The Role of Microtubules and Tau Proteins in Neuronal Excitability

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Abstract

For more than three decades, the evidence (Matsumoto and Sakai, 1979) that microtubules might play a role in neuronal excitability has been largely ignored. This situation was changed a few years ago, when several groups provided direct evidence for the involvement of microtubule-associated protein Tau in excitability. Since then, further evidence continues to accumulate. The microtubule-depolymerizing agent nocodazole has been demonstrated to reduce burst activity and seizure severity. Very recently, Hatch et al. (2017) showed that the hyperphosphorylated Tau could reduce excitability by modulating AIS in a microtubule-dependent manner. These findings support the microtubule (MT) model proposed in Paper 1. This paper will extend the MT model to explain (1) how excessive 4-repeat Tau may cause hyperexcitability, and (2) how seizures can be terminated by the detyrosinated tubulins which are disassembled from microtubules at AIS.

Introduction

Hyperexcitability is an early sign of Alzheimer's disease (Dickerson et al., 2005; Putcha et al., 2011), Parkinson's disease (Blandini et al., 1996), amyotrophic lateral sclerosis (van Zundert et al., 2012) and Huntington's Disease (Klapstein et al., 2001). The microtubule-associated protein Tau is implicated in all of these neurodegenerative disorders (Braak et al., 1993; Wills et al., 2010; Mimuro et al., 2007; Fernández-Nogales et al., 2014). In Paper 1, it was mentioned briefly that the Tau protein may modulate neuronal excitability by interacting with the microtubules at the axon initial segment (AIS). Further details are discussed in this paper.

The Tau protein has six isoforms produced from a single gene through alternative RNA splicing (Figure 1). They differ in the number of inserts at the N-terminal half and the number of repeats at the C-terminal half . The number of inserts may be 0, 1 or 2, depending on whether the exon 2 and/or 3 are included during RNA splicing. The

number of repeats may be either 3 or 4. The 4-repeat (4R) Tau includes the second repeat encoded by exon 10. In a healthy adult brain, **the levels of 4R and 3R Tau proteins are approximately equal. Distortion of the balance toward 4R Tau may lead to neurodegeneration**, as observed in Huntington's Disease (Fernández-Nogales et al., 2014), Alzheimer's disease (Yasojima et al., 1999; Ginsberg et al., 2006; Glatz et al., 2006), Parkinson's disease (Caffrey et al., 2006), and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) (Ghetti et al., 2015). Their pathogenic mechanisms will be discussed in later papers. This article focuses on the role of Tau proteins in excitability. It will be shown that the 4R Tau increases excitability.

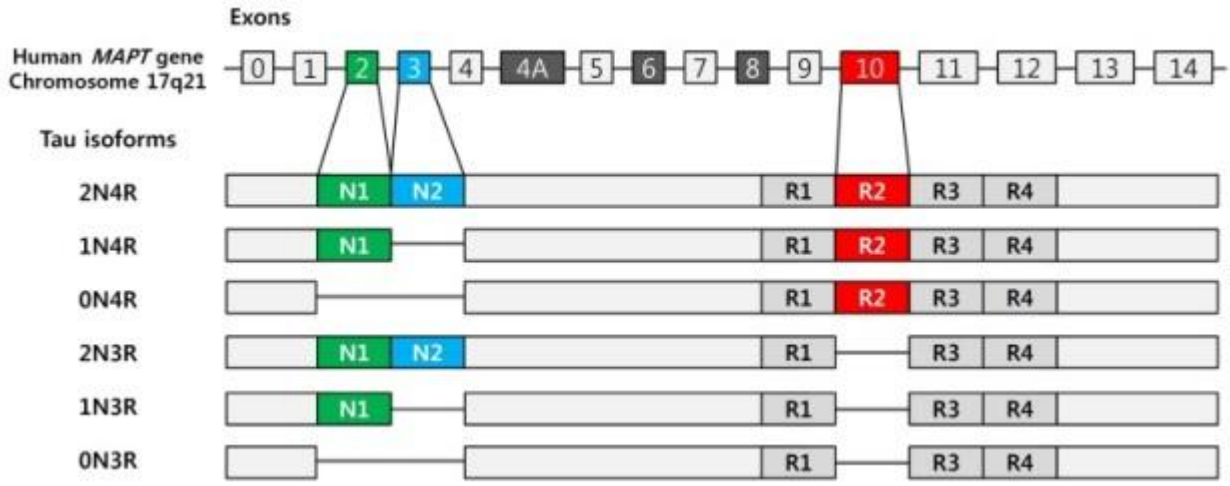

Figure 1. Tau isoforms. An isoform is designated as xNyR, where x is the number of inserts and y is the number of repeats. [Source: Park et al., 2016]

