Of microtubules and memory: implications for microtubule dynamics in dendrites and spines

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ABSTRACT Microtubules (MTs) are cytoskeletal polymers composed of repeating subunits of tubulin that are ubiquitously expressed in eukaryotic cells. They undergo a stochastic process of polymerization and depolymerization from their plus ends termed dynamic instability. MT dynamics is an ongoing process in all cell types and has been the target for the development of several useful anticancer drugs, which compromise rapidly dividing cells. Recent studies also suggest that MT dynamics may be particularly important in neurons, which develop a highly polarized morphology, consisting of a single axon and multiple dendrites that persist throughout adulthood. MTs are especially dynamic in dendrites and have recently been shown to polymerize directly into dendritic spines, the postsynaptic compartment of excitatory neurons in the CNS. These transient polymerization events into dendritic spines have been demonstrated to play important roles in synaptic plasticity in cultured neurons. Recent studies also suggest that MT dynamics in the adult brain function in the essential process of learning and memory and may be compromised in degenerative diseases, such as Alzheimer's disease. This raises the possibility of targeting MT dynamics in the design of new therapeutic agents.

DYNAMIC MICROTUBULES IN NEURONAL DENDRITES
It is difficult to comprehend how dynamic cells are at the molecular level. The cytoplasm of a living cell consists of a tightly packed assemblage of proteins, lipids, organelles, and nucleic acids in a constant dance. Certain proteins within cells—most notably tubulin, actin, intermediate filaments, and septins—have the ability to form polymeric structures that give rise to what is termed the cytoskeleton. Although the name suggests a rather stable network of filaments, the cytoskeleton is anything but (Uchida and Shumyatsky, 2015). The cytoskeleton is in constant motion through subunit exchange, assembling and disassembling via intrinsic mechanisms inherent in each type of polymer and regulated by myriad polymer-associated proteins. Many years of research on the cytoskeleton have led to the appreciation of its dynamics. Here I focus on one cytoskeletal polymer, the microtubule (MT), and discuss how MT dynamics might play an important role in brain function. I then discuss how MT dynamics in dendrites might be part of the mechanism that influences memory and the implications of this for neurodegenerative disease.

MTs are polar polymers comprising α/β-tubulin dimers that assemble end to end at centrosomes with the aid of the γ-tubulin complex (Oakley et al., 2015) to form tubules usually consisting of 13 protofilaments. The plus ends of MTs stochastically switch from growing to shrinking and back to growing via a process termed dynamic instability, and the minus ends are generally associated with the centrosome (Mitchison and Kirschner, 1984). Dynamic instability is believed to allow MTs to interrogate all regions of a cell in a very efficient manner (Mitchison and Kirschner, 1984). Although MT dynamic instability is stochastic, it is regulated by many MT-associated proteins (MAPs). Different MAPs can either stabilize or destabilize MTs and help convert growing MTs into shrinking MTs (catastrophe) or visa versa (rescue; Akhmanova and Steinmetz, 2015; Bowne-Anderson et al., 2015). Of importance, MTs also serve as the primary substrate for trafficking material throughout the cell. This transport is accomplished through activation of motor proteins. Generally speaking, kinesin motor proteins transport cargo toward the plus...
ends of MTs, whereas cytoplasmic dynein moves cargo toward the minus ends of MTs (Verhey et al., 2011; Cianfrocco et al., 2015).

