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Review

Review

The involvement of *N*-methyl-D-aspartate receptor (NMDAR) subunit NR1 in the pathophysiology of schizophrenia

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Abstract

Schizophrenia is a severe mental illness that afflicts nearly 1% of the world population. Although the exact pathophysiology of schizophrenia is unknown, the *N*-methyl-D-aspartate receptor (NMDAR), a major glutamate receptor subtype, has received great attention. The NR1 subunit is often considered indispensable for functional NMDAR assemblies, abnormal modulation of which is found in patients with schizophrenia. In this review, we discuss how disrupted function of NR1 subunits in NMDAR leads to the progression and development of symptoms of schizophrenia-like behaviors in a variety of genetically modified mouse models. We also discuss some of the susceptible genes and shared signaling pathways among the schizophrenia, and how their mutations lead to NR1 subunits hypofunction. Finally, we suggest that the subunit-selective modulators of NR1 subunits in NMDA receptors may be promising tools for the therapy of schizophrenia.

Key words: NR1 subunit, NMDA receptor, schizophrenia

Introduction

Schizophrenia is a severe brain-disabling disorder with a prevalence of 0.5%–1% of the population [1]. Core features of schizophrenia are classified into positive symptoms (e.g. hallucinations and delusions), negative symptoms (e.g. anhedonia, alogia, apathy, and poor self-care), and cognitive symptoms (e.g. executive function deficits, working memory, and recognition memory). Symptoms of schizophrenia typically emerge during adolescence or early adulthood. The positive symptoms often fluctuate, while the negative and cognitive symptoms usually cause great disability and deterioration [2]. In order to develop effective therapies, much effort has been made to further understand the core molecular alterations involved in schizophrenia.

Several abnormal neurotransmitter systems have been implicated in the pathophysiological processes underlying schizophrenia. The prominent theory is the dopamine (DA) dysfunction [3], and current antipsychotics that target DA system have been proved to be effective. Those drugs usually show success in relieving the severity of positive symptoms, but they have limited efficacy in ameliorating negative and cognitive deficits, indicating that mechanisms other than DA are likely to be involved in schizophrenia [4]. In addition to the dopaminergic abnormalities, glutamate system that mediates most of the excitatory neurotransmission in the brain has also been proposed to be involved in schizophrenia, including but not limited to *N*-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) [5], vesicular glutamate transporter 1 and 2 (VGLUT1 and VGLUT2) [6,7], glial glutamate and aspartate transporter (GLAST) [8], etc. Each subtype of glutamate receptor has distinct functional and anatomical properties that affect glutamate neurotransmission in a functionally selective manner [9]. Up to now, substantial evidence has supported that NMDAR may be an important factor in schizophrenia pathogenesis, which is widely considered as a disorder of brain development and neural connectivity [10,11]. Due to the complex subunit composition of the receptor, the properties of NMDARs are very diverse and complicated. Subunit-specific molecules are thus important tools for understanding the heterogeneous functional and pharmacological characteristics of NMDARs.

The NR1 subunit is often considered indispensable for functional NMDAR assemblies, but the remaining subunits can vary [12]. Recent advances in clinical and preclinical pharmacological, genetic, and brain studies have put forward some direct support for the importance of impaired NMDAR-NR1 subunit-mediated glutamatergic pathways in the pathophysiology of schizophrenia [9,13,14]. These findings have important translational implications that the blockage of NMDAR-NR1 subunits or NR1 subunit gene mutation can potentially increase our understanding of how schizophrenia occurs.

In this review, we describe some significant preclinical and clinically relevant findings related to NR1 subunits in schizophrenia model. These findings reveal that the hypofunction of NR1 subunits contributes to abnormalities in several genes and neurotransmitter systems implicated in the biological mechanism underlying schizophrenia. We would like to recapitulate here the central role of NMDAR-NR1 subunits in mediating the signaling network of schizophrenia. Overall, this review will provide further understanding of how NR1 subunit plays a role in the etiology of schizophrenia and, in turn, give us the possibility to develop new tools that are more selective and specific for schizophrenia treatment.

Structure and Functional Organization of NR1 Subunit in NMDAR

Accumulated evidence substantiates an important role of NR1 subunits in a variety of functions and processes in the brain. NR1 subunits can be found in all NMDARs [15], which are mainly heteromeric complex with two other subunits of NR2 (2A-2D) and/or NR3 (3A-3B) [16,17]. Hence, NR1 subunits are essential components of all NMDARs, and thus important for understanding the distribution and functions of NMDARs. The NR1 family is encoded by a single gene, *Grin1*. One N terminal (exon 5) and two C terminals (exon 21 and exon 22) of the NR1 subunit gene can undergo alternative splicing, leading to eight NR1 splice variants [18]. Additional heterogeneity is conferred by the eight alternative splice variants of the NR1 subunits that have different expression patterns within the brain. It should be noted that NR1 subunits are distributed ubiquitously in the brain and no single cassettes of NR1 subunits are differentially enriched in the postsynaptic densities. In addition, the NR1 subunit mRNA is expressed throughout the different stages of neurodevelopment, normally in excess in the endoplasmic reticulum (ER) [19–21]. On the subcellular level, C1 terminal cassettes possess several phosphorylation sites and reside primarily in the ER, while splice variants containing N terminal cassettes are equally distributed between the cell surface and the cytoplasm. C2 variants have been observed mainly in the intracellular pools of cells and may participate in long-term potentiation-dependent insertion of NMDAR into the membrane. These splice variants may alter NMDARs under either pathological or physiological condition.

