Anchoring of Calcineurin to GluN2B Differentiates Its Contributions to LTD from GluN2A

Frank Lee
eMail: frank@geon.us
Website: http://www.geon.us

Posted on: December 11, 2017

Abstract

GluN2A and GluN2B are the major subunits of NMDA receptors (NMDARs). It has been well documented that GluN2A and GluN2B contribute differentially to NMDAR-dependent long-term potentiation (LTP) and long-term depression (LTD). This paper shows that the key factor is the association of calcineurin with GluN2B, not GluN2A, via the A-kinase anchoring protein (AKAP79 in humans or AKAP150 in rodents). This fact explains why the brief tetanic stimulation induces LTP whereas the prolonged low frequency stimulation produces LTD. The paper also contrasts the calcineurin-mediated LTD with recently discovered CaMKII-mediated LTD, which is induced by prolonged agonist binding to NMDARs, but does not require Ca^{2+} influx through NMDARs.

Introduction

An NMDA receptor (NMDAR) consists of two GluN1 (also known as NR1) subunits and two additional subunits which are predominately either GluN2A (NR2A) or GluN2B (NR2B). Other subunits, GluN3, GluN2C and GluN2D, are relatively rare. Decades of intensive research has revealed that either GluN2A- or GluN2B-containing NMDARs are capable of inducing long-term potentiation (LTP), provided they can mediate sufficient Ca^{2+} influx (Shipton and Paulsen, 2013). However, GluN2B is critical for the NMDAR-dependent long-term depression (LTD) as the GluN2B-selective antagonist, ifenprodil or Ro 25-6981, abolishes LTD, but not LTP (Liu et al., 2004; France et al., 2017).

Experimentally, LTP can be induced by several different protocols (Shipton and Paulsen, 2013, Table 1). One of them, referred to as "tetanus", applies strong 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) (Paper 16). The most commonly used protocol to induce LTD is a weaker low frequency (~ 1 Hz) stimulation on the presynaptic neuron for about 15 minutes. Their underlying mechanisms are discussed in this paper. It will be shown that the two opposing synaptic modulations are determined mainly by two properties:

1. The kinetics of GluN2B-containing NMDAR currents is slower than that of GluN2A-containing NMDAR currents (Erreger et al., 2005).
2. The A-kinase anchoring protein is recruited to GluN2B-, not GluN2A-containing NMDARs (Colledge et al., 2000).

The Roles of AKAP79/150

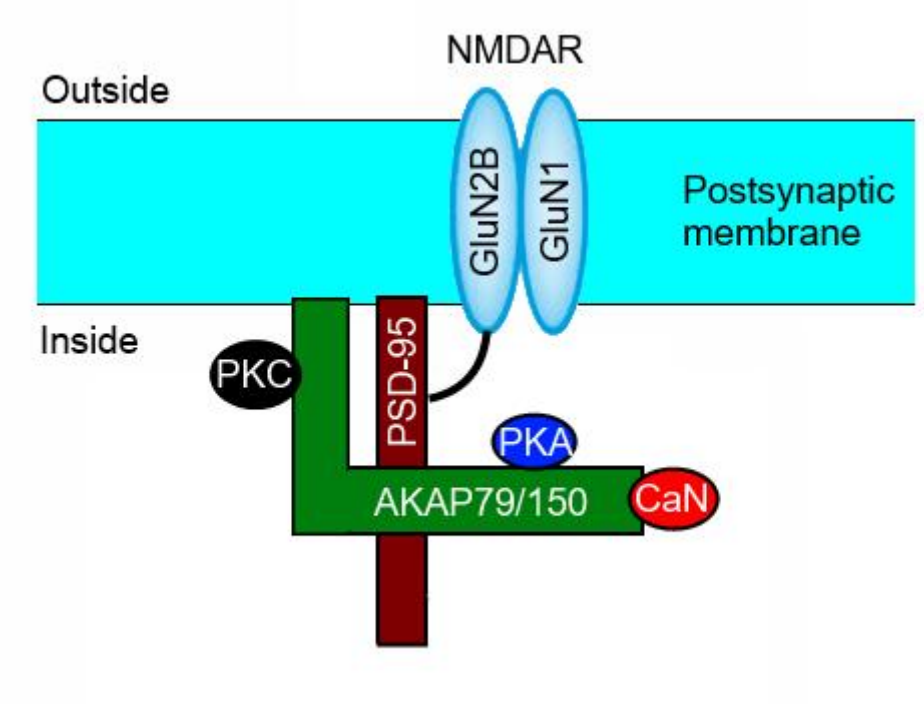

Figure 1. Schematic drawing of the organization among GluN2B-containing NMDAR, PSD-95, AKAP79/150, and anchored enzymes: PKA, PKC and CaN (calcineurin, also known as PP2B). The vertical configuration of PSD-95 is based on the observations by electron microscope tomography (Chen et al., 2008; Chen et al., 2015).

