Aspects of the NMDA receptor hypofunction hypothesis of schizophrenia

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Neurobiology in Schizophrenia

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Abstract

In this review the contributions of dopaminergic, glutamergic and GABAergic neuronal pathways to the pathophysiology of schizophrenia is discussed. Hypofunction of the N-methyl-D-aspartic (NMDA) receptor at a subpopulation of the cortical GABAergic interneuronsmay explain, at least in part, schizophrenia like symptoms. During development, NMDA receptor hypofunction at subpopulation of GABAergic interneurons is an important feature for developing the pathophysiology of schizophrenia. This subpopulation of GABA interneurons seems important for temporal control of cortical inhibition and the generation of synchronous oscillations, specifically at the gamma range. In schizophrenia these oscillations seem to be disturbed, and this disturbance may be underlying for many of the symptoms of the disease. Furthermore, the disturbances could be a downstream effect of NMDA receptor hypofunction in the cortical GABAergic interneurons.

Contents

List of abbreviations

AMPA receptor - Alpha-amino-3-hydroxy-5methyl-4-isoxazole-propionic acid receptor

DLPFC - Dorsolateral Prefrontal Cortex

GABA - γ-aminobutyric acid

NMDA receptor - N-methyl-D-aspartic receptor

PCP – Phencyclidine

PET – Positron emission tomography

PFC – Prefrontal Cortex

PV- Parvalbumin

SPECT – Single photon emission computed tomography

VTA - Ventral Tegmental Area

1 What is Schizophrenia

Schizophrenia is a severe mental illness that afflicts 0,5-1% of the world population (1). The affected individuals frequently come to clinical attention during late adolescence or early adulthood. 5-10% eventually die by suicide and most experience a lifetime of disability (2). As a result, Schizophrenia ranks as one of the leading causes of years of life lost to disability and premature mortality (3).

1.1 Definition of Schizophrenia – diagnostic criteria (4)

A. Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):

- delusions
- hallucinations
- disorganized speech (e.g., frequent derailment or incoherence)
- grossly disorganized or catatonic behavior
- negative symptoms, i.e., affective flattening, alogia, or avolition

Note: Only one Criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behavior or thoughts, or two or more voices conversing with each other.

B. Social/occupational dysfunction.

C. Continuous signs of the disturbance must persist for at least 6 months. This 6-month period must include at least 1 month of symptoms that meet Criterion A, and may include periods of prodromal or residual symptoms.

D. Schizoaffective and Mood Disorder is excluded.

E. Substance/general medical condition exclusion: The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. If there is a history of Autistic Disorder or another Pervasive Developmental Disorder, the additional diagnosis of Schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month.

1.2 Symptoms and descriptions of schizophrenia

Schizophrenia is the most common and best known psychotic illness, though it is not synonymous with psychosis but rather one of many subtypes. Symptoms of schizophrenia have over time been divided into positive and negative symptoms. See tables 1-1 and 1-2 (5). Numerous studies have recently subcategorized the symptoms of schizophrenia into five dimensions. Rather than just “positive” and “negative” symptoms they also include cognitive symptoms (table 1-3) (5), aggressive symptoms and affective symptoms. There is a substantial overlap between these dimensions (figure 1.3-1) (5).

Table 1-1: Positive symptoms of schizophrenia	Table 1-2: Negative symptoms of schizophrenia
Delusions	Blunted affect
Hallucinations	Emotional withdrawal
Distortions or exaggerations in language or communication	Alogia (restrictions in fluency and productivity of thought and speech)
Disorganized speech	Passivity
Disorganized behavior	Apathetic social withdrawal
Agitation	Difficulty in abstract thinking
	Lack of spontaneity
	Stereotyped thinking
	Poor report
	Avolition (restrictions in initial of goal-directed behavior)
	Anhedonia (lack of pleasure)
	Attentional impairment

Table 1-3: Cognitive symptoms of schizophrenia
Problems representing and maintaining goals
Problems allocating attentional resources
Problems focusing attention
Problems sustaining attention
Problems evaluating functions
Problems monitoring performance
Problems prioritizing
Problems modulating behavior based upon social cues
Problems with serial learning
Impaired verbal fluency and difficulty with problem solving

1.3 Brain circuits and symptom dimensions in schizophrenia

The various symptoms of schizophrenia are hypothesized to be localized in unique brain regions. More specific, the symptoms of schizophrenia are thought to originate from dysregulation of the neurotransmission coming into and out of these regions. These areas have unique neurotransmitters, enzymes and genes that regulate them with some overlap (figure 1.3-1). This will be accounted for later in this article.

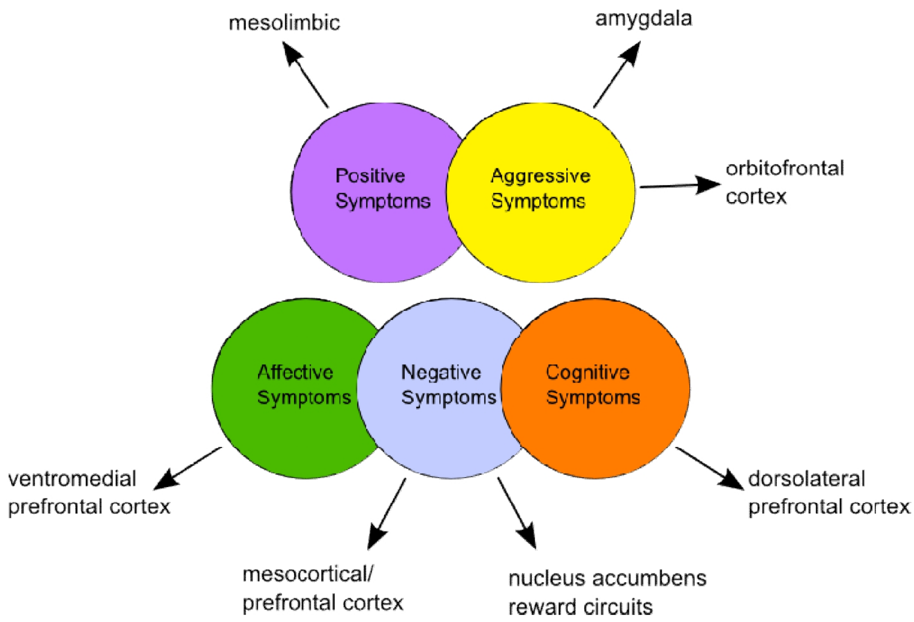


Figure 1.3-1: Symptom dimensions and suspected area of origin. Malfunction in the mesolimbic circuits have been hypothesized to account for positive symptoms. Aggressive and impulsive symptoms are thought to arise from the orbitofrontal cortex and its connections to the amygdala. Ventromedial and mesocortical prefrontal cortex connections are hypothesized to account for affective and negative symptoms. Nuclues accumbens is considered being a part of the brains reward circuit, and dorsolateral prefrontal cortex is assumed to play an important role in cognitive symptoms. The figure is modified from (5).

