The CABT Hypothesis of Schizophrenia

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

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Abstract

Decades of intensive investigations have revealed that the hypofunction of NMDA receptors (NMDARs) could be the convergent point of schizophrenia (SCZ). The NMDAR Hypofunction Model stems from the findings that the NMDAR blockers, ketamine and phencyclidine, can induce a broad range of SCZ-like symptoms. This paper shows that the SCZ-inducing effects of ketamine are not caused by the blockade of NMDARs *per se*, but by the ultimate upregulation of brain-derived neurotrophic factor (BDNF) and synaptic GluN2B-containing NMDARs in layer 3 pyramidal neurons of dorsolateral prefrontal cortex. GluN2B-NMDARs, but not GluN2A-NMDARs, are subject to the occlusion of the CABT complex, which consists of a CRMP2 monomer and a tubulin heterodimer. The CABT occlusion of GluN2B-NMDARs can be prevented by protein kinase A (PKA). This mechanism is supported by the emergence of phosphodiesterase (PDE) as a therapeutic target for SCZ. PDE catalyzes the breakdown of cAMP. Its inhibitors may elevate cAMP level, thus enhancing PKA activity.

Introduction

Schizophrenia (SCZ) is a mental disorder characterized by positive symptoms (hallucinations, delusions), negative symptoms (anhedonia, avolition) and cognitive impairment. The cognitive impairment may appear in early childhood, and often precedes the development of positive symptoms in adolescence (Tamminga et al., 1998; Bowie and Harvey, 2006). Decades of intensive investigations have revealed that the hypofunction of NMDA receptors (NMDARs) could be the convergent point of SCZ (Olney et al., 1999; Kantrowitz and Javitt, 2010; Snyder and Gao, 2013; Nakazawa et al., 2017). This paper will present evidence to corroborate this model and further propose that the NMDAR hypofunction could arise from the occlusion of GluN2B-containing NMDARs by the **CABT complex** which consists of a **C**RMP2 monomer, an **a**lpha and a **b**eta **t**ubulin. The occlusion can be prevented by protein kinase A (PKA). This hypothesis is supported by the emergence of phosphodiesterase (PDE) as a therapeutic target for SCZ (Duinen et al., 2015; Snyder and Vanover, 2017; Heckman et al., 2018). PDE catalyzes the breakdown of cAMP and/or cGMP. Its inhibitors may elevate cAMP level, thus enhancing PKA activity.

The NMDAR Hypofunction Model of Schizophrenia

The NMDAR Hypofunction Model stems from the findings that the NMDAR blockers, phencyclidine and ketamine, can induce a broad range of SCZ-like symptoms (Jentsch and Roth, 1999). Before the advent of the NMDAR Hypofunction Model, theories for the pathogenesis of SCZ were dominated by the Dopamine Hypothesis (Davis et al., 1991), which posits that SCZ is characterized by abnormally low prefrontal dopamine (DA) activity (causing cognitive impairment and negative symptoms) and excessive DA activity in the striatum (causing positive symptoms). However, subsequent studies did not find direct evidence for low DA levels in the frontal cortex. On the other hand, substantially more evidence has been provided about the multiple routes (genetic, neurodevelopmental, environmental, social) that lead to the striatal hyperdopaminergia (Howes and Kapur, 2009).

