Published in final edited form as:

Dev Neurobiol. 2015 April ; 75(4): 423–434. doi:10.1002/dneu.22227.

Signaling to the microtubule cytoskeleton: An unconventional role for CaMKII

Derrick P. McVicker¹, Matthew M. Millette², and Erik W. Dent^{1,2}

¹Department of Neuroscience, University of Wisconsin, Madison, WI 53705, USA

²Neuroscience Training Program, University of Wisconsin, Madison, WI 53705, USA

Abstract

Synaptic plasticity is a hallmark of the nervous system and is thought to be integral to higher brain functions such as learning and memory. Calcium, acting as a second messenger, and the calcium/calmodulin dependent kinase CaMKII are key regulators of neuronal plasticity. Given the importance of the actin and microtubule (MT) cytoskeleton in dendritic spine morphology, composition and plasticity, it is not surprising that many regulators of these cytoskeletal elements are downstream of the CaMKII pathway. In this review we discuss the emerging role of calcium and CaMKII in the regulation of MTs and cargo unloading during synaptic plasticity.

Keywords

CaMKII; Kinesin; Microtubules; Neuron; Plasticity

Introduction

A key characteristic of the brains of higher organisms is their ability to undergo plasticity throughout their lifespans. Synaptic plasticity encompasses the strengthening and weakening of existing synaptic connections and formation of entirely new ones. Pre-synaptic axonal boutons and post-synaptic dendritic spines are regulated in tandem and distinct changes at the cellular and molecular level affect the strength of these connections. It is widely believed that these changes comprise the basis of learning and memory. In the laboratory, these plastic changes can be induced through manipulations termed long term potentiation (LTP) and long term depression (LTD). Many changes occur following induction of LTP. A critical early event is the influx of calcium into the dendritic spine through NMDA receptors (Fink and Meyer, 2002). Subsequent signaling events downstream of calcium influx result in major rearrangement of the actin cytoskeleton (Cingolani and Goda, 2008; Fifkova and Delay, 1982; Kasai et al., 2010), increased spine size (Harris et al., 1992; Kasai et al., 2010), and increased post-synaptic density (PSD) components such as AMPA receptors (Brown et al., 2010; Makino and Malinow, 2009; Shi et al., 1999). Emerging evidence has also begun to implicate a direct role for microtubules (MTs) in synaptic plasticity (Gu et al., 2008; Hu

Corresponding author: Erik W. Dent, Department of Neuroscience, University of Wisconsin, School of Medicine and Public Health, 5431 Wisconsin Institute for Medical Research, 1111 Highland Ave., Madison, WI 53705, USA.

The authors declare they have no conflicts of interest.

et al., 2008; Jaworski et al., 2009; Kapitein et al., 2011; Merriam et al., 2011; Merriam et al., 2013). One such role of MTs is likely to involve transport of material directly into dendritic spines.

Calcium serves as an important second messenger in all cell types. Concentrations of free intracellular calcium are generally extremely low due to regulation by buffering proteins and sequestration by various organelles. This tight control enables elevations in calcium to exert both spatial and temporal control over many signaling cascades. For example, NMDA receptor-mediated calcium influx can result in opposite net effects, regulating proteins important for both long-term potentiation (LTP) and depression (LTD), depending on the duration and concentration of calcium influx. Many effects downstream of calcium are the result of calcium-dependent kinases. Of major importance and continued research focus is calcium/calmodulin-dependent kinase II (CaMKII). CaMKII is a serine/threonine protein kinase and, of its four known isoforms, α and β are brain-enriched. These isoforms can consist of multimers and freely hetero-oligomerize (Lantsman and Tombes, 2005; Lin and Redmond, 2008) to form active complexes. CaMKII β can act as a targeting molecule to localize CaMKII α/β oligomers to F-actin in dendritic spines, where it is highly enriched (Shen et al., 1998). Upon activation of spines during plasticity, CaMKII can translocate from actin in the spine head to the intracellular tail of NMDA receptors, thus becoming more enriched at the PSD.

In neurons, CaMKII remains in an inactive state through an autoinhibitory mechanism, but can be activated by an influx of calcium, that binds calmodulin and activates CaMKII, which allows for autophosphorylation of CaMKII at T286 and constitutive activation (Colbran and Brown, 2004; Coultrap et al., 2010). Induction of this constitutively active state may persist hours after the initial calcium signal has passed (Fukunaga et al., 1993; Ouyang et al., 1997; Ouyang et al., 1999). Activation of CaMKII β triggers its separation from actin and enables it to associate with other binding partners and continue its signaling cascade. In terms of the neuronal cytoskeleton and synaptic plasticity, CaMKII has been best studied in relation to its effects on actin. Both profilin (Ryan et al., 2005) and cofilin (Xie et al., 2007) can be phosphorylated by CaMKII through independent pathways, the net effect of both being an enhancement of actin polymerization. Evidence supports a role for inactive CaMKII, in cooperation with alpha-actinin (Nakagawa et al., 2004), as an actin crosslinking protein which inhibits polymerization (Hoffman et al., 2013) and gives rise to highly branched networks in a manner similar to the Arp2/3 complex (Hell, 2014). In total, it has been proposed that activation of CaMKII serves to trigger a period of dramatic actin reorganization following calcium influx, while an eventual return of CaMKII to its basal, autoinhibited state could be viewed as a process stabilizing these changes (Lin and Redmond, 2008; Okamoto et al., 2007). The importance of these structural effects of inactive CaMKII is further supported by studies showing that CaMKII β knockdown negatively impacts both dendritic spine morphology and density, while expression of a catalytically inactive CaMKII β rescues these effects (Okamoto et al., 2007). Pharmacological disruption of CaMKII β or deletion of CaMKII β in transgenic mice result in animals that exhibit severe learning impairment and are unable to undergo LTP or LTD (Borgesius et al., 2011).

