Neuropathological Background of MK-801 for Inducing Murine Model of Schizophrenia

25

Ane Murueta-Goyena Larrañaga,
Arkaitz Bengoetxea Odrioizola,
Pascual Ángel Gargiulo,
and José Vicente Lafuente Sánchez

Abstract

Schizophrenia is a complex psychiatric disorder with a developmental component that compromises neural circuits. Understanding the neuropathological basis of schizophrenia remains a major challenge for establishing new therapeutic approaches. In this review, causal factors for abnormal brain development in schizophrenia are discussed, with particular focus on N-methyl-D-aspartate (NMDA) receptor hypofunction and GABAergic circuit-mediated neurotransmission. Changes in interneuron structure and function have been reported in schizophrenia, and current evidence points to a specific involvement of interneuronal NMDA receptor signaling. Furthermore, altered gamma-band oscillations in schizophrenic patients drew attention to a possible deficit in fast-spiking parvalbumin-expressing interneurons, which play an essential role in regulating complex interaction between pyramidal cells, and represent a key to the understanding of network operations. Here, we describe the major biochemical, neuropathological, and cognitive deficits present in schizophrenic human individuals, and the faithfulness of animal models for mimicking those impairments. In NMDA receptor antagonism-based animal models, repeated injections of MK-801 (dizocilpine) during early postnatal brain development, disrupt the excitation/inhibition balance.

A. Murueta-Goyena Larrañaga, MSc (✉)
Laboratory of Clinical and Experimental Neurosicence (LaNCE), Department of Neuroscience, University of the Basque Country UPV/EHU, Leioa, Spain

University of the Basque Country,
Laboratory of Clinical and Experimental Neuroscience, Department of Neuroscience,
Faculty of Medicine and Nursing,
Barrio Sarriena s/n, Leioa, Bizkaia 48940, Spain
e-mail: ane.muruetagoyena@ehu.eus

A.B. Odrioizola • J.V. Lafuente Sánchez
Laboratory of Clinical and Experimental Neurosicence (LaNCE), Department of Neuroscience, University of the Basque Country UPV/EHU, Leioa, Spain

P.Á. Gargiulo
Laboratory of Neurosciences and Experimental Psychology, CONICET, Area of Pharmacology, Department of Pathology, Faculty of Medical Sciences, National University of Cuyo, Mendoza, Argentina

P.Á. Gargiulo, H.L. Mesones-Arroyo (eds.), *Psychiatry and Neuroscience Update - Vol. II*,
DOI 10.1007/978-3-319-53126-7_25

A unifying hypothesis to explain the altered brain function in this model is a specific perturbation of GABAergic cells that results in long-term structural brain changes and modified network activity in adulthood, especially when MK-801 is administered during neurodevelopment. Subsequent impairment in cognition, particularly working memory and associative memory, are extremely relevant for schizophrenia research.

Keywords
Schizophrenia • MK-801 • NMDA • Animal model • Neurodevelopment

Schizophrenia is a disabling psychiatric disorder whose etiopathogenesis is still unclear. Brain research has been mostly focused on neurological diseases but in recent decades, research on the neurobiological basis of mental illnesses has emerged. This lack of information has restrained research from advancing in the understanding of the neurobiological basis of schizophrenia, and has led clinicians to make the diagnosis based on some symptom clusters. Schizophrenia is characterized by positive, negative, and cognitive symptoms. Positive symptoms represent abnormal mental functions, such as hallucinations and delusions. Negative symptoms include social isolation, decreased motivation, and flattened affect. Cognitive symptoms are related to poor executive function, particularly that involving attention and memory. The typical onset of these symptoms starts between late adolescence and early adulthood [1], although neurodevelopmental processes play an important role in schizophrenia. Currently, antipsychotic drugs are effective in reducing positive symptoms but have minimal beneficial effects on cognition. Poor cognitive functions affect the everyday life of schizophrenic patients and contribute most to chronic disability and unemployment [2–4]. However, no current treatment or therapy can successfully manage cognitive deficits.

Within the past two decades, numerous efforts in understanding the underlying etiology of cognitive dysfunction of schizophrenia have been made. For that purpose, understanding the neurobiology and circuitry of the forebrain, which support cognitive processes, is of major interest. Several theories regarding the etiology of schizophrenia have been proposed, including, but not limited to, genetic predisposition [5–9], prenatal infection [10, 11], environmental influences [12, 13], or a combination of these [14]. Brain imaging and post-mortem studies have shown anatomical changes in schizophrenic patients, primarily in prefrontal cortex and temporal lobe structures, such as decreased cortical volume [15, 16], altered circuitry and connectivity [17], and changes at the neuronal level [18] that contribute to core cognitive dysfunctions in schizophrenia.

Cognitive dysfunction is pervasive and is independent of other symptoms [3]. The most characteristic finding is the decreased ability in working memory tasks, especially when a high degree of information needs to be processed [19, 20]. The dorsolateral prefrontal cortex (DLPFC) is implicated in working-memory deficits, and brain imaging studies have consistently demonstrated alterations in activation of DLPFC in schizophrenic individuals, particularly during cognitive tasks [21–23]. In addition, hippocampal circuitry is altered both regionally and extra-regionally [24]. Within these circuits, GABAergic connections are of particular interest. Local interconnections of GABAergic interneurons onto pyramidal cells show alterations at the synaptic level, resulting in cognitive deficits [25, 26]. Furthermore, afferent and efferent connections between hippocampus and DLPFC also seem to be affected [27, 28]. The aberrant plasticity of the hippocampal–prefrontal cortex pathway may explain

the deficits of cognitive processes that require spatial and temporal information [28, 29].

Postmortem studies further support the idea that GABAergic alterations in the prefrontal cortex and hippocampus are implicated in the etiology of schizophrenia. In particular, immunostaining studies reveal a selective decrease of the calcium-binding protein parvalbumin in brains of schizophrenic patients compared with controls, with no concurrent loss of calretinin- or calbindin-immunoreactive interneurons [30–32]. The reason why the decrease of GABAergic interneurons is mostly limited to parvalbumin-containing cells is unclear, but it has been suggested that developmental changes in parvalbumin (PV) expression could make them vulnerable to dysfunction. Recent new studies of postmortem brains indicate that GABAergic interneurons containing the neuropeptide somatostatin are also decreased in orbital regions of the forebrain in schizophrenic patients [33].

Maturation of GABAergic Interneurons

GABAergic interneurons are the main source of cortical inhibition in the mammalian brain [34] and account for 10–25% of total number of neurons, depending on the brain region [35]. The high diversity of GABAergic interneurons in their morphology and functional properties has made the classification challenging. Suffice it to say that GABAergic interneurons play an important role in regulating and orchestrating the activity of pyramidal cells. They also shape cortical plasticity, synaptic wiring, and oscillations during prenatal and postnatal development [35]. An important characteristic of GABAergic circuit development is its long duration. The maturation begins early in embryonic stages and proceeds in several steps before fully-developed cell features are acquired. This involves the maturation of GABA release and reuptake, the ability of neurons to form synapses at defined developmental stages, and the expression of particular proteins that regulate cell signaling to eventually acquire mature electrophysiological properties.

Interneurons containing the calcium-binding protein PV seem to be the most affected GABAergic interneurons in schizophrenia. In particular, the selective downregulation of PV and GAD67 (glutamic acid descarboxilase 67) in the prefrontal cortex is the most consistent finding [36–38]. PV+ interneurons can be classified into two morphologically differentiable groups: basket cells and chandelier cells. Basket cells usually synapse in pyramidal cell somas and proximal dendrites, and chandelier cells form axo–axonic connections with the axon initial segment of pyramidal cells. One single basket cell can target large population of pyramidal cells, thereby exerting powerful postsynaptic modulation of excitatory output. In turn, pyramidal cells provide feedback input to parvalbumin positive cells, and this closed loop seems to evoke gamma oscillations, the physiological correlate for proper sensory integration and cognitive functions [39, 40]. Although another mechanism for gamma oscillations generation has also been proposed, the involvement of basket cells in gamma oscillation generation is known to be critical [41]. Schizophrenic patients show reduced power in gamma oscillation in frontal lobe during working memory tasks, in auditory cortex after a train of clicks, and in visual cortex when the scenery needs a perceptual organization [42]. Rhythmic brain activity in gamma-band seems to be necessary to transfer information between brain regions, and a lack of proper communication between brain regions is believed to underlie the pathophysiology of schizophrenia [42]. Given that PV and GAD67 expression is regulated by cortical activity [43, 44], the absence of both in PV+ neurons of schizophrenic patients indicates a lack or decreased activity of PV+ neurons. This would shift brain activity balance towards excitation. The dysregulation of local circuitry could be explained by abnormal neurodevelopmental changes.

