Review: NMDA Receptor Hypofunction and Its Involvement in Excitotoxicity in Schizophrenia

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Abstract

Schizophrenia is a severely debilitating psychiatric condition that has long been associated with altered neurotransmission in the brain, especially in dopaminergic transmission. The dopamine hypothesis of schizophrenia, while it has been the main focus of research in the past decades, fails to explain the underlying pathogenesis. A more recent hypothesis dealing with altered central glutamate signaling in schizophrenia may help to ameliorate the deficit in understanding. Blockade of the glutamatergic NMDA receptors produces schizophrenic behaviours and phenotypes almost indistinguishable from the disease itself in animal and human models. It has been elucidated that hypofunction of NMDA receptors on the parvalbumin-containing interneurons in the cortex and subcortical regions leads to a downstream increase in cortical glutamate release, which may be causing functional connectivity issues through mechanisms of excitotoxicity. A number of studies have pointed to the hypofunction of these NMDA receptors leading to excitotoxicity and cellular degeneration, which may be implicated in the disease pathology. This review will evaluate and highlight this forthcoming evidence.

Keywords: schizophrenia, NMDA hypofunction, glutamate, excitotoxicity, AMPA, kainate, neurodegeneration

Schizophrenia

Schizophrenia, a chronic neurological disease that affects approximately 1% of the world's population (14), severely affects a person's thoughts, feelings, and behaviours. Three classes of symptoms accompany schizophrenia. The first is positive symptoms, which are seen in patients with schizophrenia, but not in the general population, and encompass hallucinations, delusions, thought disorders, and increased or agitated movements. Second, schizophrenia's negative symptoms are behaviors normally seen in the general population, but absent in patients with schizophrenia, and are associated with social withdrawal, which includes flat affect (reduced emotional expression), lack of pleasure, and lack of motivation. Third, the cognitive symptoms of the disease involve poor executive function and deficits in working memory, which have been associated with functional deficits in the prefrontal cortex (22).

Socially, the symptoms of schizophrenia can be chronically debilitating, which not only affects the individual, but also all of those around them. The disease's toll on an individual can be drastic as the positive symptoms can completely alter the patient's reality. Hallucinations and

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psychoses can lead a person to believe in things that are not based in reality and drive them to do things that a healthy individual would never imagine doing. The negative symptoms of schizophrenia can cause a person to become isolated and withdrawn from those things that once comprised their identity. Additionally, the cognitive deficits can rob people with schizophrenia of mental faculties, which previously helped them carry out day-to-day tasks. The sum of these symptoms can lead a person to require one or more caregivers, which can put heavy burden on the family and others within the support system of persons with schizophrenia. In addition, this condition requires a plethora of resources from the health care system, which is already overburdened and may lead to substandard care for those most at risk, including patients with schizophrenia. For these reasons, it is crucial that researchers gain an understanding of this disease in order to develop better treatments and work towards early intervention.

The onset of schizophrenia usually occurs in the period from the end of adolescence and the end of early adulthood, although children are seldom diagnosed with the condition as well (22). This period also accompanies significant neural changes and development, which is a reason schizophrenia is thought to occur from aberrant processes impairing such development.

As defined in the Diagnostics and Statistical manual V (DSM-V), for a diagnosis of schizophrenia a patient must meet the following criteria: **A**: two or more of the following with at least one of the first three symptoms for at least one month (delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour, or negative symptoms), **B**: disturbance for a significant period of the time in work, interpersonal relations, and/or self-care, **C**: Continuous signs of disturbance for at least 6 months **D**: Other disorders such as schizoaffective disorder, depression, or bipolar disorder with psychosis are not present, **E**: Not caused by substance usage (1).

A large body of research in schizophrenia has investigated the role of dopamine and its alterations in schizophrenic pathology. While this endeavor has resulted in treatments and supports for schizophrenic patients, it has uncovered little in terms of the underlying pathogenesis of the disease and may be a more downstream result of the underlying, mostly-unknown cause. There have been more recent advancements made in a new hypothesis, known as the NMDA receptor hypofunction hypothesis of schizophrenia, which has relations to glutamate excitotoxicity and subsequent neurodegeneration. This paper will explore and evaluate this glutamate-centric hypothesis and whether excitotoxicity has a role to play in the underlying pathology of schizophrenia.