2 The The etiology of schizophrenia

Neurodevelopmental hypothesis of Schizophrenia:

The neurodevelopmental theory of schizophrenia was first posted by Weinberger in 1987 (6) suggesting that the course of schizophrenia was more consistent with a neurodevelopmental model in which a fixed "lesion" from early life interacts with normal brain maturational events that occur much later. Today we hypothesize that schizophrenia is caused due to three events (“Three strikes theory”). A genetic predisposition (i) interacting with an environmental development insult (ii) that occurs at the “right” stage (iii), all needed for developing schizophrenia. Several of the susceptibility genes for schizophrenia are in fact linked to neurodevelopment (5, 7). It has also been hypothesized that there is a limited neurodegenerative process of developmentally regulated synaptic elimination during the prodromal and first phases of schizophrenia (7), and maybe even throughout the life of the schizophrenic subject.

Most neurons form, are selected, migrate, differentiate, and myelinate before birth, but the process of neurogenesis continues for a lifetime in selected brain areas. Synaptogenesis, synaptic strengthening, elimination, and reorganization continue over a lifetime. Competitive elimination of “weak” but critical synapses during adolescence could explain why schizophrenia has onset at this time. Almost half the brain’s synapses are eliminated during adolescence (7).

2.1 Genetics of Schizophrenia

There is substantial evidence for additive genetic effects in liability to schizophrenia, estimating the heritability to 81% (95% confidence interval, 73% - 90%) (8). At least 45 susceptibility genes have been linked to schizophrenia at present time (9), but individual effect sizes are not consistent. High heritability has not translated into a satisfying search for genetic lesions.

2.2 Environment

It seems that many- pre and perinatal risks factors are involved in increasing the risk for developing schizophrenia later in life. Prenatal exposure to influenza and other respiratory infections, rubella, obstetric complications and low birth weight is well-documented factors increasing the risk for schizophrenia. The effect sizes for the pre- and perinatal risk factors are all small with odds ratios or relative risks of approximately 2. A series of social factors have been reported to be implicated in the etiology of schizophrenia, with the more substantial evidence on urban environment, social isolation and discrimination. Social factors seem to be a factor that can impact the brain development and some social factors are predisposing for psychological vulnerabilities. Finally, there is increasing evidence that the use of psychostimulants and cannabis, both of which have major effects on dopamine systems, are associated with schizophrenia (7).

2.3 Brain imaging studies

Structural brain imaging studies over the last two decades have provided evidence that schizophrenia is associated with enlargement of the lateral ventricles and loss of volume in the prefrontal cortex (PFC), the temporal cortex, the hippocampus, the amygdala and the thalamus (10, 11). There are indications that the reduced cortical volume is present at the first psychotic episode, and can progress during the first several years of illness (12).

Functional imaging studies enhanced the evidence of hypofrontality in schizophrenic subjects and PET scans have showed that performance of memory tasks in schizophrenic subjects with deficits exhibited less activation of the frontal cortex. Similar findings have been reported in otherwise healthy relatives of schizophrenic subjects. Hippocampal activation seems to be elevated.

3 The Neurobiology of Schizophrenia

3.1 The Dopamine hypothesis of Schizophrenia

The hypothesis attempts to explain all of the major symptoms of the disorder by dysregulation of either the mesolimbic dopamine pathways or the mesocortical dopamine pathways -this with relative preservations of functioning in the remaining dopamine pathways. Positive symptoms are hypothesized to originate from hyperactivity in the mesolimbic dopamine pathways/the nucleus accumbens. Negative symptoms are thought to be linked to hypoactivity in the mesocortical dopamine pathways (5).

3.1.1 Dopamine – Synthesis, storage, release, termination and receptors

Synthesis, storage, release and termination: Dopamine is a monoamine neurotransmitter utilized by dopaminergic neurons. It is synthesized inside dopaminergic neurons and stored inside synaptic vesicles. After release into the synapse, dopamine action is terminated by either a presynaptic reuptake pump -for vesicular storage and subsequent reuse in another neurotransmission, or dopamine can be destroyed by intracellular or synaptic enzymes (Figure 3.1-1).

Receptors: There are at least five pharmacological subtypes of dopamine receptors – the most investigated being the dopamine-2 receptor (D2). D2 receptors can function as presynaptic autoreceptors, either at the axon terminal or in the somatodendritic area, where they inhibit further dopamine release through negative feedback (5). The main target for dopamine release (and antipsychotic blockade) is however, the postsynaptically situated D2 receptors.