The highly elongated and polarized shape of neurons has resulted in a distinctive MT array. Instead of being attached to the centrosome, MTs are severed from centrosomes (Yu et al., 1993; Roll-Mecak and McNally, 2010) and exist in axons and dendrites as autonomous structures with a dynamic plus end and a stable minus end. Once released from the centrosome, the minus ends of MTs are stabilized by the CAMSAP/Patronin family of proteins (Marcette et al., 2014; Richardson et al., 2014; Yau et al., 2014). New research also indicates that MTs can be nucleated throughout the neuron via the augmin and γ-tubulin ring complexes (Sanchez-Huertas et al., 2016). In axons, MTs are oriented with the plus ends away from the cell body, whereas in dendrites, MTs are of a mixed polarity, with both minus and plus ends oriented toward the cell body (Baas et al., 1988; Figure 1). Neurons are postmitotic, highly polarized cells that are maintained throughout the life of an organism. In addition, neurons are particularly rich in structural MAPs that stabilize and bundle MTs (Dehmelt and Halpain, 2005; Halpain and Dehmelt, 2006). Hence it has generally been believed that the MT cytoskeleton in neurons was stable, in order to provide both structural stability and tracks on which to transport important cargoes long distances in axons and dendrites.

Nevertheless, studies from two decades ago by Black and colleagues indicated that both dendrites and axons contain a dynamic fraction of MTs, with dendrites having preferentially more dynamic MTs (Baas et al., 1991; Brown et al., 1993). Although the plus ends of MTs were shown to be the exclusive end of the MT that incorporated tubulin dimers (Baas and Ahmad, 1992), it was not until the discovery and fluorescent labeling of +TIP proteins, which specifically label the growing ends of MTs, that we truly appreciated just how dynamic MTs could be in cells (Perez et al., 1999). The latter study, however, was conducted with an immortalized cell line. Therefore it was not obvious that MTs in primary neurons would behave in a manner similar to this cell line because of the morphological and physiological constraints of highly polarized neurons. In the first report of labeled +TIPs in neurons, an EB3–green fluorescent protein (GFP) fusion protein was used to track polymerizing MTs. Results indicated similar velocities of MT polymerization in axons and dendrites of 2– to 6–d in vitro (DIV) primary hippocampal neurons, 10– to 17–DIV Purkinje neurons, and Cos1 cells (Stepanova et al., 2003). Further studies in mature cultured primary hippocampal and cortical neurons (63DIV) demonstrated substantial EB3–GFP–labeled polymerizing MTs in dendrites (Hu et al., 2008). Of importance, recent studies showed that EB3-labeled MTs are also dynamic in cortical neuron dendrites in living brains of adult mice (Kleele et al., 2014; Yau et al., 2016). Together these data indicate that CNS neurons in culture and in vivo maintain a proportion of their dendritic MTs in a dynamic state.

None of these studies quantified the percentage of MTs in dendrites that are dynamic, which cannot be accomplished with EB3 labeling because EB3 labels only growing MTs, not stable or depolymerizing MTs. In addition, the packing of MTs within the cylindrical dendrite makes it difficult to tease out individual MTs even with superresolution microscopy techniques. Nevertheless, based on the foregoing work, it is likely that most, if not all, individual MTs in dendrites (and axons) have a stable minus end and a dynamic plus end but may differ in the length of stable and dynamic regions along individual MTs (reviewed in Baas et al., 2016). Further advances in superresolution imaging, computational methods, and correlative electron microscopy may have to be implemented to determine the dynamic/stable state of the population of MTs in mature neurons.

**Microtubule Dynamics in Learning and Memory**

The fact that MTs are dynamic in mature neurons raises the question of what function their dynamics might serve in a highly polarized and relatively stable neuronal structure. One possibility is that neurons, like all cells in the body, must respond to changes that occur within the organism, and MT dynamics may be an important factor in this cellular plasticity. Plasticity is especially important for neurons because they must maintain their extreme polarity but also undergo changes throughout the life span of the organism. Given that most neurons in the mammalian brain are postmitotic, one area where plastic changes occur is through the synapse, the junction between the presynaptic axonal bouton and the postsynaptic dendritic spine (Figure 2). Morphological and molecular changes to the synapse, both presynaptically and postsynaptically, are widely regarded as the substrate for learning and memory (Xu et al., 2009; Yang et al., 2009; Hubener and Bonhoeffer, 2010). Thus the plasticity of dendritic spines undoubtedly plays a key role in the proper functioning of the brain, and defects in spine plasticity are often associated with disease and neurodegeneration (Sala and Segal, 2014).

