plus-end-out microtubules to reach their destination quickly. For this reason, the increase in $MFPT/\tau$ in the presence of polarity flaws ($p_+ < 1$) is less pronounced for small $\frac{k_{off}}{\tau^{-1}}$ (Fig. 3B, blue data markers). For less processive motors (larger $\frac{k_{off}}{\tau^{-1}}$), polarity flaws substantially increase the $MFPT/\tau$, because the motors undergo frequent directional changes (Fig. 3B, red, yellow, and purple markers). The corresponding average velocity for molecular motors traversing the axon is reduced in the presence of polarity flaws (Fig. 3C). For high motor dissociation frequencies ($\frac{k_{off}}{\tau^{-1}}$ on the order of 10^2 or higher), the average velocity increases linearly as a function of p_+ , as predicted analytically in Eq. 1 (Fig. 3C, yellow and purple data markers).

Considering the theoretical limits of the model, we can reason that $k_{off} = 0$ (motors never switch tracks) means that molecular motors initialized on a plus-end-out microtubule will traverse the axon in time τ , while motors initialized on a minus-end-out microtubule will move to

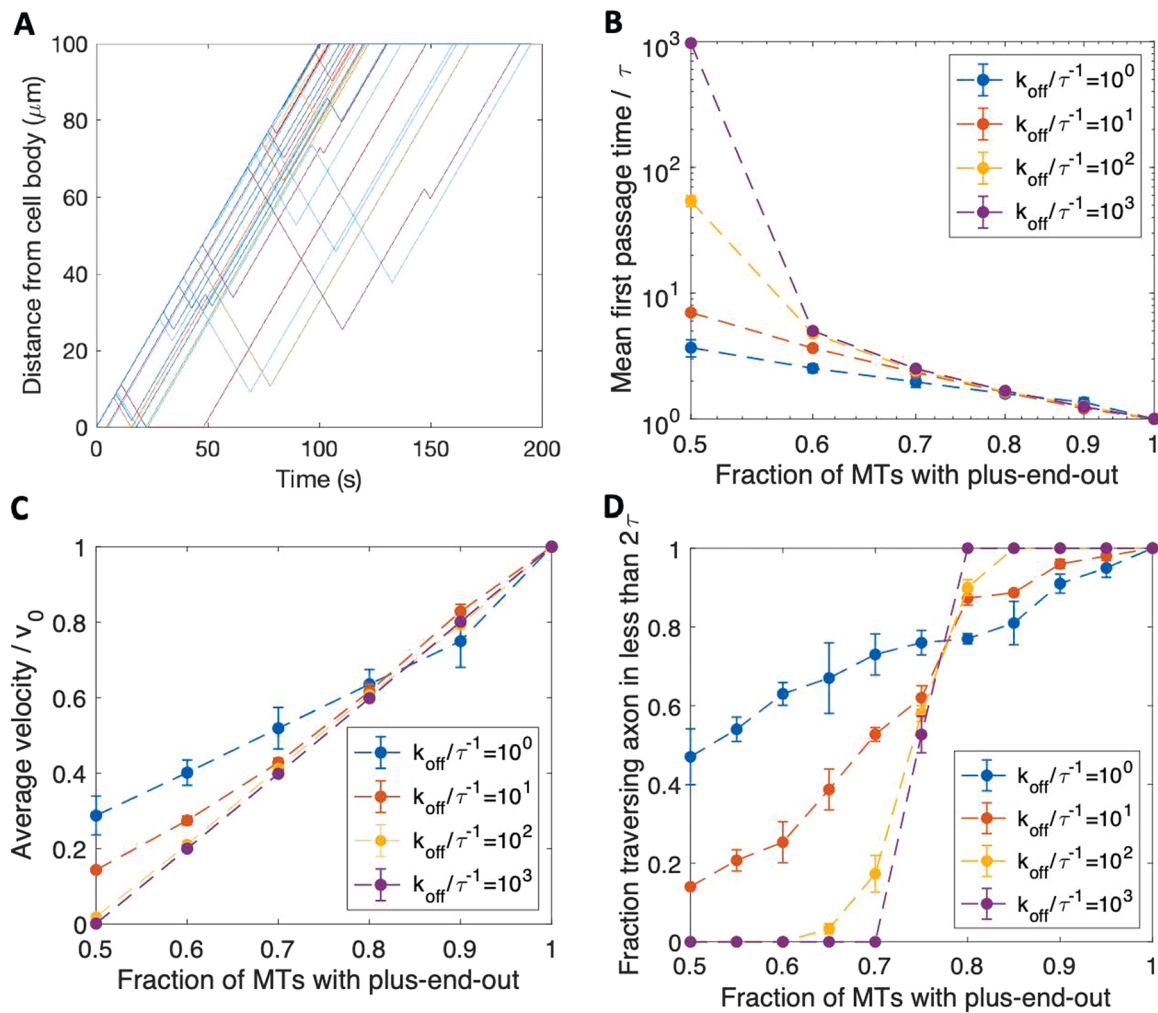


Fig. 3. Simulations demonstrate that microtubule polarity flaws disrupt organelle traffic. (A) Sample trajectories of molecular motors starting at the cell body for an axon with 5% of microtubules oriented with minus-end-out. Parameters: $p_+ = 0.95$, $k_{off} = 0.1s^{-1}$, $v_0 = 1\mu m/s$, $L_{axon} = 100\mu m$. (B) Simulated mean first passage time for molecular motors to reach the axonal terminal, $\frac{MFPT}{\tau}$, as a function of p_+ , for several values of the normalized motor dissociation rate, $\frac{k_{off}}{\tau^{-1}}$. (C) Average velocity of organelle transport, $\frac{v}{v_0}$, as a function of p_+ , for several values of $\frac{k_{off}}{\tau^{-1}}$. (D) The fraction of molecular motors that reach the axonal terminal in a time $\Delta t < 2\tau$ as a function of p_+ , for several values of $\frac{k_{off}}{\tau^{-1}}$. Each data point in panels B, C, and D represents the average of $N = 150$ independent molecular motor simulations, and the error bars represent standard error. In the figure, MTs = microtubules.