Dopamine Hypothesis

The dopamine hypothesis arose over fifty years ago and has been the predominant focus of schizophrenic research and directions for therapeutic interventions. The first iteration of the dopamine hypothesis was based on the proposition of increased dopamine transmission in the central nervous system (CNS) (15). This idea came from the fact that stimulants such as methamphetamine increase dopamine levels in the brain and produce psychotic behaviours similar to those observed in patients with schizophrenia. Another reason that a hyperdopaminergic state was thought to be the basis of schizophrenia was because neuroleptic drugs used to treat schizophrenia work in a way that block the dopamine D2 receptor in the brain. (15, 29). This blockade of D2 receptors works well at treating the positive symptoms of schizophrenia, but poorly in the negative symptoms and cognitive deficits, which has led the dopamine hypothesis to be revised.

Current treatments for schizophrenia are mostly confined to a class of medications known as antipsychotics, or more formally as neuroleptics. These are subdivided into two classes: typical neuroleptics and atypical neuroleptics. The older typical neuroleptics have their mechanism of action through blockade of dopamine D2 receptors in the CNS. They include haloperidol, prochlorperazine, and fluphenazine. This sub-class does not have the greatest efficacy for treatment and has a multitude of side effects including severe adverse effects to motor functioning (extrapyramidal symptoms), as they can modulate dopaminergic transmission in the basal ganglia, which is responsible for refining quantity of motor output (31). The newer atypical neuroleptics are seen to have higher efficacy and lower prevalence of extrapyramidal symptoms. The mechanism of actions for these is once again through D2 blockade, but also have serotonin receptor (5-HT2A) blocking ability. These atypical neuroleptics include drugs such as clozapine, olanzapine, quetiapine, and risperidone (31).

Both typical and atypical neuroleptics are quite efficacious at ameliorating the positive symptoms of schizophrenia such as hallucinations and delusions, but the negative symptoms and cognitive deficits are not as responsive to medication (31). Since these drugs are mainly antagonists to dopaminergic transmission, much of the research into the pathogenesis of schizophrenia has tended to focus on this neurotransmitter and its related circuitry in the CNS, although its incomplete efficacy indicates this is not the only underlying factor.

The second iteration of the dopamine hypothesis in schizophrenia was revised to include regional specificity of abnormal dopamine levels in the brain. The hyperdopaminergic conditions in schizophrenia are known to be localized to the subcortical limbic system and its related structures such as the striatum and the nucleus accumbens (15, 29). This was found to be especially true in the striatum where abnormally high dopamine levels, as seen in patients with schizophrenia, seem to be strongly implicated in the emergence of psychoses and other positive symptoms (11). There is also a high level of D2 receptors in these subcortical regions, and so is hypothesized to be where neuroleptics exert their efficacy at treating positive symptoms of schizophrenia (29). The prefrontal cortex on the other hand, which is implicated in the negative symptoms and cognitive deficits of schizophrenia, has been shown to have low levels of dopamine in a schizophrenic condition compared to healthy individuals (15, 29). This hypodopaminergic state has been clinically documented through radioactive imaging, but is not fully established and more evidence is required to confirm its role in schizophrenia (29).

Dopamine and its established role in schizophrenia are extremely important, as it is currently the basis of treatment for the disease. While this is a suitable explanation for some of the symptoms of schizophrenia and gives a basis for current treatment it fails to explain any of the underlying pathogenesis. A more recent hypothesis involving glutamate may help explain the neurodevelopmental origin of the disease and the pathology seen in patients and post-mortem samples.

NMDA Hypofunction Hypothesis

Because the dopamine hypothesis of schizophrenia has revealed little in terms of the pathogenesis of the disease, a new circuit-based mechanism has come to light, helping us to understand the basis of this chronically debilitating condition. Theoretically, understanding the mechanism underlying schizophrenia will enable further research into treatments that could cover the other two-thirds of the symptoms and possible preventative measures as well.

Olney and Farber in 1995 (24) revealed a piece of information that implicated glutamate signaling in schizophrenic pathology. Glutamate is one of the main excitatory neurotransmitters in the brain and its distribution is widespread. Glutamate transduces its chemical signals into electrical activity through a number of different means. The main mechanism, through which it quickly causes responses in postsynaptic neurons, is through one of three ionotropic glutamate receptors: N-methyl-D-aspartate (NMDA) receptors, α-amino 3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, or kainic acid (kainate) receptors, which are all named after the ligand specific to them. While these receptors and their physiological function are required for normal neuronal processes, their over-excitation (especially the NMDA receptor) has been well documented to lead to neuronal damage and possible subsequent neuronal death via triggering of cellular death cascades.