Several excellent reviews have been published highlighting roles of CaMKII and actin in spine plasticity (Hell, 2014; Lee and Yasuda, 2009; Okamoto et al., 2009). In this review we will discuss roles of CaMKII in synaptic plasticity but will focus on a less conventional role, its signaling to MTs. CaMKII has recently been shown to bind to MTs near active synapses, indicating CaMKII may play a role in both the modification of the actin and MT cytoskeleton during plasticity (Lemieux et al., 2012). We will begin by discussing the possible functional consequences of this behavior and outline conditions that could preferentially direct CaMKII to MTs instead of the PSD or other locations. We will then discuss an additional means by which CaMKII controls MT stability through phosphorylation of specific MT associated proteins (MAPs). Several studies have also begun to illuminate the role of CaMKII signaling in MT-based motor protein transport. We will examine this possibility in mature neurons as it concerns NMDA receptor trafficking. Interestingly, the exact process underlying the delivery of cargo to specific dendritic spines or retrieval of material from spines undergoing plasticity is not well studied. Specifically, how is cargo that is shuttled through the dendrite released from MTs and directed into specific dendritic spines? We will outline two predominant theories, diffusion of materials into spines from the dendritic shaft, and a “hand-off” model involving coordination between MT and actin-based motor proteins. We will then summarize evidence in support of a third, “direct-deposit” mechanism which could be operating in parallel or in concert with these other means of trafficking

CaMKII regulation of MTs in dendritic shafts and spines

While the majority of literature focuses on the ability of CaMKII to modulate actin activity during LTP, there is also an emerging role for CaMKII in the regulation of MTs during synaptic activation (Lemieux et al., 2012). The activation of NMDA receptors by Gly-0Mg²⁺ treatment leads to the accumulation of expressed GFP-CaMKII at active postsynaptic sites where calcium levels are high, including the head and neck of the dendritic spine and regions of the dendrite at the base of active spines (Lemieux et al., 2012) (Figure 1A). This translocation is reversible and can be reduced by blocking NMDA receptors, sodium channels, and efflux of calcium from intracellular stores (Lemieux et al., 2012). When calcium levels are globally increased within the cell with a short pulse of KCl, it was noted that expressed GFP-CaMKII formed distinct fiber patterns in the soma and thicker proximal dendrites (Lemieux et al., 2012). After global activation, CaMKII appears to co-localize with MTs throughout the somatodendritic domain by immunocytochemistry and when mCherry-CaMKII is expressed with GFP-MAP2B, colocalization of these proteins is observed along MTs throughout this domain. In fact, the expression of MAP2B seems to enhance CaMKII localization to MTs (Lemieux et al., 2012) (Figure 1B). Fluorescence recovery after photobleaching (FRAP) experiments demonstrate that CaMKII is stably bound to the MT network after KCl treatment, compared to basal conditions and the MT depolymerizing drug nocodazole decreases CaMKII stability (Lemieux et al., 2012) (Figure 1C). These data, taken together, indicate CaMKII is directly and/or indirectly associating with MTs during synaptic activity. It is not known what CaMKII is doing at these locations, but it is likely physiologically relevant because the ability of the kinase to translocate to these areas leads to increased spine size and incorporation of AMPA receptors

into the spine heads (Lemieux et al., 2012; Opazo et al., 2010). It is possible that in response to local calcium influx, CaMKII binds to MTs and is then capable of phosphorylating MT associated proteins or molecular motors.

Lemieux *et al.* reports that CaMKII is localized to both the head of the spine and MTs at the base of active spines upon synaptic stimulation (Lemieux et al., 2012), but it has also been reported that after stimulation CaMKII translocates to the PSD and is retained in the spine head (Ding et al., 2013; Otmakhov et al., 2004). Additionally, CaMKII has been documented to propagate in a wave throughout the entire somatodendritic compartment (Rose et al., 2009). Currently it is not clear what conditions dictate the localization of CaMKII during plasticity but in the studies above the CaMKII concentrations appear to mirror high calcium signals. If the calcium signals are localized to individual spines, CaMKII only concentrates in those spines (Lemieux et al., 2012), whereas global calcium activation causes CaMKII to localize throughout the dendritic arbor (Lemieux et al., 2012; Rose et al., 2009). It is also not known, in cases where CaMKII is binding to MTs, if these are direct interactions or if CaMKII is binding more indirectly through another partner, such as MAP2. Furthermore, it is not clear if CaMKII affects MT structure or dynamics under these conditions. Although it has been reported that CaMKII can directly phosphorylate tubulin (Wandosell et al., 1986), it is probably more likely that any effect of CaMKII on MTs is indirect, by controlling of the phosphorylation state of various MAPs or motors, such as MAP2 and/or kinesin.

The Regulation of Kinesin by CaMKII

In addition to interacting with MTs, CaMKII has been shown to be a regulator of MT based motors of the kinesin superfamily. KIF17, a Kinesin-2 family member, is important for learning and memory (Wong et al., 2002) and is known to associate with vesicles containing NMDA receptors in neurons via the adaptor protein Mint1 (Setou et al., 2000). Co-immunoprecipitation experiments reveal that CaMKII is capable of binding KIF17 both *in vitro* and *in vivo* (Guillaud et al., 2008). Using fluorescence resonance energy transfer (FRET) techniques, Guillaud *et al.* also demonstrated that phosphorylation of KIF17 by CaMKII can disrupt the interaction between KIF17 and Mint1 (Guillaud et al., 2008), resulting in the release of cargo. Point mutations demonstrate that S1029 on KIF17 can act as a molecular switch, where S1029A mutants are incapable of releasing Mint1, while the phosphomimetic mutant S1029D cannot bind Mint1 (Guillaud et al., 2008). It has also been shown that upregulation of CaMKII in mice disrupts KIF17 transport and the trafficking of NMDA receptors (Liu et al., 2014). This evidence demonstrates that CaMKII can directly bind KIF17 and act as a cargo release mechanism allowing dissociation of vesicles from the motor.

In addition to KIF17, CaMKII is important for the regulation of another kinesin-2 family member KIF3. Phang *et al.* demonstrated that in NIH 3T3 or HeLa cells, the phosphatase POPX2 interacted with the KIF3 motor complex and overexpression of POPX2 caused a dramatic decrease in the velocity of the KIF3 cargo N-cadherin (Phang et al., 2013). Previous work had identified S690 on the C-terminal tail of KIF3 as a major phosphorylation site (Ballif et al., 2004). To test if this site was important for transport the

authors transfected cells with KIF3-S690A mutants and detected a decrease in the velocity of N-cadherin, while S690D mutants maintained velocities similar to WT KIF3 motors, demonstrating phosphorylation of this residue was important for motor function (Phang et al., 2013). *In vitro* kinase assays identified CaMKII as the kinase responsible for phosphorylating KIF3 at S690 and pharmacological inhibition of CaMKII also resulted in decreased KIF3 velocities (Phang et al., 2013). Unlike KIF17, however, phosphorylation of KIF3 had no effect on cargo binding (Phang et al., 2013). Although these experiments were not performed in neurons, KIF3 has been shown to be important for establishment of neuronal polarity (Nishimura et al., 2004), neurite extension (Setou et al., 2000) and regulation MT dynamics in growth cones (Gumy et al., 2013).