the cell body and never reach the axonal terminal. In the opposite limit ($\frac{k_{off}}{\tau^{-1}} \gg 1$), molecular motors in a mixed microtubule polarity pattern ($p_+ = 0.5$) undergo unbiased random walks, and the mean first passage time is proportional to L_{axon}^2 (Berg (1934)), suggesting that polarity flaws will be more disruptive to organelle transport in longer axons. To estimate a biologically reasonable order of magnitude for $\frac{k_{off}}{\tau^{-1}}$, we note that the movement of the kinesin motor KIF1A in neuronal axons has been reported at an average velocity of $1\mu m/s$ for an average duration on the order of 10 s (Lee et al., 2003). This suggests a typical dissociation rate of $k_{off} = 0.1s^{-1}$. For an example axon of length $L_{axon} = 100\mu m$, and corresponding characteristic time $\tau = \frac{100\mu m}{1\mu m/s} = 100s$, the normalized dissociation frequency is $\frac{k_{off}}{\tau^{-1}} = 10$. For this biologically realistic estimate of $\frac{k_{off}}{\tau^{-1}}$, reducing the plus-end-out percentage from $p_+ = 1.0$ to $p_+ = 0.5$ leads to an almost tenfold increase in the MFPT (Fig. 3B, red data markers) and a more than 80% reduction in the average velocity (Fig. 3C, red data markers).

The efficiency of organelle delivery to the axonal terminal, illustrated by the fraction of motors arriving at the axon terminal in $\Delta t < 2\tau$, drops off significantly as the fraction of minus-end-out microtubules in

the axon is increased (Fig. 3D). For highly processive motors ($\frac{k_{off}}{\tau^{-1}} = 1$) (Fig. 3D, blue data markers), the fraction of molecular motors that arrive at the axonal terminal in $\Delta t < 2\tau$ is proportional to p_+ , because molecular motors initiated on a plus-end-out microtubule are likely to move distally across the length of the axon without changing directions. For higher $\frac{k_{off}}{\tau^{-1}}$, there is a step-like dependence on the polarity pattern (Fig. 3D, yellow and purple data points), suggesting that reliable delivery of organelles can be sustained for small polarity pattern flaws ($0.8 < p_+ < 1$), while the efficiency of organelle delivery is more severely impacted if more than 20% of microtubules have minus-end-out polarity ($p_+ < 0.8$). For a typical motor dissociation rate on the order of $\frac{k_{off}}{\tau^{-1}} = 10$, small polarity flaws lead to mild impairments in the reliability of cargo delivery, while a mixed microtubule polarity pattern ($p_+ = 0.5$) exhibits an approximately 80% reduction in molecular motor arrival compared to the expectation for a uniform microtubule polarity pattern (Fig. 3D, red data figures).

Another means by which microtubule polarity flaws might be even more detrimental would be if they appeared in clusters. For example, the axon could still have mostly plus-end-out microtubules, but discrete patches of minus-end-out microtubules would create local traffic jams