Several recent studies have begun to suggest that MT dynamics in dendrites play important roles in brain function and disease. Long-term potentiation (LTP), a stimulation protocol that mimics memory formation ex vivo and can modify memories in living mice (Nabavi et al., 2014), is affected by changes in MT dynamics. Pharmacological stabilization of MTs with paclitaxel...
FIGURE 2: Microtubules are capable of polymerizing into dendritic spines. A group of mature CNS neurons showing dendritic spines located along both apical and basal dendrites. The axon is colored green. Green horizontal rods indicate axons from other neurons growing perpendicular to apical dendrites. Inset shows two spines along a dendrite synapsing onto two perpendicular axons. Presynaptic spines are shown in axons. In this example, one dynamic microtubule polymerizes into the right dendritic spine, extending well into the head of the spine.

(Shumyatsky et al., 2005) or inhibition of MT polymerization with nanomolar (Jaworski et al., 2009) or micromolar (Barnes et al., 2010) concentrations of nocodazole abrogates LTP. One important caveat of these studies is that they all used bath application of MT drugs, which affects both axonal (presynaptic) and dendritic (postsynaptic) MTs, as well as glial MTs. It is therefore not possible to determine exactly what MT-dependent processes affect LTP. Nevertheless, these data suggest that MTs might play a key role in memory formation or retention.

Studying MT dynamics directly in living animals is exceedingly difficult; however, ingenious methods have been used to determine whether MT dynamics function in learning and memory in the intact organism. By knocking out stathmin, a protein that binds tubulin and inhibits MT polymerization, Shumyatsky et al. (2005) demonstrated that affecting MT stability results in deficits in LTP, in addition to both learned and innate fear in mice. A subsequent study by another group implemented a stable isotope method to label newly synthesized tubulin, which is incorporated into MTs undergoing dynamic instability (Fanara et al., 2010). By purifying the tau-associated (a proxy for axonal MTs) and MAP2-associated (a proxy for dendritic MTs) MT fractions, as well as cold-stable MT fractions (hyperstable MTs generally only present in neurons), this group showed that contextual fear conditioning resulted in increased turnover of the MAP2 and cold-stable fractions (Fanara et al., 2010). Inhibition of MT polymerization by nocodazole injection inhibited freezing in the contextual fear paradigm, whereas inclusion of paclitaxel (to stabilize MTs) or brain-derived neurotrophic factor (BDNF) rescued freezing. Similarly, nocodazole inhibited the increase in spine density in hippocampus and cortex after contextual fear conditioning, and these effects were rescued by paclitaxel or BDNF. Although it is not possible to pinpoint directly which cells and what subcellular changes in MT stability occur in this study, it is still intriguing that disrupting MTs can affect learning and memory.

More recent evidence, focused on a specific MT-associated protein, showed that learning can induce a stathmin phosphorylation–dependent biphasic change in MT stability (Uchida et al., 2014). Using contextual fear conditioning, followed by synaptosomal preparations and quantitative blotting, this group showed that there was an increase in tyrosinated tubulin, indicative of new/dynamic MTs, 30 min after training, followed by an increase in detyrosinated tubulin, indicative of older/stable MTs, 8 h after training. Of interest, they could pharmacologically disrupt memory formation by intra-hippocampal injection of paclitaxel in the early phase and inhibit or enhance the late phase of MT hyperstability by injecting nocodazole or paclitaxel, respectively. Furthermore, they showed that stathmin regulates transport of the GluA2 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor via KIF5, resulting in increased GluA2 at synaptic sites, which promoted long-term memory. This idea of an early phase in which MTs are hyperdynamic, followed several hours later by a phase in which MTs are hyperstable is intriguing and suggests, like the Fanara et al. (2010) study, that manipulating MT dynamics can affect learning and memory.