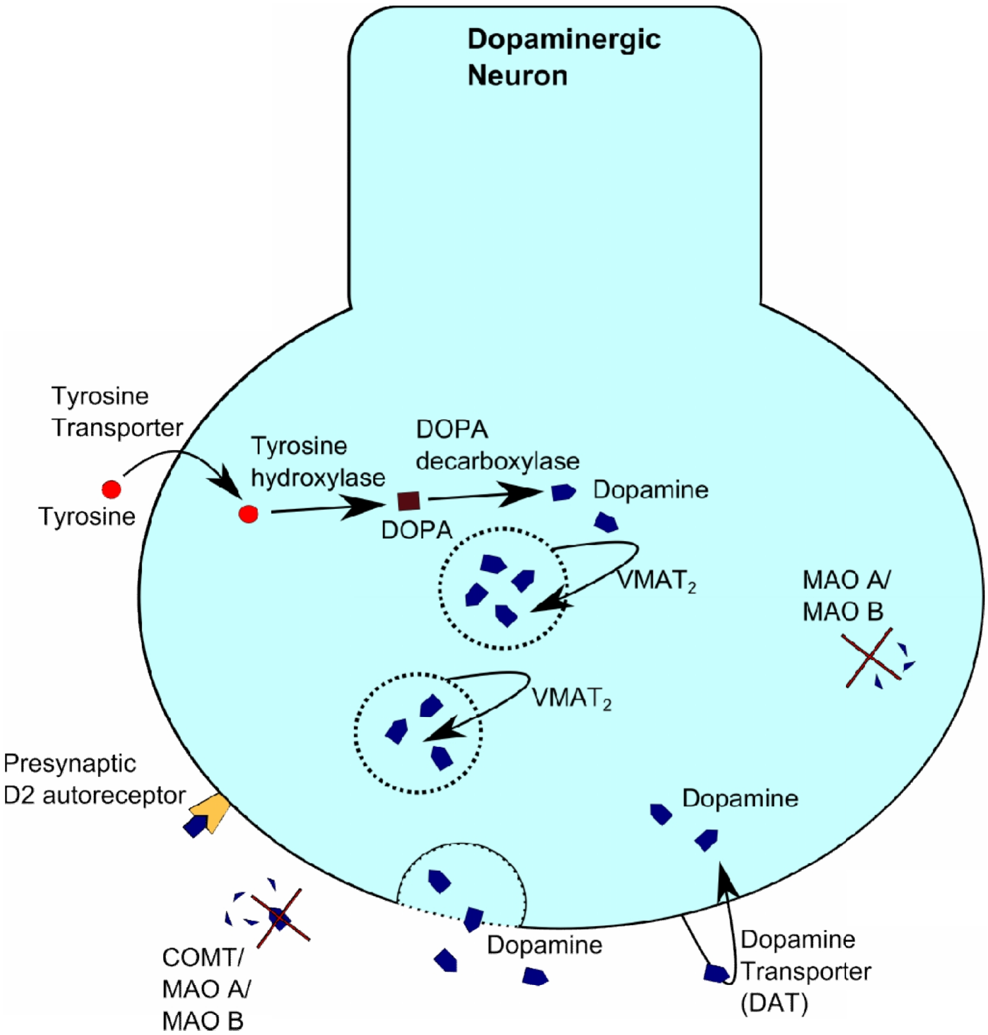


Figure 3.1-1: The dopaminergic neuron: Dopamine is synthesized from *tyrosine* by the rate-*limiting tyrosine hydroxylase* to *DOPA*. *DOPA* is then transformed by *DOPA decarboxylase* to dopamine. Dopamine is taken up into synaptic vesicles by a *vesicular monoamine transporter* ($VMAT_2$). Dopamine action is terminated by reuptake into the presynaptic neuron by *dopamine transporter* (DAT), or the *norepinephrine transporter* (NET) as a false substrate. Once back inside the dopaminergic neuron, dopamine can once again be stored inside vesicles for subsequent release or it can be destroyed within the neuron by the enzymes *monoamine oxidase* (MAO) A or B. Outside the neuron dopamine can be destroyed by MAO A or B, but also by the enzyme *catechol-O-methyl transferase* (COMT). The figure is modified from (5).

3.1.2 Key dopamine pathways of interest in schizophrenia

The Mesolimbic pathway projects from dopaminergic neurons with cell bodies in the ventral tegmental area (VTA) of the brainstem to the nucleus accumbens in the ventral striatum. The nucleus accumbens is thought to be responsible for several emotional behaviors, motivation and reward (5).

The mesocortical pathway projects from cell bodies in the VTA to areas of the prefrontal cortex (PFC). Branches to the dorsolateral prefrontal cortex (DLPFC) are thought to regulate cognition and executive functions, while branches into the ventromedial parts of PFC are hypothesized to regulate emotions and affect (5).

3.1.3 History of the dopamine hypothesis

The dopamine hypothesis of schizophrenia first emerged from the discovery of antipsychotic drugs in 1952, (13) and the identification that antipsychotics, when administered to animals, increased the metabolism of dopamine (14). In 1957 it was discovered that *reserpine* was found to block reuptake of dopamine and other monoamines

(15). Lieberman et al. found evidence that amphetamine, which increases synaptic monoamine levels, can induce psychotic symptoms (16). The original dopamine hypothesis of excessive dopaminergic neurotransmission was refined during the 1970s with the findings that clinical effectiveness of antipsychotics was related to their affinity for dopamine receptors.

In 1991, an article was published modifying the dopamine hypothesis of schizophrenia based on findings in postmortem studies, PET, neuroleptic drug action, plasma levels of dopamine metabolite homovanillic acid and cerebral blood flow (17). The findings were incompatible with the rather simple hypothesis of excessive dopaminergic transmission throughout the CNS. Evidence was found for different dopamine receptor distribution throughout the brain, more specifically that D2 receptors predominantly are expressed in the subcortical areas while D1 receptors are expressed predominantly in the cortical areas. The major innovation of this revised hypothesis for schizophrenia was moving from the hyperdopaminergic neurotransmission hypothesis to a hypothesis linking symptoms of schizophrenia to a regionally specific prefrontal hypodopaminergic neurotransmission and a subcortical hyperdopaminergic neurotransmission.

3.1.4 The dopamine hypothesis in 2011

In 2009 the dopamine hypothesis was once again revised, now proposing that the dopamine hypothesis of schizophrenia has 4 distinctive components (18).

- Dopamine dysregulation is caused by "multiple hits (genetics, environment)" – "the final common pathway to psychosis in schizophrenia".
- The locus of dopamine dysregulation moves from being primarily at the D2 receptor level to being at the presynaptic dopaminergic control level.
- Dopamine dysregulation is linked to psychosis, rather than schizophrenia
- Dopamine dysregulation is hypothesized to alter the appraisal of stimuli, perhaps through a process of aberrant salience.

3.1.5 Empirical evidence for the dopamine hypothesis coming from two sources

Neurochemical Imaging: Techniques that provide indirect indices of dopamine synthesis, release and putative synaptic dopamine levels (radiobelled L-dopa) have provided an index of dopamine synthesis and storage in presynaptic terminals of striatal dopaminergic neurons (19). 7 of 9 studies in subjects with schizophrenia have reported elevated presynaptic striatal dopamine synthesis capacities in schizophrenia (18). Two of the studies showed a small, nonsignificant increase. Using PET and SPECT scanning, several studies have found moderate to large elevation of dopamine release in striatal areas (20).

Moncrieff (21) questions the findings mentioned above, claiming results are not consistent. The author points at the low number of drug-naïve participants and that the part of striatum, in which the elevation was found, varied across studies. The study finding the strongest effect was conducted exclusively on subjects treated concurrently with neuroleptic drugs (22). Notably, the largest study found a statistically significant decrease, rather than an increase in DOPA uptake in ventral striatal area. It is also noted that the studies varied in use of stable subjects and subjects with "acute symptoms".