that would impede the transport of organelles and create pileups (Fig. 4). To illustrate this concept, we considered the following modification of our coarse-grain cargo transport model: We assumed that a $10\mu\text{m}$ region in the central axon has a completely mixed polarity pattern with 50% of microtubules oriented plus-end-out and 50% of microtubules oriented minus-end-out. Outside of this discrete cluster of mixed polarity, we set $p_+ = 1$ to simulate the case where the axon maintains a uniform plus-end-out polarity pattern along much of its length, except for a cluster of aberrantly oriented microtubules in one region. Sample trajectories illustrate that some fraction of molecular motors become temporarily stalled in the central region of the axon, where they alternate between anterograde and retrograde transport (Fig. 5A, B), thus reducing the average velocity associated with motors crossing the axon (Fig. 5C). We note that the impact of the localized cluster of mixed polarity microtubules is highly sensitive to the motor processivity, with high k_{off} associated with smaller average velocities (Fig. 5C). This is because highly processive motors (small $\frac{k_{\text{off}}}{v}$) are likely to enter the region of mixed polarity and change directions a small number of times before emerging from this region and continuing along the axon (Fig. 5A), whereas motors with lower processivity (large $\frac{k_{\text{off}}}{v}$) enter a region of mixed polarity and become effectively “trapped” for a longer time as they undergo frequent reversals of direction (Fig. 5B).

A central mechanistic take-away point from this model is that even small flaws in the typically uniform plus-end-out microtubule polarity pattern in axons are detrimental to the speed and reliability of motor-based organelle transport. Highly processive motor-based transport is less susceptible to the detrimental effects of microtubule polarity flaws, and therefore we may expect that a reduction in either the motor processivity or the average length of microtubules in the axon will be associated with a higher likelihood of pathology associated with microtubule polarity pattern flaws. We also note that the time scale for a molecular motor to traverse the length of the axon through processive transport is proportional to L_{axon} while the time scale for purely diffusive transport over this distance scales with L_{axon}^2 , suggesting that polarity flaws will produce more significant impairment in longer axons than in short axons (or dendrites).

3.2. Pathology and potential treatment strategies

Ascertaining the contribution of microtubule polarity flaws to various neurodegenerative diseases will not be an easy task for

researchers, given that conducting the work on post-mortem human tissue will be difficult, if not impossible, with the methods described here. There are other methods, such as high-resolution electron microscopy, that can distinguish microtubule orientation on the appearance of the ends of the microtubules or the skew of the microtubule lattice (Foster et al., 2022). Second Harmonic Microscopy can reveal whether a microtubule array is uniform or mixed in orientation (Baas and Lin, 2011), but whether it can quantify the level of corruption of a normally uniform microtubule array remains unclear. Animal and cell culture models (such as human brain organoids) are likely the best approach but obviously do not undergo decades of degeneration as with human patients. The strength of such models, however, is that therapeutic strategies can be readily tested and if safe and effective, can be translated to the clinic.

How to correct or prevent the microtubule polarity flaws is perhaps less challenging, given the booming interest in the mechanisms underlying how microtubule polarity patterns are established and maintained in axons and dendrites. Because mechanisms of microtubule polarity sorting are ongoing throughout the life of the neuron, ridding the axon of flipped microtubules may be as straightforward as improving the efficiency of that machinery. For example, Kinesin-5 is known to act as a “brake” on microtubule movements in the axon, and there are many anti-Kinesin-5 drugs already available, generated for cancer chemotherapy. Such drugs, when applied to neurons, consistently improve their vitality, causing axons to grow faster (Myers and Baas, 2007; Haque et al., 2004), regenerate better (Lin et al., 2011; Xu et al., 2015), and overcome toxicity (Bobylev et al., 2017). Perhaps these positive effects are attributable, at least in part, to enhancement of the machinery of microtubule polarity sorting. The fact that axons typically have a small percentage of flipped microtubules (that are in the process of being cleared) suggests that enhancing the clearance of those flipped microtubules (even if there is not a pathological increase in their number) might also boost the vitality of an axon undergoing challenges such as regeneration after injury.

3.3. Summary and conclusions

Given the vulnerability of the axon and probably the dendrites as well to microtubule polarity flaws arising and accumulating, we speculate that this mechanism contributes significantly to degeneration of the nervous system in diseases such as Hereditary Spastic Paraplegia, Amyotrophic Lateral Sclerosis and tauopathies such as Alzheimer’s

