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Review

Mechanics of axon growth and damage: A systematic review of computational models

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ABSTRACT

Normal axon development depends on the action of mechanical forces both generated within the cytoskeleton and outside the cell, but forces of large magnitude or rate cause damage instead. Computational models aid scientists in studying the role of mechanical forces in axon growth and damage. These studies use simulations to evaluate how different sources of force generation within the cytoskeleton interact with each other to regulate axon elongation and retraction. Furthermore, mathematical models can help optimize externally applied tension to promote axon growth without causing damage. Finally, scientists also use simulations of axon damage to investigate how forces are distributed among different components of the axon and how the tissue surrounding an axon influences its susceptibility to injury. In this review, we discuss how computational studies complement experimental studies in the areas of axon growth, regeneration, and damage.

1. Introduction

Mechanical forces influence the nervous system across both temporal and spatial scales. At longer time scales, mechanical forces govern folding patterns during brain development [1,2], and at shorter time scales, forces applied at high rates cause traumatic brain injury [3]. Though mechanical forces and their effects are more easily observed at the larger spatial scale, brain folding and other larger-scale phenomena are intimately connected to cell-level responses and behaviors. For example, mechanical forces promote axon growth during development [4], and studies suggest that axon growth could play a role in directing brain folding [5]. Similarly, the macroscale forces involved in traumatic brain injuries cause microscale damage in individual axons [6] (Fig. 1).

Within the axon, the cytoskeleton acts to both generate forces and to provide structural support. Microtubules run discontinuously along the length of the axon, and crosslinking proteins bundle these microtubules together to create the core of the axonal cytoskeleton [7]. While passive crosslinks like tau contribute mechanical support in response to external loading, the motion of active crosslinks like dynein generates active forces within the axon [8]. Neurofilaments, another major component of the cytoskeleton, form an extensive network and regulate the axon diameter [9]. Surrounding the microtubules and neurofilaments, spectrin alternates with actin rings to compose the actin cortex [10]. Actomyosin contraction within the cortex supplies another source of active

force generation [11]. These various cytoskeletal elements cooperate in a delicate balance of forces to maintain the structural integrity and biological function of the axon (Fig. 2).

In relating subcellular phenomena to larger scale behaviors, computational models work together with experimental studies to provide additional insight. Analytical and numerical models allow scientists to probe the isolated effects of various cytoskeletal parameters that are difficult to study using experimental methods alone. In axon growth, experimental studies have highlighted several sources of mechanical force generation [12–14], and computational models have investigated how these forces might interact to generate emergent behaviors of axon elongation and contraction [15–17]. Similarly in traumatic brain injury, experimental studies have discovered evidence of subcellular damage [18–20], and models have studied how mechanical loads of different magnitudes and rates could cause the observed damage [21,22]. Insight from these computational studies guides the development of new experimental approaches and predicts important areas for future research.

In this review, we discuss the use of mathematical models in studying axon mechanics. Starting at longer time scales, we present models related to understanding the role of mechanical forces in axon development. Next, we discuss models studying the use of tension to promote axon growth and regeneration. Finally, we examine injury-level loads and models of axon damage.

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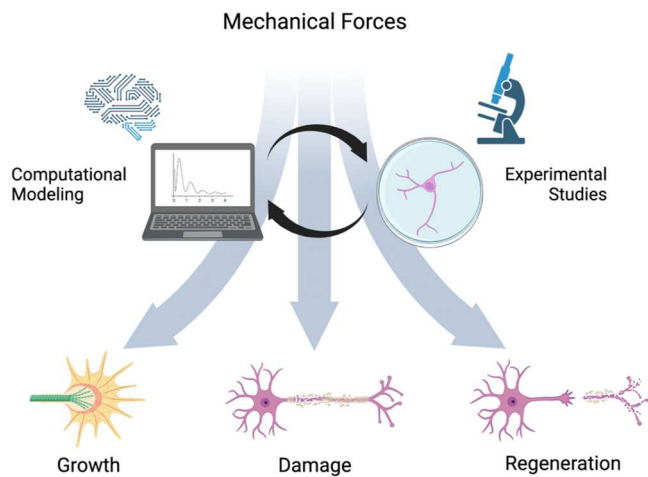


Fig. 1. Computational models and experimental studies work together in the study of axon mechanics. Scientists use a combination of *in silico*, *in vitro*, and *in vivo* approaches to elucidate the role of mechanical forces in axon growth, damage, and regeneration.

2. Intracellular forces govern axon growth

At the end of a developing axon, the growth cone both drives the forward elongation of the axon and directs growth in response to guidance cues [23]. While the growth cone plays a key role in axon growth, the axon does not elongate passively [24]. Rather, forces within both the growth cone and the axon contribute to driving growth [24].

