Short Term Potentiation May Result from Tubulin Inhibition of NMDA Receptors

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Abstract

The synaptic potentiation induced by tetanic stimulations includes not only the well-known long-term potentiation (a sustained phase), but also a transient phase referred to as short-term potentiation (STP). The mechanism of STP remains elusive. This paper shows that STP could originate from microtubule invasion into spines, resulting in the inhibition of NR2B-containing NMDA receptors by tubulin and CRMP2 (Paper 15). The Tubulin Inhibition Hypothesis leads to the following experimentally testable predictions:

1. The major cargoes transported by the dynamic microtubules into spines could be tubulin and CRMP2.
2. Phosphorylation of Serine 1166 in NR2B (also known as GluN2B), or pseudophosphorylation by mutating this residue to glutamate, may prevent tubulin binding to NR2B, thereby suppressing STP.
3. Tubulin could also bind to NR2D (GluN2D), as STP is also sensitive to the NR2D-selective antagonist UBP145.

Introduction

Experimentally, long-term potentiation (LTP) can be induced by several different protocols (Shipton and Paulsen, 2013, Table 1). One of them, referred to as "tetanus", applies high frequency (~100 Hz) stimulation on the presynaptic neuron for about 1 second. This leads to postsynaptic potentiation as monitored by field excitatory postsynaptic potentials (f-EPSPs). In most cases, the time course of f-EPSP consists of two phases: an initial decaying phase and a stable phase (Figure 1). LTP refers to the stable phase while the

initial decaying phase is known as short-term potentiation (STP) (Volianskis et al., 2013; Park et al., 2013).

The mechanism of STP is not known. Several lines of evidence suggest that it differs from LTP (Schulz and Fitzgibbons, 1997). This paper will show that STP could originate from microtubule invasion into spines, resulting in the inhibition of NR2B-containing NMDA receptors (NMDARs) by tubulin and CRMP2 (Paper 15).