Early in neurodevelopment, GABA acts as a depolarizing neurotransmitter, due to chloride accumulation in the cytoplasm. Around the end of the first postnatal week, the expression of

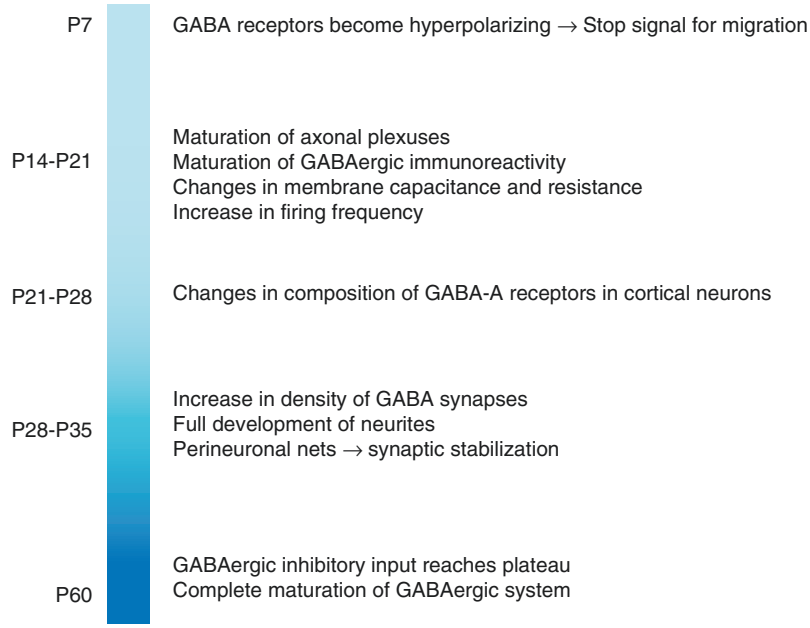

Fig. 25.1 Major steps of GABAergic interneuron maturation and neurotransmission

potassium-chloride cotransporter KCC2 dramatically increases in the forebrain, and GABA becomes hyperpolarizing [45]. The expression of KCC2 is regulated by local neuronal activity, so it subserves as a stop signal for further migration [46]. Between the second and third postnatal weeks, several changes in GABAergic interneurons take place: (1) maturation of GABAergic immunoreactivity [47], (2) appearance of adult-like electrophysiological properties (increase in firing frequency, high-frequency subthreshold membrane potential oscillations, and changes in membrane resistance) [35, 48], and (3) maturation of axonal plexuses of cortical interneurons [48]. Parvalbumin-expressing cells fully develop neurites at approximately 4–5 weeks [48]. In the first month, there is an increase in GABAergic synapses. The composition of $GABA_A$ receptors also changes during development in cortical neurons, and adult form subunits are found at 3–4 weeks [49]. Subunit switch is paralleled with the maturation of GABAergic inhibitory postsynaptic potentials (IPSPs) in the neocortex and hippocampus of parvalbumin-expressing fast-spiking cells [48]. $GABA_B$ receptors, a G protein-coupled receptor, also express different subunits during postnatal development [50] (Fig. 25.1). Developmentally regulated subunit expression has important functional implications in physiological properties.

Inhibition carried out by GABAergic interneurons is low during neurodevelopment, and acquires adult features in late adolescence or early adulthood [35]. The increase in inhibitory tone is correlated with the development of perineural nets (PNN) [51]. Perineural nets are proteoglycans that wrap certain type of neurons, and are thought to give homeostatic balance to highly active neurons. They are developed in an activity-dependent manner and act as physiological buffers for ions. PNN are particularly present in fast-spiking (FS) neurons for their high activity patterns. The opening and closure of critical periods are regulated by the level of maturation of inhibitory neurons, which in part is determined by the presence of PNN, as they offer synaptic stability [51].

There is evidence of perturbed maturation of GABAergic interneurons in schizophrenia. Impaired interneuron migration during development has been demonstrated through several findings. Various authors have found increased density of interneurons

in the superficial white matter of schizophrenic patients [52, 53]. Following knockdown of DISC-1, a gene implicated in schizophrenia susceptibility, tangential migration of MGE-derived interneurons is altered [54]. In addition, neuroregulin-1 (NRG-1) and its receptor ErB4 are also associated with increased risk of schizophrenia, and ErB4 is exclusively expressed in inhibitory interneurons, particularly in PV+ cells [55]. Altered connectivity in local excitation/inhibition circuitry has also been shown: less expression of the α1 subunit at basket cell–pyramidal cell synapses, and overexpression of the α2 subunit at chandelier cell–pyramidal cell synapses [56]. There are decreased axo–axonic synapses and less glutamatergic synaptic input onto parvalbumin-expressing basket cells in mice models [57]. These findings support the hypothesis that parvalbumin-expressing cells get improperly connected during development, resulting in aberrant network activity and plasticity in adulthood. Moreover, the substantial changes that GABAergic system undergoes at late adolescence and the onset of schizophrenic symptoms have the same age-dependency profile.

Animal Models of Schizophrenia

Animal models have been useful for unraveling the pathophysiological mechanism and treatment development in many areas of medicine. The critical obstacle in using animal models for studying psychiatric disorders is rooted in the poor understanding of their neural basis. Moreover, schizophrenia is considered a uniquely human disorder, as it mostly affects perception, thinking, language, and attention. Modeling those features in lower mammals has been controversial, but the high prevalence of schizophrenia (approximately 1% of general population), and the debilitating effects of the disease justify a great effort to study it. In this way, animal models are an indispensable tool.

Several approaches have been taken to model schizophrenia in rodents. Animal models can represent diseases from three different perspectives: (1) reproducing etiopathogenetic factors, (2) simulating signs and symptoms, or (3) predictability of response to treatment. We refer to these approaches as construct validity, face validity, and predictive validity respectively. The faithfulness of each type of validity varies considerably, and the utility of the proposed model depends greatly on the goals of each study. Oftentimes, models with high predictive validity are used for the development of new pharmacological treatments, but animal models with high construct validity provide a better framework for studying pathological processes and outcomes. Given the complexity of the nervous system and the lack of valid pathognomonic biological markers, phenotypes, or genotypes of schizophrenia, a heuristic model that would encompass different aspects of schizophrenia would be desirable [58]. These aspects should include anatomical, neurochemical, behavioral, and cognitive features of schizophrenia, but it is rare that a single model addresses multiple phenomena [58]. The best replicated neurobiological findings are thinning of prefrontal and temporal region cortices [59], and decreased expression of calcium-binding protein parvalbumin and GAD67 enzyme in cortical interneurons [60–64].

Animal models of schizophrenia have been categorized to date in three main groups: neurodevelopmental models, genetic models, and pharmacological models [58, 59, 65–69]. Neurodevelopmental models include obstetrical complications such as gestational malnutrition or prenatal exposure to influenza virus. Early stressors such as maternal separation and social isolation [70–72] have also been used, but usually combined with genetic or pharmacological approaches, also named "two-hit models" [73]. Neonatal brain lesions in ventral hippocampus have been widely performed, and present face validity in terms of damaged brain structures, although the disturbance is far more severe than in schizophrenia [74–77]. In any case, the causative role of any of these approaches is dubious, and thus the construct validity. Schizophrenia is highly heritable, and a genetic component of the disease is beyond discussion. Genes interact with environmental factors, and depending on that interaction

the disorder may or may not emerge. Animal genetic models have been perfectly reproduced for some diseases, which give strong construct and face validity. However, given the large number of genes involved in schizophrenia, and their complex interplay [78] with stochastic and environmental factors, it is unlikely that a faithful model can be built based entirely on this approach. Genes that have been involved with increased risk of schizophrenia are dysbindin, neuroregulin-1 (NRG-1), and disrupted-in-schizophrenia 1 (DISC-1), among others [35, 68]. With regard to pharmacological approaches, early studies showed that D2 dopamine receptor antagonists reduced positive symptoms of schizophrenia. Therefore, a dysfunction of dopaminergic neurotransmission has been the most enduring theory as the underlying cause of schizophrenia. Despite the longevity of the dopaminergic hypothesis and its face validity in schizophrenia research, it is now believed that dopaminergic dysfunction is a consequence rather than the cause [79]. Animal models of non-competitive NMDA antagonists are currently the most characterized pharmacological approach. Phencyclidine (PCP) and ketamine have been shown to induce psychosis in healthy humans and to exacerbate positive symptoms of schizophrenic patients [80]. This suggested the involvement of NMDA receptors in the pathophysiology of schizophrenia. The effect of non-competitive NMDA antagonists is not completely understood, but seems to have complex interactions in glutamatergic, dopaminergic, and GABAergic neurotransmission [81]. Altered glutamate transmission and NMDA receptors have been related to negative and cognitive symptoms observed in schizophrenia. NMDA antagonists, unlike dopamine, have strong construct validity for studying cognitive and attention deficits of schizophrenia [65, 67, 71]. Some authors have suggested that NMDA antagonists fail to take into account neurodevelopmental processes, mainly because acute doses of NMDA antagonist have been used in the literature, and short-term consequences measured rather than long-term effects. Nevertheless, in recent years repeated subchronic/chronic administration of NMDA antagonists has been used during the early postnatal period to model cognitive deficits of schizophrenia, and long-term behavioral aspects evaluated (during adolescence and adulthood) [69, 82]. MK-801, also known as dizocilpine, is the most potent and selective drug among non-competitive NMDA antagonists, and therefore widely used to model schizophrenia in rodents [83].

