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The synaptic life of microtubules

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Abstract

In neurons, control of microtubule dynamics is required for multiple homeostatic and regulated activities. Over the past few decades, a great deal has been learned about the role of the microtubule cytoskeleton in axonal and dendritic transport, with a broad impact on neuronal health and disease. However, significantly less attention has been paid to the importance of microtubule dynamics in directly regulating synaptic function. Here, we review emerging literature demonstrating that microtubules enter synapses and control central aspects of synaptic activity, including neurotransmitter release and synaptic plasticity. The pleiotropic effects caused by a dysfunctional synaptic microtubule cytoskeleton may thus represent a key point of vulnerability for neurons and a primary driver of neurological disease.

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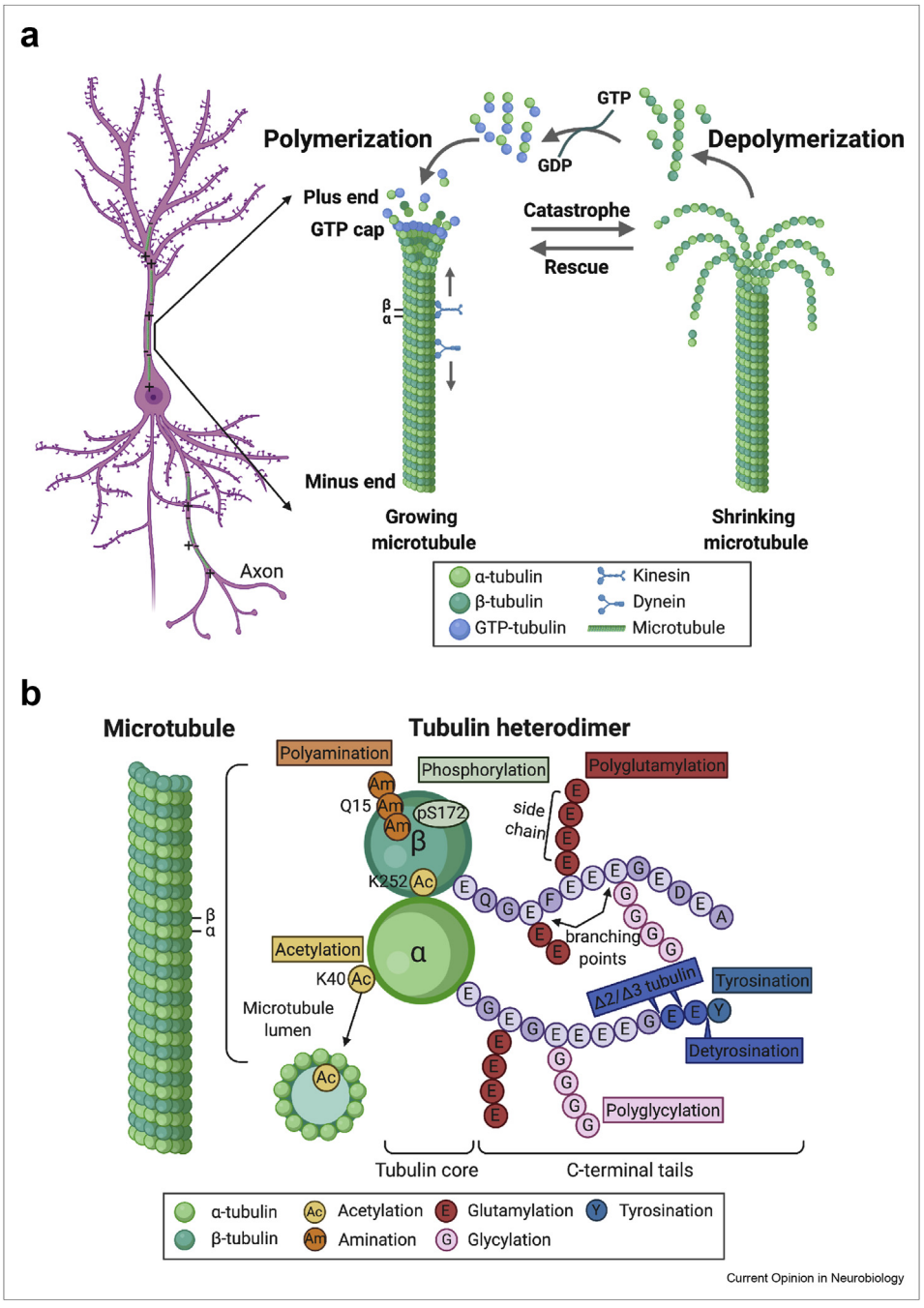
Introduction