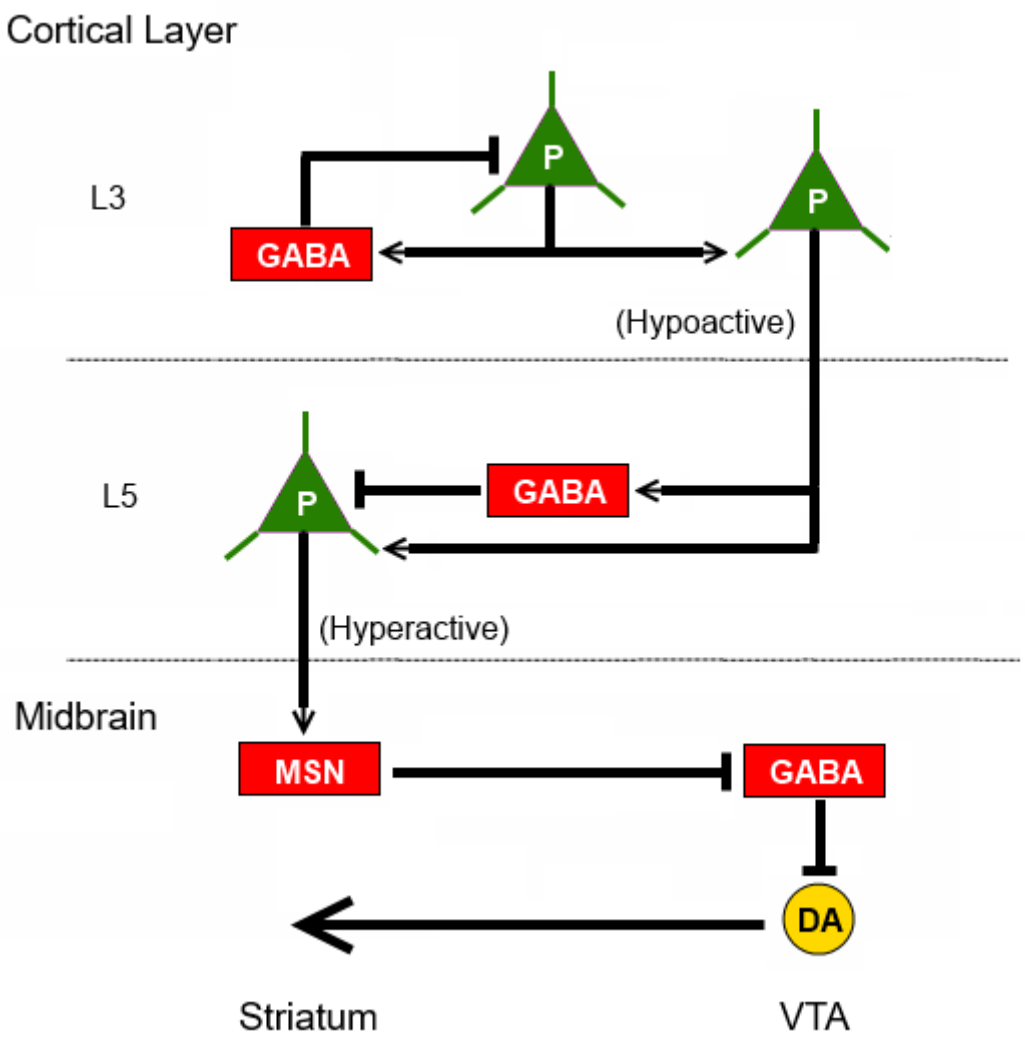

Figure 1. The NMDAR Hypofunction Model of SCZ. NMDAR hypofunction in L3 pyramidal neurons results in cognitive impairment. Hyperactive L5 pyramidal neurons (resulting from blockade of L5 GABAergic neurons, hyperactive GSK-3 activity or other factors) lead to hyperactivity of medium spiny neurons (MSNs) in the striatum, which in turn triggers excessive dopamine (DA) release from ventral tegmental area (VTA) to the striatum via disinhibition. The excessive DA activity in the striatum results in positive symptoms (psychosis). These abnomal alterations also disrupt the connectivity between the prefrontal cortex and the striatum, thereby resulting in the negative symptom (lack of motivation).

The NMDAR Hypofunction Model assumes that SCZ could originate from NMDAR hypofunction in the prefrontal cortex, which then leads to excessive dopamine activity in the striatum (Figure 1). More specifically, the NMDAR hypofunction could originate in the cortical layer 3 (L3) of dorsolateral

prefrontal cortex (dlPFC). Both GABAergic interneurons and pyramidal neurons in L3 of dlPFC exhibit significant abnormalities in SCZ subjects (Gonzalez-Burgos et al., 2015). These neurons play a central role in the generation of gamma rhythms (30-80 Hz) (see this article). The gamma synchronization is thought to be crucial for cognition (Gonzalez-Burgos and Lewis, 2012). Therefore, hypoactive L3 circuits may alter gamma oscillations, resulting in cognitive impairment.

The L3 pyramidal neurons are connected to layer 5 (L5) pyramidal neurons either directly or via GABAergic interneurons (Arnsten, 2015). Thus, the hypoactive L3 pyramidal neurons do not necessarily lead to hyperactive L5 pyramidal neurons which are the cause of positive symptoms. It depends on whether the direct or GABA-mediated pathway dominates. Ketamine has been shown to increase the activity of L5 pyramidal neurons (Wang et al., 2013), possibly by blocking L5 GABAergic interneurons (Homayoun and Moghaddam, 2007). Many other factors can also cause L5 pyramidal neurons hyperactive, such as the hallucinogenic drugs that target 5-HT2A receptors or the psychological stress that activates glycogen synthase kinase-3 (GSK-3) (discussed in Paper 26).