NR1 subunits seem to play a pivotal role in determining the voltage and ligand-gating properties of NMDARs. Assembly of the NR1 subunits with one of the four NR2 subunits is necessary for forming active NMDAR ion channels that only open in the presence of glycine and L-glutamate [22–24]. NR1 subunits also confer different intracellular interactions that affect NMDAR signaling and transport. For example, the D-serine/glycine binding sites on the NR1 subunits must be occupied for enhancing the affinity and efficacy of glutamate to activate the NMDAR [25,26]. Studies have also revealed that activation of D-serine/glycine site delays receptor desensitization, increases the duration and frequency of the open channel state [27], and promotes NMDAR turnover through priming of the receptor for internalization [28]. Additionally, NR1 splice variants also influence other characteristics, such as pH sensitivity [24].

Apart from being related to important characteristics of NMDARs, NR1 splice variants are also indispensable for functional NMDAR complexes under various physiological conditions, such as synaptic plasticity, synapse formation underlying memory and learning, as well as formation of neural networks during development. They are also important for a variety of pathological states, including acute and chronic neurological disorders, psychiatric disorders, and neuropathic pain syndromes, etc. For example, chronic developmental Pb^{2+} exposure could produce the deficits in synaptic plasticity and in learning in young adult rats by altering the cell surface expression of NMDAR-NR1 subunits (lacking C1 cassettes) [29]. Reduced expression of NR1 splice variants of NMDARs (lacking N terminal splice cassettes) could interfere with spatial reference memory in the aging process [30]. Since NR1 splice variants also affect synaptic plasticity, it is no surprise that changes at their mRNA and protein levels were found in the disease of long-term synaptic plasticity-like chronic pain, as well as in cocaine and alcohol abuse [31,32]. Taken together, NR1 subunits play an important part in processes regulating and controlling the structure and function of synapses. Their dual roles in physiological and pathological functions make it necessary to develop NR1 subunit- and site-specific drugs for precise and selective therapeutic interventions.

Abnormal Modulation of NR1 Subunit in Patients with Schizophrenia

Abnormalities of NR1 subunits in NMDARs have been linked to schizophrenia in a series of studies, among which postmortem studies can provide direct evidence in the brains of schizophrenia patients. The study of NMDARs in postmortem brain samples from schizophrenia patients began in 1989, using radio ligand binding techniques with [^{3}H]MK801 which binds to the phencyclidine (PCP) site of the NMDAR channel. No diagnostic changes were found in the prefrontal cortex or hippocampus when compared with the controls [33]. Since then, a number of studies have continued to probe the idea of abnormal NMDAR function by examining the binding and gene transcription levels of individual NMDAR subunits in various brain regions.

Several studies have identified changes in the binding site (glycine binding site) on NR1 subunits in schizophrenia, which demonstrated binding deficit of NR1 subunits in particular brain areas in schizophrenia. Nudmamud *et al.* [34] identified an increase in radio ligand binding to the glycine site on the obligate NR1 subunits of the NMDARs in superior temporal cortex in schizophrenia patients. Furthermore, strychnine-insensitive [^{3}H]glycine binding was also increased in six regions of the cerebral cortex [35]. The binding of [^{3}H]L-689,560, a potent antagonist at the glycine site in NR1 subunits, has also been reported to be increased in the putamen and in the superior temporal cortex in a small number of schizophrenia patients [36,37]. In addition to the binding deficit of NR1 subunits, it has also been demonstrated that the brains of individuals with schizophrenia display gene expression alterations of the NR1 subunits [38,39]. Some postmortem studies have revealed changes of NMDAR-NR1 gene expression in the cortex in schizophrenia patients. Although the mRNA level of the NR1 subunits was found to be similar to that in the control subjects in the prefrontal, cerebellum, and parietal–temporal cortices, it is reportedly higher in the left superior temporal cortex, the dorsolateral prefrontal cortex, the occipital cortex, and the substantia nigra in schizophrenia patients [12,40,41]. Significantly increased expression of NR1 subunits was also found in the

anterior cingulated cortex at the protein level in postmortem samples from elderly schizophrenia patients [42]. However, the expression of *NR1* mRNA was found to be lower in the frontal cortex, hippocampus, and thalamus in schizophrenia patients than those in the controls [39,43,44]. These results derived from postmortem brains suggest that alterations in obligatory NR1 subunit expression may underlie schizophrenic pathology. However, caution should be taken in interpreting these results because the patients may have been chronically treated with antipsychotic drugs, not to mention the varied moribund state, the postmortem delay, and tissue handling. Further studies with a greater number of samples will be necessary.