Synaptic transmission and plasticity play crucial roles during development and neuronal circuit remodeling, as well as in many neurodegenerative diseases. Although it is widely accepted that the neuronal cytoskeleton underlies the maintenance and plasticity of synaptic connections, most work has focused on the function and regulation of actin in dendritic spines. Remarkably, the localization of microtubules at synapses has only recently become widely accepted after decades of controversy, and the functional roles of synaptic microtubules have just begun to emerge.

Neurons uniquely possess acentrosomal microtubules composed of the polarized head-to-tail addition of α- and β-tubulin heterodimers, arranged laterally to form a hollow tube (Figure 1a). The polarity of the α/β tubulin heterodimer gives the microtubule an intrinsic polarity with a plus (β tubulin) and a minus (α tubulin) end. However, while microtubule plus ends are uniformly oriented toward the distal tip of axons, microtubules are arranged with mixed polarity in dendrites [1,2]. Dynamic microtubules differ from stable microtubules in their ability to undergo stochastic transitions from depolymerization to polymerization and *vice versa*, a process termed dynamic instability [3,4]. While a cap of more stable GTP-tubulin at plus ends maintains the microtubule in a growth state, loss of the cap exposes the less stable GDP-tubulin core, resulting in polymer disassembly. In cells, microtubule dynamic properties can be further modulated by tubulin isoform diversity, microtubule-dependent motors of the dynein and kinesin families, microtubule-associated proteins (MAPs), and tubulin post-translational modifications (PTMs). Tubulin PTMs preferentially accumulate on stable microtubules and except for α-tubulin acetylation, which takes place in the luminal side of microtubules [5], all other modifications occur on the exposed carboxyl-terminal tails of α- or β-tubulin subunits (Figure 1b). The combinatorial nature of these covalent modifications gives rise to a 'tubulin code' that regulates a variety of neuronal functions [6–10]. Both dynamic microtubule end binding proteins and tubulin PTMs on stable microtubules control the binding of microtubules to motors, microtubule severing enzymes, and MAPs [6,10–18], and these events regulate vesicle, organelle, RNA, and multiprotein complex flux into and out of synapses [19].

Despite compelling recent evidence for microtubule regulation of synaptic function, it is still unknown whether and how alterations in microtubule dynamics and/or tubulin PTMs at synapses precipitate the induction of neurological disease. In this review, we will summarize the evidence supporting key roles for synaptic microtubules in regulating neurotransmitter release and synaptic plasticity at glutamatergic synapses, and briefly discuss a few examples of how a