There have been at least 19 studies investigating striatal D2/D3 receptors in schizophrenic patients (PET, SPECT), and 3 meta-analyses (23, 24, 25). The meta-analyses are concluding that there is a modest (10-20%) elevation in striatal D2/3 receptors, independent of antipsychotic drug effect, in schizophrenic subjects. The changes have not been found in extrastriatal regions. Dopaminergic transmission in the PFC is mainly mediated by D1 receptors; dysfunction has been linked to negative symptoms (such as cognitive impairment) in schizophrenia. Three studies have investigated D1 receptor expression in drug-naïve schizophrenic subjects, reporting conflicting results. One reporting reduced D1 receptor density (26), another no difference from controls (27), and a third reporting elevated D1 receptor density in the PFC (28). The latter was using a different radiotracer, and findings of elevated D1 receptor density are consistent with chronic low levels of dopamine in the PFC (showing compensatory upregulation of D1 receptor density). Further studies are needed to clarify.

Structural differences prior to the onset of schizophrenia have been reported, though to a lesser degree. This is also the case for relatives of schizophrenic subjects. The abnormalities are located in the frontotemporal regions (29).

Advances in understanding genetic etiology of schizophrenia: After numerous studies it seems clear that no single gene encode for schizophrenia. Four of the top gene variants most strongly associated with schizophrenia are thought to be directly involved in dopaminergic neurotransmission. The strongest association being with a gene variant of the VMAT protein which is acting to accumulate dopamine and other monoamines into vesicles (figure 3.1-1). This fitting with the PET studies showing elevated radiolabeled dopamine accumulation in striatal vesicles in schizophrenia (18).

3.2 The glutamate hypothesis and NMDA receptor hypofunction hypothesis of schizophrenia.

This hypothesis can potentially explain all the five symptom dimensions of schizophrenia (figure 1.3-1), as well as how the dopamine pathways become dysregulated as a consequence of NMDA receptor hypofunction, leading to mesolimbic dopamine hyperactivity and mesocortical dopamine hypoactivity (5).

3.2.1 Glutamate – Synthesis, storage, release, degeneration, cotransmitters and receptors

Synthesis, storage, release and degeneration: Glutamate is an amino acid considered as the major excitatory neurotransmitter in the central nervous system (CNS). It is synthesized inside glutamergic cells from glutamine, which in turn is synthesized in glial cells surrounding the glutamergic neurons. Glutamate is stored within synaptic vesicles for release during glutamergic neurotransmission. After release, glutamate action is mainly terminated by reuptake into the neighboring glial cells (figure 3.2-1) (5).

Receptors: One of several groups of receptors is the postsynaptical ligand-gated ion channel receptors for glutamate. This group is subdivided into Alpha-amino-3-hydroxy-5methyl-4-isoxazole-propionic acid (AMPA) receptor, kainate receptor and N-methyl-d-aspartate (NMDA) receptor (Figure 3.2-2). The two first are known to mediate fast, excitatory neurotransmission via Na^+, while the latter is some kind of “coincidence detector”, mediating long-term potentiation, synaptic plasticity and neuronal cell degeneration (as a result of hyperactivity) (5).

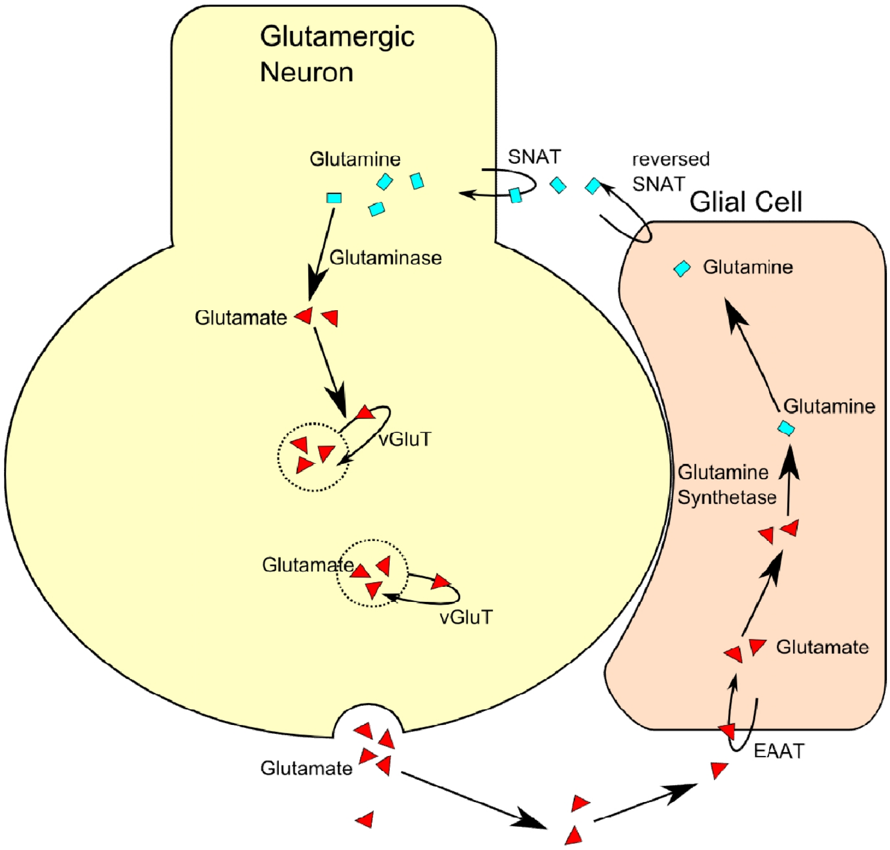


Figure 3.2-1: The glutamergic neuron: Glutamate is synthesized from *glutamine* via a mitochondrial enzyme named *glutaminase*. Glutamate is then stored inside synaptic vesicles via *a vesicular glutamate transporter* (vGluT). When released from synaptic vesicles, glutamate action is terminated by uptake into neighboring glial cells *by excitatory amino acid transporter* (EAAT). EAATs is also found on presynaptic glutamate neurons and on postsynaptic sites of the neurotransmission, but these does not seem to play an important role in the synaptic glutamate termination. Inside the glial cell, glutamate is converted back into *glutamine* by the enzyme *glutamine synthetase*. Subsequently *glutamine* is released from the glial cell *via reversed specific neutral amino acid transporter* (reversed glial SNAT). SNATs can work in both directions, and *glutamine* is transported into the glutamergic neuron in a reuptake manner. Once *glutamine* is back into the glutamergic neuron it can be converted into glutamate for subsequent storage and release. The figure is modified from (5).

