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NR2B subunit in the prefrontal cortex: A double-edged sword for working memory function and psychiatric disorders

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Abstract

The prefrontal cortex (PFC) is a brain region featured with working memory function. The exact mechanism of how working memory operates within the PFC circuitry is unknown, but persistent neuronal firing recorded from prefrontal neurons during a working memory task is proposed to be the neural correlate of this mnemonic encoding. The PFC appears to be specialized for sustaining persistent firing, with N-methyl-D-aspartate (NMDA) receptors, especially slow-decay NR2B subunits, playing an essential role in the maintenance of sustained activity and normal working memory function. However, the NR2B subunit serves as a double-edged sword for PFC function. Because of its slow kinetics, NR2B endows the PFC with not only “neural psychic” properties, but also susceptibilities for neuroexcitotoxicity and psychiatric disorders. This review aims to clarify the interplay among working memory, the PFC, and NMDA receptors; demonstrate the importance of the NR2B subunit in the maintenance of persistent activity; understand the risks and vulnerabilities of how NR2B is related to the development of neuropsychiatric disorders; identify gaps that currently exist in our understanding of these processes; and provide insights regarding future directions that may clarify these issues. We conclude that the PFC is a specialized brain region with distinct delayed maturation, unique neuronal circuitry, and characteristic NMDA receptor function. The unique properties and development of NMDA receptors, especially enrichment of NR2B subunits, endows the PFC with not only the capability to generate sustained activity for working memory, but also serves as a major vulnerability to environmental insults and risk factors for psychiatric disorders.

Keywords

Prefrontal cortex; working memory; NMDA receptors; neuron development; schizophrenia; psychiatric disorders

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Conflict of interest

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Introduction

In order to act appropriately in response to environmental demands, humans can organize their thoughts and behaviors into functional responses by integrating information from the current context with past experiences. The prefrontal cortex (PFC), including its foremost working memory function, is essential to this integrative process.

Working memory is the ability to receive a stimulus, hold that information temporarily in mind, and subsequently respond in a goal-directed manner (Baddeley, 2010). Organisms are exposed to chaotic surroundings, incessantly bombarded with external stimuli; a system must be adept in filtering out irrelevant details and focus on pertinent information so that an appropriate response can be provoked. Working memory acts as the hub in memory processes necessary for higher cognitive and executive function, including thinking, planning, and comprehension (Baddeley, 1992; Goldman-Rakic, 1996). This form of short-term memory is provisionally active and serves as a middleman between the dynamic environment and long-term consolidation. Continual presentation of a relevant stimulus can lead to more permanent retention of that memory, while previously important information can be retrieved and returned to working memory manipulations to update mental schemas (D'Esposito et al., 1995; Goldman-Rakic, 1995) (Figure 1). Working memory is an extremely important neurological process that allows organisms to appropriately manage the continuously changing world in a feasible manner. The ability to read a story or navigate to a new destination are typical examples of working memory application. Engaging in such activities requires an individual to rapidly recall stored information from prior experiences and at the same time, focus on the influx of new information in order to understand and accomplish the current task. Listening to a lecture also serves as a good example for working memory function; novel information from the lecture is transmitted to the PFC, where the information is integrated with the student's acquired knowledge from past experiences. Thus, the student is able to place this new information in some preconceived context. During this process, each sentence carries unique and important information and as the lecture proceeds, novel information integrates with prior schemas so that it is easily placed in the developing context. Overall, working memory can be considered a cerebral sieve in which the PFC first sorts incoming stimuli into essential and disposable information, recruiting and coordinating with other brain regions to manage the information. Working memory is thus a delicate process steering us through the daily chaos of modern life.

Working memory depends on the PFC

What is the neural basis of working memory in the brain? This question has been extensively studied and a wealth of data supports that working memory is integrally linked to prefrontal function. The PFC is a molecularly, neuroanatomically, and functionally distinct brain region that governs higher cognitive tasks, including working memory, affective recall, attention, and behavioral inhibition (Goldman-Rakic, 1996). Seeing that several executive processes involve PFC functioning, this area is thus crucial for guiding efficient and appropriate behavior. As discussed above, working memory is particularly necessary for properly navigating through one's own environment. In order to accomplish

the working memory function, information needs to be transiently held in mind for processing, manipulation, and ultimately leading to the initiation of a behavioral response. A growing body of literature has demonstrated the PFC's paramount role in working memory, providing evidence by a variety of methodologies (Goldman-Rakic, 1995). Lesion studies have repetitively confirmed that damage to the PFC results in working memory deficits, as assessed by performance on spatial delayed-response tasks (Buckley et al., 2009; Goldman-Rakic, 1996; Goldman et al., 1971; Mishkin and Manning, 1978; Passingham, 1975).

Electrophysiological recordings have revealed preferential sustained activity in the PFC during engagement in a delayed-response working memory task. Information perceived as important is retained transiently in mind, which requires continual robust firing in order for the PFC to properly execute top-down control (Arnsten et al., 2010). Prefrontal neuronal firing increases following presentation of a salient cue, continues throughout a delay period in which stimuli are completely absent, returning to baseline once a response has been generated (Figure 2). This specialized activation in prefrontal cortical neurons reflects an „on-line? sustained activity, which fosters mnemonic encoding (Fuster and Alexander, 1971; Goldman-Rakic, 1996). This unique firing property in a subpopulation of PFC neurons, referred to as Delay cells, is postulated to be the molecular underpinning of working memory. The local PFC circuitry is specialized for such sustained activity, providing a neural correlate for working memory function (Goldman-Rakic, 1995). However, a fundamental question raised herein is what is the molecular and cellular basis for this unique neural sustained activity that is necessary for PFC-dependent working memory function.

Prefrontal cortical circuitry displays unique properties and delayed postnatal development

At first glance, the PFC circuitry appears to be quite similar to other cortical regions; it consists of excitatory glutamatergic pyramidal neurons and inhibitory GABAergic interneurons. These two types of neurons form a local microcircuit where recurrent excitation occurs among pyramidal neurons and is regulated by feedback inhibition via GABAergic interneurons. However, the cortical circuitries of the PFC are specialized to encode working memory. Specifically, this circuitry seems to be specialized to generate persistent action potentials for working memory function. Furthermore, pyramidal neurons (e.g., layer V cells) in the PFC reach functional maturity when sodium- and calcium-dependent regenerative potentials become prominent in the apical dendrites. This allows functional coupling of the apical dendrite to the soma and promotes integration of synaptic inputs from neighboring neurons and brain regions (Flores-Barrera et al., 2014). These unique properties link the PFC to higher cognitive capabilities. This raises the fundamental questions: how and by what mechanisms? A strong possibility for these questions is that the PFC circuitry is uniquely specialized during brain development.