Figure 1



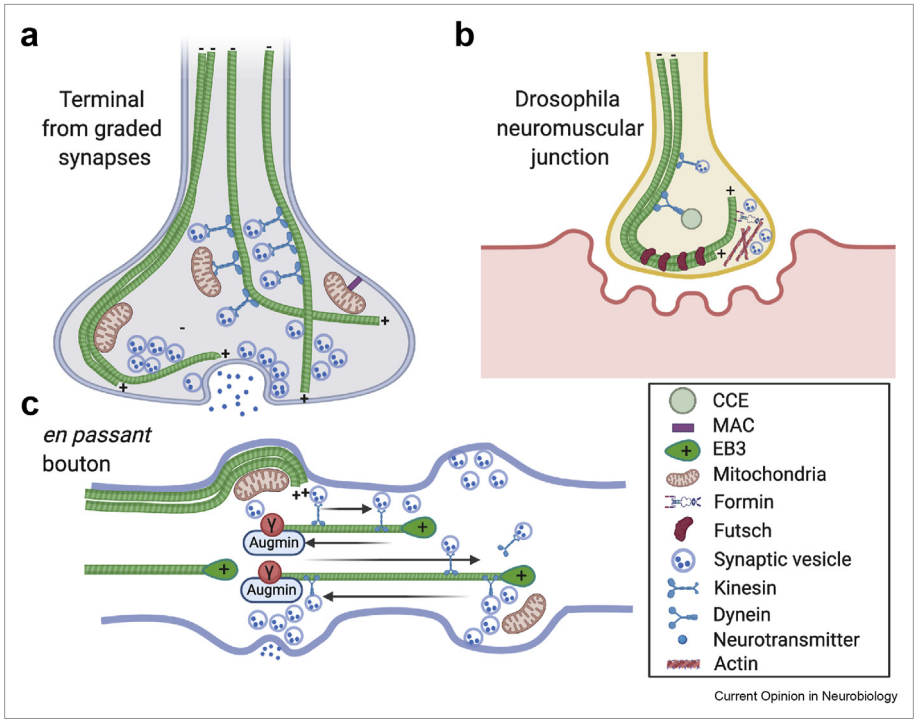
Microtubules are dynamic polymers that can be modified post-translationally. (**a**) Neurons contain acentrosomal microtubules composed of the regulated addition of α- and β-tubulin heterodimers arranged in a head-to-tail fashion, giving rise to a polarized polymer with a plus (β-tubulin) and a minus end (α-tubulin). Microtubule plus ends are uniformly oriented toward the distal end of axons, while microtubules are arranged with mixed polarity in dendrites. Microtubules undergo dynamic instability, the property of switching between growing (polymerizing) and shrinking (depolymerizing) states. Dynamic instability is defined by the combination of four parameters: the rates of growth and shrinkage and the frequency of the transitions between these two states, known as catastrophe (polymerization to depolymerization) and rescue (depolymerization to polymerization). (**b**) When stabilized, dynamic microtubules are substrates of numerous post-translational modifications to their α- and β-tubulin subunits, preferentially on C-terminal residues exposed to the surface of the microtubule lattice. The combinatorial nature of these modifications gives rise to a 'tubulin code,' an incompletely understood collection of molecular rules regulating the affinity of microtubule-binding proteins and microtubule turn-over. The location and variety of tubulin post-translational modifications (de-tyrosination, Δ2/Δ3, acetylation, polyamination, phosphorylation, polyglutamylation, polyglycylation) on α- and β-tubulin are shown.

dysfunctional synaptic microtubule cytoskeleton may drive neurological disease.

Neurotransmitter release

The role of microtubules in mammalian presynaptic terminals has remained unexplored until very recently (Figure 2). F.O. Schmitt in 1968 [20] was the first to suggest that synaptic vesicles (SVs) are translocated to sites of release by microtubules, and D.S. Smith later showed that clusters of SVs are closely associated with microtubules near presynaptic terminals in cyclostome larvae [21,22]. In agreement with these findings, tubulin was observed in subcellular fractions from nerve endings and in association with the presynaptic membrane [23–25]. It was not until the advancement of various electron microscopy (EM) fixation techniques, however, that E.G. Gray reported the existence of different subsets of microtubules at presynaptic boutons in rat cortex and cerebellum. By pretreating with albumin before fixation, Gray serendipitously preserved labile microtubule structures and was able to detect a system of SV-associated microtubules attached to the active zone (AZ) of the presynaptic membrane [26–33]. These early observations indicated that microtubules participated in the translocation of SVs and the formation and maintenance of the AZ [34]. EM analysis of synaptosome fractions from adult rat cerebral and cerebellar cortex revealed another subset of coiled microtubules that were not in close association with SVs or the AZ [32], suggesting a different function. Indeed, microtubules had been observed to surround mitochondria in synaptosomes and intact terminals isolated from the cortex [35], and findings in goldfish retinal bipolar neurons and giant calyceal terminals of Held confirmed a function for these presynaptic microtubules in mitochondrial anchoring. While in retinal bipolar neurons a band of stable and post-translationally modified microtubules plays a role in mitochondrial organization [36], in the Calyx of Held presynaptic microtubules facilitate the anchoring of mitochondria to