To date, kinesin-2 family members are the only kinesins known to be regulated by CaMKII, while the kinesin-1 family member, KIF5, is more directly influenced by calcium. Glutamate activation of NMDA receptors and the entry of calcium into the cell can recruit mitochondria to active synapses (MacAskill et al., 2009) by inhibiting both retrograde and anterograde transport of mitochondria near active spines (Rintoul et al., 2003). KIF5 is the major motor protein involved in the anterograde transport of mitochondria (Tanaka et al., 1998) and several adapter proteins, most notably Milton (Glater et al., 2006; Stowers et al., 2002) and Miro (Guo et al., 2005), are involved in linking kinesin to mitochondria. In dendrites, overexpression or siRNA-mediated knockdown of Miro1 increased or decreased the percentage of moving mitochondria, respectively (MacAskill et al., 2009). Furthermore, this group demonstrated that Miro1, upon binding calcium, caused the dissociation of the kinesin-1/Miro1 complex, releasing mitochondria from MTs (MacAskill et al., 2009).

CaMKII regulation of MT dynamics via MAPs

While many factors can affect MT polymerization and stability in neurons, the MT stabilizing proteins MAP2 and tau are key regulators of MT dynamics in the axon and dendrite, respectively. In dendrites, MAP2 binds MTs and is thought to be involved in increasing MT stability, reducing catastrophe events, and bundling MTs (Kim et al., 1979; Lewis and Cowan, 1990; Weisshaar et al., 1992). MAP2 has also been shown to associate and bundle F-actin, but it is not clear if it is capable of crosslinking actin and MTs (Dehmelt and Halpain, 2005; Roger et al., 2004). The regulation of these proteins is quite important during development and in response to cellular cues. Specifically, phosphorylation of MAP2 and tau is generally thought to reduce their association with MTs. Interestingly it has been shown that depolarization of neurons by treatment with KCl leads to activation of CaMKII and a robust phosphorylation of MAP2 (Vaillant et al., 2002). Contrary to much of the literature, this phosphorylation was shown to increase binding of MAP2 to microtubules, increase microtubule stability, and cause dendrite formation in cultured sympathetic neurons (Vaillant et al., 2002). Although MAP2 and tau can be phosphorylated by several kinases, CaMKII phosphorylates both MAPs, and MAP2 is phosphorylated and dephosphorylated in response to the activation of NMDA receptors during synaptic plasticity (Halpain and Greengard, 1990; Quinlan and Halpain, 1996).

This regulation of MAP2 by CaMKII is physiologically relevant for synaptic plasticity. A study by Yuen *et al.* demonstrates that the serotonin receptor 5-HT_{1A} can negatively

regulate NMDA receptors in neuronal cell cultures, as the pharmacological activation of 5-HT_{1A} with the agonist 8-OH-DPAT results in a dramatic decrease in NMDA currents (Yuen et al., 2005). Interestingly, the ability to modulate NMDA current with 8-OH-DPAT is dependent on MT dynamics. Application of 8-OH-DPAT increases the amount of free tubulin in neurons, and nocodazole mimics the effect of 8-OH-DPAT, with subsequent addition of 8-OH-DPAT having no additional effect (Yuen et al., 2005). Latrunculin B causes a slow decrease in NMDA current; however, 8-OH-DPAT induces an additional reduction of current, demonstrating the actin cytoskeleton is not involved. However, taxol, a MT stabilizing drug, completely occludes the effects of 8-OH-DPAT on NMDA current (Yuen et al., 2005). siRNA mediated knockdown of KIF17, which transports NMDA receptors, has similar effects to nocodazole. A reduction of NMDA currents was observed and addition of 8-OH-DPAT had no additional effect. Moreover, both knock down of KIF17 and activation of 5-HT_{1A} led to a decrease in NMDA receptors incorporated into the membrane of spines (Yuen et al., 2005). Given the importance of MT dynamics in this process, this group focused on MAP2 as a possible regulator of MTs. They demonstrated that the activation of 5-HT_{1A} failed to reduce NMDA currents in neuronal cultures treated with siRNA for MAP2 (Yuen et al., 2005). Additionally, inhibition of either CaMKII or ERK, another kinase, caused an increase in free tubulin and reduced NMDA receptor currents, like 5-HT_{1A} (Yuen et al., 2005). Although this study did not investigate the direct phosphorylation of KIF17 by CaMKII (see Guillaud et al., 2008 above), to explain their results, the authors do present convincing evidence for a model where the activation of 5-HT_{1A} leads to the phosphorylation of MAP2, either directly by CaMKII and/or downstream of CaMKII by ERK (Yuen et al., 2005). The phosphorylation of MAP2 would undoubtedly lead to the dissociation of MAP2 from MTs, causing a decrease in MT stability, and may disrupt KIF17-mediated transport of NMDA receptors. Thus, CaMKII appears to be at the centerpiece of transport and plasticity whether by a direct interaction with motors and cytoskeletal elements or via a more indirect route through MT-associated proteins such as MAP2.

Models of CaMKII mediated cargo unloading in dendritic spines

Many known components of the PSD and other cargos destined for dendritic spines are transported down the dendrites along MTs by various kinesin family members, but it is unclear how these cargos make the transition from the dendritic shaft into the head of the spine. Currently there are two predominant models for how this may occur. The first model proposes kinesin moves cargo along MTs in the shaft and cargo is unloaded directly under the dendritic spine, allowing cargo to diffuse into the spine head (Guillaud et al., 2008) (Figure 2A). The second model is similar, but proposes the cargo is transferred to actin based motors, such as myosin V, prior to or after cargo unloading and actively transported into the spines along actin filaments (Correia et al., 2008; Lemieux et al., 2012). Both of these models would require the action of CaMKII for cargo unloading, and as previously mentioned, CaMKII may associate with MTs in the dendritic shaft directly beneath spines experiencing calcium influx, allowing for efficient cargo unloading in the vicinity of the spines (Lemieux et al., 2012). Although both of these models are possible mechanisms for transferring cargos from the shaft into the spine head, the diffusion model would be the less

efficient of the two models. The typical diameter of dendritic spine necks are approximately 0.15 μm (Harris and Stevens, 1989) while the diameter of the dendritic shaft is typically more than an order of magnitude larger and can range from 0.5–5 μm (Bannister and Larkman, 1995). Moreover, under basal conditions CaMKII movement out of spines has a time constant on the order of minutes, compared to seconds for GFP (Lee and Yasuda, 2009; Sharma et al., 2006). Given these facts, after unloading from the MTs in the dendrite any diffusing cargos would likely remain in the dendrite rather than diffusing into adjacent dendritic spines.