Layer 5 is the main output layer of the cortex (Brown and Hestrin, 2009), while Layer 2/3 pyramidal cells in the frontal cortex send axons mainly to other cortical areas (Ueta et al., 2013). A major target of L5 pyramidal neurons is the striatum (Ueta et al., 2013), which consists of primarily medium spiny neurons (MSNs). MSNs are long GABAergic neurons, projecting to various brain areas, including ventral tegmental area (VTA) where nearly two-thirds of all cells are DA-releasing neurons, while about 35% are GABAergic interneurons (Nair-Roberts et al., 2008) which inhibit DA neurons. It has been demonstrated that the GABAergic MSNs of striatum innervate the GABAergic interneurons in VTA, not DA neurons (Xia et al., 2011). Therefore, activation of MSNs will cause disinhibition of DA neurons, releasing dopamine to both cortical and subcortical regions, including the striatum (Creed et al., 2014). Consequently, hyperactive L5 pyramidal neurons may lead to excessive DA activity in the striatum, resulting in psychosis.

To date, all antipsychotic drugs are full or partial antagonists of dopamine D2 receptors (D2R) (Stępnicki et al., 2018). D2R has been shown to increases the excitability of MSNs via downregulation of the potassium channel Kir2 (Cazorla et al., 2012; Simpson and Kellendonk, 2017). This explains how D2R antagonists may alleviate psychosis.

Elevation of the striatal dopamine level is known to increase motivation (Berridge and Robinson, 2016). Then, how can SCZ be characterized by both striatal hyperdopaminia and negative symptoms (lack of motivation)? Recent studies suggest that motivation depends not only on the striatal

dopamine level, but also on the synchronization (connectivity) between the prefrontal cortex and the striatum (Reckless et al., 2015; Shukla et al., 2018). Hence, the hypoactive L3 circuits could disrupt the frontostriatal connectivity, resulting in negative symptoms.

The Ketamine Model of Schizophrenia

Ketamine is an open channel blocker of NMDARs. An NMDAR consists of two obligatory GluN1 (formerly NR1) subunits and two other subunits which, in adults, are mostly GluN2A (NR2A) and GluN2B (NR2B). Some NMDARs may contain GluN2C, GluN2D or GluN3 subunits. In the telencephalon (cerebrum), GluN2C is predominately expressed in glial cells while GluN2D exists in GABAergic interneurons (Alsaad et al., 2019). Ketamine blocks all types of NMDARs (Zorumski et al., 2016). However, it lost SCZ-inducing capacity in GluN2D knockout mice (Sapkota et al., 2016; Yamamoto et al., 2016), suggesting that ketamine could induce SCZ-like symptoms via GluN2D. Since GluN2D exists primarily in GABAergic interneurons, these results indicate that ketamine targets preferentially GABAergic interneurons.

The SCZ-inducing capacity of ketamine is not due to the blockade of NMDARs in L3 GABAergic interneurons *per se*, as brief administration of NMDAR blockers is sufficient to cause long-lasting symptoms (Jeevakumar et al., 2015; Jeevakumar and Kroener, 2016). Rather, the persistent NMDAR hypofunction could result from upregulation of brain-derived neurotrophic factor (BDNF).

It has been well documented that ketamine induces rapid synthesis of BDNF (Autry et al., 2011) which is required for its antidepressant effects (Lepack et al., 2014). The underlying mechanism has been largely unveiled. By blocking GABAergic interneurons, ketamine enhances glutamate release from pyramidal neurons via disinhibition. The glutamate then stimulates AMPA receptors on other pyramidal neurons, leading to activation of voltage-dependent Ca^{2+} channels and the release of BDNF from vesicles to the extracellular side. Subsequently, the extracellular BDNF may act on TrkB, triggering the MEK–ERK and PI3K-Akt pathways which converge to the activation of mechanistic target of rapamycin (mTOR) for the synthesis of BDNF within pyramidal neurons (Aleksandrova et al., 2017; Duman, 2018). Importantly, the stress-induced depressive behaviors are also modulated by layer 2/3 pyramidal cells (Shrestha et al., 2015), indicating that ketamine may exert both pro-SCZ and antidepressant effects by blocking the same GABAergic interneurons in L3.