MICROTUBULE DYNAMICS AND DENDRITIC SPINES

If dendritic spine plasticity and MT dynamics are both important for learning and memory, is there a direct connection between MTs and spines? It is well established that MTs form a dense, overlapping array in the dendrite shaft. In fact, one would be hard pressed to find a single MT entering a dendritic spine in fixed cultures either by fluorescence light microscopy or electron microscopy (EM), whereas actin filaments are highly concentrated within dendritic spines but sparse within dendrite shafts. Previous research corroborated the idea that these cytoskeletal elements maintain separate domains within the dendrite. Early studies using fluorescently labeled actin and the MT-associated protein MAP2C in both live and fixed hippocampal neurons found that these two cytoskeletal elements did not overlap (Kaeck et al., 1997, 2001). Similarly, EM studies demonstrated that MTs could enter the complex—branched CA3 spines that synapse onto mossy fibers in the hippocampus (Chicurel and Harris, 1992)—but this seemed to be the exception rather than the rule. In addition, brains from perfused animals never contained MTs in dendritic spines (Fiala et al., 2003). Early EM studies did document the presence of MTs in dendritic spines of cortical neurons, but these studies were discounted based on the unusual technique of dissecting out brain sections into 20% bovine serum albumin/water before fixation (Westrum et al., 1980, 1983; Gray et al., 1982). Thus the bulk of data favored the interpretation that MTs and actin filaments did not overlap, suggesting the transport of any cargo by kinesin and dynein along MTs in the dendrite shaft must be "handed off" to actin filaments below the base of spines to be transported via myosin into the spine (Ryan et al., 2005; Guillaud et al., 2008).

Could these studies have missed otherwise dynamic MTs interacting with actin filaments in dendritic spines? Recent data from several labs suggest that this is in fact the case. Four independent studies published within a year of one another demonstrated that MTs indeed enter spines in a synaptic activity–dependent manner (Gu et al., 2008; Hu et al., 2008; Mitsuyama et al., 2008; Jaworski et al., 2009). It is also clear, as demonstrated by live-cell microscopy, that all of the MT polymerization events into spines are transitory, averaging only a few minutes at a time (Hu et al., 2008, 2011;
Jaworski et al., 2009; Kapitein et al., 2011; Merriam et al., 2011, 2013; Wagner et al., 2011; McVicker et al., 2016). Moreover, the presence of MTs in spines in fixed neurons required rapid fixation and the use of MT-stabilizing buffer (Gu et al., 2008; Hu et al., 2008; Mitsuyama et al., 2008). Thus the prevalence of dynamic MTs in dendrites allows them to specifically polymerize into individual spines throughout the life of the neuron. Such behavior by dendritic MTs opens up the exciting possibility that they could be directly influencing selected dendritic spines through the host of proteins that associate with actively polymerizing MTs and through the cargo transported along these MTs into and out of dendritic spines.

Why did previous studies fail to detect these dynamic MTs? In regard to the live-cell imaging studies mentioned earlier, overexpression of MAP2C may have stabilized dendritic MTs. Furthermore, the sensitivity of the camera and the infrequent imaging interval that was used also may have limited the likelihood of detecting these dynamic MTs (Kaecch et al., 1997, 2001). The more recent studies used GFP-labeled +TIP proteins (EB3) and/or tubulin, which provided enhanced signal-to-noise ratio to detect MT dynamics, and, unlike classical MAPs, would not overstabilize MTs. In addition, standard EM techniques may be insufficient for detecting MTs in dendritic spines. As mentioned earlier, each MT in a mature dendrite is likely to have a stable region at the minus end and a dynamic region at the plus end. Perfusion of brains in preparation for EM requires time for the fixative to perfuse through the capillaries and into brain tissue. It is likely that during this time, the most dynamic regions of MTs will depolymerize. Thus standard animal perfusion protocols will cause depolymerization of the most dynamic ends of MTs and result in the lack of MTs in spines in electron micrographs, even though the more stable sections of MTs appear in the dendritic shaft. It is unclear whether perfusing brains with MT-stabilizing fixative would be sufficient to preserve dynamic MTs entering spines. Future studies using two-photon confocal microscopy and cranial window imaging of fluorescently labeled MTs in neurons in the intact brain will have to be performed to determine whether MTs enter dendritic spines in living animals.