Figure 2



Microtubule functions in neurotransmitter release. (**a**) In terminals from highly active and graded synapses such as those at the Calyx of Held and retinal bipolar neurons, presynaptic microtubules are rate limiting for high-frequency neurotransmission, serving to replenish the readily releasable pool of synaptic vesicles from the reserve pool. Presynaptic microtubules also facilitate mitochondria organization and anchoring to the membrane by forming stable peripheral bundles or maintaining mitochondrion-associated adherens complex (MAC) superstructures. (**b**) The *Drosophila* neuromuscular junction contains bundled loops of microtubules held in proximity of the AZ by MAP futsch/MAP1B, as well as pioneer microtubules, a subset of dynamic microtubules regulated by formin activity. These microtubules may provide the tracks for kinesin-mediated delivery of SVs and dynein/BicD-mediated recycling of clathrin-coated endosomes (CCE) into and out of the synaptic terminal. (**c**) Mammalian glutamatergic *en passant* boutons are hotspots for γ-tubulin- and augmin-dependent *de novo* nucleation of dynamic microtubules, labeled by the $^{+}$TIP EB3. Here, γ-tubulin regulates nucleation density and augmin coordinates the uniform, plus-end directed growth toward the distal axon. The nucleation of dynamic microtubules at boutons is stimulated by neuronal activity and regulates neurotransmission by providing the tracks for interbouton delivery of rate-limiting synaptic vesicles to sites of release.

the plasma membrane via the mitochondrion-associated adherens complex superstructure [37]. Both retinal bipolar neurons and calyceal terminals are specialized glutamatergic synapses that must communicate sustained and graded signals, suggesting that in these highly active synapses, microtubules perform an essential role in maintaining the synaptic energy supply by ensuring close proximity between the source of ATP and the SV cycling machinery.

After Grey's pioneering EM studies, the characterization of microtubules at mammalian presynaptic boutons stalled for decades, likely limited by the visualization of small mammalian cortical and hippocampal boutons using conventional light microscopy. Until recently, the glutamatergic *Drosophila* larval neuromuscular junction (NMJ) [38] was the model of choice, offering large presynaptic terminals that could be easily observed in an organism amenable to genetic manipulation. Early studies reported that a small proportion of these NMJ terminals contained bundled loops of microtubules identified by the MAP1B-like protein Futsch, and these microtubule bundles marked future sites of bouton bifurcation and synaptic growth [38–40]. Futsch appeared to link presynaptic microtubules and the AZ, and post-PTMs of Futsch regulated microtubule dynamics at these boutons [40–42]. In addition to this subset of stable microtubules, a population of dynamic microtubules was also observed. Defects in the ability of this dynamic population to invade presynaptic boutons affected synaptic growth in fly mutants of *Diaphanous*, a member of the formin family [43]. Although the role of these presynaptic microtubules remains unclear, they may provide the tracks for kinesin and dynein/BicD-mediated SV recycling [44]. Interestingly, the protein encoded by *Diaphanous* also nucleates unbranched actin filaments, and its functional mammalian homolog mDia regulates both actin and microtubule dynamics in non-neuronal and neuronal cells [45,46]. Furthermore, growing evidence supports the notion that formin-dependent actin polymerization modulates SV endocytosis, synaptic recycling, and presynaptic remodeling in mammalian and *Drosophila* synapses [47–51], suggesting that formin-mediated coordination of presynaptic microtubule and actin dynamics represents a conserved function.

Recent studies on giant calyceal terminals and *en passant* boutons in mammalian neurons have begun to shed light on the function of microtubules in SV cycling. Live imaging analysis of large calyceal boutons revealed that microtubules are inserted into presynaptic terminals and regulate the transport of SVs between terminal swellings [52]. Furthermore, while disturbances in F-actin polymerization affected the fast-recycling of SVs, microtubule depolymerization mostly disrupted SV slow-recycling. These data suggest that at these large synapses, presynaptic microtubules are rate limiting for high-frequency neurotransmission by replenishing the readily releasable pool of SVs from the reserve pool [53]. In agreement with these findings, Guedes-Dias et al. recently reported that in cultured hippocampal neurons, dynamic microtubules are enriched at *en passant* boutons and allow for the targeted delivery and unloading of SV precursors by the kinesin-3 motor KIF1A [54]. These observations were confirmed and extended by a parallel study demonstrating that excitatory *en passant* boutons are hotspots for γ-tubulin- and augmin-dependent *de novo* microtubule nucleation, with γ-tubulin regulating the nucleation density, and augmin directing the uniform, plus-end directed growth toward the distal end of the axon [55]. Notably, *de novo* nucleation of dynamic microtubules at boutons was conserved in an intact circuit, stimulated by neuronal activity, and regulatory for neurotransmission by providing the tracks for targeted bidirectional interbouton delivery of a rate-limiting supply of SVs to sites of stimulated release.