The disruption of various cytoskeletal proteins *via* the administration of cytoskeletal drugs in *in vitro* studies has highlighted the essential role of intracellular forces in regulating axon growth [12,13,25–27]. These studies have pinpointed several sources of force generation within both the growth cone and the axon itself. In the growth cone, a central domain filled with microtubules and organelles is surrounded by a peripheral domain dominated by actin networks [28]. Sources of force generation within the growth cone include the action of motor proteins such as myosin II [25] and kinesin [26]. The polymerization of actin against the cell membrane in the peripheral domain acts as another source of force within the growth cone [27]. In the axon, sources of force generation include microtubule polymerization [14], microtubule sliding *via* the action of molecular motors [29], and myosin contraction [30]. As a supplement to these experimental observations, scientists

have turned to computational modeling to evaluate hypotheses of how these various sources of force generation cooperate to drive axon growth.

2.1. Growth cone forces

One role of the growth cone is to act as an engine pulling the axon forward during development [31]. The key driver behind this forward motion is F-actin retrograde flow [23,32]. F-actin polymerization at the periphery of the growth cone is balanced by myosin II contraction and membrane tension, which pull and push the filaments in towards the central domain [25]. The presence of adhesions, which connect the actin network to the substrate, is required to exploit the retrograde flow to generate forward motion [32]. The adhesions allow the myosin motors to exert traction forces on the substrate, pulling the growth cone and axon forward [33].

To synthesize these experimentally-observed forces into one unified framework, scientists have developed a quantitative model of actin treadmilling in the growth cone [34]. After calibration with experimental measurements of retrograde flow rates [25,35,36], this model was used to analyze the relative contributions of myosin contractility, membrane tension, and actin turnover to the mechanical force balance in the actin treadmill. Results of the study [34] predicted numerical values for the forces involved and highlighted the need for active transport of G-actin to maintain the force balance and to reproduce experimentally-observed concentration gradients [35]. While this actin treadmill model provided a conceptual framework for the mechanics of a stationary growth cone, it did not incorporate the influence of adhesions, which are essential for forward motion [32]. Another theoretical model has explored a potential positive feedback mechanism between adhesion and Rac1 activation dynamics in governing growth cone advancing and paused states [37]. However, this model does not address how adhesions would mechanically interact with the actin network within the growth cone. Future studies could address this question by drawing from work in other motile cells [38].

Beyond pulling the axon forward, the growth cone also steers the growth of the axon in response to guidance cues [39]. Experimental observations show that in combination with actin networks, microtubules play a key role in growth cone turning [40,41]. Studies have highlighted the importance of both microtubule polymerization dynamics [42] and interaction between microtubule and actin filaments [28]. Treatment with low doses of taxol to inhibit microtubule dynamics suppressed growth cone turning response towards a guidance cue [42],

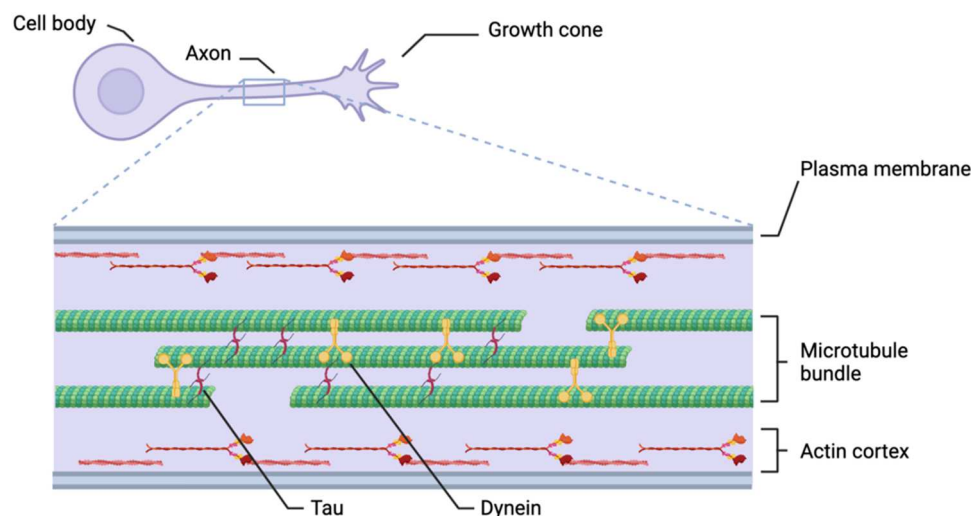


Fig. 2. Overview of the cytoskeletal structure within the axon. Passive crosslinks (tau) and active motor crosslinks (dynein) bundle microtubules together to form the core of the axon cytoskeleton. Just below the plasma membrane, the actin cortex surrounds the microtubule bundle.

and *in vitro* observations showed that microtubules extend farther into the growth cone when actin retrograde flow is reduced [28,43,44].