NMDA Hypofunction and Brain Maturation

Glutamate activates intracellular cascades via ionotropic and metabotropic receptors. Glutamate is integral in neurodevelopment. It regulates synaptogenesis, network plasticity, dendritic arborization, neuronal progenitor propagation, and migration [84]. NMDA receptors are the only glutamatergic excitatory receptors postnatally, as functional AMPA receptors are absent at the beginning of the postnatal period [85]. Glutamate can act via ionotropic and metabotropic receptors. Glutamate ionotropic receptors, N-methyl-D-aspartate receptor (NMDAR), are the target in schizophrenia research. Hypofunction of NMDAR plays a role not only in psychiatric diseases like schizophrenia, but also in Alzheimer's disease or autism [86]. The differences in clinical and neuropathological presentations might account for the timing and cause of NMDAR hypofunction.

NMDARs are made of four subunits, forming a heterotetramer composed by NR1 subunit and the facultative NR2 (A, B, C or D) or NR3 (A or B). NR3 subunits are mainly found in early development. NR2 subunits regulate the channel gating. NR2A subunit is the most abundant throughout the nervous system, but NR2B is predominant in forebrain and hippocampus. Depending on the combination of NMDA subunits, the electrophysiological properties of NMDA receptors vary. NR1-NR2B combinations have longer excitatory postsynaptic potentials in vitro than NR1-NR2A complexes [87]. NMDA receptor subunits are also involved in synaptic plasticity: a shift in subunit expression in a particular receptor potentially changes its functional properties. In fact, NR2B incorporation could increase the time period for synaptic coincidence, thereby enhancing synaptic efficacy and probably memory function. NMDAR

subunits also differ in their binding sites: NR1 subunits have glycine binding sites, and NR2 subunits glutamate binding sites. Glycine acts as a co-agonist, meaning that its binding to NMDAR is a prerequisite for the activation of NMDA receptor, together with the removal of the magnesium block. D-serine can also function as a co-agonist when it binds to glycine-B sites of NMDAR. At resting membrane potentials, magnesium ions enter the channel pore and prevent further ion permeation. A membrane depolarization is necessary to dislodge and repel magnesium block, to allow ion flow through the channel. In addition to the heterotetramer, NMDA receptors have postsynaptic densities (PSD), a set of proteins that give structural and functional stability to glutamatergic synapses.

NMDA receptors are tightly related to brain maturation. In PFC, functional NMDARs are expressed in tangentially migrating interneuron precursors [88]. Depending on the electrophysiological properties of interneurons, NMDAR mediated-currents vary. Regular-spiking (RS) interneurons maintain NMDA-mediated currents constant through development, whereas fast-spiking (FS) interneurons have a large decay [89]. This decrease is more prominent from postnatal weeks 2–4 and from weeks 12–15 [89]. NMDAR mediated currents of FS-cells decrease approximately from 75% in juvenile rats to 25% in adult animals. This is probably secondary to changes in NR2 subunits. In fact, brain circuitry maturation usually coincides with NMDAR subunit switch, marking the transition from young to adult neural processing. NR2 subunit switch is cell type-specific in prefrontal cortex, with NR2B levels remaining constant until adulthood in pyramidal cells, but with a gradual replacement from NR2B to NR2A in fast-spiking interneurons, particularly in adolescence [89, 90]. Subunits switch makes NMDARs extremely vulnerable to genetic risk factors and environmental perturbations, and both interact to affect normal brain development [91]. Similarly, NMDAR subunit expression and function in hippocampus is necessary for proper hippocampal development, with NMDAR dysregulation resulting in failures in synaptogenesis and circuit maturation [92–95].

Recent evidence supports the finding of abnormal glutamatergic transmission and NMDAR hypofunction in schizophrenic individuals [84]. Firstly, multiple genes involved in increased risk for schizophrenia are known to alter NMDAR-mediated signaling [96, 97]. Susceptibility genes for schizophrenia therefore regulate neuronal proliferation, migration, and synaptogenesis. Secondly, dysregulation of NMDAR subunits in postmortem brains of schizophrenic patients, in which NR1 subunits are decreased, further indicates perturbed NMDA function. Thirdly, transgenic mice with low NMDAR expression and animal models of NMDA antagonism present symptoms reminiscent of schizophrenia. NMDA antagonism not only produces behavioral changes, but also patterns of metabolic and neurochemical alterations of the disease [98]. Taken together, it is increasingly recognized that schizophrenia is a neurodevelopmental disorder, in which early brain development is affected [99].

Effects of MK-801 on NMDA Receptors

As stated in a previous section, failure in glutamatergic neurotransmission is known to play a role in the pathophysiology of schizophrenia. MK-801 is a noncompetitive NMDA receptor antagonist that physically blocks the receptor by inserting in the channel pore, binding to several PCP-s binding sites, and preventing the flow of cations through the channel pore. Blocking NMDA receptors results in an excessive release of glutamate that can have an impact on the blocked neuron itself and on downstream brain regions. In fact, early-life MK-801 administration has pro-apoptotic effects shortly after exposure. The ability to activate apoptotic pathways depends on the duration and severity of NMDA blockade. Doses higher than 0.25 mg/kg are necessary to induce irreversible degeneration and cell death [100]. The mechanism for activating apoptotic cell death is not well established, although NMDA receptor coupling to ERK1/2-CREB in early brain development has been proposed to be vital for neurotrophic action of

NMDA receptor. MK-801-induced apoptotic injury is believed to result from the dissociation of the NMDA receptor from the ERK1/2-CREB signaling pathway [82]. Ikonomidou et al. [100] reported that only neuronal cells committed apoptosis with no glial cell activation. Studies from the last decade, however, suggest glial impairment in the cerebral cortex of schizophrenics, including reduced glial cell size and density, and glial dysfunction in prefrontal cortex and hippocampus [101, 102]. Astrocytic glutamate metabolism, more specifically glutamate–glutamine–GABA cycle, is also perturbed after MK-801 administration. Glutamate and glutamine levels after repeated injections of high doses of MK-801 (0.5 mg/kg during 6 days) were comparable to those found in first-episode patients of schizophrenia [103]. The susceptibility for MK-801-induced damage correlates with the highest expression of NMDA receptors (around week 2) and growth spurt (peaks at P10). The excessive release of glutamate after NMDA antagonism, mediates excitotoxicity that goes beyond apoptotic pathways, affecting growth cone activity, and neurite extension and branching [104]. Neuronal injury, such as dendritic atrophy, is also seen in postmortem brains [17].

Glutamate-mediated excitotoxicity has considerable functional consequences. GABAergic interneurons are claimed to be 10 times more sensitive to NMDA receptor antagonism than pyramidal cells [105]. In particular, fast-spiking cells that express the calcium-binding protein parvalbumin (PV) seem to be the most vulnerable to damage after NMDA blockade, and their alteration is sufficient to induce behavioral traits that resemble symptoms of schizophrenia. Several studies have demonstrated that repeated MK-801 administration decreases PV immunoreactivity. Chronic NMDAR blockade in juvenile and adult rats diminishes PV+ densities in hippocampus, especially in the dentate gyrus and CA1 region, shortly after exposure [60, 62]. Neurodevelopmental models further support this finding, but usually doses higher than 0.5 mg/kg are needed to induce long-term structural and anatomical changes [106]. Such large doses not only alter hippocampal PV densities, but also PV cells in mPFC, a region involved in higher cognitive functions [61, 63, 64]. Moreover, Nakazawa et al. [41] demonstrated in transgenic mice that the lack of cortical and hippocampal NMDA receptors in GABAergic interneurons was sufficient to evoke schizophrenia-like features. Most of the altered GABAergic interneurons had, in fact, parvalbumin-positive immunoreactivity. The mechanisms by which PV+ FS-cells can be selectively susceptible to damage after MK-801 administration are unclear, although a number of hypotheses have been proposed. FS-cells express Kv1.3 channels that allow a fast repolarization of the membrane, and the ability to fire the next action potential very rapidly. The high frequency of action potential firing means that the open probability of NMDAR in FS-cells is much higher than in any other GABAergic cell or excitatory cells that fire at a slower rate. As MK-801 is an uncompetitive drug and needs the ion channel to be opened for blocking the receptor, the chances of blocking NMDAR of FS-cells is much higher. Another mechanism is the one described by Wang & Gao [107]. They demonstrated that NMDAR of presynaptic glutamatergic terminals targeting pyramidal cells and FS interneurons were distinctly affected after subchronic MK-801 exposure. Presynaptic NMDAR are critical to modulate and facilitate neurotransmitter release. Interestingly, MK-801 completely blocked presynaptic NMDA receptors in glutamatergic terminals that targeted FS interneurons, whereas new NMDA receptors were inserted in presynaptic terminals that made synaptic contact with pyramidal neurons. This mechanistic approach further confirms that synaptic mechanisms of NMDA blockade are cell-type specific. These two hypotheses are not mutually exclusive, and probably MK-801 alters NMDAR by several mechanisms, but all resulting in PV+ FS-cell underactivation, with overall disinhibition of pyramidal cell activity in cerebral cortex.