Recently, the prevalence of several anti-NMDAR antibody subtypes has been found in the serum of patients who were initially diagnosed as schizophrenia. Expressions of functional heteromers (NR1/NR2A and NR1/NR2A/NR2B) are required for antibody binding, and reactivity can hardly be identified when NR1 subunits are expressed individually [45,46]. Thus, the prevalence of those anti-NMDAR antibody subtypes in schizophrenia could be considered as a weak etiologic feature that is related to the glutamate hypothesis. The effect of disease state and the time of serum acquisition may arise; therefore, more studies are needed to quantify anti-NMDAR antibodies in both sera and cerebrospinal fluid by employing standardized assay. Moreover, larger cohorts need to be analyzed by including more patients with the psychiatric diagnoses of schizophrenia to substantiate this conclusion with sufficient statistical power [47]. This might render some schizophrenia patients susceptible to new specific glutamate-modulating therapies.

Behavioral Manifestations of Genetically Modified NR1-mutant Mice as a Model of Schizophrenia

Mice with global deletion of NR1 subunits

The glutamate hypothesis posits a deficit in NMDAR-mediated glutamate neurotransmission underlying much dysfunction seen in schizophrenia. Since the presence of NR1 subunit is indispensable for a functional heteromeric NMDAR, genetic animal models of aberrant NR1 function have been established for gaining insight into their functional role (**Table 1**). Mice with complete and global loss of the NMDAR NR1 subunits died neonatally (only survive 8–15 h after birth) [48]. To overcome the neonatal lethality of NR1-knockout (KO) mice, mice in which the NR1 KO was partially compensated by ectopic transgenic expression of NR1 splice variant were generated [50–52]. Furthermore, NR1-knockdown mice have also been generated. Previous studies have shown that decreased expression of the NR1 subunits which were required for normal NMDAR function caused a series of schizophrenia-like phenotypes. This phenomenon was consistently observed in different laboratories (**Table 1**). Mice expressing significantly reduced levels of NR1 subunits ($NR1^{neo}$–/– mice, expressing 5%–10% of the normal level of NR1 subunit in NMDAR) survived to adulthood and displayed behavioral abnormalities, including increased spontaneous locomotor activity and stereotypy [53]. Subsequent studies revealed that the NR1-knockdown model mice also showed negative and cognitive symptoms in the continuum of schizophrenia. $NR1^{neo}$–/– mice exhibited deficits in social interaction and mating behaviors; they also displayed deficits in hippocampal and prefrontal cortical-dependent spatial cognitive performance [53–56]. Each of these behaviors was found to have a unique developmental trajectory in mutant mice. For example, abnormalities of working memory and sociability in $NR1^{neo}$–/– mice were only evident in adult mice. Additionally, hyperlocomotion was most evident in juvenile mice, and stereotypy worsened progressively with aging [89]. Moreover, some schizophrenia-like behavioral alterations can be reversed by antipsychotic treatments, such as clozapine and haloperidol treatment. However, pharmacological data were not consistent or sensitive to amphetamine [53,57,58]. A decrease in prefrontal cortical metabolism was also observed in $NR1^{neo}$–/– mice, consistent with imaging studies that showed reduced frontal cortical metabolic activity in schizophrenia [59]. Taken together, these studies support that disturbances in the NMDAR-NR1 subunits produce phenotypes that are potentially relevant to the symptoms of schizophrenia. These models have the advantage of reproducing the chronic and developmental nature of NMDAR hypofunction theorized to occur in schizophrenia, representing standard behavioral readouts for the evaluation of animal models of schizophrenia. These studies suggest that $NR1^{neo}$–/– mice may model some behavioral and physiological aspects of schizophrenia.

Disruptions in the prepulse inhibition (PPI) are well-validated behavioral tests that are often used for modeling classic schizophrenia-associated deficits in sensorimotor gating. The $NR1^{neo}$–/– mice would exhibit the decreased sensorimotor gating through the paradigm of the PPI of acoustic startle responses [54,57,60]. These mice also modeled the deficits of schizophrenia patients in auditory gating in the paired tone paradigm [90,91]. Further studies demonstrated that parvalbumin (PV) interneurons in the NR1 mutants were suggested to be the crucial neuronal populations mediating the sensory gating function involved in the pathophysiology of schizophrenia [79]. Event-related potentials (ERPs) were found to be abnormal in NR1-hypomorphic mice, and thus were used as a paradigm to investigate cognitive processes, such as selective attention [61]. Analysis of ERPs evoked by visual stimuli has also been performed. A similarly increased response to visual stimuli was recorded in $NR1^{neo}$–/– mice, thereby confirming an abnormal sensory processing across modalities [55]. This result suggests that reductions in NR1 subunits lead to a pattern of dysfunction that is common to multiple sensory modalities. Collectively, data from PPI and ERP tests suggest the model characteristics of the $NR1^{neo}$–/– mouse strain for schizophrenia. Hence, mice with homozygous constitutive reductions in NR1 subunit expression showed stable behavioral changes, although many of these phenotypes might be more severe than the human disease. In contrast to what has been reported in $NR1^{neo}$–/– mice, $NR1^{neo}$+/– mice showed no change in obligatory ERP measurement. Alternatively, they showed a marked reduction in response to a deviant auditory tone, consistent with deficits of deviance-related mismatch negativity among family members of schizophrenia patients and among prodromal patients [92]. Together, these studies provide support for the view that chronic global NR1 dysfunction can induce attention, response inhibition, social interaction deficits, etc.