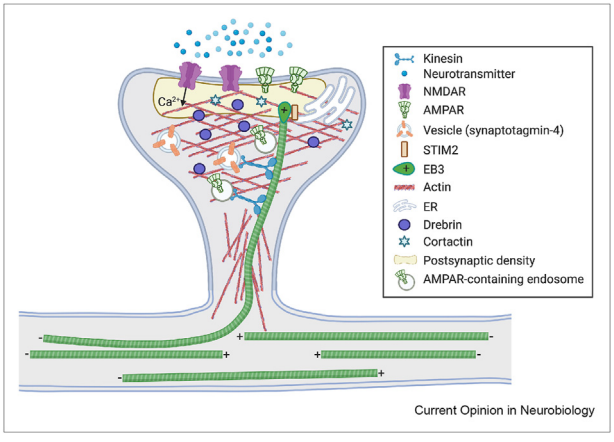
In addition to their postulated function in mitochondria anchoring at active sites of release, evidence suggests that dynamic microtubules may be directly regulating Ca^{2+} handling at terminals through the interaction of EB1/3 to the endoplasmic reticulum (ER)-Ca^{2+} sensor, stromal interacting molecule 1 and 2 (STIM1/2) [56–58]. Store-operated Ca^{2+} entry (SOCE) is a ubiquitous mechanism that allows ER/Ca^{2+} store refill from the extracellular space and is mediated by the ER Ca^{2+} sensors STIM1/2 and the plasma membrane (PM) Ca^{2+} channel Orai1 [59]. EB1 binding to STIM1 is regulated during ER Ca^{2+} store depletion and inhibits STIM1 translocation to ER–PM junctions and Orai1 recruitment [60,61], revealing an unexpected role of dynamic microtubules in preventing Ca^{2+} overload. Indeed, *in vivo* microtubule stabilization in motor neurons inhibited SOCE and reduced ER Ca^{2+} content [62] and STIM1 association with EB1/3 was critical for ER remodeling and spatial localization of Ca^{2+} signals in growth cones [63]. Interestingly, STIM1 has also been implicated in the regulation of voltage-gated Ca^{2+} channels [64–66] and activation of STIM1 at presynaptic terminals by ER Ca^{2+} store depletion inhibited presynaptic Ca^{2+} influx and SV exocytosis [67]. The role of dynamic microtubules in the modulation of this process through STIM1 binding is unknown.

Plasticity

As the major sites of excitatory synaptic input, dendritic spines have a critical role in signal reception and processing. Their ability to undergo rapid structural and functional changes in response to different patterns of input also makes them the primary encoders of synaptic plasticity. Similar to the situation in presynaptic boutons, our understanding of the role of microtubules in dendritic spines has evolved with the tools for microtubule visualization. Early EM studies observed

microtubules most prominently in spine stalks [68,69], but occasionally also associated with the postsynaptic density in spine heads [70,71]. Biochemical purification studies correspondingly indicated that tubulin was a component of postsynaptic junctions [72–76] and reported interactions between microtubules and the PSD scaffold proteins PSD-93 and PSD-95 [77–79]. These studies indicated that microtubules were static elements of spines required for maintaining their structural integrity. In contrast, similar imaging analyses indicated that actin, the other major cytoskeletal component of spines, was essential for their morphological plasticity [80–83]. This view only changed with the use of microtubule plus-end binding proteins such as EB3 to visualize growing microtubules in neurons [84] (Figure 3). By capturing microtubule polymerization, EB3 imaging revealed that dendritic microtubules were in fact highly dynamic, occasionally invading spines and altering their morphology [85–87]. Microtubule invasion of spines depended on F-actin and actin-binding proteins such as drebrin, cortactin, and the nucleator ARP2/3 complex [87–89], suggesting a role for F-actin in capturing growing microtubule plus-end binding proteins (+TIPs), such as EB3, at recently activated spines. Indeed, spine targeting by dynamic microtubules was promoted by NMDA receptor activation and Ca^{2+} influx, with chemical or tetanus-induced long-term potentiation (LTP) significantly increasing spine invasion [88,90,91]. Not only were microtubule dynamics essential for BDNF- and activity-induced spine and PSD enlargement [85,86,90,92], but inhibition of microtubule polymerization with nocodazole further impaired hippocampal LTP [87,93], demonstrating a critical role for dynamic microtubules in synaptic plasticity and the accompanying transitions in spine morphology.