The Microtubule (MT) Model for Excitability

The MT model was originally proposed for wireless communication in the brain, where microtubules at the AIS may serve as the receiving antennas for converting electromagnetic (EM) waves into neuronal excitability. A microtubule is highly negatively charged. Its association with the AIS membrane should have the same effects as hyperpolarization, i.e., inhibitory. Dissociation from the AIS membrane is equivalent to depolarization.

Ankyrin-G, together with the microtubule end binding protein EB1 or EB3 (denoted by EB1/3), play a crucial role in anchoring microtubules to the AIS membrane (Leterrier et al., 2011). Higher Ankyrin-G level will provide more "anchor points", which restrict the microtubule to bend away from the membrane, thereby reducing excitability

(Figure 2). During long range synchronization, the EM waves can induce microtubule vibration to disrupt the anchoring, resulting in microtubule bending away from the membrane. If all anchor points are disrupted, the entire microtubule will detach from the membrane.

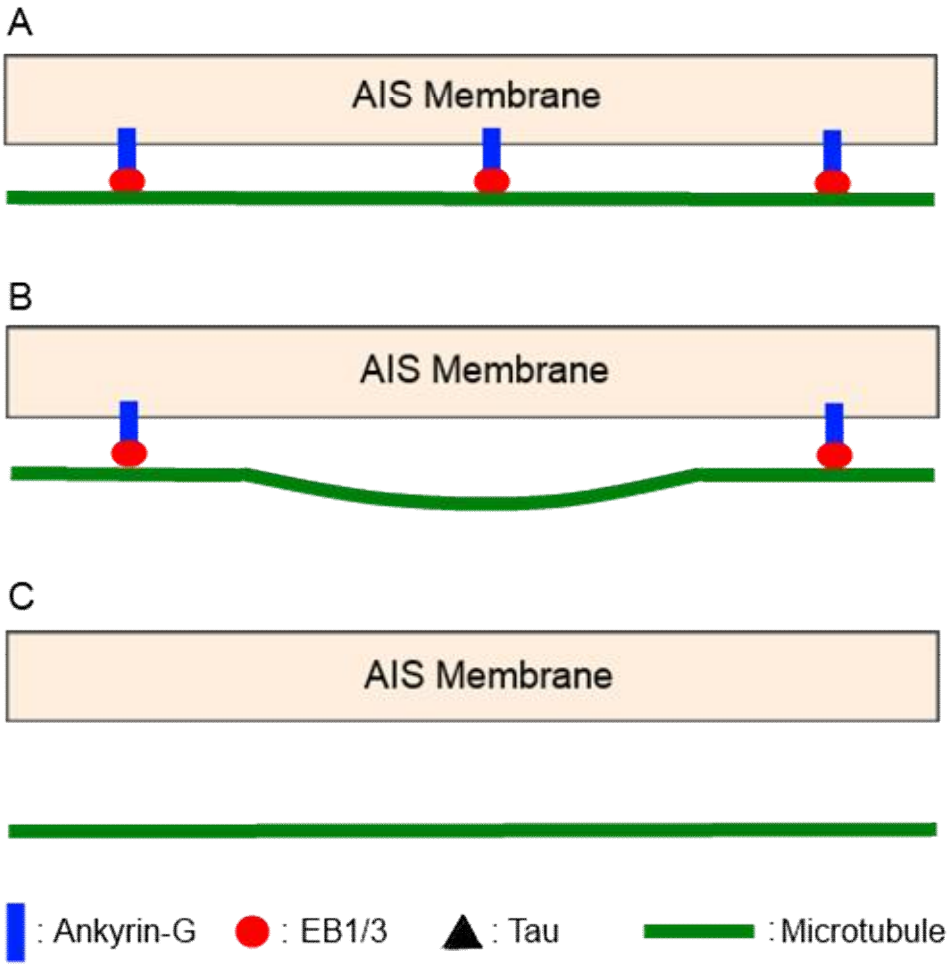

Figure 2. The effects of Ankyrin-G on excitability.
(A) The association of the negatively charged microtubule with the membrane is mediated by Ankyrin-G and EB1/3. This should reduce excitability.
(B) The loss of an anchor point causes a segment of the microtubule to bend away from the membrane, thereby increasing excitability.
(C) The loss of all anchor points causes the entire microtubule to detach from the membrane.