Sensory cortices and other cognitive centers, such as the hippocampus, undergo discrete maturation processes where neuroanatomical changes precede the completion of functional development (Dumas, 2005) (Figure 3). Prefrontal maturation is one of the latest stages of brain development (Kolb et al., 2012) and corresponds to the fruition of working memory function (Crone et al., 2006; Goldman and Alexander, 1977; Luna et al., 2004). During

postnatal development, including both juvenile and adolescent periods, an overabundance of synapses is present throughout the brain. In order to refine the circuit and facilitate cognitive maturation, these synapses are gradually pruned over time. The PFC has the greatest synaptic density during early development, while synaptic pruning is the most gradual compared to other regions (Elston et al., 2009). This unique combination promotes molding of the PFC local circuitry by environmental factors, thus affecting logical thinking, decision making, and cognitive capabilities.

The juvenile and adolescent periods constitute developmental time points of great adaptability, but also are a time during which onset of mental illnesses reaches its peak (Lee et al., 2014). Environmental or physiological insults during this period therefore can lead to maladaptive behavioral phenotypes (Braun and Bock, 2011), possibly even resulting in more severe functional impairments such as the development of neuropsychological disturbances like schizophrenia, bipolar disorder, and depression, among others. Brain development during juvenile and adolescent stages is characterized by several critical processes that affect dendrites, synapses, cortical firing patterns, and neurochemical systems, along with their receptors (Andersen, 2003; Lee et al., 2014; Lewis, 1997). Maturation of white matter tracts during this stage sharpens communication between regions (Lee et al., 2014), enhancing overall brain connectivity and function. Therefore, during postnatal development, the brain is vulnerable to imbalances in this circuitry. These imbalances can be exacerbated by environmental and genetic factors, and finally culminate into a significant mental health risk. **Understanding the intricacies of the developing brain** can facilitate our knowledge of the evolution of neurodevelopmental disorders. Furthermore, such understanding can promote discovery of effective treatments to directly target neurobiological dysfunctions that emerge during this delicate developmental period.

The delayed maturation of the PFC makes the juvenile and adolescent stages critical periods for cortical development during which many molecular and cellular processes play essential roles in the maturation of the local prefrontal circuitry. One of these critical players, as shown in Figure 3, is the N-methyl-D-aspartate (NMDA) receptor located in excitatory glutamatergic synapses. We highlight the NMDA receptor specifically because it is not only critical to the generation of persistent activity for working memory function, but also subject to regulation by stress as well as modulation by dopamine, psychostimulants, antipsychotic drugs, and many other biological agents. There are many questions about the roles of NMDA receptors in the PFC. For example, is the NMDA receptor indeed essential for normal working memory function, which is prefrontal-dependent? What evidence supports that NMDA receptors are specialized for the unique persistent activity in prefrontal neurons that is purportedly responsible for working memory? Is this due to unique NMDA receptor subunit composition at prefrontal synapses compared to that in other cortical regions? How are NMDA receptors regulated by dopamine and other neuromodulators in normal and pathological states? Further, does this regulation occur in a bi-directional manner; i.e. up- or down-regulating NMDA receptor function leads to cognitive vulnerability or perhaps enhanced cognitive capabilities? Finally, how do NMDA receptor subunits in the PFC subserve mental illness, such as schizophrenia, throughout postnatal development? We will

address these questions below by reviewing the recent progress on the study of NMDA receptor function in the PFC.

The role of NMDA receptors, specifically the NR2B subunit, in prefrontal-dependent cognitive functioning

Delving deeper into the cellular mechanisms underlying working memory, pharmacological experiments have revealed the importance of NMDA receptors for this type of mentation. NMDA receptors are heterotetrameric complexes composed of a mandatory homodimer of NR1 and homodimers of either NR2 (NR2A-D) or NR3 (NR3A-B) subunits, or heterodimers of NR2 and NR3 subunits (Ogden and Traynelis, 2011). The NR1 subunit is vital for targeting NMDA receptors to discrete regions of the cell surface as well as membrane insertion; therefore, receptors lacking this subunit are not functional (Cull-Candy and Leszkiewicz, 2004). The NR2 subtype, however, confers functional heterogeneity to the NMDA receptor complex. NR2 subunits dictate such functional characteristics as open channel time, calcium permeability, decay time, and sensitivity to pharmacological agents (Paoletti et al., 2013). NR2A and NR2B are predominantly expressed in the postnatal brain with both subunits integral for synaptic plasticity and maturation mechanisms (Monyer et al., 1994; Sheng et al., 1994).

Blocking NMDA receptor activity has been shown to diminish working memory across several different mammalian species, reinforcing the impact of these glutamate receptors. Working memory performance in conscious monkeys was impaired following chronic administration of the NMDA receptor antagonist MK-801 (Tsukada et al., 2005). Rats treated with MK-801 show disrupted working memory performance in a delay-independent manner, indicating that NMDA receptors are necessary for the inception of mnemonic processing (Aultman and Moghaddam, 2001). Induction of cognitive deficits by NMDA receptor antagonism has also been reliably produced in human subjects (Hetem et al., 2000; Krystal et al., 1994; Malhotra et al., 1996; Newcomer et al., 1999; Parwani et al., 2005). Given the constitutive role of working memory for higher cognitive processes, NMDA receptors are imperative for several forms of cognition (Collingridge et al., 2013).

Although NMDA receptors are critical, not all subunits are created equal when it comes to working memory function. The importance of NR2B subunits in learning and memory processes was first explored in transgenic mice. NR2A- and NR2B-selective antagonists were useful in beginning to parse out the contribution of these individual subunits. Long-term potentiation (LTP) was attenuated following NR2A antagonism in slices from transgenic mice, but completely blocked by an NR2B antagonist; thus demonstrating that both NR2A and NR2B contribute to prefrontal synaptic plasticity, but NR2B plays a more dominant role (Cui et al., 2011; Plattner et al., 2014).