As discussed by Gonzalez-Burgos et al. (2015), NMDAR hypofunction could originate in either L3 GABAergic or pyramidal neurons. The following

sections will show that elevated BDNF level can lead to NMDAR hypofunction in pyramidal neurons.

The PKA-Dependent NMDAR Desensitization

NMDAR hypofunction may arise from two possibilities: (1) loss of synaptic NMDARs, and (2) desensitization of synaptic NMDARs. In mice, knockout of obligatory GluN1 in L3 GABAergic neurons was shown to exhibit SCZ-like symptoms (Belforte et al., 2010). However, this does not imply that natural development of SCZ arises from lack of GluN1. In fact, ketamine has been demonstrated to **increase** both GluN2B (Burgdorf et al., 2013) and GluN1 (Liu et al., 2013), but not GluN2A (Jeevakumar and Kroener, 2016). The loss of NMDARs is not supported by postmortem studies either. Since dlPFC plays a key role in SCZ (Yoon et al., 2008), it has been the focus of postmortem studies over the last two decades. Only one study reported reduced GluN1 subunits (Catts et al., 2015). Two studies found normal levels of GluN1 and GluN2 subunits (Kristiansen et al., 2006; Henson et al., 2008). Another study showed that NMDAR complexes are **increased** in the postsynaptic density of SCZ cases (Banerjee et al., 2015).

NMDAR desensitization is defined as reduction of NMDAR currents in the continuous presence of glutamate. There are several forms of NMDAR desensitization: glycine-dependent (Cummings and Popescu, 2015), calcineurin-dependent (Krupp et al., 2002), Ca^{2+}-dependent (Sibarov and Antonov, 2018), and PKA-dependent (Aman et al., 2014). Memantine is a drug approved for treatment of Alzheimer's disease. Both memantine and ketamine are open channel blockers of NMDARs, but they have distinct effects on NMDAR desensitization. Memantine enhances Ca^{2+}-dependent desensitization of GluN2A-containing NMDARs while ketamine accelerates recovery from desensitization of GluN2B-containing NMDARs (Glasgow et al., 2017).

Interestingly, the PKA-dependent NMDAR desensitization is specific for GluN2B-NMDARs. NMDAR currents decrease when GluN2B is dephosphorylated at Ser-1166 located in the C-terminal domain (CTD). Phosphorylation of Ser-1166 by PKA increases NMDAR currents (Murphy et al., 2014). Since GluN2A-NMDARs are not subject to PKA-dependent NMDAR desensitization, higher GluN2B/GluN2A ratio at the synapse may lead to smaller NMDAR currents. As mentioned above, ketamine increases GluN2B without changing GluN2A level. This could be a key effect for ketamine to induce NMDAR hypofunction in SCZ. In agreement, aripiprazole (a third-generation antipsychotic) has been demonstrated to reduce the GluN2B/GluN2A ratio (Segnitz et al., 2011).

The importance of PKA in NMDAR hypofunction is underscored by the emergence of phosphodiesterase (PDE) as a therapeutic target for SCZ (Duinen et al., 2015; Snyder and Vanover, 2017; Heckman et al., 2018). PDE catalyzes the breakdown of cAMP and/or cGMP (Murthy and Mangot, 2015). Its inhibitors may elevate cAMP level, thus enhancing PKA activity. In rats, inhibitors of PDE type 1 (PDE1) have been demonstrated to improve memory performance as evaluated by novel object recognition (NOR) paradigm (Snyder et al., 2016). NOR is a valuable tool for the study of cognitive impairment in SCZ (Rajagopal et al., 2014). By using NOR and other methods, several PDE2 inhibitors have also been shown to exert beneficial effects on cognition (Gomez and Breitenbucher, 2013; Maehara et al., 2017).

PDE4 selectively hydrolyze cAMP, not cGMP. Its inhibitors have cognition enhancement and antidepressant action (Richter et al., 2013; Bolger, 2017). Furthermore, both PDE4 isoforms (PDE4A and PDE4B) interact with disrupted in schizophrenia 1 (DISC1) which, as the name implies, is strongly implicated in SCZ (Millar et al., 2005; Arnsten et al., 2012). PDE4 itself is associated with SCZ (Deng et al., 2011; Feng et al., 2016).