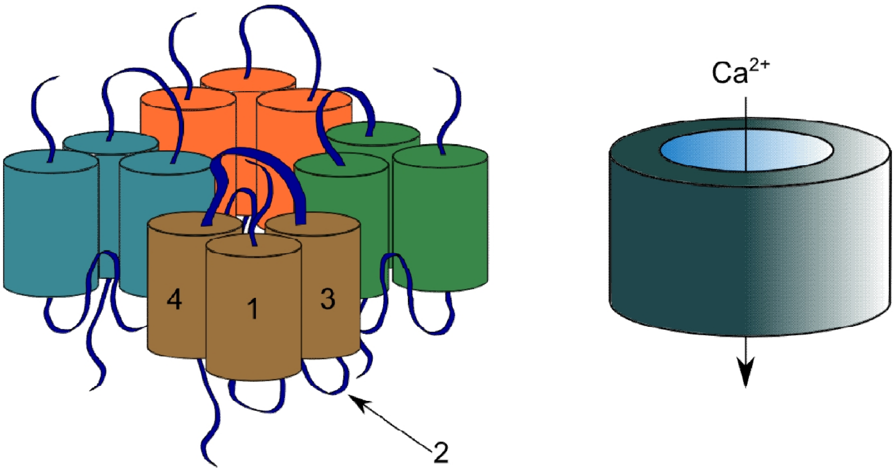
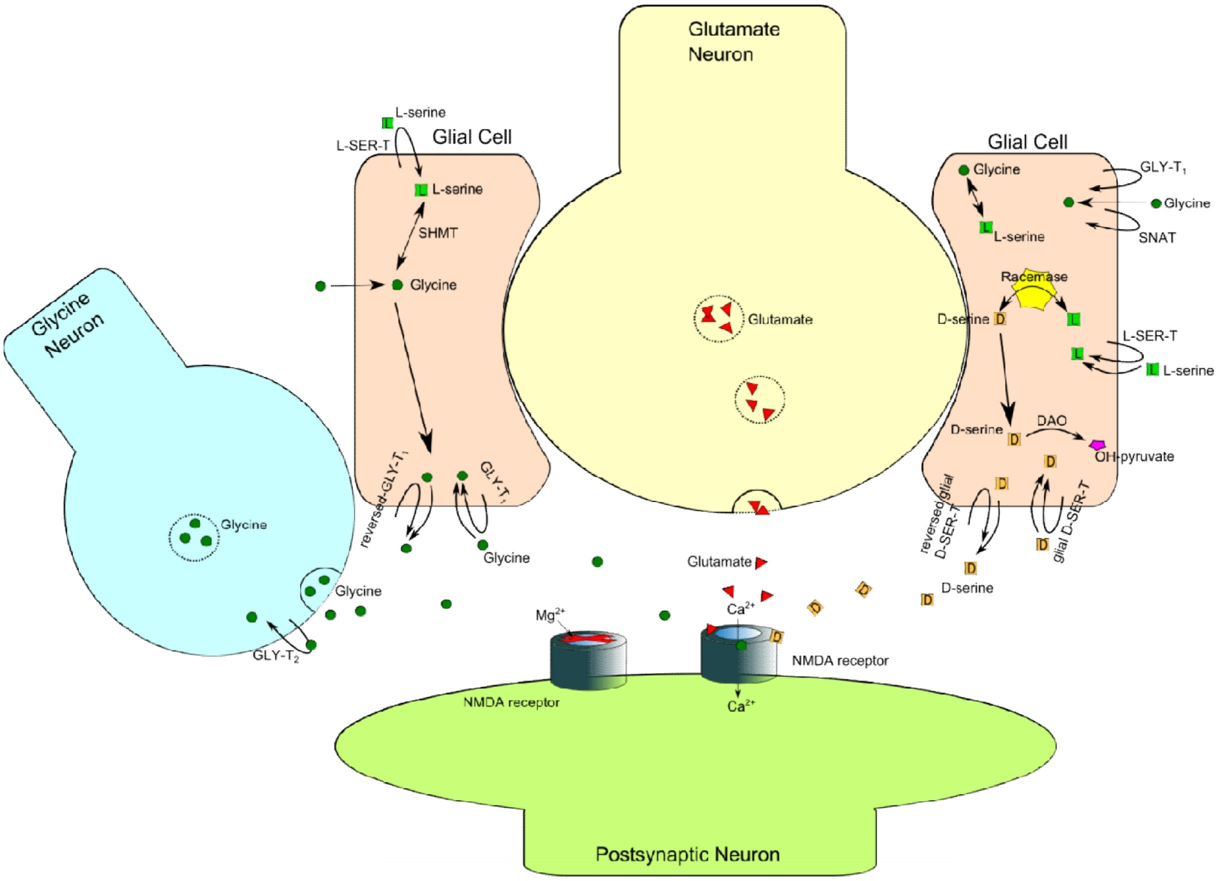


Figure 3.2-2: *N-methyl-d-aspartate receptor* (NMDA receptor) is a ligand-gated ion channel of the tetrameric subtype (also known as ionotrophic receptor). This meaning it has three full transmembrane regions (1,3,4) and a fourth reentrant loop(2). The three transmembrane regions is clustered together forming a subunit (1-4). NMDA receptor is made up by four of these subunits forming a fully functional channel in the middle. The receptor sites are in various locations on each of the subunits, and some are even inside the ion-channel. NMDA receptors can are in the resting state blocked by magnesium, and can only open to let Ca^{2+} in when three things occur at the same time. Glutamate occupies the binding seat, *glycine* or *d-serine* binds to its site and depolarization occurs allowing the Mg^{2+} “plug” to be removed. The figure is modified from (5).

Cotransmitters glycine and d-serine; synthesis, storage, release and degeneration: Cotransmitters glycine and d-serine are necessary for NMDA receptor function. Glycine is synthesized in glycine neurons and glial cells, and is not known to be stored in intracellular vesicles. The other cotransmitter, D-serine, has high affinity for the glycine site on NMDA receptor. D-serine is converted from L-serine inside glial cells, or it can be derived from glycine and stored inside glial vesicles (Figure 3.2-3) (5).



Figur 3.2-3: Metabolism of NMDA receptor cotransmitters *Glycine* and *D-serine: Glycine* is synthesized in from glycine neurons or in glial cells. It is taken into glial cells via *Glycine transporter* (Gly-T1) or *via glial specific neutral amino acid transporter* (glial SNAT) from extracellular matrix or the bloodstream. Glycine escapes to the synapse via a reversed Gly-T1. It can also be synthesized from L-serine (transported into glial cells by L-SER-T) by the glial enzyme *serine hydroxyl methyl transferase* (SHMT). *D-serine* is converted from *L-serine* via *D-serine racemase*, and back to *L-serine*. Both L- and D-serine are transported into glial cells by their own transporters. Release of D-serine is via *reversed glial D-serine transporter* (d-SER-T) for neurotransmission. *D-serine* action is terminated by inwardly acting glial d-SER-T and the *enzyme d-amino acid oxidase* (DAO) inside glial cells. The figure is modified from (5).