As determined by studies utilizing NR2B overexpression methods, this subunit heavily contributes to memory function. In adult animals, NR2B overexpression restricted to the forebrain (Cui et al., 2011; Tang et al., 1999) results in extended NMDA receptor channel opening in conjunction with enhanced NMDA receptor activation, thereby facilitating learning and memory across an entire cognitive spectrum and enabling the mice to exhibit

“smarter” performance in cognitive tasks compared to their wild-type counterparts (Cui et al., 2011; Tang et al., 1999). Moreover, overexpression of NR2B also altered synaptic plasticity, with selective enhancement in LTP while long-term depression (LTD), basal synaptic transmission, and paired-pulse depression remained comparable between transgenic and wild-type animals (Cui et al., 2011; Tang et al., 1999). These data demonstrate the important role NR2B plays across a gamut of memory functions and that expression of this subunit strongly impacts intellectual capabilities. Then, can NR2B serve as a biomarker to be tapped into as a means to alter intelligence and improve cognition? Specifically, overexpression of NR2B in the forebrain not only affected long-term synaptic plasticity at a cellular level, but also translated into a behavioral change (Cui et al., 2011; Tang et al., 1999). Remarkably, altering the expression levels of this one protein can dramatically impact PFC-dependent working memory function. Conceivably, alteration of NR2B levels can offer a promising avenue to explore a therapeutic approach to improve cognition, especially in disorders presenting with cognitive deficits, such as schizophrenia (Plattner et al., 2014; Snyder and Gao, 2013).

The NR2B subunit dictates prefrontal mnemonic encoding for working memory function

NMDA receptors are an important molecular substrate for cognitive processes, with the NR2B subunit particularly implicated in working memory function (Cao et al., 2007; Cui et al., 2011; Lett et al., 2014; Wang et al., 2009; Wang et al., 2013). Computational modeling and work in the primate dorsolateral PFC (dlPFC) has been integral in identifying the specific nature of NR2B's role in working memory. It has been proposed that NR2B grants the physiological requirements necessary for working memory function because of its slower decay time compared to NR2A. NR2B-containing receptors can conduct a large number of calcium and sodium ions during their intrinsically longer open channel state (Wang, 2001; Wang, 1999), thereby prolonging depolarization events essential for working memory function (Cull-Candy et al., 2001; Wang, 2001) and synaptic summation (Wang et al., 2008). More efficient temporal summation patterns help bring the neuron closer to the spike-firing threshold of the cell (Kumar and Huguenard, 2003), which in turn promotes cognitive capabilities. The delay period between perception of a stimulus and the manufactured response employs neurons to stay online, actively engaged. The NR2B subunit affects NMDA receptor channel kinetics so that the persistent firing pattern necessary for PFC-dependent cognitive functions, such as working memory and cognitive flexibility, can thus be maintained (Goldman-Rakic, 1995; Wang et al., 2013; Wang, 1999).

This long-held and plausible hypothesis was supported by a recent elegant study, in which Wang et al. (2013) provided the first direct evidence that NMDA receptor functioning underlies persistent firing specifically in primate dlPFC Delay cells (Wang et al., 2013) (Figure 4). Generalized blocking of NMDA receptor activity depressed Delay cell firing and working memory function. Importantly, selectively blocking NR2B activity significantly suppressed Delay cell firing, demonstrating a direct action of NR2B in the working memory task. In contrast, blockade of AMPA receptors showed a lag effect in which activity was decreased later in the Delay cell firing period, not immediately. These data suggest that

AMPA receptor activity may serve to support depolarization events in Delay cells, whereas NMDA receptors, especially NR2B subunits, are required for the moment-to-moment firing necessary for working memory function (Wang et al., 2013). This receptor system and its associated signaling molecules comprise a complex regulatory network that encodes working memory. However, some other questions remain to be addressed. For example, if sustained activity is NR2B-dependent, then why does blocking NR2A also affect firing responses? We can conjecture that NR2B channel kinetics are ideal for supporting persistent activity, while NR2A could be necessary to regulate other activity such as rapid retrieval and recycling of information from other brain regions. This intriguing idea will be further elaborated upon in the future directions section.

The dynamics of NR2B expression differ during development of associative learning and cognitive abilities

NMDA receptor subunit expression is regionally and developmentally restricted, thereby contributing to the brain's complexity by imposing unique physiological and functional properties within individual brain regions (Cull-Candy and Leszkiewicz, 2004). A subunit switch from mostly NR2B- to NR2A-containing NMDA receptors occurs at a time coincident with circuit refinement necessary for maturation of synapses and learning capabilities (Dumas, 2005). Physiological changes, such as a reduced decay time constant and enhanced temporal summation properties as well as reduced sensitivity of the NMDA receptor to NR2B-specific antagonists, such as ifenprodil and Ro25-6981, are also evident (Figure 5). The PFC is unique relative to other brain regions, because an NR2B-to-NR2A subunit switch that occurs in several other neuroanatomical regions (Dumas, 2005) appears to be absent in the PFC local circuitry. As shown in Figures 3 and 5, over the course of neurodevelopment, NR2B levels do not decline, but remain persistently high in the adult rat PFC (Wang et al., 2008). Lack of a NR2B subunit physiological switch serves to fit PFC function for working memory; in other words, the molecular composition of prefrontal neurons suits the operating characteristics of this structure. During the execution of working memory function, stimulation of glutamatergic pyramidal neurons results in persistent activity, and the temporal summation of these inputs is facilitated by the presence of NR2B subunits (Flores-Barrera et al., 2014). Seeing that the PFC retains high expression of NR2B, this brain region is ideal for supporting mnemonic encoding.

The NR2B/NR2A ratio is a critical regulator of synaptic plasticity function in addition to its role in associative learning (Figure 3). Many brain regions, including the hippocampus and primary sensory cortices, undergo this subunit switch from primarily NR2B- to NR2A-containing NMDA receptors, which eventually results in a **higher** ratio of NR2A/NR2B (Gonzalez-Burgos et al., 2008; Paoletti et al., 2013; Sheng et al., 1994; Wang et al., 2008; Williams et al., 1993). The maturation of these regions and the increased role of NR2A allow rapid responses to incoming environmental stimuli. In mature primary visual cortex (V1), the **high NR2A/NR2B** ratio endows the neural circuitry with rapid temporal summation properties and receptive field maturation (Cho et al., 2009; Philpot et al., 2001). Similarly, in the mature hippocampus where the **NR2A/NR2B** ratio is also **high**, LTP is heavily mediated by NR2A-containing receptors, and can be significantly diminished in the

presence of NR2A-specific, but not NR2B-specific, antagonists (Zhao et al., 2005), although this issue remains controversial (Yashiro and Philpot, 2008). Thus, in sensory cortices, hippocampus, and many other cortical regions, NR2A-containing NMDA receptors may dictate cognitive functions associated with long-term synaptic plasticity in adulthood.