It is not known whether the NMDAR on astrocytes are blocked, how this could influence glutamate metabolism and transport or glycine release and uptake, and thereby local circuitry [108]. What has been demonstrated so far is that

astrocytes affect the glutamatergic system. Notably, the upregulation of glutamate transporter-1 (GLT-1) mRNA [109], protein, and activity [110] in astrocytes has been found in prefrontal cortex of schizophrenic patients. Animal studies revealed that NMDAR antagonist phencyclidine provokes similar findings, although the effects of MK-801 have not been studied yet [111]. Furthermore, a selective deletion of astroglial A2AR, which tightly regulates GLT-1 activity [112], decreases working memory in rodents, as measured in radial arm maze [113]. Glycine transporters (GlyT) are present in both astroglial cells and neurons. It is well established that glycine plays a pivotal role in NMDAR neurotransmission, and GlyT are closely associated with NMDAR. Although it was believed that glycine levels in synaptic cleft were enough to saturate the glycine-B sites of the NMDA receptor, it is now known that these levels are below saturation. Under physiological conditions, glycine is actively removed by the action of presynaptic and postsynaptic glycine transporters (GlyT). Augmenting glycine availability in the synaptic cleft could therefore facilitate NMDAR function, and drugs that inhibit GlyT action have been proved to be effective in improving cognition in animal models of schizophrenia induced by MK-801 (Table 25.1) [114–118].

Table 25.1 Pharmacological compounds and behavioral tasks used to demonstrate that glycine transporter inhibition improves cognitive function in MK-801-induced rodent model of schizophrenia

	GlyT inhibitor	Behavioral paradigm
Harada et al. (2012) [114]	ASP2535	Working memory in Y-maze
Shimazaki et al. (2010) [115]	NFPS	Social memory
Black et al. (2009) [116]	NFPS	Latent inhibition
Manahan-Vaughan et al. (2008) [117]	SSR103800 SSR504734	Reference memory in RAM
Karasawa et al. (2008) [118]	NFPS	NOR

NFPS N[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine, *RAM* radial arm maze, *NOR* novel object recognition test

Effects of MK-801 on Brain Circuits and Activity

Acute systemic administration of MK-801 increases mPFC activity, and the decreased signal-to-noise ratio could account for the deficits in mPFC-dependent executive functions. Unraveling the mechanisms by which this occurs could contribute to a better understanding of the dysfunctional brain. As previously suggested, a local disinhibition of mPFC secondary to PV+ FS GABAergic cell underactivation could explain increased excitability of this region. Nevertheless, it has been shown that local infusion of MK-801 in CA1 of hippocampus augments mPFC neural activity in a similar manner to systemic administration [119]. According to these findings, local disinhibition of pyramidal cells in CA1 that send glutamatergic projections to mPFC through hippocampal-PFC pathway could contribute to the overexcitation of mPFC. The hippocampal–PFC pathway supports high-order cognitive functions [120–122]. Blot et al. [29] demonstrated an aberrant form of plasticity in this pathway after acute MK-801 administration that correlated with impaired working memory and learning flexibility in rodents. NMDA receptors are involved in brain plasticity, and cellular models of learning and memory formation such as LTP or LTD are NMDAR-dependent. Alterations in plasticity could underlie the pathophysiology of schizophrenic symptoms. In fact, human studies indicate that there is impaired glutamatergic plasticity in schizophrenic brains [123], and the hippocampal–prefrontal pathway is crucial in the pathophysiology [28]. Blot et al. [29] demonstrated that a single dose of MK-801 (0.1 mg/kg) evoked long-lasting response to synaptic input from the ventral hippocampus that was independent of synchronized afferent inputs — necessary for standard LTP. The authors suggested that this was an aberrant form of plasticity. For LTP formation in mPFC of hippocampus–prefrontal pathway synapses, concurrent activation of dopaminergic and NMDA receptors is necessary. As suggested by the authors, an excessive release of glutamate and dopamine by the action of MK-801 in mPFC could be the mechanism by which the aberrant form of plasticity

takes place. Furthermore, subchronic administration of MK-801 during 14 days hindered LTP induction. According to the results of Manahan-Vaughan et al. [117], LTP induction and expression was also profoundly impaired in the dentate gyrus 1 week after administering MK-801 acutely (5 mg/kg), and consequently learning deficits were present in MWM. LTP and learning performance were rescued by application of glycine transporter-1 inhibitors discussed before. In a subsequent study of the same group, LTP impairment was also present 4 weeks after drug administration, due to hippocampal hyperactivity and changes in expression of GABA and NMDA receptors in the prefrontal cortex and hippocampus that led to poor inhibitory control of prefrontal cortex [81]. This uncoupling of hippocampal–prefrontal communication could account for learning impairments and memory dysfunction found in MK-801-treated animals.

The nucleus accumbens has been identified as another important brain region that links several findings of schizophrenic disturbances [124–126]. The vast majority of neurons in nucleus accumbens are medium spiny neurons, a special type of GABAergic inhibitory neurons that have long-range projections. They receive glutamatergic inputs from hippocampus, prefrontal cortex, and amygdala, and dopaminergic inputs from the ventral tegmental area (VTA) (Fig. 25.2). Medium spiny neurons modulate inhibitory control on thalamocortical glutamatergic neurons. Local blockade of NMDA receptors in nucleus accumbens has been demonstrated to be sufficient to augment PFC activity and impair working memory [127]. Mesolimbic pathway connects dopaminergic neurons from ventral tegmental area with GABAergic neurons of nucleus accumbens. There is a differential dysregulation of mesocortical and mesolimbic dopaminergic pathways in schizophrenia. Mesocortical pathway is hypoactivated, whereas increased firing of dopaminergic neurons in mesolimbic pathway is found. The overexcitation of mesolimbic pathway prevents proper inhibition by accumbal neurons. This results in sensory information overload

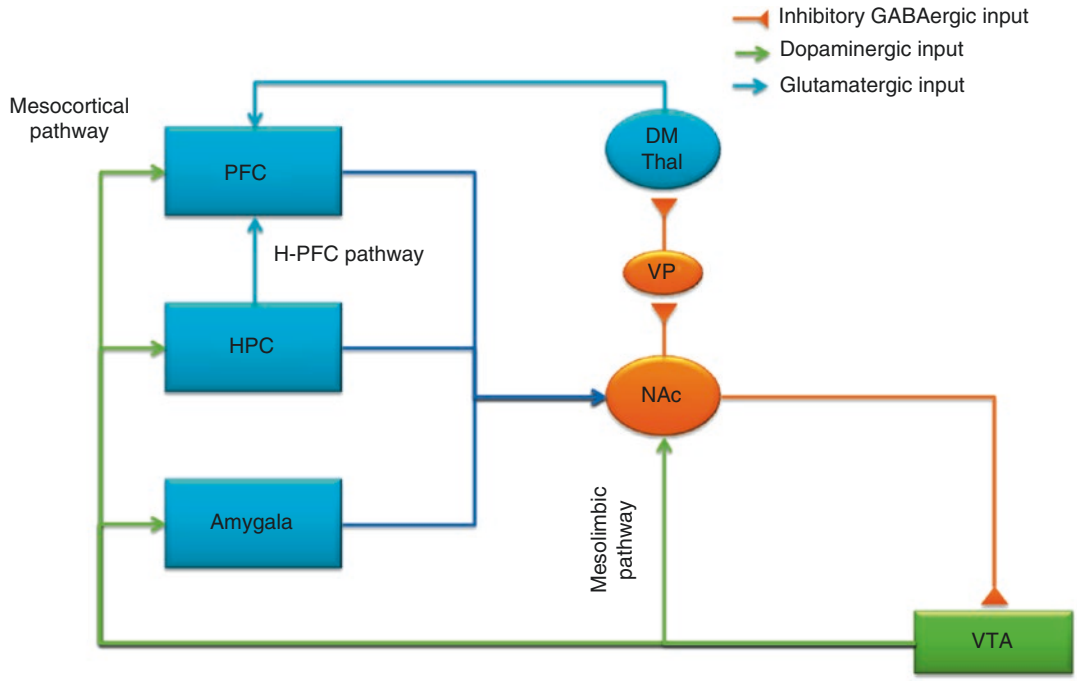

Fig. 25.2 Schematic representation of the major afferent and efferent connections of the NAc. *PFC* prefrontal cortex, *HPC* hippocampus, *DM Thal* dorsomedial thalamus, *VP* ventral pallidum, *NAc* nucleus accumbens, *VTA* ventral tegmental area

to cortex and a lack of feedback inhibition to VTA dopaminergic neurons. In turn, this will lead to an excessive release of glutamate in nucleus accumbens through the overactivation of glutamatergic neurons projecting to nucleus accumbens (Fig. 25.2).

Effects of MK-801 on Cognition

Memory function enables storage and retrieval of information over variable periods ranging from seconds to years, and is critical to daily life functioning. In schizophrenic patients, not only memory but all areas of cognition seem to be impaired, suggesting widespread cortical dysfunction. Cognitive symptoms are considered a distinct dimension of the illness, and are relatively independent of positive and negative symptoms. Cognitive deficits include impaired working, spatial, and declarative memory, attentional dysfunction, and poor cognitive flexibility. Working memory [19] and episodic memory [128] are especially sensitive to neuropsychiatric disorders, and appear to be core features of schizophrenia. Valid schizophrenia animal models should mimic at least one of these cognitive deficits.