In contrast to an over-excitation of the NMDA receptor, it has been shown that blocking glutamatergic

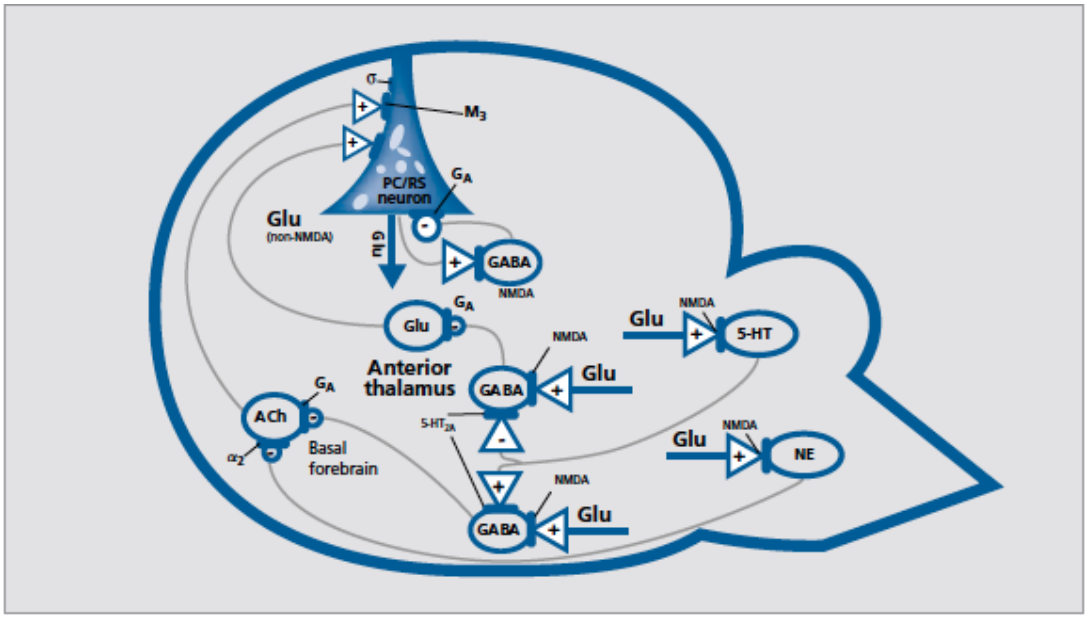

Figure 1: Depiction on NMDA hypofunction model in schizophrenia. This figure shows the various regions where GABAergic interneurons are physiologically excited by glutamate, which leads to a tonic inhibitory effect on cortical pyramidal neurons, which release excitatory neurotransmitters. Figure from Newcomer et al, 2000 (23).

NMDA receptors and causing a 'hypofunctioning' state of these receptors produces schizophrenia-like symptoms in healthy human subjects (i.e., psychosis and hallucinations). This hypofunctioning has also been found to lead to corticolimbic degeneration in rat brains after NMDA blockade. Such changes were prevented through administration of atypical neuroleptics and therefore points to NMDA receptors and their hypofunctioning being somehow involved in schizophrenic phenotypes and pathophysiology (24).

The original idea for glutamate's involvement in schizophrenia was a 1959 study that showed healthy adults given the drug phencyclidine (PCP), a known NMDA antagonist, showed symptoms of psychosis indistinguishable from those of patients with schizophrenia (25). This piece of information was not investigated much further until the 1990s, when the research into the link between glutamate and schizophrenia took ground.

The main postulate of this hypothesis is for hypofunction of NMDA receptors that are on the postsynaptic ends of GABAergic parvalbumin (PV)-containing interneurons in cortical and subcortical regions of the brain (14). These interneurons release gamma-aminobutyric acid (GABA) downstream to inhibit the excitatory pyramidal neurons in the cortex and various subcortical regions. The pyramidal neurons then go on to release glutamate onto their downstream targets again in the cortex and subcortical regions (14, 24, 21). The excitation by glutamate then causes downstream release of other neurotransmitters such as dopamine and acetylcholine (21). This circuit and how it is explained is what occurs under physiological conditions.