The Crucial Role of GluN2B in Persistent Firing of L3 Pyramidal Neurons

The importance of PKA in cognition and the specificity of PKA-dependent desensitization for GluN2B-NMDARs suggest that GluN2B could play a key role in cognitive functions. The persistent firing of L3 pyramidal neurons in dlPFC underlies cognitive functions. Both GluN2A and GluN2B subunits contribute to task-related firing. However, blockade of GluN2B-NMDARs by selective antagonists is sufficient to abolish persistent firing. This could be due to the GluN2B-NMDAR's slow kinetics that is particularly well-suited to hold "working memory". The persistent firing can also be abolished by systemic administration of ketamine (Wang et al., 2013).

Intriguingly, the activity-regulated cytoskeleton-associated protein (Arc) is required for persistent firing in slices isolated from layer 2/3 of mouse frontal cortex (Ren et al., 2014). Deletion of the Arc gene has bee shown to produce dopaminergic and behavioral abnormalities related to SCZ (Managò et al., 2016). Paper 27 will show that Arc plays an important role in regulating PKA-dependent desensitization (i.e., occlusion of GluN2B-NMDARs by CABT). Arc deficiency promotes CABT occlusion and NMDAR hypofunction, thereby disrupting persistent firing.

The GluN2B-GluN2A Switch During Development

At birth, synaptic NMDARs comprise predominately GluN2B, which is then progressively replaced by GluN2A during postnatal development (Quinlan et al., 1999). In most areas of the adult brain, synapses are dominated by GluN2A-NMDARs while GluN2B-NMDARs are enriched at extrasynaptic sites (Dumas, 2005). However, the L3 pyramidal neurons of dlPFC are an exception. Their GluN2B subunits are found exclusively within the postsynaptic densities (Wang et al., 2013).

The GluN2B-GluN2A switch occurs in the period coincident with the progression of SCZ from early childhood to adolescence. Males are more commonly affected by this disorder and exhibit psychotic symptoms earlier than females (Aleman et al., 2003; Loranger, 1984). As predicted, males display higher GluN2B/GluN2A ratio than females (Sinclair et al., 2016).

Mechanistically, the GluN2B-GluN2A switch is regulated by repressor element-1 silencing transcription factor (REST), casein kinase 2 (CK2) and tyrosine kinase Fyn:

- REST is a transcription repressor. During normal postnatal development, the activation of REST triggers experience–dependent chromatin remodeling that represses the transcription of *grin2b*, the gene encoding GluN2B (Tamminga and Zukin, 2015; Rodenas-Ruano et al., 2012). Consequently, activation of REST should reduce GluN2B level. Adverse experience in early childhood may disrupt REST activation, resulting in higher GluN2B level (Tamminga and Zukin, 2015).

- CK2 regulates the postsynaptic localization of GluN2B-containing NMDARs by phosphorylating GluN2B at Ser-1480, which is in the binding domain of PSD-95 that anchors GluN2B-NMDARs to the postsynaptic membrane. Phosphorylation of GluN2B by CK2 will disrupt their binding, leading to endocytosis. Consequently, CK2 should reduce synaptic GluN2B-NMDARs (Chung et al., 2004; Sanz-Clemente et al., 2010). In SCZ, the CK2 level is decreased (Aksenova et al., 1991), consistent with the notion that excess synaptic GluN2B could lead to SCZ. *CSNK2B*, which encodes the β-regulatory subunit of CK2, has been shown to be a risk gene for SCZ (Yang et al., 2018; Yoshikawa et al., 2018).

- While phosphorylation of GluN2B at Ser-1480 promotes internalization of GluN2B-NMDARs, phosphorylation at tyrosine 1472 (Y1472) by Fyn promotes their membrane insertion (Prybylowski et al., 2005; Chen and Roche, 2007). The abundance of Fyn in both frontal cortex and hippocampus depends on age and sex: (1) Fyn decreases with age as GluN2B switches to GluN2A and (2) males exhibits greater Fyn abundance than females (Sinclair et al., 2016, Figure 5). In SCZ

subjects, there was an increase in Fyn mRNA content (15.7%,) relative to controls (Ohnuma et al., 2003), which corroborates the hypothesis that elevated GluN2B/GluN2A ratio at the synapse is prone to develop SCZ.