The second model, however, posits a more efficient way to ensure cargos enter spines, and there is some evidence to support the hypothesis that myosin motors are indeed important for plasticity and transport of cargos (Figure 2B). By expressing a fragment of the myosin Va cargo-binding globular tail region, which acts in a dominant negative fashion, Correia *et al.* demonstrated that there was less GluR1 accumulation in dendritic spines *in vitro*, suggesting myosin Va could directly move these receptors into spines as cargos (Correia et al., 2008). In addition, this group demonstrated that expressing a constitutively active form of CaMKII α increased the amount of GluR1 receptors in spines and co-expression of CaMKII α and the dominant negative myosin Va tail led to partial rescue of the dominant negative expression alone (Correia et al., 2008). A separate group, using a dominant negative myosin Va mouse strain called Flailer, was able to demonstrate *in vivo* that myosin Va plays a significant role in the delivery of PSD-95 to dendritic spines (Yoshii et al., 2013). These Flailer mice had abnormal spine morphology and PSD-95 and AMPA receptors localized to dendritic shafts instead of spines (Yoshii et al., 2013).

Although CaMKII forms complexes with MTs in the dendrite near dendritic spines during LTP (Lemieux et al., 2012), some studies suggest CaMKII may be more widely distributed throughout the neuron (Ding et al., 2013; Rose et al., 2009). Variances in CaMKII distribution may be largely due to differences in experimental conditions or the level of synaptic activation. The majority of research suggests CaMKII is concentrated in spine heads and at the PSD (Dosemeci et al., 2007; Feng et al., 2011; Tao-Cheng et al., 2007). Nevertheless, recent EM evidence suggests there may be more CaMKII in the dendritic shaft than previously thought, and the observed higher concentration of CaMKII in spine heads and at the PSD may result from the time between saline perfusion and fixation in whole animals (Ding et al., 2013). However, this study only analyzed neurons at rest or at their basal level of activity. A second recent study using single molecule tracking and photoactivated localization microscopy (PALM), while supporting the aforementioned EM study, determined that the CaMKII in the shaft was more freely diffusible than CaMKII in the spine, suggesting CaMKII in the spine was interacting with binding partners, presumably actin (Lu et al., 2014). This study demonstrated that CaMKII rapidly concentrated in spines after NMDA receptor stimulation and doubled its concentration in the PSD (Lu et al., 2014) (Figure 3). Given that the majority of the literature supports the notion that CaMKII is predominantly localized to dendritic spines after synaptic activation, the ability of CaMKII to interact with motors and unload cargo in the dendrite could be limited. This opens the possibility for a third mechanism of moving cargo into dendritic spines.

A novel model of unloading cargo in dendritic spines

MTs can transiently invade dendritic spines, and these invasions are dependent on neuronal activity. Global stimulation of neuronal activity with KCl increases the number of invaded spines, and inhibition of activity with tetrodotoxin (TTX) reduces the number of invaded spines (Hu et al., 2008). These invasions are physiologically important because disrupting them with the MT depolymerizing drug nocodazole can lead to a disruption of LTP in hippocampal slices (Jaworski et al., 2009) and a reduction of PSD components, such as PSD-95 in dendritic spine heads of cultured neurons (Hu et al., 2011). A direct link to synaptic plasticity has also been demonstrated. Induction of chemical LTP by the synaptic activation of NMDA receptors with a Gly-0Mg²⁺ treatment leads to an increase in the number of dendritic spines invaded by MTs and the frequency of invasions (Merriam et al., 2011). After activation of NMDA receptors, spines that are invaded by MTs show a marked increase in spine size as opposed to spines that are not invaded. Furthermore, nanomolar concentrations of nocodazole, which inhibits MT dynamics and prevents MT invasion of spines, can completely abolish the NMDA-mediated increase in spine size, demonstrating that MTs are necessary for this phenomenon (Merriam et al., 2011). Importantly, it has been shown that MT invasions are mediated by both calcium and rearrangement of the actin cytoskeleton downstream of NMDA receptor activation (Kapitein et al., 2011; Merriam et al., 2013). By tracking the MT plus end binding protein EB3, which only binds to the ends of actively polymerizing MTs, Merriam *et al.* were able to demonstrate that MT entry into spines is regulated by calcium (Merriam et al., 2013). They showed MTs specifically target active spines (those experiencing transient calcium spikes) and the calcium chelator BAPTA-AM dramatically reduced the number of MTs entering spines without affecting MT dynamics in the dendritic shaft (Merriam et al., 2013) (Figure 4). Thus, calcium transients do not affect MT polymerization but are required for guiding MTs into dendritic spines.

The fact MTs invade active spines, and these invasions are important for PSD composition, opens the possibility for a third model. This model posits that kinesin-cargo complexes can directly move into active spines where the concentration of both calcium and active CaMKII is the highest and directly deliver cargos to the spine head. Currently this model is untested. However, given that MTs remain in spines for several minutes (Hu et al., 2008) and most kinesins travel at a velocity of 0.5–1.0 $\mu\text{m/s}$ (Hammond et al., 2010; Hammond et al., 2009), it is plausible, if not likely, that kinesin could directly transport material along MTs present in dendritic spines. Once the kinesin-cargo complex enters the spine along a MT, it could be phosphorylated by CaMKII in the spine head, releasing its cargo directly where needed (Figure 2C).

Although the “direct deposit” model is primarily a MT-based process, the actin cytoskeleton appears to be important for MT invasions as well. MTs preferentially enter spines that have recently increased their F-actin content, while the actin depolymerizing drug latrunculin A abolishes MT entry into spines (Merriam et al., 2013). One link between MTs and the actin cytoskeleton appears to be the actin binding protein drebrin, which can also bind the MT plus end-binding protein EB3 (Geraldo et al., 2008). During cLTP induction, drebrin concentration increases in spines and siRNA mediated knockdown of drebrin causes a

dramatic decrease in MT invasions without affecting spine morphology (Merriam et al., 2013).