The pathological condition of the aforementioned circuit occurs when the NMDA receptors present on the PV-containing interneurons become dysfunctional (or a loss of these interneurons occurs) and are unable to transmit their normal ionic current. Because NMDA receptors transduce excitatory signals to increase the membrane potential of neurons, this will lead to the interneurons to fire at lower frequencies than normal when hypofunctional. This means that they will release less GABA onto downstream pyramidal cells in the cortex and subcortical regions. By releasing the pyramidal cells of their GABAergic inhibition, they will become more likely to fire, leading to an increased release of excitatory neurotransmitters such as glutamate, dopamine, and acetylcholine (14, 21). This may then lead to the excitotoxic damage hypothesized.

Several studies show a marked decrease in the number of these PV-containing interneurons in cortical and sub-cortical regions that are implicated in the NMDA receptor hypofunction hypothesis of schizophrenia (4, 32). Observations made from post-mortem human samples comparing the hippocampi, a subcortical structure, of patients with schizophrenia and their matched controls found significant decreases in the density of these PV-containing interneurons in all hippocampal areas studied. Remarkably, these effects were also not attributable to pharmacological treatment, onset, or duration of disease, thus showing that this deficit is consistent no matter the nuances between individuals (32).

Other studies have also shown decreases of these PV-containing interneurons in the frontal cortex regions of human post-mortem samples of schizophrenic patients compared to control. The deficiency was found to not be laminar specific, but occurred in all cortical layers (4). This data of deficient interneurons in the cortex fits very well with the model for NMDA hypofunction contributing to disinhibition of the cortical pyramidal neurons leading to increased release of excitatory neurotransmitters.

It has been known for some time that blockade of NMDA receptors through pharmacologic methods such as administration of the NMDA antagonists, PCP, ketamine, and MK-801 produce increased levels of glutamate and acetylcholine in the prefrontal cortex and also causes a rise of striatal dopamine production (21). Theoretically, these may all be linked to the symptoms seen in schizophrenia; positive symptoms resulting from the increased striatal dopamine and negative symptoms from excitotoxic neuronal injury in the prefrontal cortex, as alluded to previously. This model of schizophrenia may also offer a basis of the pathogenesis and explanation for the downstream effects of schizophrenia, which are routinely treated for such as excessive dopamine.

Evidence for Excitotoxicity

Excessive release of glutamate into synapses is well known to have detrimental effects on the postsynaptic neurons. This process, called excitotoxicity, causes damage to neurons through activation of cell death pathways (leading to apoptosis) and has been heavily implicated with NMDA receptors. More specifically, it has been strongly linked to NMDA receptors containing the NR2B subunit, which has a well-established role in excitotoxicity (6). A number of intracellular signaling cascades and molecules are implicated in excitotoxicity such as p53, reactive oxygen species, NF-kB (a pro-inflammatory transcription factor), nitric oxide, and many more (6, 27). This would be a logical starting place to investigate the role of excitotoxicity in a schizophrenia model, but the models currently used (through a global NMDA antagonism) would be of little use due to null excitation of NMDA receptors anywhere in the brain. Not to say downstream NMDA overexcitation is not involved; however, there has been evidence for non-NMDA excitotoxicity in schizophrenia as well.

A study by Kristensen et al (18) looked at neuronal damage specifically in the cortex and the striatum through administration of AMPA and kainate to slices of neonatal

rat brain tissue; both areas of interest to schizophrenic pathology. Propidium iodide (PI) is used as a marker of cellular damage, which is taken up as the dying neuronal membranes become more porous. They showed significant uptake in a majority of the neurons observed after exposure to AMPA and kainate. Significant uptake occurred in both the striatum and cortex, and it was observed that the cortex was more susceptible to lower doses of the agonists. The non-NMDA glutamate antagonist NBQX, which blocks AMPA and kainate receptors, was then given simultaneously with AMPA or kainate. Even at the lowest concentration of NBQX (0.1uM), a significant reduction in PI uptake into the cells was observed, indicating blockade of non-NMDA glutamate receptors in this condition is neuroprotective (18). While this study shows that over-excitation of non-NMDA receptors is able to cause severe neuronal damage in cortical and striatal areas, it may not provide great validity because the concentrations of AMPA and kainate used were much higher than would be seen in vivo.