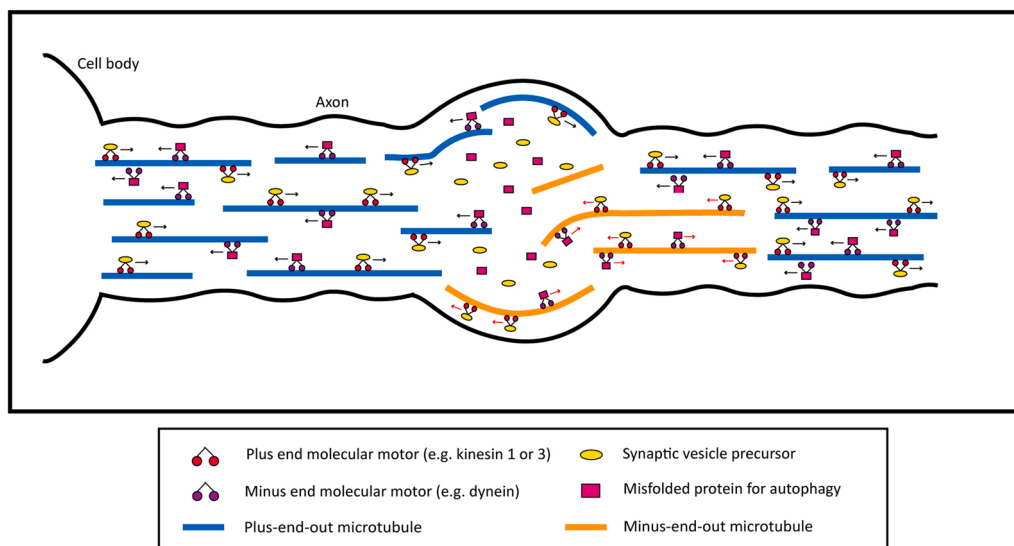


Fig. 4. Organelle “traffic jam” resulting from clusters of aberrant microtubules in the axon. Illustrated here is how a cluster of aberrantly oriented microtubules in the axon can result in a pile-up of cargoes, such as synaptic vesicle precursors and misfolded proteins for autophagy.

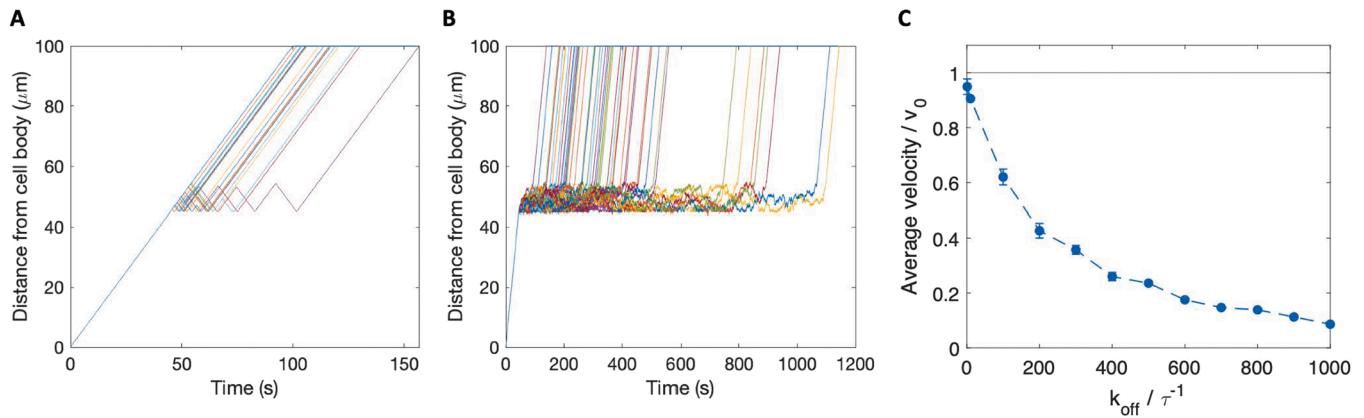


Fig. 5. Simulated organelle traffic is inhibited by clusters of aberrant microtubules in an axon. (A) Sample trajectories for an axon with a cluster of mixed polarity microtubules in the central region of the axon ($p_+ = 0.5$ for $x = 45 - 55\mu\text{m}$) and uniform polarity elsewhere ($p_+ = 1.0$). Other parameters: $k_{\text{off}} = 0.1\text{s}^{-1}$, $v_0 = 1\mu\text{m/s}$, and $L_{\text{axon}} = 100\mu\text{m}$. (B) Sample trajectories for an axon with a cluster of mixed polarity microtubules in the central region of the axon, for the same parameters as in (A), except with a higher motor dissociation rate: $k_{\text{off}} = 0.5\text{s}^{-1}$. (C) Average velocity, $\frac{v}{v_0}$, as a function of the motor dissociation rate, $\frac{k_{\text{off}}}{\tau^{-1}}$, in an axon with a cluster of mixed polarity microtubules (data markers), compared to a uniform plus-end-out polarity pattern (horizontal line), with other parameters the same as in (A) and (B). Each data point in panel C represents the average of $N = 150$ independent molecular motor simulations, and the error bars represent standard error.

disease, as well as injuries such a Traumatic Brain Injury and Spinal Cord Injury. Neurodevelopmental diseases such as Autism Spectrum Disorders may also involve this pathogenic mechanism. At present, however, there are no studies that have explored this possibility directly in diseased or injured neurons. We are of the view that filling this gap in knowledge should be a priority as work on these diseases and injuries moves forward, especially considering the likelihood of significant improvement in the status of patients if treatments can be developed to correct microtubule polarity flaws that most likely exist in their neurons.

Data availability

No data was used for the research described in the article.

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