AKAP79/150 refers to either A-kinase anchoring protein 79 (AKAP79) in humans or AKAP150 (also called AKAP5) in rodents. They have essentially the same functions. Each is a scaffold protein that organizes protein kinase A (PKA), protein kinase C (PKC) and calcineurin (CaN) at a specific subcellular location to restrict their substrate targeting. For instance, the PKA anchored to a synaptic AMPA receptor (AMPAR) should be more effective in phosphorylating the AMPAR than the PKA diffusing randomly in the cytoplasm. AKAP79/150, together with anchored enzymes, can be recruited to the postsynaptic membrane by binding phosphatidylinositol-4,5-bisphosphate (PIP2) which is regulated by PKC phosphorylation (Dell'Acqua et al., 1998). It also interacts with membrane-associated guanylate kinase (MAGUK) such as PSD-95 and SAP97 (Sanderson and Dell'Acqua, 2011). Importantly, Ca^{2+} ions can enhance the activities of all three anchored enzymes: PKA, PKC and CaN.

AKAP79/150 has been demonstrated to associate with the GluA1 (old name: GluR1) subunit of AMPARs and the **GluN2B subunit of NMDARs** (Colledge et al., 2000). LTP or LTD is fundamentally determined by the increase or decrease in synaptic AMPARs, respectively. This in turn depends on the phosphorylation state of S845 and S831 in the GluA1 subunit of AMPARs. S845 is the target of both PKA and CaN while S831 can be phosphorylated by PKC and calcium/calmodulin-dependent protein kinase II (CaMKII). Phosphorylation on S845 or S831 stimulates synaptic incorporation of AMPARs, thus promoting LTP. CaN can dephosphorylate S845, resulting in LTD (Henley and Wilkinson , 2013; Woolfrey and Dell'Acqua, 2015, Figure 2).

How the Brief Tetanic Stimulation Induces LTP

Recalling that the GluN2B-containing NMDAR currents have slow kinetics. Therefore, the Ca^{2+} influx triggered by the brief tetanic stimulation should pass through mainly the GluN2A-containing NMDARs which are NOT associated with AKAP79/150. Hence, the major enzyme that induces LTD, CaN, is not significantly affected. The other two enzymes anchored by AKAP79/150, PKA and PKC, will also remain inactive. In this case, LTP arises mainly from phosphorylation on AMPARs and stargazin by CaMKII (Hell, 2014).

Without contribution from PKA and PKC, a single tetanic stimulation typically generates lower levels of potentiation (Huganir and Nicoll, 2013). Multiple tetanic stimulations separated by a few minutes may induce PKA-dependent LTP through phosphorylation of GluA1 at S845, leading to the synaptic incorporation of calcium-permeable AMPARs (Park et al., 2016).

How the Prolonged Low Frequency Stimulation Induces LTD

The prolonged weaker low frequency stimulation (LFS) will be able to trigger substantial Ca^{2+} influx through GluN2B-containing NMDARs (Erreger et al., 2005; Shipton and Paulsen, 2013). These Ca^{2+} ions should have significant impact on the activities of anchored enzymes. However, there are two competing processes on synaptic plasticity. CaN catalyzes the dephosphorylation, while PKA stimulates the phosphorylation, of S845. Which will win? The fact that prolonged LFS induces LTD indicates that CaN dominates. How?

AKAP79/150 binds both PIP2 and dendritic F-actin in a calmodulin- and PKC-regulated manner. The Ca^{2+} influx through NMDARs may activate PKC to release AKAP79/150 from the membrane (Dell'Acqua et al., 1998). The translocation of AKAP79/150 away from the membrane also involves CaN (Gomez et al., 2002). It has been shown that PKA moves with AKAP79/150 to the cytoplasm of dendrite shafts and the soma (Sanderson and Dell'Acqua, 2011), where PKA may trigger the expression of plasticity-related genes (e.g., BDNF) by phosphorylating cAMP response element-binding protein (CREB). Importantly, CaN remains at the synapse so that it can dephosphorylate AMPARs. This key finding explains why prolonged NMDAR activation favors LTD (Smith et al., 2006).

The above mechanism is consistent with LTD at different developmental stages. It has been known for many years that LFS produces robust LTD in hippocampal slices from very young rodents (mice or rats), bu not from adult animals (Kemp et al., 2000; Milner et al., 2004). The hippocampal neurons express predominately GluN2B at birth, which then decreases into adulthood while GluN2A increases with age (Dong et al., 2006). Therefore, the young rodents, but not aged, may contain sufficient GluN2B-associated CaN to produce LTD.