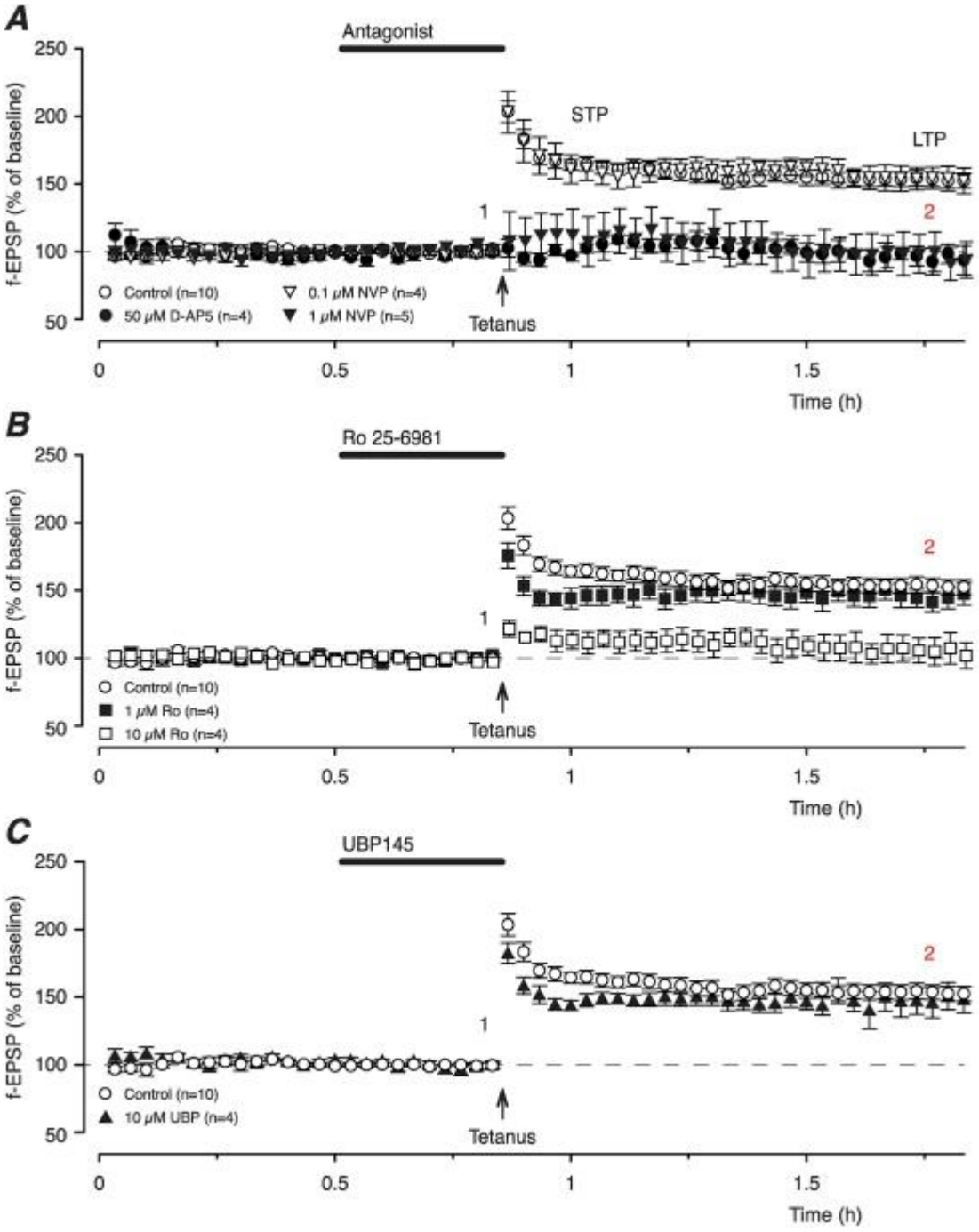

Figure 1. The time course of potentiation as manifested by f-EPSP.
(A) Tetanic stimulation induced both STP and LTP (open circles). The antagonist D-AP5 (filled circles) blocks the induction of STP and LTP; 0.1 μM NVP (open triangles) has no effect on the induction of potentiation whereas 1 μM NVP blocks both STP and LTP (filled triangles).
(B) STP is significantly reduced after pre-incubation with 1 μM Ro 25-6981 (filled squares) whereas LTP is not affected. 10 μM Ro 25-6981 (open squares) completely abolishes LTP.
(C) 10 μM UBP145 (filled triangles) reduces STP but spares LTP.

NVP, Ro 25-6981 and UBP145 target GluN2A-, 2B- and 2D-containing NMDARs respectively. D-AP5 is a non-selective NMDAR antagonist. [Source: France et al., 2017]

Microtubule Invasion into Spines

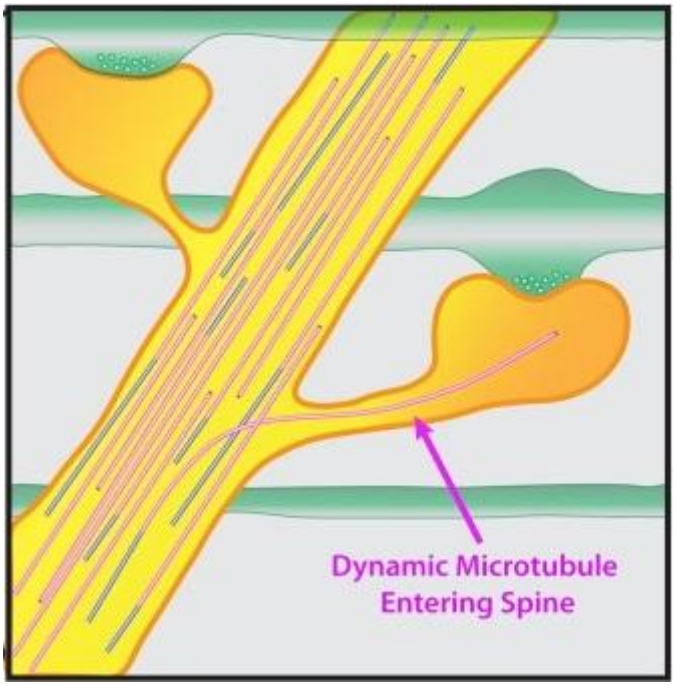

Figure 2. Illustration for the microtubule invasion into spines, which requires calcium, F-actin, and drebrin (Merriam et al., 2013). [Source: Dent, 2017]