3.2.2 Key Glutamate pathways of interest in schizophrenia

Glutamate is a ubiquitous excitatory neurotransmitter that seems to be able to excite nearly any neuron in the brain. There are five specific glutamergic pathways of particular relevance to schizophrenia.

The Corticobrainstem pathways. There are several important descending glutamergic pathways from the cortical pyramidal neurons to brainstem neurotransmitter centers. This includes the ventral tegmental area (VTA) and substantia nigra for dopamine. Normally these are regulators of neurotransmitter release acting as a brake on the dopaminergic pathway through an inhibitory GABA interneuron in the VTA, giving a tonic inhibition of dopamine release from the mesolimbic pathway (5).

The Corticostriatal pathway is projecting from pyramidal neurons to the striatum. This includes a corticoaccumbens glutamate pathway to the nucleus accumbens in the VTA. Corticostriatal glutamate projections terminate on GABA neurons in the striatum which in turn projects to the thalamus creating a “sensory filter” to prevent too much sensory traffic coming into the thalamus. Dopamine function in this projection is to inhibit GABA neurons projecting to the thalamus thus reducing the “filter” effectiveness (5).

The thalamocortical pathway is an ascending glutamate pathway rising from the thalamus innervating pyramidal neurons providing a feedback to the original pyramidal cell (or area) (5).

The CSTC loops: There are numerous glutamate projections together making up functional loops -the so called “Cortico-striatal-thalemic-cortical-circuit”. These circuits can be thought of as the brains engines for behavioral and functional outputs. The loops start and end at pyramidal cells in prefrontal cortex and are thought to regulate executive functions, problem solving, emotions, impulsivity, agitation, cognitive tasks and more (5).

The Corticothalamic pathways are descending from the cortex and directly into the thalamus, providing sensory inputs and more (5).

The Corticocortical pathways is a way for one pyramidal neuron to communicate directly with another pyramidal neuron. Normally this provides effective communication and information processing (5).

3.2.3 The NMDA receptor hypofunction hypothesis

The hypothesis arises from the observations that when NMDA receptors are made hypofunctional by their receptor antagonist phencyclidine (PCP), subjects may mimic the positive symptoms, negative symptoms, affective symptoms and cognitive symptoms of schizophrenia (30, 31). Hypothetically, a hypofunctional NMDA receptor in the corticobrainstem glutamate projection are not thought to be able to tonically excite the GABA interneurons projecting to the mesolimbic dopamine neurons, thereby making the mesolimbic dopamine pathway hyperactive. This could account for the positive symptoms of schizophrenia.

Another point of the hypothesis is that NMDA receptor hypofunction (through PCP) also mimics the cognitive, affective and negative symptoms associated with schizophrenia. Normally, the descending corticobrainstem glutamate neurons act as accelerators on mesocortical dopaminergic neurons (no GABA interneuron), having direct synapses with the dopamine neurons in the VTA that project to the cortex. This could mean that the corticobrainstem glutamate projections to the mesocortical dopaminergic neurons in VTA act as accelerators (tonically exciting). Hypothetically, a hypofunctional NMDA receptor will not be able to excite tonically and thereby create hypoactivity in the mesocortical dopamine pathways (5).

Going through a hypothetical NMDA receptor hypofunction in the CSTC loops (see above), we start considering that if the NMDA receptors in the VTA are hypofunctional, this will create mesolimbic hyperactivity as mentioned before. In CSTC loops dopamine hyperactivity reduces thalamic filter and permits the escape of excessive sensory information coming into the thalamus. By this, allowing the excessive sensory information getting into the cortex by means of the thalamocortical neurons. There is also hypothetical NMDA receptor hypofunction in the descending corticostriatal glutamate pathway as well. This reduces the excitatory drive on the GABA neurons that create the thalamic filter, causing it to fail. Too much information will escape, causing cortical manifestations of hallucinations as well as other cortical symptoms such as cognitive, affective and negative symptoms of schizophrenia (5).

In different glutamate loops, the NMDA receptors can cause both hypoactivity in an entire loop and hyperactivity in another loop. Loops can even be partially overactive and partially hypoactive in the same loop. All in all, the NMDA receptor hypofunction within the five major glutamate pathways might explain not only the five symptom dimensions accounted for above (figure 1.3-1), but also how dopaminergic neurons may become dysregulated as a consequence of NMDA receptor hypofunction. (5)

4 Cortical GABAergic interneuron origin and the NMDA receptor hypofunction theory' of schizophrenia

GABAergic neurons play a fundamental role in proper maturation of neural circuitry during postnatal development. These circuits are highly immature at birth, and GABAergic inhibition develops in a protracted postnatal period. Proper GABAergic inhibition during cortical maturation is essential for the refinement of cortical circuitry (32).

One of the most investigated areas recently is working memory deficits mapped under the cognitive symptoms dimension (figure 1.3-1). Working memory, the ability to manipulate transiently stored information, has consistently been shown to be disturbed in schizophrenia (33), and is now considered a major symptom of the disease. These deficits (due to altered DLPFC activity) might be specific for the disease process as it is present in drug naïve schizophrenic subjects, but absent in subjects with other psychotic disorders or major depression. Alterations in perisomatic inhibition of pyramidal neurons contribute to a diminished capacity for the gamma-frequency synchronized neuronal activity that is required for working memory function. This could, in part, reveal some of the pathophysiology of schizophrenia.