Scientists have developed computational models to evaluate these mechanisms of microtubule involvement in growth cone turning. Computational studies have analyzed mechanisms of actin-MT coupling [45] as well as regulation of MT dynamics [46–48] in response to chemical guidance cues. The results of these numerical studies pinpoint certain conditions that must be true for the proposed mechanisms to successfully initiate growth cone turning. For example, one model proposes that microtubules initiate turning by promoting adhesion to the substrate [45]. However, the results of the study show that this mechanism would only initiate turning for an optimum range of values for the sensitivity of adhesion formation to the presence of microtubules [45]. Future experimental observations that confirm or reject this condition would provide support for or against this theoretical model. Computational models have also been used to predict the minimum required spatial extent of a chemical guidance cue to successfully trigger turning [46]. Future work in this area could extend to models of growth cone guidance in response to not only chemical but also mechanical cues such as substrate stiffness [49–51].

2.2. Axon forces

2.2.1. Microtubules generate extensile force within the axon

While the growth cone at the end of a developing axon is thought to play a central role in driving elongation by pulling the axon forward [31], studies have shown that axons can grow even after the application of cytochalasin B has eliminated filopodial and lamellipodial activity in the growth cone [52]. Since cytochalasin B inhibits actin polymerization [53], this observation suggests that microtubules could also contribute to promoting axon growth. Both microtubule polymerization [14] and microtubule sliding via dynein motor proteins [13,29] could supply the necessary extensile force. Mathematical models have been used to analyze the feasibility of these two mechanisms and to identify key parameters governing elongation [15,54,55].

An analytical model has been used [56] to examine the possible role of microtubule polymerization and depolymerization in governing axon growth and retraction. This model uses ordinary differential equations to describe a hypothesis that the magnitude of a force applied to the end of an axon controls axon length by altering the rate of microtubule polymerization [57]. As microtubules polymerize against the cell membrane and actin networks of the growth cone, tension in the membrane and networks creates a compressive force acting on the microtubules that inhibits growth [58]. When this compression is reduced, there is a higher probability that a gap will form temporarily between the end of the microtubules and the membrane, increasing the rate of polymerization [58]. In this way, a force pulling on the end of the growing axon would promote polymerization and growth. A study using this model to predict force-elongation curves for axon growth and retraction [54] found that the model predictions closely matched *in vivo* measurements of axon elongation in response to applied forces [59]. Close agreement between model predictions and experimental measurements supports the theory of microtubule polymerization regulating axon growth. However, this model presents a limited view of the forces that contribute to axon elongation and retraction. For example, experiments have shown that dynein inhibition leads to retraction [13], but this model does not consider the action of motor protein crosslinks like dynein.

To assess the potential role of motor proteins in promoting axon elongation, scientists have simulated both unipolar and bipolar motors acting on bundled microtubules [55]. These simulations revealed two necessary conditions for elongation to occur. First, a critical concentration of unipolar motors must be present in the axon because only unipolar motors can generate coordinated extensile motion [55]. Bipolar motors, on the other hand, act as passive crosslinkers between parallel microtubules and do not drive overall extension or retraction [55].

Second, microtubules in the axon must be uniformly oriented for unipolar motors to successfully produce extensile force [55]. In bundles of microtubules with randomly assigned polarities, the actions of motor protein crosslinks sort the bundle into two groups of microtubules rather than elongating the bundle [55]. These conclusions provide support for the involvement of motor proteins in axon elongation because they agree with experimental observations. Studies have shown that dynein, a unipolar motor, is involved with transport in the axon [60], and microtubules in the axon are mostly uniformly oriented with their plus ends located distally [61]. Computational modeling serves as the link that shows these cytoskeletal observations are consistent with the proposed role for motor proteins in promoting axon elongation.

In addition to examining microtubule polymerization and motor protein crosslinking in isolation, computational studies have also investigated potential interactions between these mechanisms that could affect axon growth [62]. These studies have expanded a classical finite element framework into a dynamic simulation platform that simultaneously incorporates microtubule polymerization and dynamic crosslinking to simulate their combined effects [15]. In contrast to previous analytical models, this approach introduces a high enough spatial resolution to consider individual crosslink locations along microtubules. Its systematic parametric studies revealed that the attachment locations of the fixed domains of the dynein motors play a key role in determining the overall motion of the microtubule bundle: when the fixed domains attach near the plus ends of the microtubules, extension occurs, but when the fixed domains attach near the minus ends, contraction results instead [15]. Simulations of bundles where dynein molecules are randomly dispersed among plus and minus ends exhibited no net motion [15]. These model predictions agree with experimental findings of dynein localization at microtubule distal ends [63]. Simulations have also analyzed how polymerization and depolymerization at the plus ends of microtubules alter the extensile behavior of the bundle by influencing dynein attachment: polymerization promotes more dynein proteins to attach, and the total extensile force within the axon increases; depolymerization, on the other hand, decreases dynein attachment and extensile force [62]. This suggests that microtubule polymerization could influence axon elongation both by directly pushing forward on the distal end of the axon and by altering dynein attachment [62]. This model highlights the existence of multiple pathways through which microtubule polymerization could affect axon growth and emphasizes the need for future experiments to determine which pathways do drive axon elongation.