Mice with region-specific deletion of NR1 subunits

Alterations of NR1 subunits in certain brain regions may underlie the observed deficits in schizophrenia patients. Region-specific deletions of NR1 subunits may thereby reveal more precisely the contribution of local NR1 subunits to the development of a schizophrenia-like behavior. Working memory deficits, which have been shown to be consistently associated with reduced levels of elementary social skills and learning capacity in schizophrenia patients, were present early in the course of schizophrenia. Recent findings have demonstrated that they are associated with the deletion of NR1 subunits in animal models of schizophrenia. For example, targeted ablation of the NR1 subunits in the dentate gyrus of the hippocampus resulted in spatial working memory impairments [62,63]. Mice with loss of NR1 subunits in hippocampus CA3 pyramidal cells were unable to fully recall

Table 1. Summary of genetic mouse models targeting the NMDAR-NR1 subunits

NR1-mutant mouse model	Brain alterations	Behavioral phenotypes characterization	References
NR1 KO (NR1−/−) mice	Severe deficit in somatosensory barrel cortex formation in the brain	Perinatal lethality, N/A	[48,49]
NR1 KO mice (compensated by ectopic transgene of NR1 splice variant)	Significantly increased life span, altered somatosensory pattern, exuberant axonal arborizations, and altered dendritic differentiation; projection neurons of corpus callosum developed prematurely and faster	N/A	[50–52]
NR1 knockdown (90%–95% loss)	Alterations in the regional brain metabolism	Disrupted sensorimotor gating, abnormal ERPs, impaired social and sexual interactions, increased spontaneous locomotor activity, stereotypy	[53–61]
DG-NR1 KO (deletion of NR1 in dentate gyrus granule cells of the hippocampus)	N/A	Spatial working memory impairments, decreased ability to distinguish two similar contexts	[62,63]
CA3-NR1 KO or knockdown with AAV-Cre (in hippocampal CA3 pyramidal cells)	N/A	Deficits in recalling the associative memory; impairment in adaptive nonspatial learning and excitation, impairment in spatial working memory, decreased social approach behavior	[64–68]
CA1-NR1 KO (inducible, reversible, and CA1-specific NR1 KO of hippocampal CA1 pyramidal cells)	N/A	Deficits in spatial and nonspatial learning and memory, spatial memory consolidation and retention of contextual fear associations	[69–73]
iFB-KO of NR1 (inducible, reversible, and forebrain-specific NR1 KO of forebrain excitatory neuron with CaMK II promoter)	N/A	30-Day Dox treatment during the seventh month disrupts the 9-month-old remote fear memories, no influence on normal learning and memory function, locomotor activity and cerebellar coordination, impaired nonspatial memory retention and consolidation	[74–76]
Amygdala microinjection of rAAV-GFP-Cre into Floxed NR1 mice	N/A	No deficits in locomotor, somatosensory, or sensory-motor behaviors	[77]
Nucleus accumbens (Acb) microinjection of rAAV-GFP-Cre into Floxed NR1 mice	Decrease in the dendritic density in Acb neurons	Diminished social interaction, without affecting novel object recognition or open-field activity	[78]
Mice with NR1-specific deletion in GABAergic interneuron	Reduction the inhibitory action in GABAergic interneuron	Disruption in self-care, nest building, social short-term memory, social interactions, mating frequency; increased anxiety-like behaviors in the elevated plus maze and open-field test, impaired PPI, no significant hyperlocomotion	[79–83]
Mice with $NR1^{N598}$ (Q^{neo}/Q^{neo}, R^{neo}/R^{neo}, −/Q,−/R,+/R,+/Q) genetic modification	N/A	$NR1^{N598}$ (Q^{neo}/Q^{neo}, R^{neo}/R^{neo},−/Q,−/R) lethality; $NR1^{+/N598Q(R)}$ increased mortality, impaired maternal behaviors	[84]
Mice with $Grin1^{D418N}$, $Grin1^{D481N/K483Q}$ point mutations	$Grin1^{D481N/K483Q}$ mice: striatal dopaminergic and serotonergic hyperfunction	$Grin1^{D418N}$ mice: deficits in spatial recognition, spatial reference learning and memory, deficits in sociability, reduced anxiety and increased startle reactivity, yet normal PPI $Grin1^{D481N/K483Q}$ mice: locomotor hyperactivity, enhanced stereotypy, increased startle reactivity, impaired nest building	[85–88]