This model is supported by two studies that have demonstrated the effects of Ankyrin-G on excitability. The first study employed TsA201 cells to express Nav1.6 channels and/or Ankyrin-G. If only Nav1.6 channels are expressed, a significant

persistent sodium current I_{NaP} (conducted by Nav1.6 channels) was observed. However, co-expression with Ankyrin-G reduced I_{NaP} (Shirahata et al., 2006). Nav1.6 is abundant at the AIS, with a critical role for the regulation of excitability (O'Brien and Meisler, 2013). The second study investigated the effects of amyloid precursor protein (APP) over-expression in a transgenic mouse model. It was found that the APP over-expression up-regulates a microRNA, miR-342-5p, which in turn down-regulates the expression of Ankyrin-G (Sun et al., 2014). In agreement with the MT model, the APP transgenic mice exhibited hyperexcitability (Wesson et al., 2011; Bezzina et al., 2015).

Modulation of Excitability by Tau Proteins

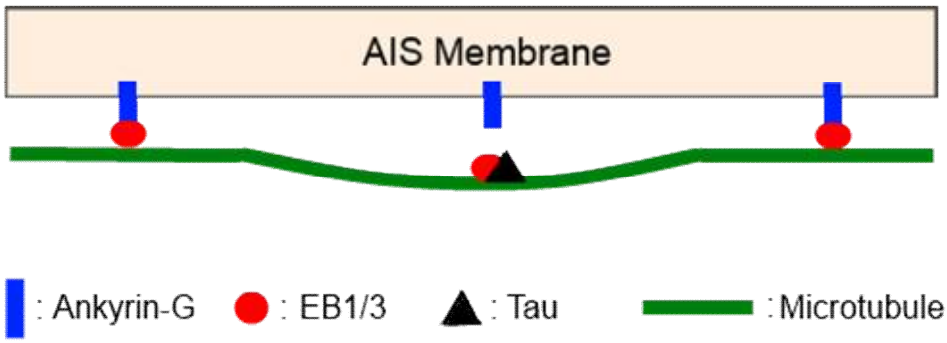

Figure 3. The Tau protein may bind with the microtubule to disrupt its association with the membrane, thereby increasing excitability.

Tau is a microtubule-associated protein. It can also interact with EB1/3 (Sayas et al., 2015). Thus, Tau may increase excitability by interfering with the association between microtubules and the membrane. This explains why in animal models Tau reduction or knockout attenuates hyperexcitability (Holth et al., 2013; DeVos et al., 2013; Li et al., 2014).

Hyperphosphorylated Tau has been shown to reduce excitability (Hatch et al., 2017). Phosphorylation is a process that adds a negatively charged phosphate group to a protein. As a result, hyperphosphorylation of the Tau protein should reduce its binding with the negatively charged microtubules. In the absence of the Tau interference, the MT model predicts that the microtubule should be more likely to associate with the membrane, thereby attenuating excitability.

Tau hyperphosphorylation is a major characteristic of Alzheimer disease (AD). During the development of the disease, the hippocampus and entorhinal cortex

first exhibited hyperexcitability, but then followed by hypoactivation as the disease progresses (Dickerson et al., 2005). The later hypoactive phase is due to Tau hyperphosphorylation. The early hyperactive phase may arise from excessive 4R Tau.

In the Tau protein, the repeat region is the microtubule binding domain. Thus, the 4R Tau should bind to the microtubule more tightly (Bunker et al., 2004), and prevent microtubule association with the membrane more effectively than the 3R Tau. As a result, the 4R Tau should have greater impact on enhancing excitability than the 3R Tau. Excessive 4R Tau may lead to hyperexcitability, which could be the origin of neurodegeneration.