2.2.2. Myosin contraction in the actin cortex opposes extensile forces in the microtubule bundle

In addition to the extensile forces within the microtubule bundle, contractile forces within the surrounding actin cortex participate in regulating axon elongation and retraction [12,13]. Nocodazole and high doses of taxol, drugs that affect microtubule polymerization, inhibit axon growth [13,64]. Similarly, the inhibition of dynein promotes axon retraction [13]. However, the simultaneous application of anti-actin drugs such as latrunculin and cytochalasin B reverses these inhibitory effects on growth [12,13]. Myosin inhibition also eliminates nocodazole-mediated retraction, implicating actin-myosin contractility as a key regulator for axon growth [13]. Mathematical models could help explain how the forces within the microtubule bundle and actin cortex might interact to produce axon behaviors of elongation, stall, and retraction [62,65].

Analytical modeling suggests that the presence of both the microtubule bundle and the actin cortex is required to produce axon growth, stall, and retraction [65]. In this model, polymerization generates extensile force within the microtubule network while myosin generates contraction in the actin cortex. Interactions between these intracellular forces and an externally applied end load govern the individual and combined responses of the microtubule and actin networks. In simulations of the microtubule network alone, a threshold force, the

microtubule stall force, delineates a transition between retraction and elongation [65]. Large compressive end loads cause microtubule depolymerization, and, as a result, retraction occurs [65]. Decreasing the compressive end load below a characteristic threshold generates axon elongation instead [65]. Looking next at simulations of the actin cortex in isolation, a similar threshold exists. Tensile forces of magnitudes larger than the stall force of the actin network trigger elongation, whereas forces below this threshold lead to retraction [65]. While the behaviors of these two networks in isolation include only elongation and retraction, a third phenomenon, stall, emerges as a result of combining the two components [65]. At intermediate force values in between the stall forces of the actin and microtubule networks, the axon exhibits neither elongation nor retraction [65]. This three-phase behavior of growth, stall, and retraction seen in the model agrees with the experimental finding of two thresholds governing axon response to an externally applied force [66]. This analytical study shows that axon behavior may be driven by the dynamic interplay of multiple cytoskeletal components. However, only microtubule polymerization was included as a source of extensile force, leaving the role of motor proteins within the microtubule bundle unexplored.

Another computational study used a finite element model to analyze the interactions between microtubule polymerization and motor protein activity in both the microtubule bundle and the actin cortex [62]. In agreement with experimental observations [13], simulations showed that the actions of dynein in the microtubule bundle and myosin motors in the actin cortex generate opposing forces [62]. Therefore, the ratio of the amount of dynein to the amount myosin activity present in the axon determines whether elongation or retraction occurs in the model. This computational study further investigated the feasibility of a theory in which microtubule depolymerization could cause axon retraction by altering the action of myosin motor proteins via the GEF-H1 pathway [67]. By coupling myosin activity to the amount of microtubule mass in the axon, the model also showed that upregulating myosin activity via the GEF-H1 pathway is a feasible explanation for axon retraction [62]. This suggests that myosin regulation via the GEF-H1 pathway may be an interesting area for future experimental studies.

2.3. The growth cone and axon act in series

Experimental observations of neurons growing *in vitro* have shown that when only the growth cone is attached to the substrate, motion of the growth cone causes the rest of the cell to move with respect to a fixed reference point [31]. This creates an image of the growth cone as an engine that drives axon elongation by pulling the axon forward. This contrasts with the more recent experimental and computational studies from Sections 2.1 and 2.2 that suggest that the axon does not elongate passively. Instead, forces generated along the axon act in combination with those in the growth cone to regulate axon growth [24,65]. Mathematical models have been used to devise new experimental techniques for measuring these forces and to investigate the cytoskeletal mechanisms behind force generation.

A simple rheological model represents the axon and growth cone as dashpots in parallel with motors [24]. The dashpots represent the viscous behavior of the two regions, and the motors represent the forces generated by proteins within the cytoskeleton. The axon and growth cone act in series with each other and an additional spring element that represents a towing needle (Fig. 3). Analysis of the rheological model demonstrates that the velocities of the axon and growth cone would be zero only when the local motor forces are equal to the applied force of the towing needle [24]. This observation inspired a new measurement technique that interprets subcellular forces in the axon and growth cone as a result of the motion of docked mitochondria in these regions [24]. Measurements using this technique suggested that both the axon and growth cone generate contractile forces and that the overall rest tension of the axon is the average of the forces in the axon and growth cone [24]. While the phenomenological model of this study facilitated the analysis