memory when recalling cues were degraded [64,65]. They were also impaired in rapid hippocampal encoding of novel information for fast learning of a one-time experience [65,66]. Alternatively, knocking down NR1 subunits with AAV-Cre virus in adult mice resulted in deficient associative memory learning in adult mice, and this kind of learning requires >70% of CA3 neurons and the expression of NR1 subunits [67]. These studies suggest that NR1 subunits in CA3 brain region play an important role in the recall of spatial reference memory by pattern completion and are required for pattern separation of spatial cues. Furthermore, NR1 subunit deletion in CA3 region was found to play a critical role in tracing eye blink conditioning, a form of nonspatial learning. In addition, CA3 NR1 subunit deletion could decrease social approach behavior [68]. Similarly, mice with the deletion of NR1 subunits in CA1 area exhibited significantly impaired spatial (nonspatial) learning and memory [69,70]. NR1 subunits in CA1 area were also crucial for the formation of temporal memories that associate events across time [71]. Results from inducible, reversible CA1-NR1-specific KO mice further indicated that NR1 subunit-dependent synaptic reinforcement was crucial for memory consolidation [72,73]. These results demonstrate that hippocampus-specific

deletion of NR1 subunits mainly causes impairment in spatial and nonspatial working memories. However, the underlying cellular mechanisms have not been revealed.

Long-term memory is a multifactorial construct, composed of different stages of information processing and different cognitive operations that are mediated by distinct neural systems, some of which may be responsible for the schizophrenia-associated deficits of memory problems. Apart from the evidence for the essential functions of the NR1 subunit in learning and memory consolidation, its role in the storage of remote memories has also been examined. Inducible and reversible, and forebrain-specific NR1-knockout (iFB-KO) mice, in which NR1 subunits can be temporarily switched off in the forebrain specifically during the information storage stage, have permitted a more precise investigation of long-term memories in a hippocampal-independent manner [74]. By temporarily switching off the forebrain NR1 subunits during the various stages of memory processes, including learning and consolidation stage, the iFB-KO mice showed severe performance deficits in taste memory retention tests. Therefore, NR1 subunits were demonstrated to play a multistage role in dynamically maintaining the long-term synaptic stability of memory storage circuits in the brain [74,75]. On the contrary, overexpression of the NMDAR composed of NR1-NR2B subunits in the mouse forebrain enhanced NMDAR-dependent synaptic potentiation and produced improvements in learning and memory [76]. Other schizophrenia-associated brain regions, such as the amygdala and the nucleus accumbens, were also examined with spatially restricted deletions of the NR1 subunit genes. Amygdala microinjection of rAAV-GFP-Cre produced a decrease in NR1 subunit gene expression, but did not affect locomotor, somatosensory, or sensory-motor behaviors [77]. Spatially restricted NR1 subunit deletion in the nucleus accumbens could induce a diminished social interaction in $NR1^{neo-/-}$ mice [78]. These findings focusing on special brain areas may be particularly relevant in deciphering the mechanisms underlying deficits in schizophrenia.

Mice with cell type-specific deletion of NR1 subunits

Cell type-specific ablation of NR1 subunits may allow a better understanding of the contribution of NR1 subunits in defined cell populations for inducing schizophrenia-like behaviors. Floxed NR1 mice were crossed with Cre transgenic mice, and thus a cell type-specific and partial deletion of NR1 subunits in GABAergic interneurons was obtained [80]. Interneuron-specific ablation of NR1 subunits resulted in a broad variety of behavioral abnormalities, such as disruption in self-care, nest building, social short-term memory, social interactions, and mating frequency in an age-dependent manner [79,81–83]. These mice also showed increased anxiety-like behaviors in the elevated plus maze and open-field test, impaired PPI, but no significant hyperlocomotion [93]. Several of these behavioral disturbances were exacerbated after social isolation stress, mediated by impairment in antioxidant defense mechanisms [80,94]. In those mice models, specific deletion of NR1 subunits might reduce the inhibitory action of GABAergic interneurons. This NR1-mediated deficit could then cause a loss of inhibitory control over multiple projections [95]. In conclusion, these studies demonstrate that selective loss of the obligatory NMDAR subunit NR1 on PV interneurons may reduce the activation of inhibitory GABAergic interneurons and lead to abnormalities similar to negative symptoms of schizophrenia. These findings have led us to hypothesize that NR1 subunits may play a role in schizophrenia by controlling neuronal patterning and some behavioral abnormalities. The disruption of NR1 subunit function may result in either a net reduction of inhibition or an increase of excitation. Impaired NMDAR signaling on GABAergic interneurons may have the potential to mediate several aspects of schizophrenia, such as negative symptoms.