Behavioral paradigms that evaluate working memory are non-matching to sample of objects or odors, operant tasks, and paradigms that use spatial information, such as maze tasks (delayed alternation, radial-arm maze, Morris water maze) [129]. Using these devices, deficits in working memory have been consistently shown after MK-801 administration [130–134]. Morris water maze (MWM) and radial arm maze (RAM) are the most widely performed tasks for spatial learning and memory assessment. MK-801 impairs spatial learning and memory. Concretely, acquisition, reversal learning, and working memory performance are affected in hidden-platform trials, although reference memory is spared [106, 135–139]. Spatial learning tasks are readily available both in humans and animals, and allow a direct translation of findings. In an attempt to bridge the gap between human and animal research, a variant of Morris water maze has been used in schizophrenic patients, showing decreased navigational abilities in human patients, and further confirming spatial impairment [140]. Deficits in cognitive or behavioral flexibility have been documented in schizophrenic patients [141], a type of executive function carried out by prefrontal cortex. MK-801-induced animal models have also displayed problems in cognitive flexibility measured by reversal learning in MWM, as stated previously, and by active place avoidance tasks in radial arm maze [29, 136]. It is well established that hippocampal neurons are essential for spatial navigation. Nevertheless, the neurocircuitry involved in spatial learning and memory contains different systems that collaborate in serial or parallel fashion. In this context, the role of medial prefrontal cortex is essential. Neurons from cornus ammonis 1 (CA1) send projections to frontal areas, mainly the prelimbic and cingulated cortices [142]. Disconnecting hippocampus from the medial prefrontal cortex impairs spatial memory and spatial working memory in rodents [120, 121]. It seems that spatial information is acquired by the hippocampus and then transferred to mPFC.

Novel object recognition (NOR) is one of the most widely performed preclinical cognitive tests for schizophrenia [143], and it has long been considered the analog of human episodic memory. Multiple schizophrenia-relevant studies have used NOR for exploring cognitive impairment in rodents [144, 145]. NOR is based on a rodent's natural tendency to explore new stimuli and environments. Following acute administration of MK-801, NOR is severely disrupted [146, 147, 118] but in neurodevelopmental models these results failed to be replicated. In fact, early-life repeated injections of MK-801 have no long-term consequences in NOR, using delays of 1.5 h [148], 2 h [149] and 5 h [150] between acquisition and test trials. Using NOR for evaluating declarative memory has raised some concerns, as it seems that NOR is a familiarity-based test, which is not affected in schizophrenic patients, rather than a recollection-based test [151, 152]. Tests that use associative or relational information are more closely related to human episodic memory. Such

tests need temporal and spatial precision of object memory. Li et al. [64] demonstrated that associative recognition memory was impaired in adolescence and adulthood after early-life NMDA blockade. The dissociation of results between NOR and tests that use relational information in MK-801 rodent model could be explained by neural circuitry. Brain wiring for rodent recognition memory involves several structures, but perirhinal (Prh) cortex plays a major role [153]. Although hippocampus (HPC), medial prefrontal cortex (mPFC), mediodorsal thalamus (MD) and postrhinal cortex (PostRh) participate in recognition memory, NOR is particularly sensitive to perirhinal cortex dysfunction, and not to hippocampal alterations. Rather, the role of hippocampus is to integrate object information with spatial or contextual information. Similarly, mPFC integrates spatial information from CA1 subfields of HPC with object information of Prh cortex, using NMDA-dependent synaptic plasticity [154]. Therefore, associative tasks require network interdependency across multiple structures, in which HPC–mPFC-Prh circuits are essential for memory acquisition and retrieval. Furthermore, associative memory depends on NMDA receptor neurotransmission, and hippocampal NMDAR are required for acquisition, but not retrieval, of associative memories [154]. mPFC and HPC are two critical brain regions in the pathophysiology of schizophrenia, so assessing cognitive functions dependent on the interaction of both regions will preferably reveal cognitive deficits in rodents.

Conclusions

Neonatal administration of MK-801 instills a hypofunction of NMDAR that results in a widespread apoptotic injury during synaptogenesis. The GABAergic system is particularly sensitive in the developing brain to transient NMDA blockade. Among subpopulations of GABAergic interneurons, PV+ cells seem to provide the most potent inhibitory input on pyramidal neurons by formation of synaptic contacts in soma, proximal dendrites, and axon initial segment. A decrease in PV+ interneuron population following MK-801 administration therefore has a significant impact on neuronal synchrony and information processing. Disrupted synaptic integration in early brain development results in modified network activity and plasticity in adulthood. This is corroborated by behavioral data that show a constellation of neurobehavioral sequelae that resemble symptoms of schizophrenia.

Mimicking behavioral and cognitive deficits of schizophrenia in animal models is a real challenge, and the validity of cognitive deficits in animal models largely depends on the appropriate behavioral paradigm. Cognitive impairment is best assessed by means of tasks that require mPFC–HPC interactions, a circuit that underlies episodic, working, and spatial memory. Although deficits elicited by repeated NMDA antagonism are unlikely to represent an animal model of schizophrenia *per se*, a strong body of evidence supports an MK-801-induced neurodevelopmental model of schizophrenia as a valid model for some of the essential deficits occurring in this condition.

Acknowledgements This study was supported by grants from the University of the Basque Country UPV/EHU (UFI 11/32), (EHU 14/33), and by the Government of the Basque Country (GIC IT 901/16). Murueta-Goyena A is financed by a predoctoral fellowship of the University of the Basque Country (UPV/EHU).

References

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. PLoS Med. 2005;2(5):e141.
2. Green MF, Kern RS, Braff DL, Mintz J. Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"? Schizophr Bull. 2000;26(1):119–36.
3. Gold JM. Cognitive deficits as treatment targets in schizophrenia. Schizophr Res. 2004;72(1):21–8.
4. Bouwmans C, de Sonneville C, Mulder CL, Hakkaart-van RL. Employment and the associated impact on quality of life in people diagnosed with schizophrenia. Neuropsychiatr Dis Treat. 2015;11:2125–42.
5. Bergen SE, Petryshen TL. Genome-wide association studies of schizophrenia: does bigger lead to better results? Curr Opin Psychiatry. 2012;25(2):76–82.
6. Kukshal P, Thelma BK, Nimgaonkar VL, Deshpande SN. Genetics of schizophrenia from a clinicial perspective. Int Rev Psychiatry. 2012;24(5):393–404.