In the PFC, however, **the NR2A/NR2B** ratio is stable during development, which is distinctly different from the mature state of these aforementioned brain areas with associative learning capabilities (Dumas, 2005; Wang et al., 2008). In the adult rat PFC a significant NR2B-component is evident (Wang et al., 2008). Indeed, the maintenance of NR2B-containing NMDA receptors aid synaptic and functional maturation of the PFC. In late adolescence/early adulthood, but not earlier developmental stages, LTP can be induced in the PFC upon stimulation of the ventral hippocampus. This is a time corresponding to the peak contribution from NR2B-containing NMDA receptors of the PFC, indicating this molecular component is required for circuit level maturation. Blockade of the NR2B subunit or NMDA receptors in the PFC at this later developmental stage abolishes LTP (Flores-Barrera et al., 2014). These data indicate that the NR2B subunit intimately regulates circuit refinement and functional maturation of the PFC, which is necessary for normal functioning of working memory.

Working memory is a dynamic and transient process compared to long-term memory consolidation and storage; thus, it is not surprising that the molecular mechanisms **governing these** processes are fundamentally different (Arnsten and Jin, 2014; Taylor et al., 1999). Differing from the PFC, NR2B protein levels decline in other cortical areas during development and at adulthood reach a level of expression that will be maintained until aging (Dumas, 2005) (Figure 3, 5). NR2A protein levels rise in parallel with this process such that mostly NR2A-containing receptors are present post-synaptically. However, the molecular mechanisms that drive this switch are still poorly characterized. In sensory cortices and hippocampus, this switch is strongly dependent on experience and neuronal activity (Yashiro and Philpot, 2008). These two factors do not appear to guide prefrontal development in a similar fashion as the PFC lacks direct thalamocortical innervation from the sensory thalamus with the exception of the mediodorsal nucleus of the thalamus (Conde et al., 1990; Ferguson and Gao, 2015; Giguere and Goldman-Rakic, 1988; Ray and Price, 1992).

Although physiological properties and cognitive functions associated with the PFC are unique from other brain regions, we can utilize reports on the development of these other regions as a framework to guide our understanding of prefrontal development. Rodenas-Ruano et al. (2012) have delineated the epigenetic mechanisms that drive the subunit switch in the hippocampus (Rodenas-Ruano et al., 2012). Epigenetic processes are uniquely poised to dynamically regulate gene expression in response to a variety of environmental and genetic factors. Repressor element 1 silencing transcription factor (REST; neuron-restrictive silencer factor) specifically targets the promoter region of the gene encoding NR2B, *Grin2b* (Ballas et al., 2005; Qiang et al., 2005). REST binds to two repressor sites of the *Grin2b* promoter, NRSE2 and NRSE3 (Qiang et al., 2005), in association with other co-repressor proteins CoREST and Sin3A (Yu et al., 2011). Shortly after enrichment of REST **and its co-repressors at these sites**, there is a reduction of NR2B protein. The presence of REST at the

Grin2b promoter region is accompanied by epigenetic readouts, including changes in histone trimethylation (Roopra et al., 2004) and DNA methylation. These epigenetic modifications culminate in reduced NR2B protein levels, while NR2A protein is independently upregulated (Desai et al., 2002; Rodenas-Ruano et al., 2012). Thus, the developmental switch can occur and cognitive capabilities associated with learning and other hippocampal functions are able to reach their fully functional state. Whether epigenetic mechanisms contribute to the persistent expression of NR2B in the PFC, however, has yet to be explored.

The preceding argument is based on experiments conducted in both rat and nonhuman primate PFC, but there is a long-standing debate about whether rats have what could be a homologous to the primate PFC (Preuss, 1995; Seamans et al., 2008). Thus, the questions still remain: do rats have Delay cells as have been observed and studied in the primate dlPFC, and how can findings in rodents be extrapolated to primates? In order to evaluate whether rats have a region comparable to the primate dlPFC, a set of criteria are commonly used to study the homology of these species' cortices. For our purposes, we will focus on (1) the pattern and relative density of specific connections among the PFC and other brain regions, as well as (2) the electrophysiological and behavioral properties that make up the functional PFC (Uylings et al., 2003). Firstly, based on anatomical definitions, it has been shown that both nonhuman primate and rat prefrontal cortices receive their most dense projections from the mediodorsal nucleus of the thalamus (Ferguson and Gao, 2015), a major PFC identifier. Furthermore, both rat and primate PFC receive vast afferent projections from other cortical areas, primarily those of sensory and limbic origins (Van Eden et al., 1992). Anatomical evidence supports the notion that rat medial PFC (mPFC) is related to primate anterior cingulate cortex (ACC) as well as dlPFC (Seamans et al., 2008). Thus, previous literature suggests that rats do have an anatomical region relevant to the study of prefrontal cortical cognitive functioning. However, it is still unclear whether rat PFC contains a discrete region comparable to primate dlPFC, and therefore, whether rats can provide a useful tool to model dorsolateral function and dysfunction, specifically (Preuss, 1995). Comparing the neuroanatomical correlates of behavior among species is more difficult given the unique survival mechanisms of each species (Uylings et al., 2003). Behavior is essentially the expression of an animal's capability to respond to environmental demands (Uylings et al., 2003). Through this simplified definition, it becomes easier to identify the brain regions specifically responsible for weeding out distracting stimuli, placing incoming information in a previously developed context, and allowing for an appropriate behavioral response, be it new or old. We cannot deny that this function serves a critical role in the survival of both rats and nonhuman primates.

In primates, lesions to dlPFC result in deficits to working memory function. In rats, lesions to the medial PFC (mPFC), also result in severe working memory deficits, specifically in acquisition and retention of a task (Uylings et al., 2003). We can therefore expect that neurons with properties similar to Delay cells as described in the dlPFC may also exist in the rat mPFC. In fact, such cells have recently been identified in the rat mPFC via electrophysiological recordings made during a modified delayed alternation Y-maze task (Yang et al., 2014). Through this technique, Yang et al. (2014) identified a subset of

pyramidal neurons within the mPFC that respond in a transient, but not persistent, manner during the delay period of this paradigm of Y-maze. Interestingly, they identified three types of delay-like cells: those that fire during the early, middle, or late stages of the delay period (Yang et al., 2014). Therefore, from an electrophysiological perspective, we can conclude that rats do indeed possess a cellular correlate for encoding working memory information. It should be noted that in the eight-arm radial maze, a more cognitively complex task, such delay-like cells were not identified in the rat mPFC (Jung et al., 1998). Thus, these characteristic firing patterns in the rat mPFC may be dependent on the cognitive load imposed by each behavioral task. Interestingly, the activity of discrete cells throughout the delay stage of a working memory task has also been identified in primate dlPFC (Funahashi and Inoue, 2000; Rainer et al., 1999). Thus, there appears to be a network of cells in the rat mPFC capable of encoding working memory-relevant information (Yang et al., 2014), as in the primate dlPFC. However, it remains to be determined whether these cells have NR2B-containing NMDA receptors, and whether they are similarly sensitive to NR2B antagonism during a working memory task, as those described in primate dlPFC.