**FIGURE 3:** Events resulting in microtubule polymerization into a dendritic spine. (1) In the basal state, microtubules, tipped by “comets” of +TIP proteins (EB3 shown here), actively polymerize throughout the dendrite (as well as the axon; not shown). Actin and the actin-binding protein drebrin are concentrated in spines. (2) As the action potential makes its way down an axon (red in second frame), resulting in neurotransmitter release at the synapse, calcium influx in the postsynaptic spine occurs. (3) Within seconds to minutes after calcium spikes in the spine, actin polymerization occurs in the spine head and neck and can extend into the dendritic shaft. The spine head increases in size due to the increased actin polymerization. Increased actin also concentrates drebrin, which interacts with EB3 protein at the tips of polymerizing microtubules in the vicinity of the spine. (4) This drebrin–EB3 interaction results in the increased probability that the polymerizing microtubule will enter the spine (on the right). The microtubule can extend well into the spine head, often to the postsynaptic density.

**HOW DO MICROTUBULES ENTER SPINES?**

The dynamicity of MTs in dendrites does not necessarily predict that they would polymerize into dendritic spines. After all, dendritic spines are generally oriented perpendicular to the dendrite shaft, and the necks of spines are an order of magnitude thinner than the parent dendrite, with diameters ranging from 50 to 250 nm (Bourne and Harris, 2008; Tonnesen et al., 2014). Moreover, MTs generally polymerize in a relatively straight line, unless they encounter organelles or other noncompliant structures within cells. It is likely that MTs that enter dendritic spines polymerize from a stable portion of an existing MT. However, this would require the correct orientation (perpendicular to the dendrite shaft) of the polymerization event. When Merriam et al. (2013) recorded plus-end MT dynamics at rapid intervals with the +TIP protein EB3, they found that most of the MTs that polymerized into dendritic spines originated within 2 μm of the spine they invaded, suggesting that their entry into spines was locally regulated. Indeed, both a spike of calcium within the spine head and neck and subsequent actin polymerization were required for MT polymerization into spines (Merriam et al., 2013; Figure 3). Therefore MTs appear to specifically target spines that are undergoing activity-dependent changes in a regulated manner rather than polymerizing into a small subset of spines at random.
Actin polymerization in the spine neck and protrusion into the dendritic shaft might provide a sufficient “gate” to allow MTs polymerizing near a spine to follow the actin filaments into the spine (Figure 3). There is precedent for actin filaments interdigitating with dendritic MTs in platinum replica electron micrographs (Korobova and Svitkina, 2010), potentially providing a substrate upon which MTs could enter spines. Although MTs may polymerize along actin filaments, the interaction is unlikely to be direct. Instead, actin–MT interactions occur through many different actin- and MT-associated proteins (Coles and Bradke, 2015; Cammarata et al., 2016). The +TIP protein EB3, upon entering spines at the tip of polymerizing MTs, stabilizes the actin-associated protein p140Cap in the postsynaptic density (Jaworski et al., 2009). In a separate study, EB3 and the actin-bundling protein drebrin were found to interact in developing axonal growth cones (Gerald et al., 2008). Drebrin is highly expressed in dendritic spines and is enhanced in the hippocampus after LTP (Fukazawa et al., 2008; Ivanov et al., 2009). Recent experiments in dendrites confirmed that drebrin was a key actin-associated protein involved in MT polymerization into spines (Merriam et al., 2013; Figure 3). Thus there is likely to be a complex interplay among actin- and MT-associated proteins in the spine neck and head. Of importance, polymerizing MTs must be recruited into specific dendritic spines rather than stochastically polymerizing into any spine. This recruitment of MTs into spines involves N-methyl-D-aspartate–dependent calcium influx, signaling cascades resulting in actin polymerization, and interaction of MT +TIP proteins (EB3) with actin-associated proteins in the spine neck (drebrin) and head (p140Cap; Jaworski et al., 2009; Merriam et al., 2011, 2013; McVicker et al., 2015). Further research will be required to determine whether other proteins associated with actin–MT interactions are involved in MT entry of spines.