BDNF Increases Surface GluN2B/GluN2A Ratio

BDNF has been demonstrated to increase synaptic GluN2B-NMDARs through Fyn-mediated phosphorylation on Y1472 (Xu et al., 2006; Hildebrand et al., 2016). Fyn is under the regulation of striatal-enriched protein tyrosine phosphatase (STEP) which has several isoforms (Braithwaite et al., 2006). The longest one, STEP61, can inactivate Fyn by dephosphorylation at Y420 (Nguyen et al., 2002) or promote GluN2B-NMDAR endocytosis directly by dephosphorylating GluN2B at Y1472 (Trepanier et al., 2012). Moreover, the BDNF signaling may activate phospholipase C gamma (PLCγ) (Paper 4) to induce STEP61 degradation through the ubiquitin-proteasome system (Saavedra et al., 2016). This explains how BDNF may increase synaptic GluN2B-NMDARs (Figure 2).

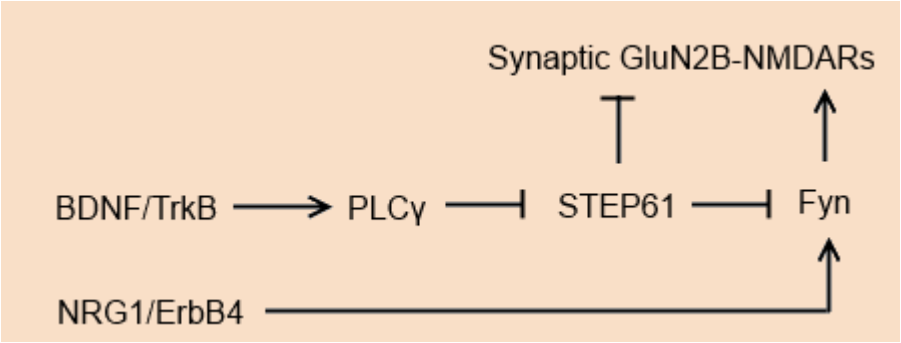

Figure 2. Regulation of synaptic GluN2B-NMDARs. The BDNF/TrkB signaling activates PLCγ to induce STEP61 degradation. Since Fyn is inactivated by STEP61, lower STEP61 level should increase Fyn activity. Hence, BDNF may augment Fyn activity, thereby increasing synaptic GluN2B-NMDARs. STEP61 can also directly promote GluN2B-NMDAR endocytosis by dephosphorylating GluN2B at Y1472. The NRG1/ErbB4 signaling can activate Fyn directly to enhance the surface expression of GluN2B-NMDARs.

ErbB4 is the tyrosine kinase receptor for Neuregulin 1 (NRG1). In the prefrontal cortex of SCZ subjects, the NRG1/ErbB4 signaling increases substantially, resulting in NMDAR hypofunction (Hahn et al., 2006). The increase of NRG1 signaling was also observed in treatment-resistant SCZ (Mostaid et al., 2017). There are two possible mechanisms: (1) direct

activation of Fyn (Bjarnadottir et al., 2007) and (2) mediated by TrkB (Pandya and Pillai, 2014).

It is important to note that SCZ could be caused by NMDAR hypofunction in cortical L3 pyramidal neurons. Thus, excess BDNF level in L3 may promote SCZ. However, at other brain regions, BDNF may have beneficial effects. For instance, L5 pyramidal neurons are hyperactive in SCZ. Therefore, the elevated GluN2B/GluN2A ratio resulting from BDNF upregulation in these neurons could be protective. While increased BDNF level in L3 pyramidal neurons promotes SCZ, reduced BDNF level in L5 pyramidal neurons predisposes to positive symptoms. This may reconcile the conflicting results of postmortem studies which reported both increased and decreased BDNF level in SCZ subjects without separating L3 from other brain areas (Gören, 2016). A recent study found that higher serum levels of BDNF are associated with greater severity of negative symptoms (Binford et al., 2018).

The CABT Hypothesis of NMDAR Hypofunction

What is the underlying mechanism for PKA-dependent NMDAR desensitization? The CABT Hypothesis posits that the NMDAR could be blocked by the CABT complex (Figure 3). Both CRMP2 and tubulin interact with the GluN2B subunit of NMDARs, not GluN2A (van Rossum et al., 1999; Al-Hallaq et al., 2007; Brittain et al., 2012; Brustovetsky et al., 2014). Therefore, the CABT complex can occlude only GluN2B-NMDARs, while GluN2A-NMDARs are spared, consistent with GluN2B-selective PKA-dependent NMDAR desensitization. This selectivity explains why NMDAR hypofunction should depend on the GluN2B/GluN2A ratio: the higher the ratio, the more synaptic NMDAR currents could be reduced by CABT occlusion.