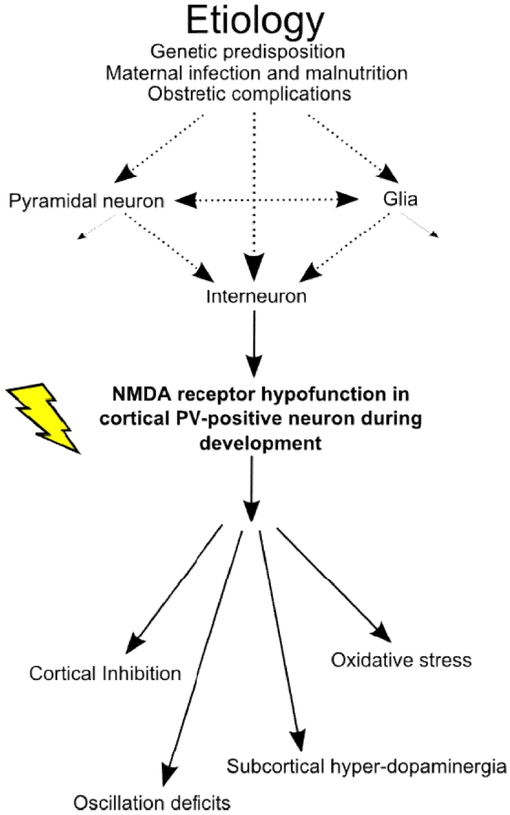


Figure 4-1: The GABAergic neuronal dysfunction hypothesis of schizophrenia pathophysiology. Diagram from (69) modified from (70).

4.1 GABAergic neurons of the Chandelier subclass are affected in schizophrenia

4.1.1 GABAergic interneurons are divided into subtypes

GABAergic interneurons are subdivided into distinct subtypes based on morphology, electrophysiology, synaptic connectivity and gene expression (34). These subtypes appear to support cortical circuit function – including network oscillations (35) and the balancing of excitation and inhibition (36). The chandelier subpopulation of GABA neurons seems to have a specialized function in regulating pyramidal neuron activity, and they express the calcium-binding protein parvalbumin (PV) (32). Parvalbumin is a slow calcium buffer that does not affect the amplitude, but accelerates the decay of calcium transients in GABA nerve terminals (37). Thus, PV decreases the residual calcium levels that normally accumulate in nerve terminals and facilitate GABA release during repetitive firing (38). The axon terminals of PV-positive chandelier neurons principally target the axon initial segments (AIS) of pyramidal neurons (39). Another subclass of the GABA neurons is the calretinin containing double-bouquet cells that tend to synapse on the dendrites of other GABA cells (40*).* The calretinin-positive GABA neurons in primate DLPFC appear to be unaffected in schizophrenia (41).

The cortical PV-positive interneurons often display fast-spiking patterns whose activity is essential to generating gamma oscillations (42). These are believed to be of major importance for organizing of the functional neural ensembles in addition to keeping a tight temporal control of cortical inhibition.

4.1.2 Alterations in Chandelier neurons in schizophrenia

Accumulating evidence suggest disturbances in the synchronized oscillatory activity, in particular in the gamma range, is a major physiological feature of schizophrenia (43, 44). Alterations in the PV-positive neurons in schizophrenia could be secondary to NMDA receptor hypofunction. For example, NMDA receptor antagonists decrease the mRNA expression for PV and the GABA-synthesizing enzyme glutamic acid decarboxylase67 (GAD67 encoded by GAD1) (45). This may reflect the measured decrease of PV and GAD67 in schizophrenic subjects (chapter 4.4) (32). Furthermore, although GAD1 mRNA is undetectable in approximately 25-30% of the DLPFC GABA interneurons in schizophrenia, the remaining GABA interneurons all have normal levels of GAD1 mRNA expression (46). *The affected GABA interneurons include those that express parvalbumin, which is found in approximately 25% of GABA interneurons in primate DLPFC.* Note that the expressed levels of PV mRNA are reduced, but the number of PV-positive interneurons appears to be unchanged. Furthermore, 50% of the PV-positive neurons lack detectable levels of GAD67 (41).

Levels of mRNA for the GABA membrane transporter (GAT1), responsible for GABA reuptake, also seem reduced in the axon terminals of the chandelier class of PV-positive neurons (47). The $GABA_A$ receptors in the cortex contain one of six α subunits with different subcellular distribution and physiological properties (48). The α2 subunit is overall present in 15% of the $GABA_A$ receptors, but it is present in more than 95% of the inhibitory synapses onto pyramidal neuron AIS (49). In addition, the $GABA_A$ α2 subunit seems to have higher affinity for GABA, which in turn results in faster activation and slower deactivation compared to other subunits (50). Thereby, the $GABA_A$ α2 subunit receptor seems to be specialized in mediating a potent inhibitory influence on the output of pyramidal neurons (32). Furthermore, the postsynaptic AIS on the pyramidal neurons have increased immunoreactivity for the $GABA_A$ receptor α2 subunit in schizophrenia. These changes are specific for the disease (41).

The reduced levels of GAT1 in the chandelier axon terminals, and the postsynaptic increased immunoreactivity of the α2 subunit are inversely correlated, and several lines of evidence suggest that the reduction in presynaptic GABA markers (PV and GAT1) and the increased postsynaptic expression of $GABA_A$ receptors could be a compensatory response to deficits in GABA release from chandelier neurons (51). Combined reduction of PV and GAT1 proteins and upregulation of postsynaptic $GABA_A$ receptors would both act to increase the efficiency of GABA neurotransmission at pyramidal neuron AIS. It has been suggested that both alterations are downstream consequences of deficits in GAD67 mRNA expression (32).

4.1.3 PV-positive GABA interneurons and NMDA receptor

NMDA receptor hypofunction, at GABAergic interneurons in particular may explain, at least in part, schizophrenia like symptoms.

Several lines of evidence suggest that PV-positive GABA neurons are particularly sensitive to reductions in excitatory transmission through NMDA receptors (53), and that these cells receive a much larger number of excitatory inputs than other GABA neurons (54). In addition, the PV-positive GABA neurons are particularly sensitive to changes in excitatory signaling through NMDA receptor, being more vulnerable to cortical disinhibition and toxic neuronal cell degeneration (as a result of hyperactivity). This is because they function at a high metabolic cost, and their mitochondrias produce much more reactive oxygen species (free radicals) (55) leading to oxidative stress. Thus, reduced signaling through NMDA receptors could be an upstream event that selectively affects the PV-positive GABA neurons that are critical for the entrainment of DLPFC neuronal networks at the gamma frequency.

The ERB receptor is member of the receptor tyrosine kinase and neutrophin signal transduction system, and is co localized with the NMDA receptor. ERB may be involved in mediating the neuroplasticity triggered by NMDA receptor (5). Alterations in NRG1-ErbB4 signaling could directly contribute to the disturbances in GABA neurotransmission in schizophrenia by altering the expression of $GABA_A$ receptor subunits or the strength of GABA-induced currents (56). ErbB4 is expressed in nearly 90% of the PV-positive neurons, but only 15% of the calretinin containing neurons in the adult rat neocortex (57) which might suggest that NRG1-ErbB4 signaling in schizophrenia would be most prominent in the PV-positive subclass of GABA neurons. This is all converging on the hypothesis that selective alterations in GABA neurotransmission in PV-positive neurons in schizophrenia is a downstream consequence of NMDA receptor hypofunction, and that genetically driven changes in NRG1-ErbB4 signaling could affect PV-positive neurons both directly and through NMDA-mediated mechanisms.