The evidence that microtubules might play a role in synaptic plasticity came in 2008, when three independent groups reported that microtubules could enter spines in an activity-dependent manner (reviewed in Dent, 2017). Upon strong synaptic

stimulation, microtubules were shown to polymerize all the way to postsynaptic density (PSD) (Figure 2) and, within 20 seconds to 30 minutes, depolymerize back to the dendritic shaft (Hu et al., 2008). The functional role of the transient entry into spines is not clear.

The major function of microtubules is to transport various cargoes such as proteins and mitochondria. Presumably, during the short visit to spines, microtubules must bring in certain cargoes that are important for synaptic plasticity. However, the particular cargoes remain elusive. CaMKII is an essential protein in spines, but its entry into spines and PSD depends on F-actin, not microtubules (see this figure). PSD-95, another crucial protein in spines, is regulated by brain-derived neurotrophic factor (BDNF). Although the BDNF-mediated increase of PSD-95 in spines requires dynamic microtubule invasion, PSD-95 was not directly transported along microtubules into dendritic spines (Hu et al., 2011).

The Tubulin Inhibition Hypothesis suggests that the major cargoes transported by the dynamic microtubules into spines could be tubulin and CRMP2. Tubulin is the canonical binding partner of CRMP2. It has been demonstrated that the tubulin/CRMP2 complex can be transported by microtubules via the motor protein Kinesin-1 (Kimura et al., 2005).

The Mechanism of STP

STP might originate from microtubule invasion into spines, resulting in the inhibition of NR2B-containing NMDARs by tubulin and CRMP2. The NR2A-containing NMDARs will not be affected, contributing to the sustained phase of f-EPSPs (i.e., LTP). This hypothesis is supported by the following findings.

1. The tetanic stimulation used to induce LTP has been demonstrated to cause microtubule invasion into spines (Mitsuyama et al., 2008), which may carry tubulin and CRMP2 into spines to inhibit NR2B-containing NMDARs.
2. STP can be separated into two pharmacologically and kinetically distinct components: STP1 and STP2. STP2 decays more slowly than STP1. Pharmacologically, STP1 is similar to LTP, but STP2 can be abolished by Ro 25-6981, which selectively inhibits NR2B-containing NMDARs (Volianskis et al., 2013; Park et al., 2013).
3. The time for microtubules to invade spines takes minutes (Hu et al., 2008), which is approximately the same as the duration of STP.

The Binding Site of Tubulin

Paper 15 proposes that the NR2B-containing NMDARs can be inhibited by tubulin. Experiments have revealed that tubulin binds to the C-terminal domain of NR2B (van Rossum et al., 1999), but the exact binding site was not elucidated. It has been known for over two decades that protein kinase A (PKA) regulates the Ca^{2+} influx through NMDARs:

1. The NMDAR-mediated excitatory postsynaptic currents (EPSCs) are enhanced by PKA phosphorylation (Raman et al., 1996).
2. PKA blockers markedly inhibited NMDAR-mediated Ca^{2+} rises (Skeberdis et al., 2006).

More specifically, PKA inhibition decreased the open probability of NR2B-, but not NR2A-containing NMDARs (Aman et al., 2014), suggesting that PKA targets NR2B (also known as GluN2B), not NR2A (GluN2A). Therefore, PKA seems to counteract the inhibition of NR2B-containing NMDARs by tubulin. If this is indeed the case, *tubulin should bind to NR2B around the PKA phosphorylation site* (Figure 3). Recently, the PKA phosphorylation site on NR2B has been identified as Serine 1166 (Murphy et al., 2014). Tubulin is a highly negatively charged protein while phosphorylation is a process that adds a negatively charged phosphate group PO_4^{3-} to a protein. Hence, phosphorylation of this site should disrupt tubulin binding, thereby increasing the open probability of NR2B-containing NMDARs.