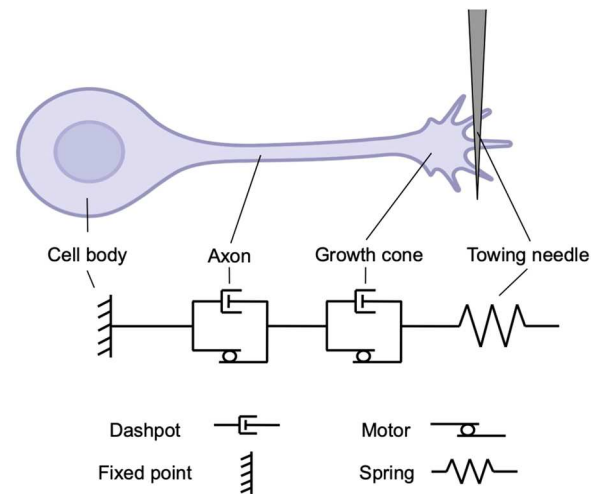


Fig. 3. Interplay of viscous and contractile forces in the axon and growth cone. The total force measured by a towing needle attached to the growth cone is a combination of the forces in the axon and growth cone. Measuring the individual active and passive contributions to the forces in the axon and growth cone remains a challenge.

of forces in the axon and growth cone by simplifying the complex cytoskeleton into a collection of dashpots and motors, this model does not provide insight into the underlying mechanisms that generate these forces.

More recently, scientists have started to incorporate cytoskeletal mechanisms such as actin polymerization and active contraction of myosin into their analytical models [65]. As actin networks appear in both the axon and growth cone, the model introduces two parameters that characterize the action of the acto-myosin network: one governs actin polymerization in the growth cone, and the other governs the contractility of the actin cortex. This model is more mechanistic in nature and enables scientists to investigate the effect of drugs on axon elongation. Analysis of the model suggests that different concentrations of cytochalasin affect these two acto-myosin networks differently, which could explain contradicting experimental observations of the effect of cytochalasin on axon retraction [14,68]. The model proposes that lower concentrations selectively decrease the forward propulsion generated by actin polymerization, while higher concentrations disintegrate the actin network entirely and reduce the contractility of the actin cortex [65]. Therefore, lower concentrations of cytochalasin would inhibit axon elongation, but higher concentrations would promote elongation by reducing the contractile forces within the axon [65]. Incorporating the active acto-myosin network into axon modeling provides a mechanistic framework that can help interpret seemingly contradictory experimental results.

3. Externally applied tension promotes growth and regeneration

Mechanical forces in the growth cone play a key role in governing axon growth during the first stage of neuronal development [31], but once a synapse forms and the growth cone is abolished, other forces take over [69]. As an organism grows, the terminal end of the axon is pulled farther away from the neuronal cell body. The resulting tensile force stimulates growth within the axon during this second stage of development [70]. Scientists have used this mode of stretch-driven growth as an *in vitro* tissue-engineering technique to fabricate implantable nerve conduits [71] and as a method to accelerate regeneration in nerve repair [72]. In these applications, tensile force generated by pulling on the population of axons increases the speed of axon growth beyond its natural magnitude. Although applying a tensile force to an axon can promote growth, applying large forces too quickly can damage the axon

instead [69,73]. Computational models can help estimate the optimal stretch and stretch rates to maximize axon growth without damaging the axon.

A critical limiting factor to axon growth is the interplay of the production, transport, and polymerization of tubulin monomers [74]. Mathematical modeling can help explain how the formation of microtubules *via* polymerization affects the length and cross-sectional area of the axon. In this analytical model, the rate at which polymerization, and therefore growth, occurs depends on the rates of tubulin production and transport in and from the soma, the axon cell body [74]. Furthermore, this model implements a hypothetical mechanism by which externally applied forces accelerate growth by generating tension in the cell membrane. The membrane tension increases the opening probability of mechanosensitive ion channels as seen in experimental studies in other contexts [75,76]. In the model, the activity of these ion channels then increases the rate of tubulin production in the soma. If, however, the membrane tension is too high, failure will occur [77]. This implies that optimal growth would occur when the membrane tension remains just below the axon failure threshold [74]. The optimal growth curve of this approach agrees well with experimental observations of axon growth and disconnection *in vitro* [73]: elongation rates above the failure threshold result in disconnection, and elongation rates below the threshold result in the fastest possible growth. Both the predictions and the experiment confirm that absolute growth can accelerate over time as axons elongate and adjust to higher growth rates.

While this model successfully predicts disconnection at longer time scales, it does not consider the viscoelasticity of the axon, and, therefore, cannot predict disconnection at shorter time scales. In *in vitro* experiments, axons have been stretched using micro stepper motors, which apply step displacements followed by rest periods [73]. Micro stepper motors can achieve large ranges of net elongation rate by altering either the size of the step displacement or the duration of the rest period. Although the maximum possible elongation rate depends on the rate of axon growth, the maximum step size is instead governed by the viscoelastic material behavior of the axon [78]. Predicting axon disconnection based solely on growth cannot account for the shorter time scale behavior that is governed by axon viscoelasticity [78].