While it is clear calcium influx through NMDA receptors is a key regulator of MT dynamics and spine invasions, the mechanism for this is currently unknown. Given that the increase in F-actin is vital for MT spine entry, and calcium/calmodulin activation of CaMKII is known to mediate an increase in F-actin in spines during LTP (Ahmed et al., 2006; Ouyang et al., 2005), it is possible that MT invasions into spines are downstream of this CaMKII pathway as well. Current evidence suggests that MT invasion of spines occurs through modulation of the actin cytoskeleton and drebrin (Merriam et al., 2013). Interestingly, the activity of drebrin has recently been shown to be regulated by Cdk5 phosphorylation, which is also downstream of the CaMKII pathway (Dhavan et al., 2002). CaMKII directly phosphorylates Cdk5 in vitro, and that interaction is stimulated by glutamate receptor activation and calcium signaling (Dhavan et al., 2002). Activated drebrin may then act to essentially capture MT plus ends via EB3 and guide them into the spine head (Figure 2D).

With the addition of this model, there are three testable mechanisms for unloading cargo into spines during synaptic activity (Figure 2). However, this does not imply that these models are mutually exclusive, and there is a distinct possibility that different cargos may enter spines using different mechanisms. Previous work suggests that kinesin-1 may be biased to bind acetylated and detyrosinated MTs (Dunn et al., 2008; Reed et al., 2006) while no such bias has been reported for family members kinesin-2 or -3. Newly polymerized MTs in spines would presumably be tyrosinated and not yet have accumulated significant amounts of acetylation, which could preclude kinesin-1 from entering the spine while allowing other family members access. Myosin Va has been shown to play an important role in transporting PSD-95 (Yoshii et al., 2013) and GluR1 (Correia et al., 2008) into dendritic spines, but transport of other cargos, such as NMDA receptors, does not appear to rely on myosin Va function. Therefore, it is possible that kinesin-1 cargos are transported into spines via myosin and other kinesins-cargo pairs can directly enter the spine on MTs. This hypothesis is consistent with the fact that kinesin-1 cargo unloading is regulated by a more direct calcium binding mechanism, while kinesin-2 is phosphorylated by CaMKII, which most evidence suggests, accumulates in the spine head after synaptic activation.

Conclusions and Future Directions

Taken together, the above studies indicate that there is an emerging signaling cascade that exists from calcium to CaMKII to MAPs, motors, adapters and MTs themselves.

Throughout this review we have focused on the more unconventional role that CaMKII may be serving by signaling to the MT cytoskeleton, rather than to its known roles in signaling to the actin cytoskeleton. It is likely that these pathways from CaMKII to MTs are in parallel or series with the canonical associations that CaMKII has with actin associated proteins and the actin cytoskeleton. In the coming years it will be interesting to dissect these pathways in more detail to determine which effectors are upstream or downstream and what feedback loops might exist between this important kinase and the actin and microtubule cytoskeletons.

Acknowledgements

We apologize that, owing to space constraints, that we could not cite many excellent studies. This work was supported by NIH grant NS064014 and the Vilas Associates Award from the University of Wisconsin to E.W.D.

References

- Ahmed R, Zha XM, Green SH, Dailey ME. Synaptic activity and F-actin coordinately regulate CaMKII α localization to dendritic postsynaptic sites in developing hippocampal slices. *Mol Cell Neurosci*. 2006; 31:37–51. [PubMed: 16214364]
- Ballif BA, Villen J, Beausoleil SA, Schwartz D, Gygi SP. Phosphoproteomic analysis of the developing mouse brain. *Mol Cell Proteomics*. 2004; 3:1093–1101. [PubMed: 15345747]
- Bannister NJ, Larkman AU. Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: I. Branching patterns. *J Comp Neurol*. 1995; 360:150–160. [PubMed: 7499560]
- Borgesius NZ, van Woerden GM, Buitendijk GHS, Keijzer N, Jaarsma D, Hoogenraad CC, Elgersma Y. β CaMKII Plays a Nonenzymatic Role in Hippocampal Synaptic Plasticity and Learning by Targeting α CaMKII to Synapses. *J Neurosci*. 2011; 31:10141–10148. [PubMed: 21752990]
- Brown MTC, Bellone C, Mameli M, Labouebe G, Bocklisch C, Balland B, Dahan L, Lujan R, Deisseroth K, Luscher C. Drug-Driven AMPA Receptor Redistribution Mimicked by Selective Dopamine Neuron Stimulation. *Plos One*. 2010; 5
- Cingolani LA, Goda Y. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. *Nat Rev Neurosci*. 2008; 9:344–356. [PubMed: 18425089]
- Colbran RJ, Brown AM. Calcium/calmodulin-dependent protein kinase II and synaptic plasticity. *Curr Opin Neurobiol*. 2004; 14:318–327. [PubMed: 15194112]
- Correia SS, Bassani S, Brown TC, Lise MF, Backos DS, El-Husseini A, Passafaro M, Esteban JA. Motor protein-dependent transport of AMPA receptors into spines during long-term potentiation. *Nat Neurosci*. 2008; 11:457–466. [PubMed: 18311135]
- Coultrap SJ, Buard I, Kulbe JR, Dell'Acqua ML, Bayer KU. CaMKII Autonomy Is Substrate-dependent and Further Stimulated by Ca²⁺/Calmodulin. *Journal of Biological Chemistry*. 2010; 285:17930–17937. [PubMed: 20353941]
- Dehmelt L, Halpain S. The MAP2/Tau family of microtubule-associated proteins. *Genome biology*. 2005; 6:204. [PubMed: 15642108]
- Dhavan R, Greer PL, Morabito MA, Orlando LR, Tsai LH. The cyclin-dependent kinase 5 activators p35 and p39 interact with the α -subunit of Ca²⁺/calmodulin-dependent protein kinase II and α -actinin-1 in a calcium-dependent manner. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2002; 22:7879–7891. [PubMed: 12223541]
- Ding JD, Kennedy MB, Weinberg RJ. Subcellular organization of camkii in rat hippocampal pyramidal neurons. *J Comp Neurol*. 2013; 521:3570–3583. [PubMed: 23749614]
- Dosemeci A, Makusky AJ, Jankowska-Stephens E, Yang X, Slotta DJ, Markey SP. Composition of the synaptic PSD-95 complex. *Mol Cell Proteomics*. 2007; 6:1749–1760. [PubMed: 17623647]
- Dunn S, Morrison EE, Liverpool TB, Molina-Paris C, Cross RA, Alonso MC, Peckham M. Differential trafficking of Kif5c on tyrosinated and detyrosinated microtubules in live cells. *J Cell Sci*. 2008; 121:1085–1095. [PubMed: 18334549]
- Feng B, Raghavachari S, Lisman J. Quantitative estimates of the cytoplasmic, PSD, and NMDAR-bound pools of CaMKII in dendritic spines. *Brain Res*. 2011; 1419:46–52. [PubMed: 21925648]
- Fifkova E, Delay RJ. Cytoplasmic Actin in Neuronal Processes as a Possible Mediator of Synaptic Plasticity. *J Cell Biol*. 1982; 95:345–350. [PubMed: 6890558]
- Fink CC, Meyer T. Molecular mechanisms of CaMKII activation in neuronal plasticity. *Curr Opin Neurobiol*. 2002; 12:293–299. [PubMed: 12049936]
- Fukunaga K, Stoppini L, Miyamoto E, Muller D. Long-term potentiation is associated with an increased activity of Ca²⁺/calmodulin-dependent protein kinase II. *J Biol Chem*. 1993; 268:7863–7867. [PubMed: 8385124]