Dopamine terminals provide synaptic inputs to PV-positive, but not to calretinin-positive, GABA neurons in monkeys (58). Also, PV-positive cortical neurons express a combination of glutamergic receptor subunits that differs from those in other populations of GABA neurons (59). Thereby, NMDA receptor hypofunction may lead to reduced D1 mediated signaling in cortical projections. Supporting this is evidence that chronic recreational ketamine users exhibit upregulation of D1 receptors in the DLPFC, probably as a compensation for deficits in the dopamine transmission (60).

4.2 NMDA antagonist studies

In 1994 the glutamate NMDA receptor hypofunction hypothesis advanced substantially with the discovery that healthy volunteers receiving a steady dose infusion of NMDA receptor antagonist, ketamine, exhibited negative symptoms and subtle cognitive impairments reminiscent of schizophrenia and partial manifestation of positive symptoms such as illusions (61). A later study using similar methods found that ketamine induced a thought disorder similar to that observed in schizophrenia (62). NMDA receptor antagonists PCP and ketamine also increase both positive and negative symptoms in schizophrenic subjects (63).

Systemic administration of NMDA receptor antagonists increases PFC pyramidal cell firing, apparently by producing disinhibition (52). It remains to be seen whether or not impairments in interneuron networks are a primary cause of schizophrenia or secondary to effects of alterations in other neurotransmitter systems.

4.3 Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia like phenotypes (64)

As mentioned above, cortical GABAergic dysfunction may underlie the pathophysiology of schizophrenia. A recent study by Belforte et al. (64) selectively deleted the essential NR1 subunit of the NMDA receptor in 40-50% of cortical and hippocampal GABAergic interneurons (a majority which contained parvalbumin) in early postnatal development in conditional knockout mice. They also generated a conditional NR1 knockout mutant in which NMDA receptor deletion first occurs after adolescence in the same neuron population. This was preformed to assess whether adult onset of NMDA receptor deletion is critical for the emergence of schizophrenia pathophysiology.

The study found that restricted deletion of NMDA receptor in early postnatal corticolimbic interneurons were sufficient to trigger several behavioral and pathophysiological features in mice, which resemble schizophrenia in humans. The mice exhibited both positive symptoms (psychomotor agitation) and negative symptoms (reduced preference for sweet solution, deficits in nesting/mating mirroring anhedonia and social withdrawal). The mutant mice also had cognitive symptoms such as deficits in spatial working memory and short term social memory. NR1 deleted cortical GABAergic interneurons showed reduced GAD67 and PV levels as well, concurring with the reduced expressions found in postmortem studies of human schizophrenics subjects (65). Interestingly, several mutant phenotypes had a somewhat delayed onset of symptoms; aberrant behavior was first noticed after a 12 week period, resembling the premorbid stage that precedes the onset of psychosis in schizophrenia.

Notably, in the mutant knockout mice where NR1 ablation in the GABAergic neurons occurred after adolescence, the schizophrenia-like behavior was absent. Postnatal NR1 knockout in the corticolimbic GABAergic neurons contributed to an increase in excitatory neuronal activity and reduced neuronal synchrony. This was not observed in adult NR1 knockout mice, suggesting that the NR1 deletion impair the postnatal maturation of GABAergic neurons, and, in the absence of proper GABAergic inhibition, the refinement of cortical circuitry may be impaired. This idea of abnormal maturation of cortical circuits is consistent with the neurodevelopmental hypothesis of schizophrenia (6).

In conflict with findings by Belforte et al. (64), Benneyworthy at al. (66) failed to reproduce these previous findings. The study examined SR (serine racemase, see figure 3.1-4) null mutant mice to study the link between NMDA receptor hypofunction and decreased PV expression, assessed by immunoreactive (IR) cell density in the medial PFC and hippocampus and protein levels in brain homogenates from the frontal cortex and hippocampus. The SR null mutant mice showed modest elevations in PV-IR cell density and no difference in PV expression in brain homogenate. The study also investigated PV expression in mice and rats following subchronic PCP or ketamine treatments in adulthood. This failed to reproduce previous findings, concluding that the pathological deficits in PV expression are not simply a consequence of NMDA receptor hypofunction (66).

4.4 Postmortem studies

Postmortem brains from schizophrenic subjects suggest that dysfunction of GABAergic interneurons, particulary those containing the calcium-binding protein PV, may be a core feature of schizophrenia. Supporting this is evidence of reduced expression of the GABA-synthesizing enzyme GAD67 and PV in cortical interneurons in schizophrenic subjects. Several studies have confirmed the reduced GAD67 levels in postmortem brain tissues of schizophrenic subjects (65). Reduced spine density and dendritic morphology of cortical glutamergic excitatory neurons is furthermore one of the most consistent findings in schizophrenia (67).

5 Conclusions

The accumulating body of support for the NMDA receptor hypofunction hypothesis of schizophrenia has over the last years provided the first compelling alternative to the dopamine hypothesis. The pathophysiology of schizophrenia contains changes in dopamine and glutamate neurotransmission as well as changes in perisomatic inhibitory regulation of pyramidal neurons required for synchronized gamma frequency oscillations. Selective dysfunctions in corticolimbic PV-positive interneurons result in a variety of pathophysiological alterations characteristic for schizophrenia. Postmortem studies and functional scans have further confirmed disturbances in several of the neuronal systems in schizophrenia. Still, this may not rule out that GABAergic neurons (especially the PV-positive), which have a protracted developmental period and seems particularly vulnerable to developmental disruption, is the origin of the disease.

One could suggest that the disruption of synchrony from the PV-positive GABAergic fast-spiking interneurons is a downstream consequence triggered by NMDA receptor hypofunction. Evidence of this has been proposed after observations of schizophrenia like symptoms in normal humans after infusion of NMDA receptor antagonists like ketamine and PCP. Postnatal NR1 ablation in knockout mice supports these findings with schizophrenia like behavior after a 12 week delay, while postadolescent NR1 ablation did not produce aberrant behavior. These findings are consistent with the neurodevelopmental hypothesis of schizophrenia. However more research on consequences of NMDA receptor hypofunction in fast-spiking neurons are needed in order to explain the underlying impaired synchronized activity and gamma oscillations, as the evidence is not convergent (66).

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