To predict axon disconnection across both short and long time scales, scientists have developed a model that simultaneously integrates both the viscoelastic and growth behaviors of the axon [78]. This model describes a relationship between the stretch history of an axon and its membrane tension. Pulling on an axon increases the tension in the membrane, but over time, the tension relaxes due to viscoelasticity and growth [78]. Viscoelasticity is the collective result of cellular phenomena that act on a shorter time scale such as membrane unfolding [79] or cytoskeletal reorganization [15]. Growth is the result of the addition of new matter and occurs over a longer time scale [5]. Simulations predict that optimal growth occurs when the membrane tension is just below the axon failure threshold [78]. The predictions of this model agree well with other *in silico* predictions [74] and *in vitro* measurements [73]. Including viscoelasticity allows us to predict maximum displacement steps to avoid axon disconnection (Fig. 4). While a viscoelastic growth model succeeds in bridging predictions across the short and long time scales, the phenomenological nature of the model does not propose any explanation of how tension accelerates growth, and different models would be needed to study potential mechanisms.

4. Loading at high rates causes injury

Axons can withstand large strains and even grow in response to large forces when applied incrementally at low enough rates [73]. However, the high strain rates during traumatic brain injury unavoidably result in axon damage [80]. The presence and severity of traumatic axon injury are correlated with unfavorable patient outcomes in traumatic brain injury [81], so an understanding of the mechanisms of axon damage could aid the development of traumatic brain injury treatment

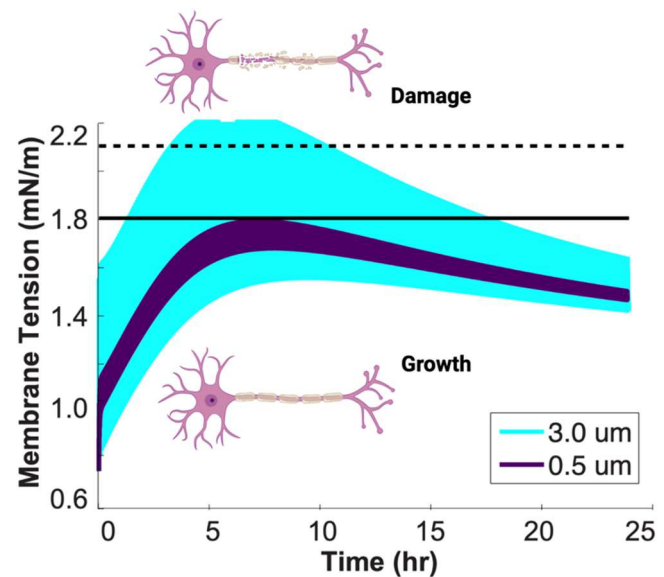


Fig. 4. Computational models predict limits on stretch-driven axon growth. A computational model can make predictions about whether a given stretch history will promote growth or cause damage within an axon. The resulting membrane tension values for a stretch history using a microstepper motor with a 3.0 μm step size exceed damage thresholds for both the peak (dotted) and average (solid) tension values [44].

strategies. Computational models provide valuable insight into how mechanical loads are distributed among different components of the axon and how the surrounding tissues influence an axon's susceptibility to injury.

4.1. Damage to axon compartments

Experimental studies of axon stretch injury provide evidence of damage to various axon components including both the cytoskeleton and the plasma membrane [18–20]. In an *in vitro* study, electron microscopy revealed ruptured microtubules in injured axons [18], and other experiments have shown loading rate-dependent increases in plasma membrane permeability after injury [19,20]. Computer models of the axonal cytoskeleton and axolemma can illustrate how these structures deform and fail under mechanical loading.

Computational studies of the microtubule bundle have found that the mechanism of failure depends on the material properties and dynamic behaviors assigned to the microtubules and crosslinks in the model (Fig. 5) [82–84]. While the different models use a similar geometry for the arrangement of microtubules and crosslinks [82–84], incorporating dynamic crosslinking or viscoelastic material properties [83,84] changes the failure characteristics compared to fully elastic models [82]. Microstructural microtubule-crosslink models delineate the different ways in which the axonal cytoskeleton could fail and can help identify critical thresholds beyond which failure occurs [82–84].

One of the first studies of this kind used an entirely elastic model for both crosslinks and microtubules and concluded that crosslinks are the weakest link in the microtubule bundle [82]. In simulations of tensile loading, strains in the crosslinks reached the critical threshold for failure while strains in the microtubules stayed well below the failure threshold [82]. Therefore, microtubules remained intact but disconnected from each other due to the failure of the crosslinks [82]. This fully elastic model explains one possible mode of axon failure; however, it does not reflect any time-dependent mechanisms and behaves similarly at all loading rates. Therefore, the elastic model cannot reproduce the loading rate dependence seen in axon injury [80]. To study the effect of loading rate on axon failure, axon models must incorporate time-dependent properties.