Figure 3



Microtubule functions in synaptic plasticity. Dynamic dendritic microtubules can invade dendritic spines, a process promoted by NMDAR activation, Ca^{2+} influx, and actin polymerization, and mediated by the neuron-specific F-actin binding proteins drebrin and cortactin. Invasion of dynamic microtubules into spines regulates spine structural plasticity, by mediating delivery of cargoes such as synaptotagmin-4 and AMPARs, as well as the synaptic localization and delivery of the Ca^{2+} sensor and ER-resident protein STIM2.

Changes in the stability of the microtubule network at synapses have been linked to hippocampal learning and memory in a study showing that synaptic microtubules are not modified and presumably highly dynamic in the early phase (15–60 min) after contextual fear conditioning of mice (a paradigm that requires hippocampal learning), but exhibit increased tubulin detyrosination and presumably are more stable in the late phase (after 8 h) [94,95]. Importantly, pharmacological suppression of either of these two microtubule transitions completely inhibited memory formation. These biphasic shifts required the microtubule destabilizing phosphoprotein stathmin, whose binding to microtubules increases in the early phase of learning and decreases in the late phase, leading to microtubule hyperstability [94,95]. Although it is unclear whether these stathmin-induced changes impact microtubule dynamics within spines, the study suggests an important role for microtubule dynamic instability in synaptic plasticity underlying learning and memory. Accordingly, defects in memory and learning were associated with inhibited microtubule dynamics in KIF21B KO mice [96]. However, the mechanisms by which dynamic microtubules facilitate plasticity remain poorly understood.

A series of recent studies indicate important functions for dynamic microtubules in cargo and organelle delivery into spines, facilitating protein turnover as well as changes in spine size, stability, and plasticity. For instance, endosomes containing AMPA-type glutamate receptors (AMPARs), instrumental for excitatory synaptic transmission and plasticity, required dynamic microtubules for their transport along dendritic shafts and into spines [97]. Indeed, the entry of AMPARs into spines was observed to coincide with microtubule invasion [97], indicating that microtubule polymerization may propel AMPARs into spine heads. Syntaptotagmin-4 (syt-4)-containing vesicles are also transported by polymerizing microtubules into spine heads, where they subsequently undergo exocytosis [98]. Interestingly, syt-4 trafficking was dependent on synaptic activity and the kinesin KIF1A, whose knockdown promoted dysregulated exocytosis of syt-4 vesicles along dendritic spines and shafts [98]. These findings indicate that kinesins such as KIF1A can restrict vesicle fusion to specific sites like spine heads, by sequestering their cargo along spine-invading microtubules. In hippocampal neurons, syt-4 localized to dense core vesicles [99] and negatively regulated BDNF release and LTP [100], indicating its important role in synaptic plasticity. Intriguingly, a recent study reports that local synaptic activity caused c-Jun N-terminal kinase–mediated phosphorylation of syt-4, disrupting its interaction with KIF1A and

microtubules, and thereby promoting dense core vesicle capture at active synapses [99]. This pathway may serve as a mechanism to prevent excessive BDNF release and synaptic strengthening. It is likely that dynamic microtubule-mediated transport of other synaptotagmins, many of which mediate Ca^{2+}-triggered vesicle exocytosis [101], as well as glutamate receptors (e.g. NMDARs and mGluRs) also contribute to the maintenance and plasticity of excitatory synapses.