Another piece of supportive evidence has shown that administration of the NMDA antagonist, MK-801, in a rat model leads to profound cortical neurodegeneration when stained for cells undergoing apoptosis through the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method, which stains for DNA damage. Regions of particularly high-susceptibility to apoptotic damage were the parietal cortex, caudate (a component of the striatum), cingulate cortex, thalamus, layer II neurons of the prefrontal cortex, hippocampus, and retrosplenial areas, which are known to be affected in schizophrenia (16). The experiments were carried out during postnatal development at times that correlates with the third trimester of pregnancy in humans. This is known to be a time when fetal insult can predispose the fetus to developing schizophrenia later in life, so it makes these results even more relevant to the condition (16). The study shows that the NMDA hypofunction in vivo model of schizophrenia can lead to cortical neuronal injury, as put forward by the NMDA hypofunction hypothesis, and that it must be due to some other mechanism than NMDA-mediated excitotoxicity as NMDA receptors were globally blocked. This downstream cortical injury could potentially be due to glutamate toxicity to the neurons through AMPA or kainate-mediated transmission as discussed previously.

A different study has brought together these two pieces of evidence and confirmed cortical neuronal damage through administration of NMDA-antagonists such as MK-801 (8). It was shown that the non-NMDA glutamate blocker, NBQX, is able to significantly prevent the glutamatergic neuronal damage from blocked NMDA receptors when compared to the saline injection control in the ipsilateral retrosplenial cortex. This means that that the neuronal damage seen is dependent on glutamate, but through receptors other than NMDA. It was also shown that administration of scopolamine, a cholinergic antagonist, was able to prevent neurotoxic damage in addition to NBQX. This correlates well with the evidence of increased acetylcholine release from NMDA hypofunction mentioned previously (8, 21). The administration of NBQX, with the NMDA receptor being blocked in both groups, meant that the glutamate-mediated toxicity most likely would have been due to AMPA and/or kainate receptor over-activation, which has been extensively reviewed elsewhere (6).

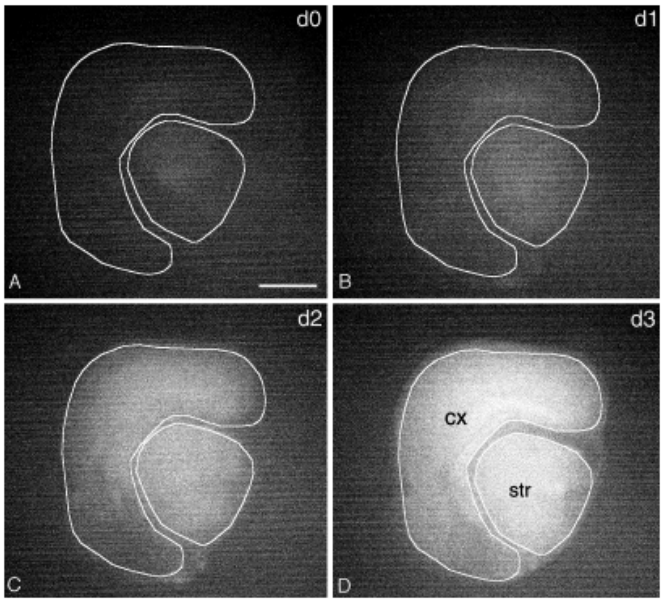

Figure 2: Imaging of brain slices showing uptake of PI before (A), 24 hours after kainate administration (B), 48 hours after kainate administration (C), and after total neuronal death (D). Results indicate kainate administration leads to cell death in striatal and cortical regions. Figure from Kristensen et al, 1999 (18).

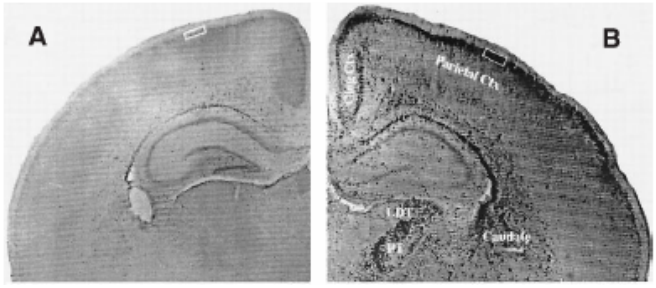

Figure 3: Post-natal rat brain hemisections showing TUNEL staining of saline control (A) and MK-801 treated (B) brains that were treated 24 hours previously. MK-801 treated brain shows significant neuronal apoptosis. Figure from Iknoomidou et al, 1999 (16).