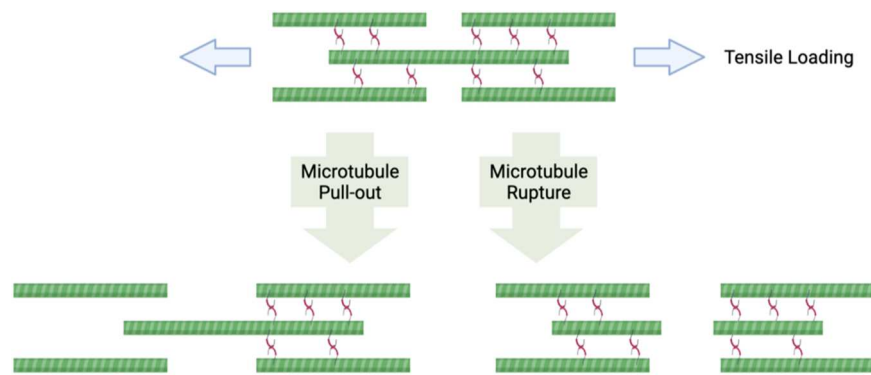


Fig. 5. Two possible failure modes for the microtubule bundle. Computational models predict two modes of failure for axonal microtubules: elastic models show microtubule pull-out only, while viscoelastic models show either microtubule pull-out or microtubule rupture depending on the loading rate.

One possibility to incorporate time-dependent effects is to include the dynamic attachment and detachment of crosslinks [83]. While the material properties of the individual microtubules and crosslinks remain elastic, crosslinks can detach and reattach dynamically, and mechanical forces can modulate the rate of crosslink detachment. Similar to the failure mode of the fully elastic axon model [82], axon damage is an emergent property of the accumulation of crosslink detachment and microtubule disconnection. However, now, the incorporation of dynamic crosslinking introduces a loading rate-dependent behavior: as the loading rate increases, detached crosslinks have less time to reattach before the individual microtubules are pulled apart [83]. Damage therefore accumulates more quickly at faster loading rates [83]. Although dynamic crosslinking introduces rate dependence to the mechanical behavior of the microtubule bundle, failure still occurs via microtubule disconnection and does not explain *in vitro* observations of microtubule fracture in axon stretch injury [18].

Another alternative for incorporating time-dependency is modeling the crosslinks as viscoelastic instead of elastic [84,85]. In the viscoelastic model, crosslinks and microtubules experience different strains depending on the overall loading rate: at low loading rates, crosslinks are more compliant and experience larger strains, allowing microtubules to slide past each other; at high loading rates, crosslinks are stiffer and deform less, resulting in larger microtubule strains to compensate [84]. At low loading rates, the failure mode of the viscoelastic model resembles the one of the elastic model [82], where intact microtubules detach from the bundle. At high loading rates, microtubules experience higher strains and fracture [84,85]. Viscoelastic crosslink models provide detailed insight into microtubule fracture in an axon subjected to tensile loading beyond the physiological range.

Since *in vitro* studies have revealed microtubule fracture as a major failure mode [18], modeling studies have focused mainly on the failure of the microtubule bundle [82–85]. Nonetheless, there is increasing interest in models that include other components of the axon to understand failure of the axon as a whole. Scientists have built a finite element model that includes both the microtubule bundle and the axolemma to compare the strains in these two compartments [22]. Simulations showed that these compartments behave differently under the same applied global axon strain. Looking at the spatial distribution of the maximum strains, the locations of maximum axolemma strain do not coincide with the locations of the maximum microtubule strain [22]. Instead, maximum axolemma strains coincide with the region in the microtubule bundle where the microtubules have been pulled the farthest apart [22]. Interestingly, the axolemma exhibits larger strains and reaches its failure threshold at a lower global axon strain compared to the microtubules [22]. This suggests that acute axolemma damage could play a key role in axon injury in addition to microtubule fracture.

While several different approaches have been taken in modeling axon damage, these models have all used an idealized geometry for the microtubules in the axon cytoskeleton [22,82–85]. Future

computational studies could take into consideration structural differences between different neuron populations and the potential basis for differences in susceptibility to injury [86].

4.2. Influence of surroundings

The computational studies presented in Section 4.1 provide valuable insight into the mechanical behavior of axons in isolation; yet, they fail to mimic the behavior of axons embedded in their *in vivo* environment. The mechanical properties of the surrounding tissues dictate how loads are transferred to the axons and influences their susceptibility to injury [87–91]. For example, experimental measurements have shown that myelin [87] and glial cells [88] contribute to the stiffness of neural tissue, and myelinated axons appear to be less vulnerable to damage compared to unmyelinated axons [89]. Researchers have also discovered that axon damage occurs frequently at the gray-white matter interface [90,91], suggesting that the mismatch in material properties at this boundary increases vulnerability to injury. Computational modeling can provide insights into how the mechanical properties of surrounding tissue influence the occurrence of axon damage.