NMDA receptor-mediated persistent activity is modulated by other receptor systems relevant to working memory

Thus far we have described that neuronal activity and working memory function in the PFC are intimately linked to the NMDA receptor; however, this receptor cannot act in solitude. NMDA receptors are at the core of a dynamic network of receptor complexes and signaling cascades, which regulate neuronal activity and ultimately, working memory and higher order cognitive capabilities (Paoletti et al., 2013). These receptor systems are poised to regulate NMDA receptor-induced activity because they are located within dendritic spines (Arnsten and Jin, 2014).

In the context of prefrontal-dependent working memory, two major neuromodulatory systems, the adrenergic and dopaminergic, are involved in regards to how they can influence NMDA receptor-mediated function. Beginning with the adrenergic system, activation of $\alpha 1$ - and $\alpha 2$ -adrenergic receptors can have bidirectional effects on prefrontal-dependent performance. That is, infusion of the $\alpha 1$ agonist phenylephrine into the primate dlPFC impairs delay-response performance; however, guanfacine, an $\alpha 2$ agonist, improves working memory performance (Mao et al., 1999; Wang et al., 2007b). When norepinephrine, the endogenous agonist of adrenergic receptors, is experimentally or naturally depleted, spatial working memory performance can be restored following administration of $\alpha 2$ agonists (Arnsten and Goldman-Rakic, 1985; Rama et al., 1996). These findings suggest an important role of norepinephrine-mediated signaling in prefrontal-dependent working memory function. More specifically, adrenergic receptor activation has been found to reduce NMDA currents in prefrontal cortical neurons (Liu et al., 2006), but it remains to be determined how unique subtypes of adrenergic receptors regulate NMDA receptors to affect working memory function and their possible links to neuropsychiatric diseases.

The dopamine system plays an integral role in both normal cognitive performance as well as in schizophrenia pathology (Seamans and Yang, 2004). The hyper-dopaminergic state of the cortex was one of the first discoveries regarding the underlying pathology associated with

schizophrenia. To this day, the dopamine hypothesis plays a major role in drug development and treatment paradigms. Vijayraghavan et al. (2007) demonstrated that dopamine D1 receptor (D1R) stimulation effects working memory performance in an inverted U dose-response manner (Arnsten et al., 1994; Vijayraghavan et al., 2007). Moderate levels of D1R stimulation sharpen neuronal firing patterns underlying PFC-dependent cognitive processes; non-preferred direction firing is reduced while preferred direction firing is sustained, thereby increasing the signal-to-noise ratio. In contrast, high levels of dopamine induce an overall suppression of delay-related firing in both preferred and non-preferred responses (Vijayraghavan et al., 2007; Williams and Goldman-Rakic, 1995), likely impairing working memory function. Because schizophrenia is characterized by a hyper-dopaminergic state at the cortical level, it is possible this excess of dopamine dampens Delay cell firing through direct or indirect modulation of NMDA receptors in the PFC. In addition, refinement of the PFC during development is accompanied by maturation of neuromodulatory signaling pathways, including the dopamine system. Co-activation of NMDA and dopamine receptors facilitates the functional maturation of the PFC (Flores-Barrera et al., 2014). Thus, NMDA receptors are essential in working memory performance, but are also strongly dependent upon the appropriate development of neuromodulatory systems in order for expression of full functionality.

A delicate balance of normal working memory functioning

The persistent firing in working memory-relevant Delay cells has been shown to be NMDA-dependent and more specifically, NR2B-dependent (Wang et al., 2013). The slow channel kinetics of NR2B subunits make them ideal for sustaining persistent firing; however, there can be negative consequences when this delicate balance goes askew. Specifically, synaptic NR2B-containing NMDA receptors mediate a large influx of calcium into the post-synaptic spine when activated. Calcium signaling regulates pyramidal cell firing in the PFC and working memory function. Calcium can promote its own accumulation in the spine through a series of local signaling molecules, including protein kinase A (PKA), cyclic AMP (cAMP) and cAMP-generating mechanisms, IP₃-dependent intracellular stores, metabotropic glutamate receptors (mGluRs), as well as activation of monoamine (such as dopamine, serotonin, and norepinephrine) receptor-mediated modulations (Arnsten and Jin, 2014; Arnsten et al., 2012; Snyder and Gao, 2013). cAMP is a major downstream effector of calcium throughout the brain. Neuronal firing patterns in the PFC are negatively modulated by the accumulation of this molecule after NMDA receptor activation; increasing cAMP-driven activity or blocking the inhibition of cAMP production results in diminished Delay cell firing in a working memory task (Wang et al., 2011b; Wang et al., 2007a), ultimately resulting in impaired working memory function (Taylor et al., 1999). Small conductance calcium-activated potassium channels are necessary for negative feedback regulation of this signaling cascade. By minimizing calcium influx through NMDA receptors, these channels promote negative regulation of working memory function (Brennan et al., 2008; Faber, 2010). Therefore, longer channel openings allow for a greater influx of calcium into the post-synaptic cell, which under controlled conditions ideally suits the physiological basis of persistent firing, but can quickly pose as a risk for excitotoxicity (Lett et al., 2014; Wang et al., 2013). In this case, NR2B serves as a double-edged sword for the PFC: it plays a critical

regulatory role in normal functional operation, but also leaves this region vulnerable to psychiatric disorders (Figure 6), as discussed below.