The Effects of Microtubule-Active Agents

The idea that microtubules could play a role in neuronal excitability has been proposed several decades ago, based on the observation that the excitability of squid giant axons correlates with microtubule assembly (Matsumoto and Sakai, 1979). It was further found that the internal perfusons which cause microtubules to depolymerize reduce the excitability and those supporting microtubule assembly increase the peak sodium current (Sakai et al., 1985). Recently, similar results were obtained from epilepsy models in the rat: the microtubule-depolymerizing agent nocodazole reduced burst activity and seizure severity whereas the stabilizing agent paclitaxel (taxol) did not have a clear influence (Carletti et al., 2016).

Nocodazole is likely to act on the microtubules at AIS, as the chemical agent has been shown to modulate AIS location (Hatch et al., 2017). A tubulin dimer contains about 50 negatively charged residues (Minoura and Muto, 2006). Thus, the disassembled tubulins could associate with the AIS membrane to inhibit neuronal firing with their strong negative electric fields. This notion agrees with the finding that, in the squid giant axon, the diminished excitability due to microtubule depolymerization can be restored by tubulin-tyrosine ligase (Matsumoto et al., 1979), which catalyzes the addition of a tyrosine to the C-terminal of α–tubulin. The α–tubulin is synthesized with a C-terminal tyrosine, but in the post-translational modification, the tyrosine may be removed by α–tubulin tyrosine carboxypeptidase, resulting in detyrosinated tubulin with a C-terminal glutamate. The detyrosinated tubulin can be re-tyrosinated by tubulin-tyrosine ligase (Szyk et al., 2011). The finding of Matsumoto et al.

suggests that only the detyrosinated tubulin may associate with the membrane to reduce excitability.

Discussion

For more than three decades, the evidence (Matsumoto and Sakai, 1979) that microtubules might play a role in neuronal excitability has been largely ignored. This situation was changed a few years ago, when several groups provided direct evidence for the involvement of microtubule-associated protein Tau in excitability (Holth et al., 2013; DeVos et al., 2013; Li et al., 2014). Since then, further evidence continues to accumulate. The microtubule-depolymerizing agent nocodazole has been demonstrated to reduce burst activity and seizure severity (Carletti et al., 2016). Very recently, Hatch et al. (2017) showed that the hyperphosphorylated Tau could reduce excitability by modulating AIS in a microtubule-dependent manner. This finding supports the MT model that the excitability may be regulated by the microtubules at AIS. However, Hatch et al. attributed the reduced excitability to the relocation of AIS away from the soma, whereas the MT model suggests that the reduced excitability is due to the negative charges on microtubules.

The shift of AIS away from the soma does not necessarily attenuate excitability. There are two competing factors. On one hand, a more distal AIS will increase the voltage attenuation from synapses to AIS which is the initiation site of action potentials. As a result, the possibility for AIS potential to reach the threshold is decreased. On the other hand, the large somatodendritic membrane area acts as a current sink for sodium current generated in the AIS. A more distal AIS would reduce the current flow, thereby increasing the local depolarization at the AIS (Hamada et al., 2016). For pyramidal neurons (used in the study of Hatch et al.), computer modeling indicates that a more distally located AIS should enhance, rather than reduce, excitability. However, due to the two opposite factors, the change in excitability by AIS relocation is only modest (Gulledge and Bravo, 2016).

In contrast to theoretical prediction, another experimental study also found that activity-dependent distal shift of the AIS is associated with reduced excitability (Grubb and Burrone, 2010). This conflict can be reconciled by the MT model. As discussed in the last section, reduced excitability may arise from detyrosinated tubulins, independent of AIS location. An axonal microtubule contains mixed populations of detyrosinated and tyrosinated tubulins, with detyrosinated tubulins

enriched in the proximal axon (i.e., AIS) (Brown et al., 1993). Thus, disassembly of microtubules at AIS should increase the level of individual detyrosinated tubulins. Before they are re-tyrosinated, they may associate with the AIS membrane to inhibit neural activities. This view is consistent with the well-established result that calcium ions (Ca^{2+}) can induce microtubule depolymerization (Matsumoto et al., 1979) and the new finding that both AIS relocation and excitability attenuation can be prevented by blocking T- and L-type calcium channels (Grubb and Burrone, 2010).