Mice with targeted point mutations in the NR1 subunits

Studies on recombinant NMDARs identified a single amino acid residue in the NR1 subunits, asparagine 598 (N598), as a critical determinant for the key properties of the NMDARs which mediates high Ca^{2+} permeability and voltage-dependent Mg^{2+} block. It was subsequently found that mice expressing these mutated NR1 alleles (**Table 1**) developed a perinatally lethal phenotype or reduced life expectancy and displayed signs of underdevelopment, such as growth retardation and impaired righting reflex [84], indicating that targeted point mutations in $NR1^{N598}$ could affect the key properties of the NMDAR and induce causal abnormal animal behavior. Mice with targeted point mutations in the D-serine/glycine binding sites of the NR1 subunits have also been studied. $NR1^{D481N}$ mice with reduced affinity for glycine displayed the anxiolytic-like phenotypes and exhibited deficits in spatial recognition, spatial reference learning and memory, reduced anxiety, and increased startle reactivity, but with normal PPI responses [85–87]. Like the NR1-knockdown mice, $NR1^{D481N}$ mice also showed selective deficits in sociability, similar to the social withdrawal aspect of the negative symptom of schizophrenia [86]. Furthermore, in the $Grin1^{D481N/K483Q}$ mice with two-point mutations in the NR1 D-serine/glycine site, more severe reductions in D-serine/glycine occupancy were found to be associated with abnormalities related to psychosis, including locomotor hyperactivity, enhanced stereotypy, increased startle reactivity, impaired nest building as well as striatal dopaminergic and serotonergic hyperfunction [88]. Therapeutic potential of endogenous D-serine/glycine binding sites agonist D-serine has been assessed through the genetic inactivation of its catabolic enzyme D-amino acid oxidase (DAO) in mice. The hypofunctional Dao1(G181R) mutation elevated brain levels of D-serine without affecting performance in the behavioral measures. Compared with animals with only the Grin 1(D481N) mutation, mice with both the Dao1(G181R) and Grin 1(D481N) mutations displayed an improvement in social approach and spatial memory retention, as well as a reversal of abnormally persistent latent inhibition and a partial normalization of startle responses [96]. Collectively, these genetic models with point mutation displayed phenotypes relevant to schizophrenia, particularly to the negative and cognitive symptoms, suggesting that the NR1 subunits may be relevant to the pathophysiology of these symptoms. The utility of these models lies not in how well they reproduce the behavioral abnormalities seen in schizophrenia, but rather in how they improve our understanding of schizophrenia pathogenesis. D-Serine or DAO inhibitors may be used to effectively increase D-serine level in the brain and be exploited for schizophrenia treatment.

Collectively, these studies in transgenic animals support the notion that perturbations in NR1 subunit-mediated signaling contribute to the behavioral phenotypes of schizophrenia. A closer look at individual mutants, however, reveals the specific relationship between mutant NR1 subunits and patient behavioral outcomes. It appears that alterations in NR1 subunit-mediated signaling can lead to different behavioral manifestations. Transgenic studies indeed helped us to evaluate the pathophysiologic roles of NR1 subunits in schizophrenia. However, it is still a challenge to correlate behavioral outcomes of mutant mice with the expression of genetic variants of NR1 subunits in patients. In our opinion, gene alterations in transgenic mice are more pronounced than in patients. Thus, behavioral manifestations of these risk genotypes in patients might be much weaker than those seen in transgenic mice. Hence, we should be very cautious to interpret the results from animal studies.

NR1 Subunits as Potential Mediators of Convergent Molecular Abnormalities for Schizophrenia

Numerous susceptibility genes have been shown to be involved in the pathophysiology of schizophrenia [97,98]. Many of these genetic variants that are associated with schizophrenia are related to the NMDAR-mediated glutamatergic system in the brain. They have been verified to be involved in neurodevelopment, and consequently contributing to the disturbed information processing in brain circuits that mediate the symptoms of schizophrenia [98,99]. The NMDAR-mediated glutamatergic model thus provides an alternate paradigm for interpreting the etiology of schizophrenia. It is therefore of great interest to clarify how hypofunction of NR1 subunits is involved in the abnormal signaling pathways and neurotransmission system in schizophrenia.

The changes of DA function in the brain might be secondary to reduced activity of NR1 subunits [100]. There is a physical interaction between DA receptor 1 and NR1 subunits, in which NR1 subunits increase plasma membrane insertion of D1 receptors and modify D1 receptor trafficking [101,102]. Thus, a decrease in NR1 subunits function can produce the DA disturbance in schizophrenia. Particularly, NR1 subunits can regulate DA neurons and DA transmission, and hypofunction of NR1 subunits may be responsible for the abnormal DA activity associated with the symptoms of schizophrenia [100,103]. Furthermore, disruption of phasic DA neuron activity by selective genetic inactivation of NR1 subunits impaired the burst firing and dramatically attenuated cognitive function-related behavioral responses together with acoustic startle behavior [78,104,105]. In addition, it has been demonstrated that the glutamatergic disturbances may lead to hypofunction of NR1 subunits on the GABAergic interneurons in the limbic circuit [106]. Therefore, it appears that NR1 subunits in NMDAR could be the convergent point underlying major neurotransmissions associated with schizophrenia. Accumulated evidence shows that convergent mechanisms targeting NR1 subunits of NMDAR may contribute to symptoms and neurocognitive dysfunction in schizophrenia.

In the following sections, we will focus on the current literatures and explain that the NR1 subunit hypofunction might be a downstream joint point for some susceptible genes in schizophrenia. We will also attempt to elucidate the possible signaling pathways related to the regulation of NR1 subunit function by high-risk genes for schizophrenia.