NR2B is a double-edged sword for cognitive function, neuropathic pain, excitotoxicity, and schizophrenia

Heightened levels of NR2B in the PFC or forebrain can improve or recover cognitive performance and synaptic plasticity. However, there is a double-edged element to this overabundance of NR2B; in addition to the pro-cognitive effects, this can increase the brain's susceptibility to excitotoxicity via a superfluity of intracellular calcium levels, as discussed above. These effects are strongly dependent on development. Variation in NR2B or NMDA receptor function during early postnatal development, such as during juvenile or adolescent stages, can have lasting effects on cognitive capabilities. Brief inhibition of NMDA receptors with a noncompetitive antagonist, such as ketamine, in early development can have long-term effects on prefrontal-dependent cognition and GABAergic interneurons of the mPFC (Jeevakumar et al., 2015). Working memory, associative learning, and attention are all impaired in adulthood following transient NMDA antagonism during juvenile development (Jeevakumar et al., 2015). Globally, an elevation of NR2B subunit levels can sensitize the brain to hyperexcitability (Jantzie et al., 2015). In epilepsy, hyperexcitability, particularly in the temporal lobe, can increase excitotoxicity. In children with treatment-resistant electrical status epilepticus, the region responsible for abnormal brain activity was removed and found to have a higher ratio of NR2B/NR2A levels compared to healthy control patients (Loddenkemper et al., 2014). During early postnatal development, stimulation of glutamate receptors with seizure-inducing doses of NMDA, a time during which NR2B is highly expressed throughout the forebrain, results in cognitive impairment in adulthood (Stafstrom and Sasaki-Adams, 2003). This evidence further confirmed that over-stimulation of NR2B-containing NMDA receptors in early postnatal development can result in cognitive impairments, likely due to an overabundance of intracellular calcium signaling. In this way, the NR2B subunit exposes the brain's vulnerability and sensitivity to environmental perturbations during early postnatal development that can have direct effects on cognition in adulthood.

Mis-expression of NR2B can be a result of its regulatory proteins becoming dysfunctional. Reelin, an extracellular matrix glycoprotein, for example, is consistently found to be diminished in post-mortem SCZ tissue. Importantly, Reelin demonstrates a regulatory role in the developmental expression of NR2B in the PFC. In mice with a haploinsufficiency for Reelin, prefrontal-dependent long-term fear memory, LTP, and spine density are all diminished in juvenile mice (Iafrati et al., 2014). This effect may be due to an overabundance of NR2B-containing NMDA receptors. Reelin-haploinsufficient juvenile animals treated with a single dose of Ro25-6981, an NR2B-specific antagonist, or ketamine, an NMDA receptor antagonist, demonstrated recovery in these outcome measures. Remarkably, a similar dosing paradigm using ketamine also resulted in recovery of spine levels, synaptic function and cognition in adolescence (Iafrati et al., 2014). These data strongly demonstrate the importance of NR2B, and more generally NMDA receptor, functioning in development. In a Reelin-deficient environment, NR2B levels become

overabundant and this can have disruptive effects on cognitive function in early development. When this overabundance is corrected by antagonism of NR2B or NMDA receptors, functioning is restored.

These data reveal the double-edged nature of developmental NR2B expression on cognition. It is critical for the expression of NR2B to be tightly regulated, particularly during early postnatal development. Aberrantly elevated levels of NR2B at this stage increase the vulnerability of the brain to excitotoxic events, which can have long-lasting effects on neuronal structure and cognitive function. However, when elevated in adulthood, NR2B exerts an enhancing effect on cognition. This highlights the importance of NR2B's diverse role throughout development. Remarkably, it appears this aberrant overabundance of NR2B can be ameliorated by even a single dose of an NR2B-specific or noncompetitive NMDA receptor antagonist (Iafrati et al., 2014). This effect highlights the extremely plastic nature of the juvenile and adolescent brain; a single attempt to normalize the neural environment can indeed result in lasting amelioration of early dysfunction.

Thus, we propose that a threshold of NR2B expression exists. In this case, age seems to set this threshold. In early development, increases in NR2B can result in lasting impairment of cognitive performance and synaptic plasticity (Wang et al., 2011a). When this same aberrant increase in NR2B is placed in the context of the adult brain, we see many pro-cognitive effects of its overexpression (Cui et al., 2011; Tang et al., 1999). This is intimately tied to the conductance of calcium ions through NMDA receptors. High calcium in early development results in excitotoxic events, whereas in adulthood, high calcium seems to restore the plasticity of the brain. How can this be? NR2B levels are relatively high in the PFC throughout development, suggesting a greater calcium influx is an integral part of PFC maturation and cognitive functioning. Thus, other critical developmental milestones, such as synaptic pruning and maturation of neuromodulatory systems, contribute to prefrontal development and eventually working memory and cognitive functioning, as described above.

The double-edged property of the NR2B subunit is further evident when this subunit is overexpressed in the brain. On one hand, the overexpression of NR2B in the forebrain enhances working memory and cognitive performance, but this comes at a price; forebrain-restricted NR2B-overexpressing mice exhibit an enhanced sensitivity to inflammatory pain (Wei et al., 2001), and behavioral sensitization after inflammation (Wu et al., 2005). Therefore, the NR2B subunit is not only important for memory, but also for chronic and neuropathic pain (Zhuo, 2009).

Moreover, NMDA receptor activation was found to initiate opposing actions in neuronal damage and survival. NR2B-containing receptors, especially those localized at extrasynaptic sites, mediate excitotoxic events that lead to neuronal damage and apoptosis (Hardingham, 2006), whereas NR2A activation stimulates pro-survival pathways. These results further demonstrate that NR2B poses a risk factor for excitotoxicity (Liu et al., 2007). Because NR2B and NR2A have dual actions in excitotoxicity this helps to explain why memory processes in the PFC are dependent on both subunits; NR2B activity mediates Delay cell firing for working memory function and NR2A activity helps to counterbalance the

sustained firing by activating pro-survival pathways to prevent overexcitation, however, this idea is speculative and future experiments are warranted.

Furthermore, any alteration to the number or composition of NMDA receptors results in profound neurodevelopmental and functional impairments (Endele et al., 2010). In disorders with a deficit in working memory, such as schizophrenia, the PFC is an exceptionally vulnerable neuroanatomical region because of NR2B expression prevalence throughout development (Lett et al., 2014; Wang et al., 2008; Wang et al., 2013). A unique gene encodes each subunit of the NMDA receptor. Polymorphisms in the genes that encode NR1, NR2A, and NR2B, in particular, are segregated with schizophrenia diagnosis. Chronically ill schizophrenia patients who are carriers of a single nucleotide polymorphism (SNP) in the NR2B gene show a significant reduction of reasoning performance compared to controls (Weickert et al., 2013). In addition, this SNP results in a greater reduction of NR1 protein and mRNA in the dlPFC compared to carrier control subjects, which may lead to overall loss of NMDA receptor insertion and therefore function. Analysis of NMDA receptor mRNA levels in post-mortem schizophrenia tissue has resulted in conflicting findings, however, and studies report increases, decreases, or no change in transcript levels. This inconsistency hinders our ability to identify whether the pathophysiological mechanism underlying schizophrenia is a direct result of loss of NMDA subunit proteins (Weickert et al., 2013). Nevertheless, mutations in *Grin2b* are consistently associated with cognitive deficits (Endele et al., 2010). This evidence suggests the mis-regulation of NR2B and the PFC in schizophrenia contributes significantly to cognitive aberrations that are characteristic of this mental illness.