- Geraldo S, Khanzada UK, Parsons M, Chilton JK, Gordon-Weeks PR. Targeting of the F-actin-binding protein drebrin by the microtubule plus-tip protein EB3 is required for neuritogenesis. *Nat Cell Biol.* 2008; 10:1181–1189. [PubMed: 18806788]
- Glater EE, Megeath LJ, Stowers RS, Schwarz TL. Axonal transport of mitochondria requires mltin to recruit kinesin heavy chain and is light chain independent. *The Journal of cell biology.* 2006; 173:545–557. [PubMed: 16717129]
- Gu J, Firestein BL, Zheng JQ. Microtubules in dendritic spine development. *J Neurosci.* 2008; 28:12120–12124. [PubMed: 19005076]
- Guillaud L, Wong R, Hirokawa N. Disruption of KIF17-Mint1 interaction by CaMKII-dependent phosphorylation: a molecular model of kinesin-cargo release. *Nat Cell Biol.* 2008; 10:19–29. [PubMed: 18066053]
- Gumy LF, Chew DJ, Tortosa E, Katrukha EA, Kapitein LC, Tolkovsky AM, Hoogenraad CC, Fawcett JW. The kinesin-2 family member KIF3C regulates microtubule dynamics and is required for axon growth and regeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2013; 33:11329–11345. [PubMed: 23843507]
- Guo XF, Macleod GT, Wellington A, Hu F, Panchumarthi S, Schoenfield M, Marin L, Charlton MP, Atwood HL, Zinsmaier KE. The GTPase dMiro is required for axonal transport of mitochondria to *Drosophila* synapses. *Neuron.* 2005; 47:379–393. [PubMed: 16055062]
- Halpain S, Greengard P. Activation of NMDA receptors induces rapid dephosphorylation of the cytoskeletal protein MAP2. *Neuron.* 1990; 5:237–246. [PubMed: 2169265]
- Hammond JW, Blasius TL, Soppina V, Cai D, Verhey KJ. Autoinhibition of the kinesin-2 motor KIF17 via dual intramolecular mechanisms. *J Cell Biol.* 2010; 189:1013–1025. [PubMed: 20530208]
- Hammond JW, Cai D, Blasius TL, Li Z, Jiang Y, Jih GT, Meyhofer E, Verhey KJ. Mammalian Kinesin-3 motors are dimeric in vivo and move by processive motility upon release of autoinhibition. *PLoS Biol.* 2009; 7:e72. [PubMed: 19338388]
- Harris KM, Jensen FE, Tsao B. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 1992; 12:2685–2705. [PubMed: 1613552]
- Harris KM, Stevens JK. Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 1989; 9:2982–2997. [PubMed: 2769375]
- Hell JW. CaMKII: claiming center stage in postsynaptic function and organization. *Neuron.* 2014; 81:249–265. [PubMed: 24462093]
- Hoffman L, Farley MM, Waxham MN. Calcium-calmodulin-dependent protein kinase II isoforms differentially impact the dynamics and structure of the actin cytoskeleton. *Biochemistry.* 2013; 52:1198–1207. [PubMed: 23343535]
- Hu X, Ballo L, Pietila L, Viesselmann C, Ballweg J, Lumbard D, Stevenson M, Merriam E, Dent EW. BDNF-induced increase of PSD-95 in dendritic spines requires dynamic microtubule invasions. *J Neurosci.* 2011; 31:15597–15603. [PubMed: 22031905]
- Hu X, Viesselmann C, Nam S, Merriam E, Dent EW. Activity-dependent dynamic microtubule invasion of dendritic spines. *J Neurosci.* 2008; 28:13094–13105. [PubMed: 19052200]
- Jaworski J, Kapitein LC, Gouveia SM, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, Di Stefano P, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. *Neuron.* 2009; 61:85–100. [PubMed: 19146815]
- Kapitein LC, Yau KW, Gouveia SM, van der Zwan WA, Wulf PS, Keijzer N, Demmers J, Jaworski J, Akhmanova A, Hoogenraad CC. NMDA receptor activation suppresses microtubule growth and spine entry. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2011; 31:8194–8209. [PubMed: 21632941]
- Kasai H, Fukuda M, Watanabe S, Hayashi-Takagi A, Noguchi J. Structural dynamics of dendritic spines in memory and cognition. *Trends Neurosci.* 2010; 33:121–129. [PubMed: 20138375]