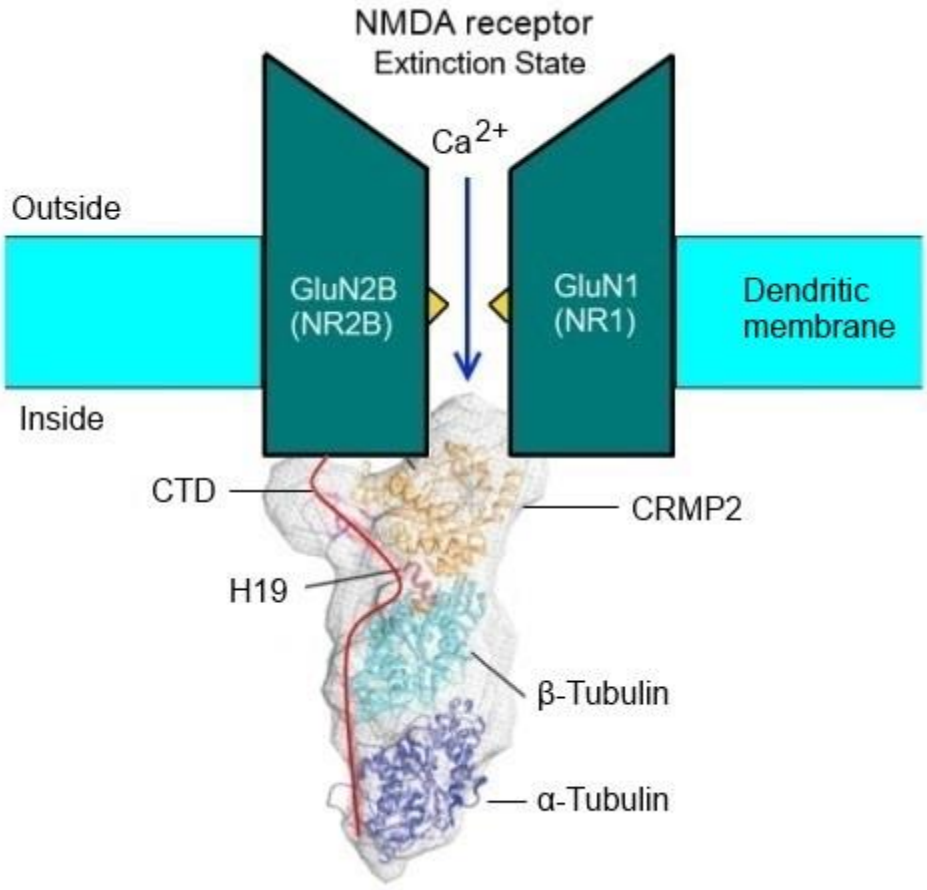

Figure 3. Occlusion of the GluN2B-containing NMDAR by the CABT complex which consist of a CRMP2 monomer, α and β tubulin. This state is called the "extinction state" because it could be the underlying mechanism for memory extinction. PKA can prevent the occlusion by phosphorylating GluN2B at S1166 located in the C-terminal domain (CTD). [The CABT structure is adapted from Niwa et al., 2017]

The CABT Hypothesis was originally proposed to explain "memory extinction" (inhibition of memory retrieval). It is supported by many studies on memory processes (see *Born to Forget, Die to Remember*). Interestingly, the hypothesis can readily explain a well known phenomenon, **infantile amnesia**, i.e., the inability for an adult to recall early childhood memories (Travaglia et al., 2016). The early childhood memories were encoded in the neurons dominated by GluN2B-NMDARs which are subject to CABT occlusion. Consequently, they were severely extinguished and very hard to retrieve. In line with this hypothesis, SCZ patients with higher GluN2B/GluN2A ratio are harder than healthy control in retrieving memories (Holt et al., 2009; Holt et al., 2012).

The NMDAR hypofunction should also depend on the binding between CABT and GluN2B, in which PKA plays a central role. Phosphorylation of GluN2B at Ser-1166 by PKA prevents CABT from occluding GluN2B-NMDARs, thereby increasing NMDAR currents (see Paper 24 for detail). Hence, PDE inhibitors may exert beneficial effects on cognition by enhancing PKA activity to prevent NMDAR hypofunction in L3 pyramidal neurons.