Future Directions

Compilation of data across the years has demonstrated that the PFC is a specialized forebrain region that stands apart molecularly, anatomically, and functionally from other cognitive centers. The PFC is a neuroanatomical **hub for** working memory, **which** has been shown by an assortment of experimental approaches to be NMDA receptor-dependent **and** more specifically NR2B-dependent. **This brain region** undergoes a unique developmental progression; at maturity the NR2B/NR2A ratio remains high as opposed to other regions in which NR2B expression gradually declines. Preservation of relatively high NR2B expression into adulthood facilitates this structure to perform **higher order** neural functions such as working memory, which requires sustained neuronal firing. Although the molecular **retention of** NR2B subunit levels ultimately determines cognitive capacity and is an integral aspect of **PFC functionality**, expression of this subunit **also serves as** a double-edged sword. Proper working memory function requires NR2B activity, but over-activation can turn disadvantageous and result in excitotoxicity that makes the PFC vulnerable to impairment, and **the development of neuropathic pain and** psychiatric disorders. Because **high** NR2B activity walks a fine line between beneficial and harmful **outcomes**, the PFC **has a greater risk of susceptibility** due to the stable expression of NR2B throughout development.

Research has come a long way in elucidating the **connections** among the PFC, working memory, and NMDA receptors, but many critical questions still remain. To continue

expanding our knowledge and start uncovering the underpinnings of psychiatric illnesses, two major topics **involving** NMDA receptors must be addressed. First, what are the differential roles of NR2A and NR2B subunits? Also, what is the relationship between NR2B and psychiatric illnesses, specifically schizophrenia?

1) Do NR2B and NR2A subunits confer different functional properties within the PFC local circuit?

First, one of the major current issues is the lack of knowledge on the functional differences between the NR2A and NR2B subunits; and with that, we should better understand the prominent roles of NR2A and NR2B within the PFC. Do these subunits offer distinctive properties to the individual neurons of the PFC, including both pyramidal neurons throughout the cortical lamina **as well as** distinct subtypes of GABAergic interneurons, especially during different postnatal developmental periods? Is NR2B more involved in working memory, **while NR2A helps mitigate** excitotoxicity? How does altering the NR2B/NR2A balance in the PFC affect cellular and behavioral outcomes? How is this ratio regulated by other neurotransmitters, such as monoamines, in the brain?

To address these questions a series of conditional and inducible knockout experiments should be conducted *in vivo*. NR2A and NR2B should be independently knocked down within the PFC of **wild-type** animals. Once knockout efficiency has been confirmed, cognitive tasks assessing working memory performance in addition to excitotoxic activity would be conducted. Based on previous literature about the dual actions of NMDA receptor subunits, we would predict differential outcomes for each knockout scenario. Prefrontal NR2B knockout animals would be predicted to result in more cognitive deficits, specifically working memory impairments due to the loss of NR2B, which mediates extended depolarization required for sustained firing of pyramidal neurons. NR2A knockout animals, however, would be predicted to exhibit greater neuronal damage and cell death due to decreased stimulation of pro-survival pathways. Conducting these experiments would help parse out the differential roles of each subunit within the PFC more specifically.

Furthermore, to gain a developmental perspective on the importance of these subunits, inducible knockout of NR2A and NR2B subunits can be restricted to juvenile and adolescent periods. In this case, we can further evaluate the role of these individual subunits throughout development. Additionally, we can assess **how NR2A and NR2B disruption** in adolescence can affect persistent firing, and thus adult PFC-dependent cognition and neuronal survival.

2) What are the connections between NR2B and schizophrenia?

Secondly, we should scrutinize the role NR2B plays in **relation to** pathological conditions such as schizophrenia. Schizophrenia is a neurodevelopmental psychiatric disorder with the major functional deficit being cognitive impairments. Seeing that the PFC is the **major** site for working memory, NR2B fluctuations within this neuroanatomical region could **be one of the** underlying etiologies of cognitive deficits in **schizophrenia**. One approach to address the role NR2B plays in the context of disease would be to overexpress prefrontal NR2B in different animal models of schizophrenia to assess whether working memory deficits can be rescued **by this restoration**. Neuregulin 1, dysbindin, and Disrupted in Schizophrenia 1 (DISC1) mutant animals, for example, could be employed as the genetic models for the

disease, while the methylazoxymethanol (MAM) or poly I:C model would serve as environmental **insult** models (Carpenter and Koenig, 2008). If working memory deficits are reversed or **ameliorated** following PFC-restricted NR2B overexpression, this would help to solidify that NR2B **indeed is** a molecular target in disease progression and **that this** subunit underlies various aspects of the pathophysiological phenotype displayed. **In addition, this would provide** solid evidence for the NMDA receptor hypofunction hypothesis (Snyder and Gao, 2013).

Schizophrenia is a neurodevelopmental disorder, in which cognitive symptoms typically occur in the juvenile stage and fully manifest during early adolescence. Therefore, it is important to elucidate the difference between normal PFC development and that which is affected by schizophrenia. It would be interesting to investigate how NR2B disruption early in development affects an organism **later on in** adulthood. An inducible knockout animal model would be **an elegant** experiment that could help answer this question. After transiently knocking out PFC-restricted NR2B protein during juvenile or adolescent periods, these animals would then undergo cognitive behavioral tasks to assess working memory **function**. In doing so, we can better shape our understanding of the neuropathology of schizophrenia, and learn whether NR2B could trigger the development of this disease.

Another informative study that would provide insightful knowledge into this concept would be determining how much is too much NR2B activation? What is the threshold for overexcitation? Does NR2A activation increasingly come on board to provide neuroprotection once the threshold is reached and surpassed? Is the level of individual NR2B and NR2A subunits, or rather the relative ratio of NR2B/NR2A, critical to cognitive dysfunction in the disease state? Although the optimal experimental designs for probing these particular questions are unclear, they remain critical to address and would offer tremendous knowledge to the field.