7. Owen MJ. Implications of genetic findings for understanding schizophrenia. Schizophr Bull. 2012;38(5):904–7.
8. Greenwood TA, Swerdlow NR, Gur RE, Cadenhead KS, Calkins ME, Dobie DJ, et al. Genome-wide linkage analyses of 12 endophenotypes for schizophrenia from the consortium on the genetics of schizophrenia. Am J Psychiatry. 2013;170(5):521–32.
9. Singh S, Kumar A, Agarwal S, Phadke SR, Jaiswal Y. Genetic insight of schizophrenia: past and future perspectives. Gene. 2014;535(2):97–100.
10. Brown AS. Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. Dev Neurobiol. 2012;72(10):1272–6.
11. Labouesse MA, Langhans W, Meyer U. Long-term pathological consequences of prenatal infection: beyond brain disorders. Am J Physiol Regul Integr Comp Physiol. 2015;309(1):R1–R12.
12. Bourque F, van der Ven E, Fusar-Poli P, Malla A. Immigration, social environment and onset of psychotic disorders. Curr Pharm Des. 2012;18(4):518–26.
13. Pishva E, Kenis G, van den Hove D, Lesch KP, Boks MP, van Os J, et al. The epigenome and postnatal environmental influences in psychotic disorders. Soc Psychiatry Psychiatr Epidemiol. 2014;49(3):337–48.
14. Uher R. Gene–environment interactions in severe mental illness. Front Psych. 2014;5:48.
15. Ordóñez AE, Luscher ZI, Gogtay N. Neuroimaging findings from childhood onset schizophrenia patients and their non-psychotic siblings. Schizophr Res. 2015;72(8):803–12.
16. Kubota M, van Haren NE, Haijma SV, Schnack HG, Cahn W, Hulshoff Pol HE et al. Association of IQ changes and progressive brain changes in patients with schizophrenia. JAMA Psychiatry. 2015;72(8):803–12.
17. Garey L. When cortical development goes wrong: schizophrenia as a neurodevelopmental disease of microcircuits. J Anat. 2010;217(4):324–33.
18. Liu J, Corbera S, Wexler BE. Neural activation abnormalities during self-referential processing in schizophrenia: an fMRI study. Psychiatry Res. 2014;222(3):165–71.
19. Silver H, Feldman P, Bilker W, Gur RC. Working memory deficit as a core neuropsychological dysfunction in schizophrenia. Am J Psychiatry. 2003;160(10):1809–16.
20. Barch DM. What can research on schizophrenia tell us about the cognitive neuroscience of working memory? Neuroscience. 2006;139(1):73–84.
21. Wolf DH, Satterthwaite TD, Calkins ME, Ruparel K, Elliott MA, Hopson RD. Functional neuroimaging abnormalities in youth with psychosis spectrum symptoms. JAMA Psychiat. 2015;72(5):456–65.
22. Brunoni AR, Vanderhasselt MA. Working memory improvement with non-invasive brain stimulation of the dorsolateral prefrontal cortex: a systematic review and meta-analysis. Brain Cogn. 2014;86:1–9.
23. Arnsten AF, Jin LE. Molecular influences on working memory circuits in dorsolateral prefrontal cortex. Prog Mol Biol Transl Sci. 2014;122:211–31.
24. Benes FM. Emerging principles of altered neural circuitry in schizophrenia. Brain Res Brain Res Rev. 2000;31(2-3):251–69.
25. Rotaru DC, Lewis DA, Gonzalez-Burgos G. The role of glutamatergic inputs onto parvalbumin-positive interneurons: relevance for schizophrenia. Rev Neurosci. 2012;23(1):97–109.
26. Volk DW, Lewis DA. Impaired prefrontal inhibition in schizophrenia: relevance for cognitive dysfunction. Physiol Behav. 2002;77(4-5):501–5.
27. Meyer-Lindenberg AS, Olsen RK, Kohn PD, Brown T, Egan MF, Weinberger DR, et al. Regionally specific disturbance of dorsolateral prefrontal–hippocampal functional connectivity in schizophrenia. Arch Gen Psychiatry. 2005;62(4):379–86.
28. Godsil BP, Kiss JP, Spedding M, Jay TM. The hippocampal–prefrontal pathway: the weak link in psychiatric disorders? Eur Neuropsychopharmacol. 2013;23(10):1165–81.
29. Blot K, Bai J, Otani S. The effect of non-competitive NMDA receptor antagonist MK-801 on neuronal activity in rodent prefrontal cortex: an animal model for cognitive symptoms of schizophrenia. J Physiol Paris. 2013;107(6):448–51.
30. Blum BP, Mann JJ. The GABAergic system in schizophrenia. Int J Neuropsychopharmacol. 2002;5(2):159–79.
31. Reynolds GP, Abdul-Monim Z, Neill JC, Zhang ZJ. Calcium binding protein markers of GABA deficits in schizophrenia — postmortem studies and animal models. Neurotox Res. 2004;6(1):57–61.
32. Gonzalez-Burgos G, Cho RY, Lewis DA. Alterations in cortical network oscillations and parvalbumin neurons in schizophrenia. Biol Psychiatry. 2015;77(12):1031–40.
33. Joshi D, Catts VS, Olaya JC, Shannon WC. Relationship between somatostatin and death receptor expression in the orbital frontal cortex in schizophrenia: a postmortem brain mRNA study. NPJ Schizophrenia. 2015;1:14004.
34. Jentsch TJ, Stein V, Weinreich F, Zdebik AA. Molecular structure and physiological function of chloride channels. Physiol Rev. 2002;82(2):503–68.
35. Le Magueresse C, Monyer H. GABAergic interneurons shape the functional maturation of the cortex. Neuron. 2013;77(3):388–405.
36. Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry. 2000;57(3):237–45.
37. Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, et al. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci. 2003;23(15):6315–26.

38. Zhang ZJ, Reynolds GP. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophr Res. 2002;55(1–2):1–10.
39. Singer W. The formation of cooperative cell assemblies in the visual cortex. J Exp Biol. 1990;153:177–97.
40. Uhlhaas PJ. Dysconnectivity, large-scale networks and neuronal dynamics in schizophrenia. Curr Opin Neurobiol. 2013;23(2):283–90.
41. Nakazawa K, Zsiros V, Jiang Z, Nakao K, Kolata S, Zhang S, et al. GABAergic interneuron origin of schizophrenia pathophysiology. Neuropharmacology. 2012;62(3):1574–83.
42. Williams S, Boksa P. Gamma oscillations and schizophrenia. J Psychiatry Neurosci. 2010;35(2):75–7.
43. Qin ZH, Zhang SP, Weiss B. Dopaminergic and glutamatergic blocking drugs differentially regulate glutamic acid decarboxylase mRNA in mouse brain. Brain Res Mol Brain Res. 1994;21(3-4):293–302.
44. Gierdalski M, Jablonska B, Smith A, Skangiel-Kramska J, Kossut M. Deafferentation induced changes in GAD67 and GluR2 mRNA expression in mouse somatosensory cortex. Brain Res Mol Brain Res. 1999;71(1):111–9.
45. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, et al. The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature. 1999;397(6716):251–5.
46. Bortone D, Polleux F. KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. Neuron. 2009;62(1):53–71.
47. Del Rio JA, Soriano E, Ferrer I. Development of GABA-immunoreactivity in the neocortex of the mouse. J Comp Neurol. 1992;326(4):501–26.
48. Doischer D, Hosp JA, Yanagawa Y, Obata K, Jonas P, Vida I, et al. Postnatal differentiation of basket cells from slow to fast signaling devices. J Neurosci. 2008;28(48):12956–68.
49. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci. 1992;12(11):4151–72.
50. Fritschy JM, Meskenaite V, Weinmann O, Honer M, Benke D, Mohler H. GABAB-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. Eur J Neurosci. 1999;11(3):761–8.
51. Bitanihirwe BK, Woo TU. Perineuronal nets and schizophrenia: the importance of neuronal coatings. Neurosci Biobehav Rev. 2014;45:85–99.
52. Eastwood SL, Harrison PJ. Interstitial white matter neurons express less reelin and are abnormally distributed in schizophrenia: towards an integration of molecular and morphologic aspects of the neurodevelopmental hypothesis. Mol Psychiatry. 2003;8(9):769. 821–31
53. Joshi D, Fung SJ, Rothwell A, Weickert CS. Higher gamma-aminobutyric acid neuron density in the white matter of orbital frontal cortex in schizophrenia. Biol Psychiatry. 2012;72(9):725–33.
54. Steinecke A, Gampe C, Valkova C, Kaether C, Bolz J. Disrupted-in-schizophrenia 1 (DISC1) is necessary for the correct migration of cortical interneurons. J Neurosci. 2012;32(2):738–45.
55. Fazzari P, Paternain AV, Valiente M, Pla R, Luján R, Lloyd K, et al. Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. Nature. 2010;464(7293):1376–80.
56. Volk DW, Pierri JN, Fritschy JM, Auh S, Sampson AR, Lewis DA. Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. Cereb Cortex. 2002;12(10):1063–70.
57. Zheng K, An JJ, Yang F, Xu W, Xu ZQ, Wu J, et al. TrkB signaling in parvalbumin-positive interneurons is critical for gamma-band network synchronization in hippocampus. Proc Natl Acad Sci U S A. 2011;108(41):17201–6.
58. Lipska BK, Weinberger DR. To model a psychiatric disorder in animals: schizophrenia as a reality test. Neuropsychopharmacology. 2000;23(3):223–39.
59. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nat Neurosci. 2010;13(10):1161–9.
60. Rujescu D, Bender A, Keck M, Hartmann AM, Ohl F, Raeder H, et al. A pharmacological model for psychosis based on N-methyl-D-aspartate receptor hypofunction: molecular, cellular, functional and behavioral abnormalities. Biol Psychiatry. 2006;59(8):721–9.
61. Abekawa T, Ito K, Nakagawa S, Koyama T. Prenatal exposure to an NMDA receptor antagonist, MK-801 reduces density of parvalbumin-immunoreactive GABAergic neurons in the medial prefrontal cortex and enhances phencyclidine-induced hyperlocomotion but not behavioral sensitization to methamphetamine. Psychopharmacology (Berl). 2007;192(3):303–16.
62. Braun I, Genius J, Grunze H, Bender A, Möller HJ, Rujescu D. Alterations of hippocampal and prefrontal GABAergic interneurons in an animal model of psychosis induced by NMDA receptor antagonism. Schizophr Res. 2007;97(1–3):254–63.
63. Coleman Jr LG, Jarskog LF, Moy SS, Crews FT. Deficits in adult prefrontal cortex neurons and behavior following early post-natal NMDA antagonist treatment. Pharmacol Biochem Behav. 2009;93(3):322–30.
64. Li JT, Zhao YY, Wang HL, Wang XD, Su YA, Si TM. Long-term effects of neonatal exposure to MK-801 on recognition memory and excitatory-inhibitory balance in rat hippocampus. Neuroscience. 2015;308:134–43.
65. Marcotte ER, Pearson DM, Srivastava LK. Animal models of schizophrenia: a critical review. J Psychiatry Neurosci. 2001;26(5):395–410.
66. Jones CA, Watson DJG, Fone KCF. Animal models of schizophrenia. Br J Pharmacol. 2011;164(4):1162–94.
67. van der Staay FJ, Arndt SS, Nordquist RE. Evaluation of animal models of neurobehavioral disorders. Behav Brain Funct. 2009;5:11.
68. Salgado JV, Sandner G. A critical overview of animal models of psychiatric disorders: challenges and