There are many risk genes associated with schizophrenia. These genes include *Dysbindin*, *Disrupted in schizophrenia 1* (*DISC1*), etc. [107–109], most of which appear to converge at synaptic sites, and are indirectly or directly involved in modulating NR1 subunit-mediated function through a variety of mechanisms (**Fig. 1**). In addition to the molecular and physiologic effects of reduced Dysbindin in the DA system [110], Dysbindin was demonstrated to regulate NR1 subunit expression and degradation in the glutamate system which correlates

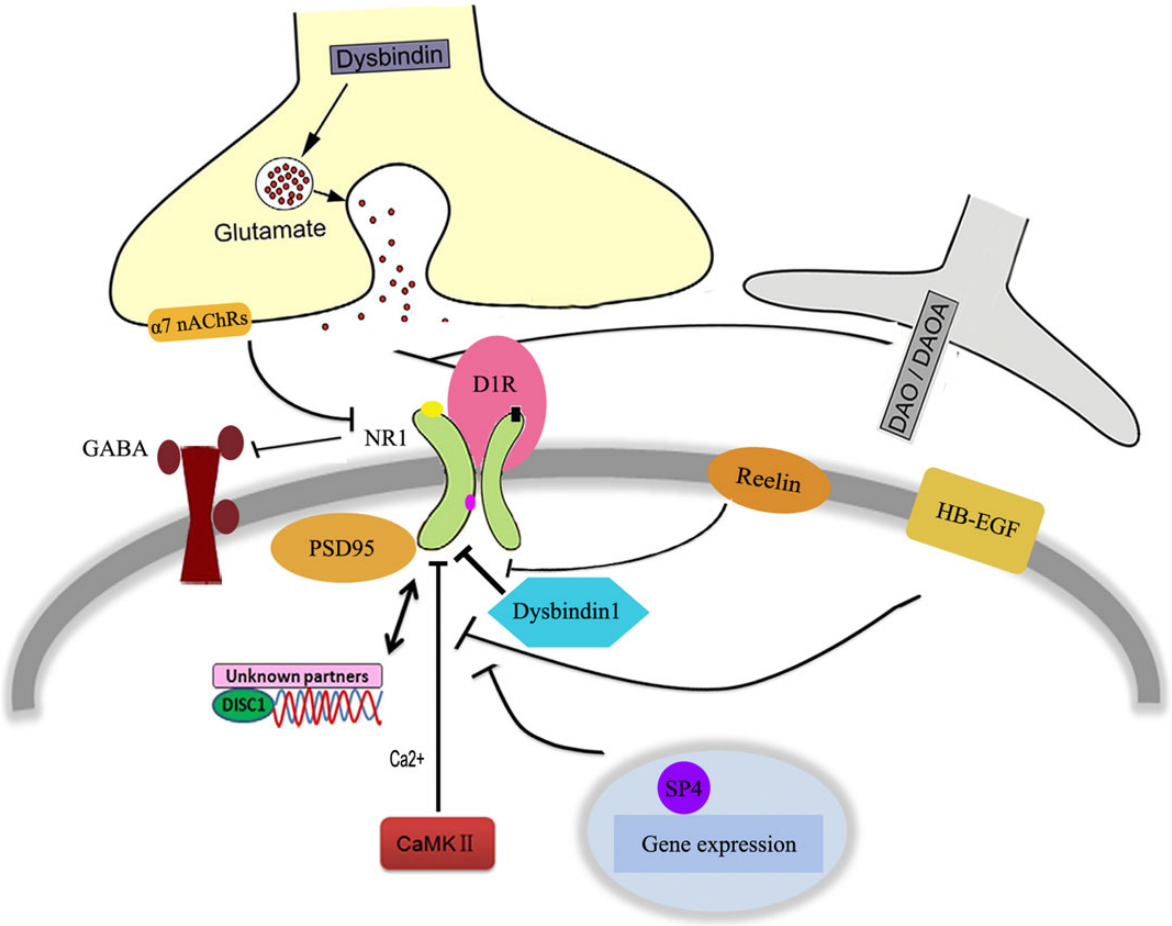

Figure 1. Schizophrenia-susceptible genes related to the hypofunction of NR1 subunits in NMDAR ↑ represents positive function, ⊥ represents negative function, and ↕ represents interaction.

with impairments in spatial working memory performance [111]. Furthermore, genetic disruption of Bloc1s8 subunit in Dysbindin was sufficient to downregulate the expression of NR1 subunits in the hippocampus [112]. Cell surface but not the cytoplasmic expression of the NR1 subunits of the NMDAR was decreased, suggesting a dysregulation of NMDAR trafficking in a mouse model carrying a large genomic deletion exclusively within the Dysbindin gene [113]. Thus, the above studies establish a link between Dysbindin expression level and the activity of NR1 subunits. The relationship between DISC1 and NR1 subunits also attracted much attention. In humanized DISC1-Boymaw mice which displayed abnormal information processing of acoustic startle, the protein translation activity of NR1 subunits was decreased [114]. On the other hand, sub-chronic administration of MK801 produced reductions in both spine density and DISC1 expression. These results indicate that synaptic levels of DISC1 and NMDAR function are reciprocally regulated [115]. However, so far none of other partners have been identified to be related to the NR1–DISC1 interaction, which means that other unknown factors may be involved in this signaling pathway.