In summary, the PFC is a specialized brain region featured with working memory function that offers an adaptive advantage to the organism. The exact mechanism of how working memory operates within PFC circuitry is unknown, but is presumed to be associated with persistent activity. Although the molecular underpinnings of sustained activity remain somewhat elusive, research efforts over the past few decades have clarified that NMDA receptors, particularly those containing slow-decay NR2B subunits, play an essential role in the maintenance of sustained activity and the normal operation of working memory function. NR2B endows the PFC with special neural psychic properties because of its slow kinetics; however, this feature in turn can become detrimental, leading to vulnerability for neural excitotoxicity and psychiatric disorders, such as schizophrenia. Because of this fine balance between favorable and harmful consequences that can result from NR2B mis-expression, this subunit serves as a double-edged sword. In this review we have raised many important questions and addressed some key recent findings that provide novel mechanistic insights into understanding the critical role NMDA receptors, particularly NR2B subunits, play in persistent activity and working memory function, as well as prefrontal function in both normal conditions and disease states.

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Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
GABA	gamma-aminobutyric acid
LTP	long-term potentiation
LTD	long-term depression
NMDA	N-methyl-D-aspartate
PFC	prefrontal cortex

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Highlights

- Interplay among working memory, the PFC, and NMDA receptors
- PFC displays unique properties and delayed postnatal development
- NR2B subunit determines prefrontal mnemonic coding for working memory function
- NR2B/NR2A ratio is correlated with associative learning and cognitive abilities
- NR2B is a double-edged sword for cognitive function and neuropsychiatric disorders

Figure 1.

Working memory processing in the PFC is a crucial function, steering us through the daily chaos of modern life by filtering out distractions and retrieving the most important information from our environment. Amidst the chaos, pertinent stimuli must be filtered as needed for efficient execution of goal-directed behaviors. For this, working memory function is critically necessary. Transiently holding such information in the PFC enables completion of tasks, i.e. (A) briefly maintaining important words from your lecture in the PFC in order to (B) retrieve relevant information from long-term memory stores so as to give the information context, or to consolidate it into long-term memory storage for future reference.

Figure 2.

The schematic illustrates neuronal firing of a Delay cell, captured by single unit electrophysiology recording, located in the primate dorsolateral PFC during the oculomotor delayed response working memory task. The persistent neuronal activity during the delay period in the absence of a visual cue is hypothesized to be the neuronal correlate of working memory.

Figure 3.

Color-coded correlation between the NR2 subunit switch (**top**) and associative learning (**bottom**) during postnatal development: this model shows the normal (solid line) and abnormal (dashed line) development and function of NMDA receptor subunits and their associations with spatial learning and working memory in the hippocampus and PFC, respectively. Green lines are representative of the hippocampus, and red and black lines represent the prefrontal cortex. The subunit shift of the NR2A/2B ratio precedes the development of associative learning; for example, spatial learning for the hippocampus (Hippo; **solid green lines**) matures immediately after the NR2A/2B ratio reaches its adult level. When the ratio is prematurely or aberrantly altered, associative learning capabilities do not reach their fully functional state, resulting in cognitive deficits (green dashed lines represent hippocampal deficits in spatial learning and red dashed lines represent prefrontal deficits in working memory). [Modified from (Dumas, 2005). Copyright 2005, with permission from the Rightslink® by the Copyright Clearance Center via ScienceDirect].

Figure 4.

Blockade of NR2B, but not AMPA receptors, completely shut down the task-related (preferred direction) firing of Delay cells in the primate dorsolateral PFC (dlPFC). A, An example of a Delay cell treated with NR2B antagonist Ro25-6981 versus AMPA receptor antagonist CNQX. Under control conditions, the neuron showed spatially tuned Delay-related firing (dark blue) induced by the visual cue stimulation. Subsequent iontophoresis of the NR2B antagonist Ro25-6981 (red) reduced the task-related firing. The iontophoretic current was then turned off and the neuron recovered normal rates of firing (light blue). After recovery, the AMPA antagonist CNQX (green) was iontophoretically delivered onto the neuron. CNQX had little effect on firing early in the Delay epoch, but reduced firing in the later portion of the Delay epoch (green). B, Average firing patterns of the eight Delay cells under conditions of control (dark blue), iontophoresis of Ro25-6981 (red), and iontophoresis of CNQX (green). Ro25-6981 produced a substantial reduction in task-related firing, and CNQX had a subtle effect, reducing firing only in the later aspect of the Delay epoch [Reprinted from (Wang et al., 2013). Copyright 2013, with permission from the Rightslink® by the Copyright Clearance Center via ScienceDirect].

Figure 5.

Excitatory synapses in the PFC exhibit a similar proportion of NR2A and NR2B subunits in both young and adult rats. A, There is no difference in the decays of NMDA receptor-mediated EPSCs between young and adult rat PFC layer 5 pyramidal neurons ($p=0.363$). B, NR2B antagonist ifenprodil effectively blocked NMDA current in the PFC. C, Similar proportion of ifenprodil-sensitive NMDA currents in both young and adult PFC synapses ($p>0.05$). D, NR2A and 2B protein levels from young and adult (3.5 month) PFC tissue samples. Lanes 1-4 denote the layout of the Western blot gel, as seen in the above representative image. E, Similar proportions of NR2A and NR2B subunits in both young and adult rats ($n=5$ for each, $p>0.05$) [Modified from (Wang et al., 2008). Copyright (2008) National Academy of Sciences, U.S.A.].

Figure 6.

At the favorable NR2B/2A ratio, the adult PFC is capable of optimal working memory performance, which sets the stage for more complex prefrontal-dependent cognition, such as cognitive and behavioral flexibility, as well as attention. In contrast, deviation from this optimal ratio results in alterations to working memory and prefrontal-dependent cognition. A reduced NR2B/NR2A ratio induced by either decreased expression levels of NR2B or increased levels of NR2A will result in lower than normal cognitive performance in all facets due to limited synaptic plasticity and Ca^{2+} -dependent signaling, including impaired working memory, cognitive and behavioral inflexibility, and diminished learning and memory capability. On the other hand, a high NR2B/NR2A ratio induced by either increased NR2B or decreased NR2A will improve working memory and cognitive performance, yet exposes the brain to hyperexcitability, neuropathic pain, and excitotoxic vulnerability.