- Kim H, Binder LI, Rosenbaum JL. The periodic association of MAP2 with brain microtubules in vitro. *The Journal of cell biology*. 1979; 80:266–276. [PubMed: 457745]
- Lantsman K, Tombes RM. CaMK-II oligomerization potential determined using CFP/YFP FRET. *Biochim Biophys Acta*. 2005; 1746:45–54. [PubMed: 16185778]
- Lee SJ, Yasuda R. Spatiotemporal Regulation of Signaling in and out of Dendritic Spines: CaMKII and Ras. *Open Neurosci J*. 2009; 3:117–127. [PubMed: 20463853]
- Lemieux M, Labrecque S, Tardif C, Labrie-Dion E, Lebel E, De Koninck P. Translocation of CaMKII to dendritic microtubules supports the plasticity of local synapses. *The Journal of cell biology*. 2012; 198:1055–1073. [PubMed: 22965911]
- Lewis SA, Cowan N. Microtubule bundling. *Nature*. 1990; 345:674. [PubMed: 2113613]
- Lin YC, Redmond L. CaMKII β binding to stable F-actin in vivo regulates F-actin filament stability. *Proc Natl Acad Sci U S A*. 2008; 105:15791–15796. [PubMed: 18840684]
- Liu Y, Liang Y, Hou B, Liu M, Yang X, Liu C, Zhang J, Zhang W, Ma Z, Gu X. The inhibitor of calcium/calmodulin-dependent protein kinase II KN93 attenuates bone cancer pain via inhibition of KIF17/NR2B trafficking in mice. *Pharmacol Biochem Behav*. 2014; 124C:19–26. [PubMed: 24836181]
- Lu HE, MacGillavry HD, Frost NA, Blanpied TA. Multiple Spatial and Kinetic Subpopulations of CaMKII in Spines and Dendrites as Resolved by Single-Molecule Tracking PALM. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2014; 34:7600–7610. [PubMed: 24872564]
- MacAskill AF, Rinholm JE, Twelvetrees AE, Arancibia-Carcamo IL, Muir J, Fransson A, Aspenstrom P, Attwell D, Kittler JT. Miro1 Is a Calcium Sensor for Glutamate Receptor-Dependent Localization of Mitochondria at Synapses. *Neuron*. 2009; 61:541–555. [PubMed: 19249275]
- Makino H, Malinow R. AMPA Receptor Incorporation into Synapses during LTP: The Role of Lateral Movement and Exocytosis. *Neuron*. 2009; 64:381–390. [PubMed: 19914186]
- Merriam EB, Lumbard DC, Viesselmann C, Ballweg J, Stevenson M, Pietila L, Hu X, Dent EW. Dynamic microtubules promote synaptic NMDA receptor-dependent spine enlargement. *Plos One*. 2011; 6:e27688. [PubMed: 22096612]
- Merriam EB, Millette M, Lumbard DC, Saengsawang W, Fothergill T, Hu X, Ferhat L, Dent EW. Synaptic regulation of microtubule dynamics in dendritic spines by calcium, f-actin, and drebrin. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2013; 33:16471–16482. [PubMed: 24133252]
- Nakagawa T, Engler JA, Sheng M. The dynamic turnover and functional roles of alpha-actinin in dendritic spines. *Neuropharmacology*. 2004; 47:734–745. [PubMed: 15458845]
- Nishimura T, Kato K, Yamaguchi T, Fukata Y, Ohno S, Kaibuchi K. Role of the PAR-3-KIF3 complex in the establishment of neuronal polarity. *Nat Cell Biol*. 2004; 6:328–334. [PubMed: 15048131]
- Okamoto K, Bosch M, Hayashi Y. The roles of CaMKII and F-actin in the structural plasticity of dendritic spines: a potential molecular identity of a synaptic tag? *Physiology (Bethesda)*. 2009; 24:357–366. [PubMed: 19996366]
- Okamoto KI, Narayanan R, Lee SH, Murata K, Hayashi Y. The role of CaMKII as an F-actin-bundling protein crucial for maintenance of dendritic spine structure. *Proc Natl Acad Sci U S A*. 2007; 104:6418–6423. [PubMed: 17404223]
- Opazo P, Labrecque S, Tigaret CM, Frouin A, Wiseman PW, De Koninck P, Choquet D. CaMKII Triggers the Diffusional Trapping of Surface AMPARs through Phosphorylation of Stargazin. *Neuron*. 2010; 67:239–252. [PubMed: 20670832]
- Otmakhov N, Tao-Cheng JH, Carpenter S, Asrican B, Dosemeci A, Reese TS, Lisman J. Persistent accumulation of calcium/calmodulin-dependent protein kinase II in dendritic spines after induction of NMDA receptor-dependent chemical long-term potentiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2004; 24:9324–9331. [PubMed: 15496668]
- Ouyang Y, Kantor D, Harris KM, Schuman EM, Kennedy MB. Visualization of the distribution of autophosphorylated calcium/calmodulin-dependent protein kinase II after tetanic stimulation in the CA1 area of the hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1997; 17:5416–5427. [PubMed: 9204925]

- Ouyang Y, Rosenstein A, Kreiman G, Schuman EM, Kennedy MB. Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1999; 19:7823–7833. [PubMed: 10479685]
- Ouyang Y, Wong M, Capani F, Rensing N, Lee CS, Liu Q, Neusch C, Martone ME, Wu JY, Yamada K, Ellisman MH, Choi DW. Transient decrease in F-actin may be necessary for translocation of proteins into dendritic spines. *Eur J Neurosci*. 2005; 22:2995–3005. [PubMed: 16367766]
- Phang HQ, Hoon JL, Lai SK, Zeng Y, Chiam KH, Li HY, Koh CG. POPX2 phosphatase regulates the KIF3 kinesin motor complex. *J Cell Sci*. 2013
- Quinlan EM, Halpain S. Postsynaptic mechanisms for bidirectional control of MAP2 phosphorylation by glutamate receptors. *Neuron*. 1996; 16:357–368. [PubMed: 8789950]
- Reed NA, Cai D, Blasius TL, Jih GT, Meyhofer E, Gaertig J, Verhey KJ. Microtubule acetylation promotes kinesin-1 binding and transport. *Curr Biol*. 2006; 16:2166–2172. [PubMed: 17084703]
- Rintoul GL, Filiano AJ, Brocard JB, Kress GJ, Reynolds IJ. Glutamate decreases mitochondrial size and movement in primary forebrain neurons. *J Neurosci*. 2003; 23:7881–7888. [PubMed: 12944518]
- Roger B, Al-Bassam J, Dehmelt L, Milligan RA, Halpain S. MAP2c, but not tau, binds via its microtubule binding and bundles F-actin domain. *Current Biology*. 2004; 14:363–371. [PubMed: 15028210]
- Rose J, Jin SX, Craig AM. Heterosynaptic molecular dynamics: locally induced propagating synaptic accumulation of CaM kinase II. *Neuron*. 2009; 61:351–358. [PubMed: 19217373]
- Ryan XP, Alldritt J, Svenningsson P, Allen PB, Wu GY, Nairn AC, Greengard P. The Rho-specific GEF Lfc interacts with neurabin and spinophilin to regulate dendritic spine morphology. *Neuron*. 2005; 47:85–100. [PubMed: 15996550]
- Setou M, Nakagawa T, Seog DH, Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science*. 2000; 288:1796–1802. [PubMed: 10846156]
- Sharma K, Fong DK, Craig AM. Postsynaptic protein mobility in dendritic spines: long-term regulation by synaptic NMDA receptor activation. *Mol Cell Neurosci*. 2006; 31:702–712. [PubMed: 16504537]
- Shen K, Teruel MN, Subramanian K, Meyer T. CaMKII beta functions as an F-actin targeting module that localizes CaMKII alpha/beta heterooligomers to dendritic spines. *Neuron*. 1998; 21:593–606. [PubMed: 9768845]
- Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science*. 1999; 284:1811–1816. [PubMed: 10364548]
- Stowers RS, Megeath LJ, Gorska-Andrzejak J, Meinertzhagen IA, Schwarz TL. Axonal transport of mitochondria to synapses depends on Milton, a novel Drosophila protein. *Neuron*. 2002; 36:1063–1077. [PubMed: 12495622]
- Tanaka Y, Kanai Y, Okada Y, Nonaka S, Takeda S, Harada A, Hirokawa N. Targeted disruption of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. *Cell*. 1998; 93:1147–1158. [PubMed: 9657148]
- Tao-Cheng JH, Gallant PE, Brightman MW, Dosemeci A, Reese TS. Structural changes at synapses after delayed perfusion fixation in different regions of the mouse brain. *J Comp Neurol*. 2007; 501:731–740. [PubMed: 17299754]
- Vaillant AR, Zanassi P, Walsh GS, Aumont A, Alonso A, Miller FD. Signaling mechanisms underlying reversible, activity-dependent dendrite formation. *Neuron*. 2002; 34:985–998. [PubMed: 12086645]
- Wandosell F, Serrano L, Hernandez MA, Avila J. Phosphorylation of tubulin by a calmodulin-dependent protein kinase. *J Biol Chem*. 1986; 261:10332–10339. [PubMed: 3733711]
- Weisshaar B, Doll T, Matus A. Reorganisation of the microtubular cytoskeleton by embryonic microtubule-associated protein 2 (MAP2c). *Development*. 1992; 116:1151–1161. [PubMed: 1338311]