Once the MT enters a spine, it is only present for a short period of time, depolymerizing out of the spine on the order of a few minutes (Hu et al., 2008). MTs can target the same spine multiple times, but each entry is transient. Moreover, MTs target spines of different shapes, including mushroom, thin, and stubby spines, as well as dendritic filopodia (Gu et al., 2008; Hu et al., 2008; Jaworski et al., 2009). It is not known whether MTs can polymerize into all spines on a dendritic tree or certain spines are specifically targeted but others are not. Nevertheless, synaptic activity influences MT polymerization into dendritic spines in a direct manner. More synaptic activity results in more spines targeted by MTs (Hu et al., 2008). Moreover, the time that MTs remain in spines can be lengthened by addition of BDNF (Hu et al., 2011), and the frequency of polymerization into spines and the percentage of spines targeted increases after the induction of LTP (Merriam et al., 2011) and decreases after induction of long-term depression (LTD; Kapitein et al., 2011). Because MTs are the primary cytoskeletal polymers that transport cargo within cells and LTP increases spine size but LTD decreases spine size (Tada and Sheng, 2006), it is possible that MTs may transport specific cargoes into or out of dendritic spines during synaptic plasticity.

**WHAT CARGOES ARE MICROTUBULES TRANSPORTING INTO OR OUT OF SPINES?**

There are a number of ways in which material could enter dendritic spines (Figure 4). Transmembrane receptors or proteins that are associated with these receptors can be exocytosed in the dendrite shaft and diffuse into the spine in the plane of the membrane (Gray et al., 2006; Huganir and Nicoll, 2013). Cytoplasmic proteins can diffuse into spines from the dendrite shaft (Rose et al., 2009). Both of these mechanisms would be efficient for entry of material into spines but may not provide a very targeted way for cargo to enter particular spines that are undergoing plastic changes versus adjacent spines that are not being potentiated.

To circumvent this lack of specificity, it is possible that cargo could associate with both MT- and actin-based motor proteins. For example, cargo could be shuttled anterogradely in a dendrite via the motor protein kinesin or dynein, given that dendrites contain MTs of mixed polarity (Baas et al., 1988). However, once the cargo reaches the spine for which it is destined, the MT-based motor could “hand off” the cargo for transport via a myosin-based motor along the actin filaments at the base or in the neck of the spine (Figure 4). The cargo would then use myosin motors to enter the dendritic spine. In fact, this type of hand-off is likely to occur for the entry of...
endoplasmic reticulum (ER) into dendritic spines. A study showed that in cerebellar Purkinje neurons, ER enters dendritic spines via myosin-V-based transport (Wagner et al., 2011). Surprisingly, although the ER is intimately associated with MTs in the dendrite shaft, MT entry of Purkinje spines was not associated with ER entry, whereas knockdown of myosin-V abolished ER entry (Wagner et al., 2011). Studies in our lab using hippocampal neurons confirmed that ER entry of spines is not a MT-based process (unpublished data). Moreover, our recent work suggests that mitochondria may also be using this actomyosin hand-off model to enter dendritic spines (McVicker et al., 2016; Figure 4). This raises a question: if MTs are not transporting ER into spines, then what are the potential cargoes entering spines along MT tracks?

Given that one of the primary functions of MTs in all cells is to act as railways for the transport of cargo throughout the cell, it is likely that transient MT polymerization into dendritic spines acts as a path for the delivery and/or removal of cargo that play a role in spine plasticity. However, MT polymerization into spines is transient, with a dwell time of only a few minutes. Is this enough time to transport cargo into or out of spines? The likely answer is yes. Both dynein and dynein motor proteins can move at speeds of ∼1 μm/s (Hammond et al., 2009), and dendritic spines are only a few micrometers long. Thus, if a MT is present in a spine for only a few seconds, the speed of dynein and kinesin-based transport would be sufficient to transport material either into or out of spines along a MT.