In addition to AMPARs and syt-4, other transmembrane cargoes of dynamic microtubules include STIM family of ER Ca^{2+} sensors [56–58] and this interaction was shown to regulate ER tubule elongation and spine morphology and stability in hippocampal neurons [102]. Given STIM2's role as a regulator of store-operated Ca^{2+} entry [103] and its links to synaptic plasticity [104] and spine loss in neurodegenerative disease [105–107], STIM2 transport into spines by dynamic microtubules appears to be critical for maintaining neuronal communication and health. Dynamic microtubules may also mediate the selective delivery of spine targeting MAPs and organelles into active spines [108–110]. For instance, as with syt-4 vesicles, lysosome entry is regulated by microtubule polymerization and local synaptic activity [110]. Moreover, lysosomes were found to colocalize with internalized AMPARs in a subset of spines [110], suggesting that they are precisely positioned to mediate the degradation of AMPARs and other recently internalized membrane proteins. This activity-dependent coupling of lysosome entry into spines with AMPAR internalization may facilitate long-term depression, implicating spine-invading dynamic microtubules in mechanisms for decreasing as well as increasing synaptic strength.

Neurological disease

Given the critical roles of microtubules in synaptic transmission and plasticity, it is not surprising that alterations in microtubule dynamics are associated with neurodevelopmental and neurodegenerative diseases characterized by synaptic dysfunction. For instance, increased microtubule stability is observed in a mouse model of fragile X syndrome, a genetic disorder and form of intellectual disability caused by silencing of the *FMR1* gene encoding the RNA-binding protein FMRP (fragile X mental retardation protein) [111]. Microtubule hyperstability in Fmr1 knockout animals is linked to de-repression of the microtubule-stabilizing protein MAP1B, one of the many neuronal and synaptic proteins whose translation is regulated by FMRP [112–114]. Pathogenic mutations in another protein important for dynamic microtubule dis/assembly, tubulin-specific chaperone D (TBCD), are associated with autism, epilepsy, and early-onset encephalopathy [115]. Although it is not known whether synaptic microtubules are affected by TBCD mutations, these genetic findings directly link loss of microtubule dynamics both to synaptic dysfunction and neurodevelopmental disease. Alterations in microtubule stability and spine loss are also notoriously associated with Alzheimer's disease (AD). A key pathogenic event in AD is hyperphosphorylation of the MAP Tau, leading to its dissociation from microtubules. Loss of Tau binding reduces the labile domain of microtubules [116], likely affecting plasticity by reducing the number of dynamic microtubules available to invade synapses. Exposure of hippocampal neurons to oligomeric Aβ, mimicking conditions in the AD brain, also promotes microtubule stabilization upstream of Aβ- and Tau-dependent spine and synapse loss [46]. Given the many synaptic roles of dynamic microtubules, these findings suggest that hyperstabilization of dynamic microtubules engaged in synaptic functions may be a key precipitating factor at the earliest stages of AD pathology.

Concluding remarks

Within the last 10 years, great strides have been made in revealing multiple functions of microtubules in both the pre- and postsynaptic compartments, yet a slew of questions remain. For instance, it is not known whether microtubule nucleation can occur on demand within spines, as has been shown within presynaptic terminals [55]. How presynaptic microtubule nucleation is regulated by synaptic activity and why specific boutons activate microtubule nucleation on neuronal firing remains to be established. Also unknown is whether presynaptic microtubule nucleation is conserved at other synapse types, an issue that may be of relevance for dopaminergic and cholinergic synapses that exhibit 'volume neurotransmission' through the extrasynaptic space that reaches multiple targets. Whether dynamic microtubules control the activity-dependent recruitment of other organelles, MAPs, RNA granules, and ribosomes to synapses also remains to be determined. Finally, how microtubule and actin dynamics are coordinated at synaptic contacts to regulate neurotransmitter release and plasticity is still poorly understood. Spatiotemporally restricted manipulation of microtubule dynamics using caged microtubule drugs [117], photoswitchable compounds [118,119], or chromophore-assisted laser inactivation will be required for functional studies aimed at dissecting the primary from secondary consequences of microtubule perturbation at synapses.

Microtubule dynamics, stability, and tubulin PTMs have been implicated in many neurodegenerative diseases, and synapse loss is often the best correlate of their clinical severity. Untangling the mechanisms of synaptotoxicity at a prodromal stage of disease is a critical challenge for developing effective therapeutics and preventive measures. Whether perturbation of

microtubule dynamics at synapses directly triggers their dysfunction and/or loss remains an open question and an exciting new area of investigation.

Author contributions

All authors have contributed to the design, literature research, writing, and editing of the review article. We apologize to all the contributors whose work has not been cited in this review because of space limitations.

Conflict of interest statement

Nothing declared.

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