perspectives. Rev Bras Psiquiatr. 2013;35(Suppl 2):S77–81.
69. Bubeníková-Valesová V, Horácek J, Vrajová M, Höschl C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. Neurosci Biobehav Rev. 2008;32(5):1014–23.
70. Jones GH, Hernandez TD, Kendall DA, Marsden CA, Robbins TW. Dopaminergic and serotonergic function following isolation rearing in rats: study of behavioural responses and postmortem and in vivo neurochemistry. Pharmacol Biochem Behav. 1992;43(1):17–35.
71. Geyer MA, Wilkinson LS, Humby T, Robbins TW. Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. Biol Psychiatry. 1993;34(6):361–72.
72. Wilkinson LS, Killcross SS, Humby T, Hall FS, Geyer MA, Robbins TW. Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. Neuropsychopharmacology. 1994;10(1):61–72.
73. Gilabert-Juan J, Belles M, Saez AR, Carceller H, Zamarbide-Fores S, Moltó MD, et al. A "double hit" murine model for schizophrenia shows alterations in the structure and neurochemistry of the medial prefrontal cortex and the hippocampus. Neurobiol Dis. 2013;59:126–40.
74. Daenen EW, Wolterink G, Van Der Heyden JA, Kruse CG, Van Ree JM. Neonatal lesions in the amygdala or ventral hippocampus disrupt prepulse inhibition of the acoustic startle response; implications for an animal model of neurodevelopmental disorders like schizophrenia. Eur Neuropsychopharmacol. 2003;13(3):187–97.
75. Rueter LE, Ballard ME, Gallagher KB, Basso AM, Curzon P, Kohlhaas KL. Chronic low dose risperidone and clozapine alleviate positive but not negative symptoms in the rat neonatal ventral hippocampal lesion model of schizophrenia. Psychopharmacology (Berl). 2004;176(3–4):312–9.
76. Marquis JP, Goulet S, Doré FY. Neonatal ventral hippocampus lesions disrupt extra-dimensional shift and alter dendritic spine density in the medial prefrontal cortex of juvenile rats. Neurobiol Learn Mem. 2008;90(2):339–46.
77. Tseng KY, Lewis BL, Hashimoto T, Sesack SR, Kloc M, Lewis DA, et al. A neonatal ventral hippocampal lesion causes functional deficits in adult prefrontal cortical interneurons. J Neurosci. 2008;28(48):12691–9.
78. Green JG, McLaughlin KA, Berglund PA, Gruber MJ, Sampson NA, Zaslavsky AM, et al. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. Arch Gen Psychiatry. 2010;67(2):113–23.
79. Grace AA. Gating of information flow within the limbic system and the. Brain Res Brain Res Rev. 2000;31(2–3):330–41.
80. Lahti AC, Weiler MA, Tamara Michaelidis BA, Parwani A, Tamminga CA. Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology. 2001;25(4):455–67.
81. Grüter T, Wieschollеck V, Dubovyk V, Aliane V, Manahan-Vaughan D. Altered neuronal excitability underlies impaired hippocampal function in an animal model of psychosis. Front Behav Neurosci. 2015;9:117.
82. Lim AL, Taylor DA, Malone DT. Consequences of early life MK-801 administration: long-term behavioural effects and relevance to schizophrenia research. Behav Brain Res. 2012;227(1):276–86.
83. Wong EH, Kemp JA, Priestley T, Knight AR, Woodruff GN, Iversen LL. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proc Natl Acad Sci U S A. 1986;83(18):7104–8.
84. Snyder MA, Gao WJ. NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia. Front Cell Neurosci. 2013;7:31.
85. Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa JL. GABAA, NMDA and AMPA receptors: a developmentally regulated 'ménage à trois'. Trends Neurosci. 1997;20(11):523–9.
86. Newcomer JW, Farber NB, Olney JW. NMDA receptor function, memory, and brain aging. Dialogues Clin Neurosci. 2000;2(3):219–32.
87. Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron. 1994;12(3):529–40.
88. Soria JM, Valdeolmillos M. Receptor-activated calcium signals in tangentially migrating cortical cells. Cereb Cortex. 2002;12(8):831–9.
89. Wang HX, Gao WJ. Cell type-specific development of NMDA receptors in the interneurons of rat prefrontal cortex. Neuropsychopharmacology. 2009;34(8):2028–40.
90. Wang H, Stradtman 3rd GG, Wang XJ, Gao WJ. A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. Proc Natl Acad Sci U S A. 2008;105(43):16791–6.
91. Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev. 2000;24(4):417–63.
92. Roberts AC, Díez-García J, Rodriguiz RM, López IP, Luján R, Martínez-Turrillas R, et al. Downregulation of NR3A-containing NMDARs is required for synapse maturation and memory consolidation. Neuron. 2009;63(3):342–56.
93. Brigman JL, Wright T, Talani G, Prasad-Mulcare S, Jinde S, Seabold GK, et al. Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. J Neurosci. 2010;30(13):4590–600.
94. Gambrill AC, Barria A. NMDA receptor subunit composition controls synaptogenesis and synapse stabilization. Proc Natl Acad Sci U S A. 2011;108(14):5855–60.

95. Gray JA, Shi Y, Usui H, During MJ, Sakimura K, Nicoll RA. Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: single-cell NMDA receptor subunit deletion in vivo. Neuron. 2011;71(6):1085–101.
96. Moghaddam B. Bringing order to the glutamate chaos in schizophrenia. Neuron. 2003;40(5):881–4.
97. Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry. 2005;10(1):40–68.
98. Morris BJ, Cochran SM, Pratt JA. PCP: from pharmacology to modelling schizophrenia. Curr Opin Pharmacol. 2005;5(1):101–6.
99. Lewis DA, Gonzalez-Burgos G. Neuroplasticity of neocortical circuits in schizophrenia. Neuropsychopharmacology. 2008;33:141–65.
100. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 1999;283(5398):70–4.
101. Cotter DR, Pariante CM, Everall IP. Glial cell abnormalities in major psychiatric disorders: the evidence and implications. Brain Res Bull. 2001;55(5):585–95.
102. Kondziella D, Brenner E, Eyjolfsson EM, Sonnewald U. How do glial–neuronal interactions fit into current neurotransmitter hypotheses of schizophrenia? Neurochem Int. 2007;50(2):291–301.
103. Kondziella D, Brenner E, Eyjolfsson EM, Markinhuhta KR, Carlsson ML, Sonnewald U. Glial–neuronal interactions are impaired in the schizophrenia model of repeated MK801 exposure. Neuropsychopharmacology. 2006;31(9):1880–7.
104. Ringler S, Aye J, Byrne E, Anderson M, Turner C. Effects of disrupting calcium homeostasis on neuronal maturation: early inhibition and later recovery. Cell Mol Neurobiol. 2008;28(3):389–409.
105. Grunze HC, Rainnie DG, Hasselmo ME, Barkai E, Hearn EF, McCarley RW, et al. NMDA-dependent modulation of CA1 local circuit inhibition. J Neurosci. 1996;16(6):2034–43.
106. van der Staay FJ, Rutten K, Erb C, Blokland A. Effects of the cognition impairer MK-801 on learning and memory in mice and rats. Behav Brain Res. 2011;220(1):215–29.
107. Wang HX, Gao WJ. Prolonged exposure to NMDAR antagonist induces cell-type specific changes of glutamatergic receptors in rat prefrontal cortex. Neuropharmacology. 2012;62(4):1808–22.
108. Takahashi N, Sakurai T. Roles of glial cells in schizophrenia: possible targets for therapeutic approaches. Neurobiol Dis. 2013;53:49–60.
109. Matute C, Melone M, Vallejo-Illarramendi A, Conti F. Increased expression of the astrocytic glutamate transporter GLT-1 in the prefrontal cortex of schizophrenics. Glia. 2005;49(3):451–5.
110. Simpson MD, Slater P, Deakin JF. Comparison of glutamate and gamma-aminobutyric acid uptake binding sites in frontal and temporal lobes in schizophrenia. Biol Psychiatry. 1998;44(6):423–7.
111. Fattorini G, Melone M, Bragina L, Candiracci C, Cozzi A, Pellegrini Giampietro DE, et al. GLT-1 expression and Glu uptake in rat cerebral cortex are increased by phencyclidine. Glia. 2008;56(12):1320–7.
112. Matos M, Augusto E, Santos-Rodrigues AD, Schwarzschild MA, Chen JF, Cunha RA, et al. Adenosine A2A receptors modulate glutamate uptake in cultured astrocytes and gliosomes. Glia. 2012;60(5):702–16.
113. Matos M, Shen HY, Augusto E, Wang Y, Wei CJ, Wang YT, et al. Deletion of adenosine A2A receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment: relevance to schizophrenia. Biol Psychiatry. 2015;78(11):763–74.
114. Harada K, Nakato K, Yarimizu J, Yamazaki M, Morita M, Takahashi S, et al. A novel glycine transporter-1 (GlyT1) inhibitor, ASP2535. Eur J Pharmacol. 2012;685(1–3):59–69.
115. Shimazaki T, Kaku A, Chaki S. D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats. Psychopharmacology (Berl). 2010;209(3):263–70.
116. Black MD, Varty GB, Arad M, Barak S, De Levie A, Boulay D, et al. Procognitive and antipsychotic efficacy of glycine transport 1 inhibitors (GlyT1) in acute and neurodevelopmental models of schizophrenia: latent inhibition studies in the rat. Psychopharmacology (Berl). 2009;202(1–3):385–96.
117. Manahan-Vaughan D, Wildförster V, Thomsen C. Rescue of hippocampal LTP and learning deficits in a rat model of psychosis by inhibition of glycine transporter-1 (GlyT1). Eur J Neurosci. 2008;28(7):1342–50.
118. Karasawa J, Hashimoto K, Chaki S. D-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. Behav Brain Res. 2008;186(1):78–83.
119. Jodo E, Suzuki Y, Katayama T, Hoshino KY, Takeuchi S, Niwa S, et al. Activation of medial prefrontal cortex by phencyclidine is mediated via a hippocampo–prefrontal pathway. Cereb Cortex. 2005;15(5):663–9.
120. Wang GW, Cai JX. Disconnection of the hippocampal–prefrontal cortical circuits impairs spatial working memory performance in rats. Behav Brain Res. 2006;175(2):329–36.
121. Wang GW, Cai JX. Reversible disconnection of the hippocampal–prelimbic cortical circuit impairs spatial learning but not passive avoidance learning in rats. Neurobiol Learn Mem. 2008;90(2):365–73.
122. Churchwell JC, Kesner RP. Hippocampal–prefrontal dynamics in spatial working memory: interactions and independent parallel processing. Behav Brain Res. 2011;225(2):389–95.
123. Hasan A, Nitsche MA, Rein B, Schneider-Axmann T, Guse B, Gruber O, et al. Dysfunctional long-term potentiation-like plasticity in schizophrenia revealed by transcranial direct current stimulation. Behav Brain Res. 2011;224(1):15–22.
124. Gargiulo PA, Siemann M, Delius JD. Visual discrimination in pigeons impaired by glutamatergic