What cargoes might use MTs to enter or exit spines? PSD-95 is an important postsynaptic scaffolding protein that is directly associated with synaptic strength (El-Husseini et al., 2000; Ehrlich et al., 2007) and is increased in spines after BDNF treatment (Yoshii and Constantine-Paton, 2007). Hu et al. (2011) showed that although PSD-95 was not directly trafficked along MTs into dendritic spines, polymerization of MTs into spines was necessary for the increase in PSD-95 after BDNF treatment. A more recent study showed that recycling endosomes could use MTs to enter dendritic spines, but their primary method of entry was through myosin-V-based transport (Esteves da Silva et al., 2015). Thus a particular cargo has yet to be demonstrated to specifically use MTs to enter dendritic spines.

Given that MTs polymerize into spines plus end leading, it is likely that kinesin motor proteins will convey cargo into spines and cytoplasmic dynein will convey cargo out of spines along MTs. One study showed cytoplasmic dynein and neurofilin leaving a dendritic spine together, but MTs were not imaged simultaneously (Schapitz et al., 2010). These data suggest that dynein can transport neurofilin out of spines along MTs, but definitive proof of motor/cargo transport out of spines along MTs will require simultaneous imaging with tubulin. However, a recent study documented a motor/cargo pair that uses MTs for entering dendritic spines. McVicker et al. (2016) showed that the kinesin 3 motor KIF1A transports the synaptic tagtomin (syt) family member syt4 directly into spines along dynamic MTs. Once the syt4-containing vesicle enters a spine, it can exocytose in the spine head (Figure 4). Entry of this KIF1A/syt4 motor/cargo pair is dependent on synaptic activity and is regulated by homeostatic plasticity. Moreover, this work showed that knockdown of KIF1A paradoxically results in increased syt4 entry and exocytosis in dendritic spines, as well as in the dendrite shaft. This increase in cargo exocytosis appears to occur because the syt4-containing vesicles are no longer tethered to the MTs in the dendrite shaft via KIF1A. The increased exocytosis that occurs throughout the dendritic arbor is followed by movement of exocytosed syt4 within the plane of the membrane. Together these results indicate that MTs are indeed capable of transporting specific cargo into targeted dendritic spines, and, of importance, this directed targeting of cargo is highly disrupted in the absence of a particular motor protein, KIF1A (McVicker et al., 2016). Thus not only are MTs important for transporting material throughout the dendritic arbor, but they are also instrumental in sequestering vesicles and cargo away from the plasma membrane while it is en route to its destination.

IMPLICATIONS FOR DISEASE AND NEURODEGENERATION

The aforementioned studies suggest that MTs change their dynamics in dendrites during episodes of learning and memory and that pharmacological manipulation of MT dynamics can have a pronounced effect by either enhancing or disrupting the process of learning and memory. If this is the case, then it follows that diseases that are known to affect MTs or microtubule-associated proteins (MAPs) in dendrites may be amenable to therapeutics that target MT dynamics. Indeed, paclitaxel has been used extensively in treatment of multiple types of cancers and is beginning to be used for nerve injury (Baas and Ahmad, 2013). Unfortunately, paclitaxel treatment results in peripheral neuropathy (Gornstein and Schwarz, 2014) and does not easily cross the blood–brain barrier (Fellner et al., 2002). However, new MT-stabilizing agents have been developed with more desirable properties (Brunden et al., 2014).

One drug that has been tested in a number of animal models is epothilone D (EpoD; BMS-231027; Kolman, 2004). EpoD has been shown to be much more brain penetrant than paclitaxel (Andriech et al., 2006; Brunden et al., 2010). Moreover, since several neurodegenerative and psychiatric diseases, including Alzheimer’s, Parkinson’s, amyotrophic lateral sclerosis, schizophrenia, and depression, appear to have etiologies associated with destabilization of MTs, drugs that are capable of entering the brain and stabilizing MTs might have a use as therapeutic agents (Brunden et al., 2014). For example, several recent studies have shown that EpoD is effective in ameliorating axonal dysfunction, neurotoxicity, cognitive deficits, and pathology in two different Alzheimer’s mouse models (Brunden et al., 2010; Barten et al., 2012; Zhang et al., 2012). These results suggest that EpoD may provide important therapeutic benefits for human patients.