Simulations of isolated and embedded axons suggest the surrounding tissue could have a protective effect on axons when subjected to tensile loads [92]. In a direct comparison of an isolated axon and an axon embedded in a glial matrix, the isolated axon exhibits larger peak strains within the axolemma [92]. The embedded axon exhibits lower peak strains and a more uniform strain distribution [92]. This is a net effect of the glial matrix that connects different regions of the axon and allows them to carry the applied load more equally [92]. Since peak strains are lower in the embedded model, the axolemma reaches its failure threshold at a higher globally applied strain compared to the isolated model [92]. These simulations suggest that the surrounding tissue provides additional mechanical support and allows the axon to withstand larger strains without injury.

A similar study examined how the myelin surrounding an axon might protect microtubules in the cytoskeleton from damage [93]. The underlying model consists of both microtubules and a combined outer layer of actin cortex and myelin. Viscoelastic crosslinks connect the microtubules to each other and to the outer layer. The simulations applied tensile loading to the outer layer only, and the crosslinks transferred the load to the microtubules inside the cell. A systematic comparison of various loading magnitudes and rates revealed the critical loading regimes beyond which the microtubule stress exceeds the failure threshold [93]. Inspired by the experimental evidence of spectrin and myelin breakdown after traumatic brain injury [94,95], the simulations also compared the original model to one with decreased stiffness in the spectrin and myelin layer to mimic spectrin-myelin damage. With reduced spectrin and myelin stiffnesses, the microtubules have to carry larger loads [93]. This results in axon damage at lower applied stresses and stress rates compared to the original undamaged model [93]. The

observations suggest that damage to the surrounding tissue could be one explanation for the compounding effect of repeated trauma [93]. After the initial trauma, softening of the myelin and actin-spectrin layers reduces their protective effect, leaving axons more vulnerable to damage from subsequent impacts [93].

Rather than purely protecting the axons, the surrounding tissues could also increase their susceptibility to injury [96]. Histological studies of injured brains have shown that axon damage preferentially occurs at the interface between gray and white matter [96]. Computational models can help explain how the mismatch in material properties across this interface creates regions of increased mechanical stress in embedded neurons [97]. A stiffer white matter matrix and a softer gray matter matrix encapsulate axons with uniform material properties. A uniform external mechanical loading results in a localized increase in stresses and strains in the portion of the axon near the material interface on the gray matter side [97]. The stiffer white matter holds the axon tightly in place while the more compliant gray matter deforms, pulls the axon along, and generates high axon stresses [97]. The mismatch of gray and white matter properties can explain an increased susceptibility to axon damage at the gray and white matter interface [97].

4.3. Functional impairment

Electrophysiological impairment accompanies structural damage in the axon [98]. At the single cell level, *in vitro* experiments have used both localized suction [99] and stretching of extensible substrates [100] to examine the effect of mechanical strain on ion channel currents and dynamics. These studies have found that stretched cells exhibit altered ion current amplitudes and thresholds for action potential initiation [99–102]. At the tissue level, studies of the guinea pig spinal cord have shown that the amplitude of the compound action potential decreases in response to mechanical strain, but some of the functional impairment recovers after injury [103]. Both the magnitude and rate of the applied strain affect the extent of the functional impairment [104].

Inspired by these experimental observations, scientists have employed computational models to simulate the connection between mechanical deformation and electrophysiological impairment. One approach that has been taken is to adapt the Hodgkin-Huxley electrophysiological model [105] by altering the inactivation and activation parameters of the sodium ion channels [106]. Simulations of these altered ion channel dynamics [106–108] have linked altered sodium currents to experimental observations of decreased action potential amplitude and conduction speed [100,103], increased sodium current [109], and spontaneous electrical activity [102]. Building on these electrophysiological models, other computational studies have directly coupled mechanical deformation to altered electrophysiological properties [110–112]. Both one-dimensional [110] and three-dimensional [111,112] models have been developed to study the effects of various mechanical loads on neuron function. While the one-dimensional models are limited to studying axial loads, three-dimensional models have been used to explore other loading methods such as crush injury [112]. Future work could extend these studies to look at other modes of injury such as blast.

5. Conclusion

Computational modeling provides a systematic framework to organize disparate pieces of experimental evidence. In axon growth, these computational models link subcellular phenomena to cell-level observations and help scientists test different hypotheses around axon elongation, retraction, and damage. In axon damage, computational models reveal intra- and extracellular factors that influence susceptibility to injury. While *in silico* experiments certainly cannot replace *in vitro* and *in vivo* experiments, computational studies can pinpoint interesting future directions for experiments and inspire new techniques. At the same time, experimental studies inspire the creation of models to facilitate the

interpretation of observations. It is critical for computational and experimental studies to work in concert to further our understanding of axon growth and damage.

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Declarations of interest

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