Implication of CRMP2 in Schizophrenia

CRMP2 is encoded by the *DPYSL2* gene which is implicated in SCZ (Liu et al., 2014; Pham et al., 2016). It has three subtypes: A, B and C. The CRMP2B protein (encoded by DPYSL2B) contains 572 amino acids (aa) while CRMP2A comprises 677 aa. In the brain, CRMP2B is ~20 times more abundant than CRMP2A (Balastik et al., 2015). Perhaps for this reason, previous studies focused on CRMP2B. However, CRMP2A, being a larger protein, could be more effective in occluding GluN2B-NMDARs than CRMP2B. Strikingly, the expression of DPYSL2A (but not DPYSL2B) is correlated with sex: higher expression in males (Liu et al., 2014), which may contribute to the higher incidence of SCZ in males.

In the 5′-untranslated region of DPYSL2, there is a CT di-nucleotide repeat (DNR) which responds to mTOR signaling. In Caucasians, this DNR most often comprises 11 CT repeats (11DNR). The polymorphic 13 CT repeats (13DNR) increases the risk for SCZ. It has been demonstrated that the 13DNR is associated with reduced CRMP2B level, but the translation of CRMP2A is not affected (Liu et al., 2014; Pham et al., 2016).

Since CRMP2 may reduce neuronal excitability by occluding GluN2B-NMDARs, the above results suggest that the dendritic spines of L3 pyramidal neurons could contain more CRMP2A than CRMP2B. Thus, higher expression of CRMP2A predisposes males to NMDAR hypofunction and SCZ. On the other hand, the L5 pyramidal neurons could be dominated by CRMP2B such that the 13DNR may increase their excitability to promote psychosis.

Future Test for the CABT Hypothesis

Compelling evidence indicates that SCZ arises from hypoactive GABAergic and pyramidal neurons in cortical layer 3 (Hoftman et al., 2017; Nakazawa et al., 2017). The effects of NMDAR blockers (e.g., ketamine and phencyclidine) further suggest that their hypoactivity could result from NMDAR hypofunction. This hypothesis has been

tested by ablation of obligatory GluN1 subunits in cortical and hippocampal GABAergic interneurons (Belforte et al., 2010). SCZ symptoms were not replicated unless under social isolation stress (Jiang et al., 2013; Bygrave et al., 2019). As pointed out by Bygrave et al., the social isolation stress may produce multiple changes in psychiatrically relevant behaviors, genes and neuronal structure on its own. Indeed, social isolation has been shown to increase GluN2A and GluN2B levels and exhibit aggressive behavior which can be abolished by knockdown of GluN2B expression (Chang et al., 2015).

Notably, postnatal GluN1 deletion largely confined to the excitatory neurons in layer 2/3 of the medial prefrontal cortex and sensory cortices displayed only cognitive deficits without additional behavioral or cellular phenotypes reflecting SCZ pathophysiology, even under chronic social isolation (Rompala et al., 2013). Removing NMDARs from the synapse is not the same as blocking synaptic NMDARs, because AMPA receptors can substitute for NMDARs at the synapse. Therefore, genetic ablation of GluN1 in excitatory neurons does not necessarily lead to reduced glutamatergic transmission and neuronal hypoactivity in L3 (see Rompala et al., 2013, Figure 8). However, cognitive functions will be impaired due to the crucial role of GluN2B-NMDARs in persistent firing (Wang et al., 2013). According to the CABT Hypothesis, the pro-SCZ effects of ketamine is not caused by the blockade of NMDARs *per se*, but by the ultimate upregulation of BDNF and synaptic GluN2B-containing NMDARs in L3 pyramidal neurons. The genetic ablation of GluN1 would not be able to upregulate GluN2B-NMDARs even under chronic social isolation. This hypothesis can be tested by overexpressing GluN2B in L3 pyramidal neurons.

In SCZ, L5 pyramidal neurons are hyperactive, leading to positive symptoms (psychosis). Psychological stress is known to activate glycogen synthase kinase-3 (GSK-3) (Jope et al., 2017) which is the convergent target of most antipsychotics (Beaulieu, 2012). Therefore, GSK-3 is likely to play a critical role in the hyperexcitability of L5 pyramidal neurons. Further details are presented in Paper 26.

Acknowledgment

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