The Farber et al study also considered whether NMDA hypofunction-related neuronal damage could be recreated through the use of receptor agonists injected into cortical regions, as to mimic the downstream effects of blocking NMDA receptors (6). They used AMPA as an AMPA receptor agonist, kainate as a kainate receptor agonist, and carbachol as nicotinic cholinergic agonist. Individually, none of these agonists was able to produce neuronal damage to the extent of MK-801 administration. They did find however that when kainate and carbachol were co-administered, significant levels of neuronal damage were induced, similar to that of MK-801 administration (6). These findings indicate that while glutamate may not be the only receptor agonist responsible for cortical damage seen in schizophrenic models, it does play a large role, and its inhibition through non-NMDA receptors may be a target for therapeutics.

While these studies point towards evidence of excitotoxicity in NMDA hypofunction, thus linking it to schizophrenia, these are in vitro and in vivo animal models. These models may be profoundly different in human patients with schizophrenia. A 2009 study conducted by Rao et al (27) performed protein and mRNA analysis on post-mortem human brain samples to look for markers of neuroinflammation and excitotoxicity in schizophrenia. Upregulation of both protein levels and mRNA levels for neuroinflammatory markers such as interleukin-1β (IL-1β), tumor necrosis factor- α (TNF-α), nuclear factor kappa-light chain enhancer of activated B cells (NF-κB), and cyclooxygenase-2 (COX-2) were found in schizophrenia patient samples when compared to control. Another important pro-inflammatory marker that was found to be upregulated in schizophrenic samples was phospholipase A_2 (PLA_2), which is involved in arachidonic acid (AA) metabolism for the production of inflammatory mediators such as thromboxane , prostaglandins, and leukotrienes (27).

This study also looked at what it called markers of excitotoxicity in the tissue samples. They found no significant difference between schizophrenic and control groups when sampling for the NMDA receptor subunits NR1 and NR2B, c-fos, and inducible nitric oxide synthase (iNOS). They concluded from these results that while there is an increase in neuroinflammation in schizophrenic pathology, there is no conclusive evidence for excitotoxicity occurring in schizophrenia (27).

The conclusions of this study seem to be an oversimplification of what their results actually show. First, AA (and its subsequent metabolism through PLA_2) is known to be upregulated in neurons through glutamate excitation and can lead to increases in intracellular levels of sodium and calcium. This in turn has been shown to lead to neuronal apoptosis through mitochondrial-apoptotic cascades (7), thus showing its involvement in excitotoxicity. Meanwhile, Rao's conclusions did not acknowledge this link to excitotoxicity. Also, if the NMDA hypofunction model of schizophrenia holds merit, it would not be wise to look at NMDA receptors in regards to excitotoxicity, as they would be altered for reasons other than excitotoxicity. Rather, AMPA and kainate receptors should be focused on. Other excitotoxic markers that should also be evaluated are neuronal nitric oxide synthase (nNOS), calpain, and death-associated protein kinase-1 (DAPK1; 20). This study also does not detail which cortical region the samples came from, or if there was any regional consistency between schizophrenic and control samples. This may lead to the non-significance seen in their analysis of NR1, NR2B, c-fos, and iNOS. If the NMDA hypofunction hypothesis is correct, excitotoxicity would be expected mostly in cortical and subcortical regions such as the prefrontal cortex, caudate, and retrosplenial cortex areas. It was also noted that majority of the patients from whom the samples came were being treated with the atypical neuroleptic risperidone. This may have some unknown mechanism by which it ameliorates the excitotoxic marker presence, and thus leading to the non-significance seen (27).

Studies have also provided evidence for an increase in glutamate levels in the brain (9, 10, 30). These have been conducted through post-mortem tissue analysis and live imaging studies. The main areas focused on, which have shown a significant increase in glutamate levels, are the prefrontal cortex and the associative striatum. These areas are greatly implicated with schizophrenia, and therefore provide further evidence towards confirmation of the NMDA hypofunction hypothesis and its possible downstream excitotoxicity. Together, these pieces of evidence cumulatively point to excitotoxicity, likely playing a role in the pathology of schizophrenia and NMDA receptor hypofunction being a probable mechanism.