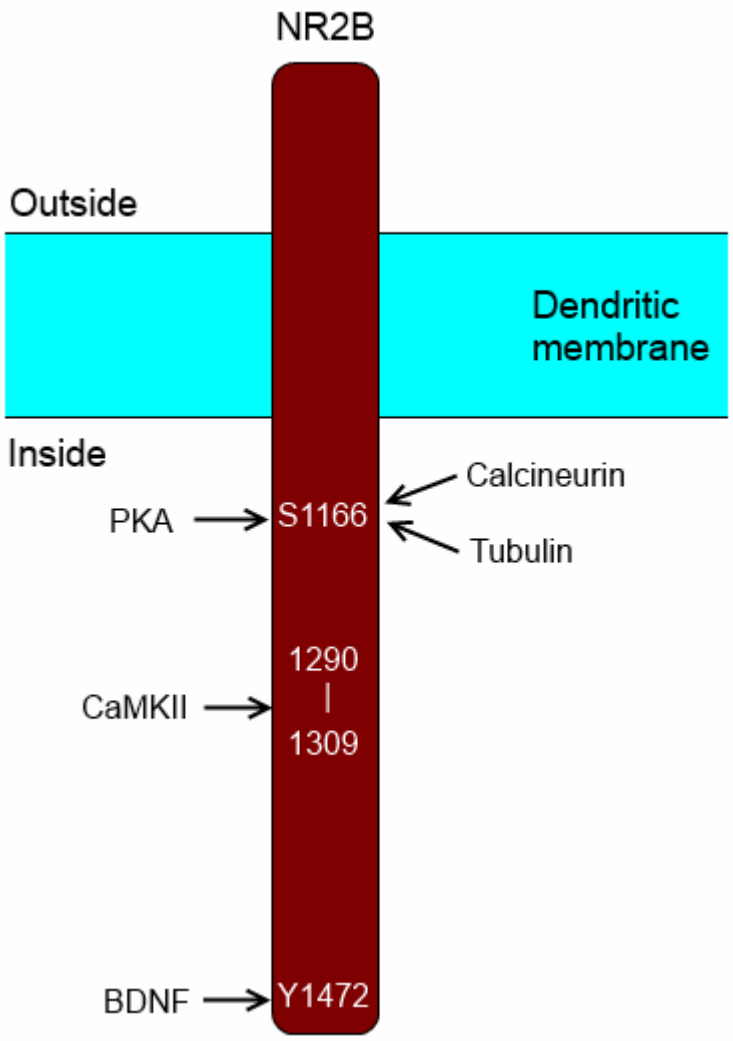

Figure 3. The crucial sites in the C-terminal domain of NR2B (GluN2B). (1) Serine 1166 (S1166) is the target of PKA phosphorylation, which prevents NMDAR inhibition by tubulin. Calcineurin may dephosphorylate S1166, thus promoting tubulin inhibition. (2) CaMKII binds to the region 1290-1309 (Strack et al., 2000). (3) BDNF promotes phosphorylation at Y1472, which prevents NR2B-containing NMDARs from being internalized, consequently increasing their localization to the membrane (Xu et al., 2006).

Memory extinction and retrieval could be fundamentally governed by tubulin inhibition of NR2B-containing NMDARs, which in turn depends on the phosphorylation state of S1166. Details are presented in the book, *Born to Forget, Die to Remember*.

Testable Predictions

1. The major cargoes transported by the dynamic microtubules into spines could be tubulin and CRMP2.
2. Phosphorylation of S1166 in NR2B, or pseudophosphorylation by mutating this residue to glutamate, may prevent tubulin binding to NR2B, thereby suppressing STP.
3. Tubulin could also bind to GluN2D (NR2D), as STP is also sensitive to the GluN2D-selective antagonist UBP145.