The Mechanism of CaMKII-Mediated LTD

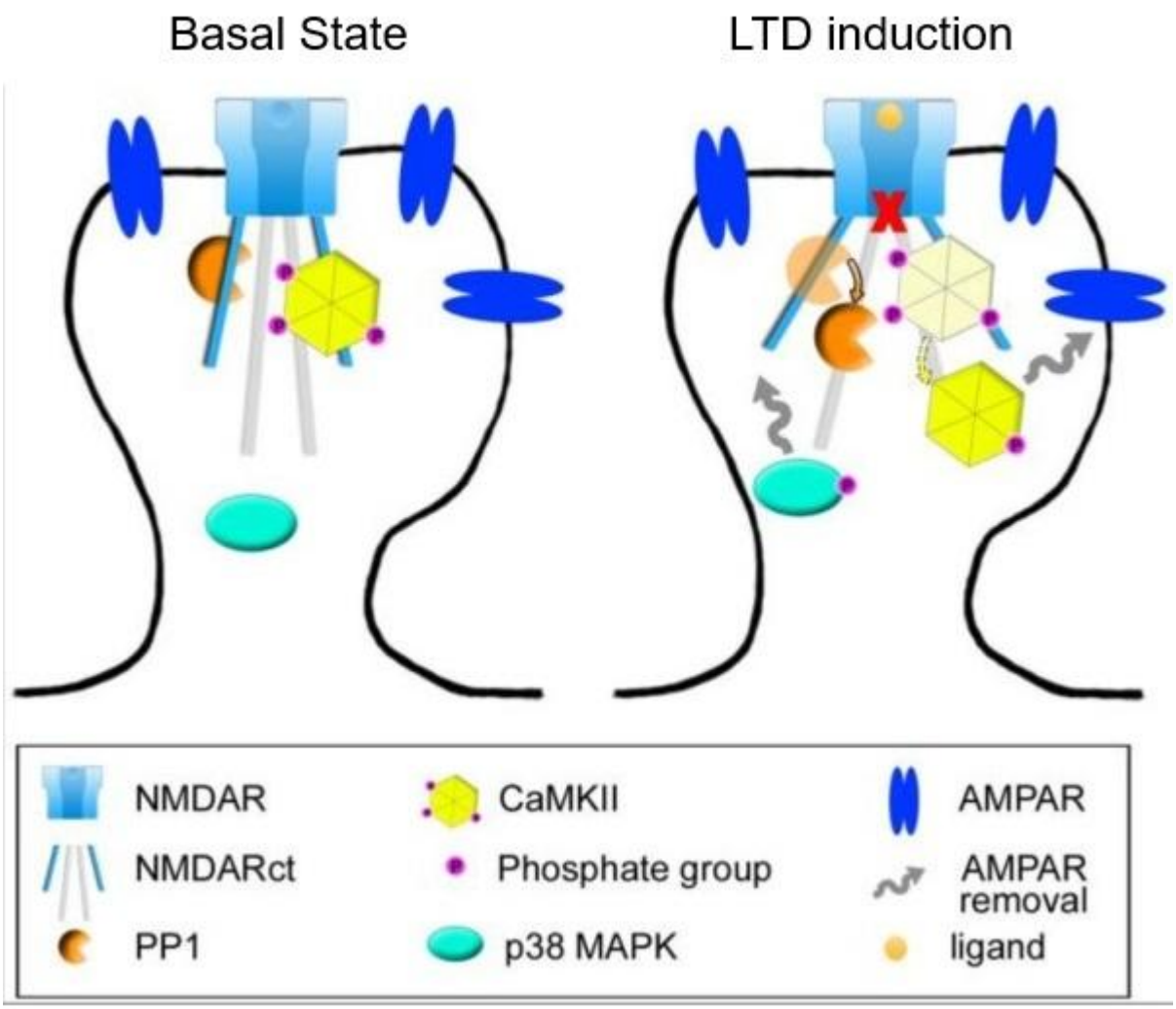

Figure 2. A model for the CaMKII-mediated LTD. In the basal state, the CaMKII associated with the NMDAR cytoplasmic domain is inaccessible to PP1. Upon agonist binding, movement in the NMDAR cytoplasmic domain permits PP1 to dephosphorylate CaMKII at T286, resulting in CaMKII relocation, which then phosphorylate the GluA1 subunit of AMPARs at S567, leading to AMPAR endocytosis and consequently LTD. [Adapted from Dore et al., 2016].

Not all forms of LTD depend on NMDARs or CaN. The best known example is the metabotropic glutamate receptor (mGluR)-dependent LTD which is not mediated by CaN (Lüscher and Huber, 2010; Kang and Kaang, 2016). Recently, an NMDAR-dependent, but

CaN-independent, LTD has been discovered (Nabavi et al., 2013). This novel LTD is induced by prolonged agonist binding to NMDARs, but does not require Ca^{2+} influx through NMDARs. In the past few years, further studies have provided compelling evidence that the Ca^{2+}-independent LTD could be mediated by relocation of CaMKII associated with the NMDAR cytoplasmic domain. The prolonged agonist binding to NMDAR causes movement of the cytoplasmic domain, leading to CaMKII relocation. This process requires dephosphorylation of CaMKII at T286 by protein phosphatase 1 (PP1) (Aow et al., 2015). The relocated CaMKII can then phosphorylate the GluA1 subunit of AMPARs at S567 (Coultrap et al., 2014), which is known to increase AMPAR endocytosis (Dore et al., 2016).

While the Ca^{2+} influx through NMDARs is not required for CaMKII-Mediated LTD, certain Ca^{2+} level is still necessary to achieve robust synaptic depression. The requirement of basal Ca^{2+} level has been found to correlate with CaN activity (Nabavi et al., 2013). These results suggest that CaMKII may act synergistically with CaN to mediate LTD during prolonged stimulation.