Cortical Degeneration and Excitotoxicity

A large body of evidence exists for a decrease in grey matter volume, which consists mainly of the neuronal cell bodies, axons, dendrites, and synapses, of the frontal cortex in individuals with schizophrenia and may be contributing substantially to the cognitive deficits of the disease (2, 3, 12, 17). While the decreasing grey matter would seem to correlate well with the excitotoxic hypothesis mechanisms present in schizophrenia, there is also evidence to show that there is no significant decrease in the number of cortical neurons in schizophrenic patients, as described in post-mortem analyses (12, 28). This seems to go against the NMDA hypofunction hypothesis, as they found no overall cellular loss, but could be due to the fact that other neuronal populations prevail in development in place of the

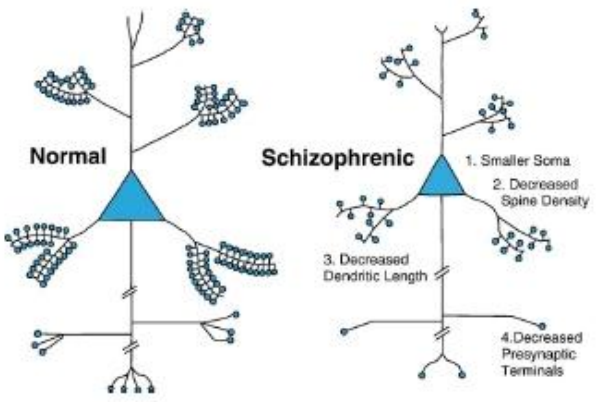

Figure 4: Depiction of neuronal excitotoxic pathologies hypothesized in schizophrenia. These include decreased somal volume, decreased spine density, decreased dendritic length, and decreased number of presynaptic terminals. Figure from Glantz et al, 2006 (12).

ones damaged by excitotoxicity. On top of this, and with data to support it, evidence has come to light that a sub-lethal form of excitotoxicity may have a role in interrupting neurotransmission in similar ways to how total apoptosis could.

There have been numerous suggestions that this decreased cortical grey matter volume could be related to a decrease in synaptic connections between neurons, and subsequently lead to the anatomical abnormalities seen uniformly in patients with schizophrenia (12, 19). The decrease in grey matter volume may also be reflective of the decreased number of PV-containing interneurons that are seen to be decreased in schizophrenia as previously mentioned (4, 32), but not in an amount significant enough to show a change in total neuronal density when compared to control.

The idea of a degeneration of the neuropil (axons, dendrites, and sites of synapse) being what is contributing to the volumetric deficits has its basis founded in work that shows significant decreases in synaptic markers such as synaptophysin in human post-mortem samples of patients with schizophrenia and their matched controls (13). This has since been confirmed and furthered by studies that have identified decreases in other synaptic proteins such as drebdin, and neurotrophic factors (that are known upon removal to lead to apoptosis) such as brain-derived neurotrophic factor (BDNF) in post-mortem samples of schizophrenic brains compared to matched controls (12, 27). These indications of decreased cortical connectivity could be associated with functional deficits in information processing that could be associated to the problems schizophrenia patients have with working memory and attention. There is also a known reduction in dendritic spine density, decreased dendritic length, and a reduction in the size of the soma in schizophrenic patients, which also fits into this paradigm (12).

There is increasing evidence for this sub-lethal and localized version of excitotoxicity or apoptosis that occurs only in the synaptic areas, and therefore could be the underlying pathology seen in decreased grey matter volume and connectivity deficits (12). Increased levels of caspases, which are involved in excitotoxic signaling, have been observed in synaptic regions, and decreases in the neuroprotective protein Bcl-2 have also been observed in the cortex of patients with schizophrenia (12). Together with increases in excitotoxic signalling and decreased neurotrophic support from BDNF, these pieces of evidence point to an environment that could be conducive to apoptosis of cortical synapses, leading to decreases in functional connectivity and able to produce deficits in executive functions seen in schizophrenia (12, 27). While this has not been directly observed, it warrants future research as it could provide an understanding of the pathology involved in the cognitive deficits seen in individuals with schizophrenia and possible targets for therapeutic intervention.