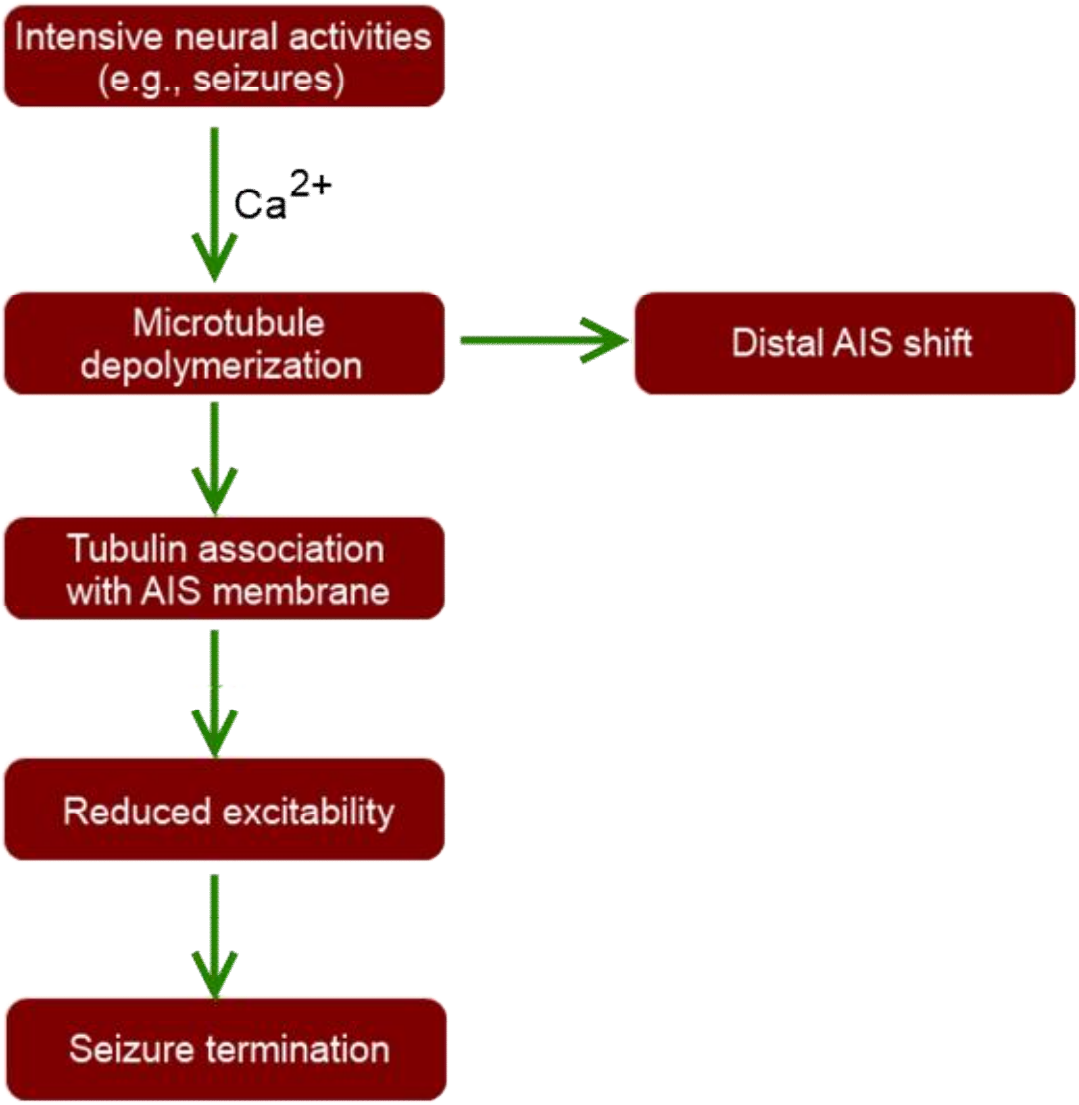

Figure 4. The role of microtubules in excitability. Seizures or normal intensive neural activities induce large Ca^{2+} influx to depolymerize microtubules, resulting in distal AIS shift. In the mean time, the depolymerized microtubules also produce individual tubulins which may be tyrosinated or detyrosinated at the

C-terminal. The detyrosinated tubulin could associate with the AIS membrane to inhibit neural activities.

The inhibition of neural activities by disassembled tubulin may play an important physiological role: providing a negative feedback on excitability. This can prevent endless neuronal firing. In the course of seizure progression, the number of neurons joining synchronized firing continue to grow (Jiruska etal., 2013). Then, how can the seizure terminate? The answer may lie in the individual detyrosinated tubulins which are disassembled from microtubules at AIS.