blockade of nucleus accumbens. Physiol Behav. 1998;63(4):705–9.
125. Gargiulo PA, Acerbo MJ, Krug I, Delius JD. Cognitive effects of dopaminergic and glutamatergic blockade in nucleus accumbens in pigeons. Pharmacol Biochem Behav. 2005;81(4):732–9.
126. Gargiulo PA, Landa De Gargiulo AI. Glutamate and modeling of schizophrenia symptoms: review of our findings: 1990–2014. Pharmacol Rep. 2014;66(3):343–52.
127. Baiardi G, Ruiz AM, Beling A, Borgonovo J, Martínez G, Landa AI, et al. Glutamatergic ionotropic blockade within accumbens disrupts working memory and might alter the endocytic machinery in rat accumbens and prefrontal cortex. J Neural Transm (Vienna). 2007;114(12):1519–28.
128. Pause BM, Zlomuzica A, Kinugawa K, Mariani J, Pietrowsky R, Dere E. Perspectives on episodic-like and episodic memory. Front Behav Neurosci. 2013;7:33.
129. Dudchenko PA. An overview of the tasks used to test working memory in rodents. Neurosci Biobehav Rev. 2004;28(7):699–709.
130. Li JT, Su YA, Guo CM, Feng Y, Yang Y, Huang RH, Si TM. Persisting cognitive deficits induced by low-dose, subchronic treatment with MK-801 in adolescent rats. Eur J Pharmacol. 2011;652(1–3):65–72.
131. Zemanova A, Stankova A, Lobellova V, Svoboda J, Vales K, Vlcek K, et al. Visuospatial working memory is impaired in an animal model of schizophrenia induced by acute MK-801: an effect of pretraining. Pharmacol Biochem Behav. 2013;106:117–23.
132. Su YA, Huang RH, Wang XD, Li JT, Si TM. Impaired working memory by repeated neonatal MK-801 treatment is ameliorated by galantamine in adult rats. Eur J Pharmacol. 2014;725:32–9.
133. Kim TW, Kang HS, Park JK, Lee SJ, Baek SB, Kim CJ. Voluntary wheel running ameliorates symptoms of MK-801-induced schizophrenia in mice. Mol Med Rep. 2014;10(6):2924–30.
134. Li ML, Yang SS, Xing B, Ferguson BR, Gulchina Y, Li YC, et al. LY395756, an mGluR2 agonist and mGluR3 antagonist, enhances NMDA receptor expression and function in the normal adult rat prefrontal cortex, but fails to improve working memory and reverse MK801-induced working memory impairment. Exp Neurol. 2015;273:190–201.
135. Stuchlik A, Rezacova L, Vales K, Bubenikova V, Kubik S. Application of a novel Active Allothetic Place Avoidance task (AAPA) in testing a pharmacological model of psychosis in rats: comparison with the Morris Water Maze. Neurosci Lett. 2004;366(2):162–6.
136. Lobellova V, Entlerova M, Svojanovska B, Hatalova H, Prokopova I, Petrasek T, et al. Two learning tasks provide evidence for disrupted behavioural flexibility in an animal model of schizophrenia-like behaviour induced by acute MK-801: a dose–response study. Behav Brain Res. 2013;246:55–62.
137. Vales K, Bubenikova-Valesova V, Klement D, Stuchlik A. Analysis of sensitivity to MK-801 treatment in a novel active allothetic place avoidance task and in the working memory version of the Morris water maze reveals differences between Long–Evans and Wistar rats. Neurosci Res. 2006;55(4):383–8.
138. Singer P, Hauser J, Llano Lopez L, Peleg-Raibstein D, Feldon J, Gargiulo PA, Yee BK. Prepulse inhibition predicts working memory performance whilst startle habituation predicts spatial reference memory retention in C57BL/6 mice. Behav Brain Res. 2013;242:166–77.
139. Nozari M, Mansouri FA, Shabani M, Nozari H, Atapour N. Postnatal MK-801 treatment of female rats impairs acquisition of working memory, but not reference memory in an eight-arm radial maze; no beneficial effects of enriched environment. Psychopharmacology (Berl). 2015;232(14):2541–50.
140. Hanlon FM, Weisend MP, Hamilton DA, Jones AP, Thoma RJ, Huang M, et al. Impairment on the hippocampal-dependent virtual Morris water task in schizophrenia. Schizophr Res. 2006;87(1–3):67–80.
141. Han K, Young Kim I, Kim JJ. Assessment of cognitive flexibility in real life using virtual reality: a comparison of healthy individuals and schizophrenia patients. Comput Biol Med. 2012;42(8):841–7.
142. Ferino F, Thierry AM, Glowinski J. Anatomical and electrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat. Exp Brain Res. 1987;65(2):421–6.
143. Young JW, Powell SB, Risbrough V, Marston HM, Geyer MA. Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. Pharmacol Ther. 2009;122(2):150–202.
144. Dere E, Huston JP, De Souza Silva MA. The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev. 2007;31(5):673–704.
145. Lyon L, Saksida LM, Bussey TJ. Spontaneous object recognition and its relevance to schizophrenia: a review of findings from pharmacological, genetic, lesion and developmental rodent models. Psychopharmacology (Berl). 2012;220(4):647–72.
146. King MV, Sleight AJ, Woolley ML, Topham IA, Marsden CA, Fone KC. 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation — an effect sensitive to NMDA receptor antagonism. Neuropharmacology. 2004;47(2):195–204.
147. Nilsson M, Hansson S, Carlsson A, Carlsson ML. Differential effects of the N-methyl-d-aspartate receptor antagonist MK-801 on different stages of object recognition memory in mice. Neuroscience. 2007;149(1):123–30.
148. Lim AL, Taylor DA, Malone DT. A two-hit model: behavioural investigation of the effect of combined neonatal MK-801 administration and isolation rearing in the rat. J Psychopharmacol. 2012;26(9):1252–64.

149. Stefani MR, Moghaddam B. Transient N-methyl-D-aspartate receptor blockade in early development causes lasting cognitive deficits relevant to schizophrenia. Biol Psychiatry. 2005;57(4):433–6.
150. Baier PC, Blume A, Koch J, Marx A, Fritzer G, Aldenhoff JB, Schiffelholz T. Early postnatal depletion of NMDA receptor development affects behaviour and NMDA receptor expression until later adulthood in rats — a possible model for schizophrenia. Behav Brain Res. 2009;205(1):96–101.
151. Achim AM, Lepage M. Is associative recognition more impaired than item recognition memory in schizophrenia? Brain and Cogn. 2003;53:121–4.
152. Danion JM, Huron C, Vidailhet P, Berna F. Functional mechanisms of episodic memory impairment in schizophrenia. Can J Psychiatry. 2007;52:693–701.
153. Winters BD, Bussey TJ. Glutamate receptors in perirhinal cortex mediate encoding, retrieval, and consolidation of object recognition memory. J Neurosci. 2005;25(17):4243–51.
154. Barker GRI, Warburton EC. Object-in-place associative recognition memory depends on glutamate receptor neurotransmission within two defined hippocampal–cortical circuits: a critical role for AMPA and NMDA receptors in the hippocampus, perirhinal, and prefrontal cortices. Cereb Cortex. 2015;25(2):472–81.