To test the therapeutic potential of EpoD, a phase 1 clinical trial was conducted in 2012–2013 by Bristol-Myers Squibb to evaluate the effects of EpoD on cerebral spinal fluid (CSF) biomarkers in patients with mild Alzheimer’s disease (clinicaltrials.gov/ct2/show/NCT01492374). One of the issues with EpoD and other MT-stabilizing drugs such as paclitaxel concerns the adverse side effects of the drug when given at doses effective for cancer treatment, which include neutropenia and peripheral neuropathy (Brogdon et al., 2014). However, for this study, EpoD was dosed less frequently and at lower concentrations than in previous cancer therapy trials and was generally well tolerated by patients. Unfortunately, EpoD showed little change in CSF biomarkers (tau N-terminal fragments) during the 9-wk dosing regimen, and further clinical trials were abandoned. In studies with Alzheimer’s model mice, EpoD took 11 wk to inhibit incorporation of newly synthesized tubulin into MTs (an indirect measure of MT dynamics) and reduce tau pathology (Barten et al., 2012). Thus it is unlikely that efficacy in humans would have been detected in only 9 wk in the clinical trial of EpoD. Several other MT-stabilizing compounds, including CNDR-51657 (Kovalevich et al., 2016), continue to be evaluated in Alzheimer’s disease mouse and cell models, with some encouraging results. However, it is unclear whether stabilizing MTs will prove to be an effective therapy for diseases such as Alzheimer’s.

Although stabilizing otherwise hyperdynamic MTs in Alzheimer’s disease may prove to be an effective treatment in the future, it is important to consider that MT dynamics in all cell types in the body
will be affected by systemic administration of a pharmacological compound like EpoD. Therefore systemic treatments need to be well tolerated and not induce other toxic side effects (e.g., peripheral neuropathy, neutropenia; Baas and Ahmad, 2013). Moreover, it is unclear how MT-stabilizing compounds affect MT dynamics in other cells of the brain, including astrocytes, oligodendrocytes, and microglia. Because MTs in glial cells polymerize faster than neurons (Stepanova et al., 2003) and have fewer markers for long-lived MTs, such as detyrosination and acetylation (unpublished data), it is likely that MT dynamics in glial cells will be substantially affected by such drugs, which may also affect brain function.

CONCLUSIONS

This is an exciting time in the field of cytoskeletal dynamics. Given that a portion of MTs remain dynamic for the life of the neuron and are capable of polymerizing into dendritic spines raises new questions of how MT dynamics may affect fundamental processes in the brain, such as learning and memory. Moreover, data indicating that MT dynamics is compromised in developmental or neurodegenerative diseases suggest novel avenues for intervention. Nevertheless, we should proceed cautiously when administering potential therapeutic agents that affect MT dynamics. Although neuronal MTs may be more labile in diseases such as Alzheimer’s, stabilization of MTs must be carefully controlled so as not to over-stabilize these polymers, which will likely compromise learning and memory. Going forward, it will also be important to understand how such pharmacological agents affect MT dynamics in other cells of the brain and how stabilizing MTs in these cells affects their functions. Although MTs and their inherent dynamics may prove to be useful targets for therapeutic agents, MT dynamic instability may prove to be too broad of a target. Instead, it might be more prudent to devote time and effort to designing compounds that target specific MT-associated proteins, which may provide more precise control and have fewer side effects. Clearly, a more thorough understanding of the interplay between MT dynamics, MAPs, and other cellular components will provide an essential foundation on which to build our knowledge and pursue the most efficacious interventions.

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