Some other genes that are associated with the functions of NR1 subunits in schizophrenia model have also been briefly reviewed (**Fig. 1**). Genetically induced reduction in the NR1 subunits expression was also observed in α7 nicotinic acetylcholine receptors (α7 nAChRs) null mice, which may underlie the cortical dysfunction in schizophrenia. Data showed that glutamatergic synapse formation and GABAergic deficits were both impaired in α7 nAChRs' deletion model [116,117]. Evidence also supported the involvement of Reelin, an extracellular matrix (ECM) glycoprotein, in the regulation of NR1 subunits in the pathophysiology of schizophrenia, thus strongly affecting synaptic maturation and stabilization. Reelin has been shown to mediate a switch in the subunits composition of NMDARs, which was considered to be the hallmark of synaptic maturation [118]. In the frontal cortex, Reelin heterozygous mice showed significant downregulation of NR1 subunits. This genotype effect was male-specific for locomotor hyperactivity [119]. In neonatal ventral hippocampus (NVH)-lesioned rats (a neurodevelopmental impairment model of schizophrenia), reduced Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) autophosphorylation and NR1 (Ser896) phosphorylation were confirmed. This result demonstrated that impaired cognition observed in NVH-lesioned rat is associated with decreased CaMK II and NR1 subunits activities in memory-related brain regions [120]. In line with this, the infusion of a CaMK II inhibitor and NR1 antisense oligonucleotide into the prefrontal cortex produced an impairment of latent learning, indicating a dysfunction of NR1-CaMK II signaling in the mouse model of schizophrenia [121].

Neurotrophic factors and cytokines have been shown to be associated with schizophrenia. A multiplatform profiling study identified peripheral changes that are potentially linked to central alterations in synaptic plasticity and neuronal function associated with NMDAR-NR1 hypofunction [122]. Besides the abnormal behaviors similar to those described in other schizophrenia mouse model [123], reduction of NR1 subunits was observed in mice with heparin-binding epidermal growth factor (HB-EGF) disruption. Additionally, NR1 subunits were considered as mediators of reduced Specificity Protein (Sp) transcription factors associated with negative symptoms in schizophrenia. As Sp may play a role in schizophrenia by controlling neuronal patterning and some behavioral abnormalities [124], an imbalance in NR1 subunit-mediated function has thus been proposed as the underlying mechanism for the complex negative symptoms in this disorder.

Taken together, these susceptible genes function to affect NR1 subunits activities through a variety of mechanisms. Those pathways might regulate NR1 subunit-dependent signaling through both presynaptic and postsynaptic mechanisms, and likely are or contribute to the downstream consequence of the NR1 deficiency. These findings provide convincing evidence for an involvement of NR1 subunits in schizophrenia, and lead to the idea that NR1 subunits may be a mediator of convergent molecular abnormalities for schizophrenia.

Perspective

In the past few years, diverse investigations including developmental studies, genetic manipulations, and postmortem analysis have identified molecular and cellular mechanisms that link the NR1 subunits in NMDAR to the etiology of schizophrenia. These studies have so far enhanced our understanding of both the structural and neurochemical bases of glutamate system that leads to symptoms of schizophrenia, and have led to the discovery of potentially novel treatments for this disorder.

Genetically modified mouse models represent valuable tools for studying the role of NR1 subunits in schizophrenia. However, all rodent models of schizophrenia suffer from the fact that the definition of a schizophrenia-like phenotype in rodents is unclear in the absence of any conclusively demonstrated neurobiological basis of this disease. Future studies should focus on identifying NR1 subunit-mediated developmental deficits associated with schizophrenia at cellular levels.

Much of our knowledge of NR1 subunits in NMDAR was from rodent models based on loss of function via genetic deletion of NR1 subunits. Although some supporting evidence from human postmortem brain analyses was obtained, it is still not known if NR1 subunits for the pathophysiology of schizophrenia can lead to cellular deficits and impaired signaling in patients' neurons. It has been widely accepted that an induced pluripotent stem cell (iPS) model for schizophrenia patients at the cellular level should be established to gain new insight into its pathophysiology. Neurons from human induced pluripotent stem cells (hiPSCs) were presumably glutamatergic. Schizophrenia hiPSC-derived neural progenitor cells and neurons showed significant perturbations of glutamate receptor signaling pathway [125,126]. Thus, we propose to use the iPS model to confirm the function of NR1 subunits derived from existing hypotheses or to verify previous results from animal models.

Due to the complex subunit composition of the receptor, the pharmacology of NMDARs is very diverse and complicated. Although several broad-spectrum competitive antagonists and channel blockers have been developed, molecules with a broad-spectrum effect on all subunit types are not likely to be clinically useful for schizophrenia. Approaches targeting the glycine binding site in NR1 subunits seem more promising because they can induce cognitive enhancement in animal models without producing excitotoxicity [127]. Clinical data showed that agonists for D-serine/glycine site in NR1 subunits (e.g. glycine, D-cycloserine, and D-serine) positively modulate NMDAR channel function and significantly improve cognitive deficits and negative symptoms when given in combination with antipsychotics [128–130]. Therefore, increasing the activation of D-serine/glycine site in NR1 subunits may offer a safer alternative with an increased therapeutic efficiency and a decreased side effect.

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