Future Directions

Taken together with all the evidence, it seems probable that NMDA receptor hypofunction is an underlying factor in the pathogenesis and pathology of schizophrenia, but as neurodegenerative diseases are often multifactorial, the dysfunction is probably not an end-all explanation of schizophrenia. The dopamine hypothesis is still an important pillar of understanding schizophrenia and is not disproven in light of this glutamate-centric hypothesis. While evidence for hypofunctioning provides a mechanism for the downstream excitotoxic (total and/or possibly sub-lethal) insults observed in schizophrenia. A first direction for future research in the field would be to better understand the etiology of NMDA receptor hypofunction. Some proposed mechanisms exist, but they are quite vague and have no substantial amount of evidence to support them, and therefore are not reviewed here (14). Using tools such as genome-wide association studies and analysis of the NMDA receptors (i.e., amino acid sequences) from patients with schizophrenia would be a good starting place for this research. Gaining better understanding of how this hypofunction occurs may lead to possible early-life interventions for high-risk individuals to prevent or minimize the severity of symptoms associated with schizophrenia.

Other avenues to be explored within this paradigm include investigations into confirming and interrupting the neuroinflammatory/excitotoxic pathways that are implicated in schizophrenia. These include the pathway of

arachidonic acid metabolism (via), NF-kB, IL-β1, caspases, Bcl-2 and others. Interrupting these pathways could most likely be done through the work of transcriptional inhibitors or interfering peptides to interrupt activity. The problem with blocking these pathways is that an early intervention would be needed, as schizophrenia is a condition that is progressive, and its pathogenesis can begin at very early life stages with no symptoms (16).

As increase in glutamate levels and glutamatergic transmission have been implicated in schizophrenic pathology, antagonizing its transmission may be another possible therapeutic direction to investigate. It has already been shown in vivo that blockade of non-NMDA mediated transmission (via NBQX) is neuroprotective in a model of NMDA-hypofunction (8). Translation into larger animal studies and possible clinical trials with schizophrenic patients should be looked into, as the preliminary in vivo results have been promising. Glutamatergic blockade may be able to replace or be an adjunct to current therapies that mostly interact within the dopamine system.

The use of optogenetics or **D**esigner **Re**ceptors **A**ctivated by **D**esigner **D**rugs (DREADDs) are other ways that could more precisely modulate and return activity of the NMDA-dependent glutamatergic system that is involved in this research. Optogenetics, a technique first developed by Stanford's Karl Deisseroth, sees certain neuronal populations that can be distinguished through unique markers (i.e., CAMKIIα for glutamatergic neurons or parvalbumin-containing neurons such as the ones implicated in this research) virally transfected with the gene for a light-sensitive opsin protein. The opsin can then be activated by a nearby light source to open an ion channel which will either depolarize or hyperpolarize a neuron, thereby giving control to if and when a neuron fires (5, 26). The technique could potentially allow a therapy whereby the PV-containing interneurons with dysfunctional NMDA receptors are specifically activated via a light source and regain normal inhibitory control over cortical pyramidal neurons. DREADDs is another technique whereby specific cells, such as optogenetics, can be transfected with a receptor that is designed to respond to a normally inert drug and open an ion channel when bound. This could again allow for specific activation of the population of PV-containing interneurons with dysfunctional NMDA receptors. While optogenetics is a more established technique on the verge of use as a human therapeutic, DREADDs is a newer technology that is still overcoming hurdles for human use. Nonetheless, these exciting techniques offer limitless opportunities for understanding and therapeutics, and will surely be of use in the field of schizophrenia research now and into the future.

Lastly, future research should also be conducted into the reductions of grey matter volume and their probable sub-lethal excitotoxic mechanisms. By gaining a greater understanding of these mechanisms and the sub-cellular events involved, possible therapeutic approaches could be identified. One possibility could be the decreased BDNF levels observed in schizophrenia, and its replacement may be an area to look further into. Optimistically, one of these directions may lead to a greater understanding of how schizophrenia occurs and the subsequent emergence of therapeutics to prevent or treat these abnormalities. This would be of great societal significance, and a considerable achievement for humanity, in helping the individuals with this severely debilitating condition.

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