- Wong RW, Setou M, Teng J, Takei Y, Hirokawa N. Overexpression of motor protein KIF17 enhances spatial and working memory in transgenic mice. *Proc Natl Acad Sci U S A*. 2002; 99:14500–14505. [PubMed: 12391294]
- Xie Z, Srivastava DP, Photowala H, Kai L, Cahill ME, Woolfrey KM, Shum CY, Surmeier DJ, Penzes P. Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. *Neuron*. 2007; 56:640–656. [PubMed: 18031682]
- Yoshii A, Zhao JP, Pandian S, van Zundert B, Constantine-Paton M. A Myosin Va mutant mouse with disruptions in glutamate synaptic development and mature plasticity in visual cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2013; 33:8472–8482. [PubMed: 23658184]
- Yuen EY, Jiang Q, Chen P, Gu ZL, Feng J, Yan Z. Serotonin 5-HT1A receptors regulate NMDA receptor channels through a microtubule-dependent mechanism. *J Neurosci*. 2005; 25:5488–5501. [PubMed: 15944377]

Figure 1. Activity dependent translocation of CaMKII to microtubules correlates with spine remodeling

(A) Mature rat hippocampal neurons co-expressing eGFP-CaMKII and mCherry were stimulated with Gly-0Mg²⁺ for 5 minutes. Arrow (red) points to a single spine while the bracket indicates a region of high CaMKII concentration in the dendrite shaft. After treatment, CaMKII translocates to subdendritic regions in the spine head and near the base of the spine and spine volume is increased. (B) Neurons expressing mCherry-CaMKII and eGFP-MAP2 were treated for one minute with KCl. After treatment there was an increase in colocalization of CaMKII with MAP2. Arrows (white) point to microtubule like-sites of

CaMKII translocation. (C) Time-series of FRAP of eGFP-CaMKII before (top) and after (bottom) a 1 minute KCl treatment. The circle (white) indicates area of photobleaching. The recovery time was significantly slower after KCl treatment indicating a less diffusive population of CaMKII. Adapted from Lemieux et al., 2012.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Models of cargo transport into dendritic spines

(A). A diffusive model where CaMKII could phosphorylate kinesin and/or adapter proteins in the dendrite shaft, releasing cargo (a vesicle in these models) to diffuse freely in the dendritic shaft or into the spine head. (B) A hand-off-model, where myosin and kinesin are bound to the same vesicle. CaMKII could phosphorylate kinesin and/or adapters releasing the vesicle from MTs. The vesicle then attaches to actin filaments in the dendrite shaft or spine neck and is transported into the spine head via myosin-driven transport. (C) A direct-deposit model where microtubules can polymerize into the spine head and kinesin can directly traffic vesicles into the spine where CaMKII and Ca^{2+} concentrations are the highest. Note that a hand-off to actin could also occur in the spine head in this model. (D) Flow diagram of the CaMKII pathway leading to microtubule invasion of spines and cargo unloading in dendritic spines.

Figure 3. Activated CaMKII concentrates in spines after NMDA receptor activation
 (A) Mature rat hippocampal neurons were transfected with eGFP-CaMKII and PSD95-mCherry. After Gly-0Mg²⁺ treatment CaMKII concentrated in the spine heads at, and away from, the PSD. (B) Enlarged view from boxed region in A before (top) and after (bottom) Gly-0Mg²⁺ treatment. (C) CaMKII localizations were binned (25 nm) to construct a map of CaMKII density before and after Gly-0Mg²⁺ treatment. (D) Quantification of CaMKII enrichment at the PSD before and after Gly-0Mg²⁺ treatment. Adapted from Lu et al., 2014.

Figure 4. MTs target spines after NMDAR activation in a calcium dependent manner
 (A) Sequential frames showing rapid synaptic calcium influx in GCaMP3 transfected mature mouse hippocampal neurons during Gly-0Mg²⁺ treatment. The elevation in GCaMP3 fluorescence is restricted to the second spine. (B) Kymographs of both spines from A. Only the second spine is invaded by the MT plus end binding protein EB3 following Gly-0Mg²⁺ treatment. (C) Kymographs of EB3 puncta moving in three different dendritic shafts before (left) and after (right) calcium chelation with BAPTA-AM. (D) Kymographs of EB3 puncta in five different dendritic spines before (left) and after (right) calcium chelation with BAPTA-AM. Calcium chelation has no effect on EB3 movement in the shaft, but drastically reduces spine invasions by MTs. Adapted from Merriam et al., 2013.