

## NMDA Receptor and Schizophrenia: A Brief History

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**Although glutamate was first hypothesized to be involved in the pathophysiology of schizophrenia in the 1980s, it was the demonstration that N-methyl-D-aspartate (NMDA) receptor antagonists, the dissociative anesthetics, could replicate the full range of psychotic, negative, cognitive, and physiologic features of schizophrenia in normal subjects that placed the “NMDA receptor hypofunction hypothesis” on firm footing. Additional support came from the demonstration that a variety of agents that enhanced NMDA receptor function at the glycine modulatory site significantly reduced negative symptoms and variably improved cognition in patients with schizophrenia receiving antipsychotic drugs. Finally, persistent blockade of NMDA receptors recreates in experimental animals the critical pathologic features of schizophrenia including downregulation of parvalbumin-positive cortical GABAergic neurons, pyramidal neuron dendritic dysgenesis, and reduced spine density.**

*Key words:* NMDA receptor/Glutamic Acid/Dopamine/GABA/Schizophrenia/Bipolar Disorder

“History is written by the victors.” Winston Churchill

### Introduction

The implication of the N-methyl-D-aspartate receptor (NMDAR) in the pathophysiology of schizophrenia is a rather recent development with the first associations appearing in print in the 1980s. According to ISI Thomson, there have been over 2000 articles published on the subject since then. This review of the hypothesized role of NMDAR in schizophrenia will focus on articles appearing prior to 2002 (ie, greater than one decade ago) unless they validate or build upon prior findings. More recent articles could be viewed as part of the current research efforts. Decisions about importance and timeliness are based upon the citation record of the articles.

The history of our evolving understanding of the pathophysiology of psychiatric disorders is complex and

affected by the changing concepts of psychiatric disease and advances in neuroscience<sup>1,2</sup>. Furthermore, as Thomas Kuhn<sup>3</sup> has emphasized, science is not a neutral observer, and new conceptual approaches are generally met with considerable resistance. This was clearly the case with regard to the role of NMDARs in the pathophysiology of schizophrenia. Early research viewed hypofunction of NMDARs through the lens of the widely held dopamine hypothesis of schizophrenia,<sup>4-6</sup> whereas recent research has focused on the NMDAR deficit as element, albeit important, in dysfunctional cortical circuit with dopamine-mediated psychosis, a downstream consequence.<sup>7-9</sup> Nevertheless, 30 years ago, investigators were already touting glutamatergic neurotransmission as a worthy target for drug development to treat schizophrenia,<sup>10,11</sup> a strategy that has only recently been embraced by the pharmaceutical industry.<sup>12,13</sup>

The NMDAR subtype of the glutamate-gated cation channels was identified 30 years ago through its selective antagonism by D-2-amino-5-phosphonovaleric acid and Mg<sup>2+</sup> of glutamate-induced neuronal depolarization, which distinguished it from AMPA (2-amino-3-[5-methyl-3-oxo-1,2-oxazol-4-yl]propanoic acid) and kainate subtypes of glutamate ionotropic receptors.<sup>20</sup> Johnson and Ascher<sup>21</sup> showed that glycine potentiates NMDAR responses, and Kleckner and Dingledine<sup>22</sup> demonstrated the obligatory role of glycine/D-serine in permitting L-glutamate's opening of the NMDAR channel. Given the fact that extracellular levels of glycine in brain were approximately 7 μM,<sup>23</sup> thereby saturating the glycine modulatory site (GMS), the GMS seemed irrelevant as a target for positively modulating the NMDAR until it was demonstrated that the glycine transporter, GlyT1, maintains subsaturating concentrations of glycine within the synaptic cleft.<sup>24</sup> Although long-term potentiation (LTP) was first identified nearly 40 years ago,<sup>25</sup> the demonstration of the key role of the NMDAR as the coincidence detector mediating LTP elevated the NMDAR's profile into being the dominant regulator of neuroplasticity.<sup>26</sup> Subsequently, NMDAR mediated

neuroplasticity was shown to also include use-dependent structural modifications of dendrites and spines.<sup>27</sup>

Kornhuber's laboratory could be credited with first proposing a role for glutamate in schizophrenia.<sup>11</sup> Their hypothesis was based upon the finding of low cerebrospinal fluid (CSF) levels of glutamate in Huntington's disease and schizophrenia compared with controls. The hypothesis was closely intertwined with the dopamine hypothesis as they reported that chronic administration of amphetamine to rats also lowered glutamate content in CSF and in several areas of the brain.<sup>4</sup> However, Perry<sup>28</sup> contested these findings with more sensitive and specific assays that revealed no differences in brain or CSF levels of glutamate in schizophrenia. Subsequent studies have not revealed significant alterations in CSF glutamate levels in schizophrenia.<sup>29,30</sup> Thus, while Kornhuber's hypothesis was prescient, the primary evidence motivating it was not substantiated.

#### *Dissociative Anesthetics and Symptoms of Schizophrenia*

The driving force in linking NMDARs to schizophrenia was the long recognized psychotogenic effect of dissociative anesthetics such as ketamine and phencyclidine (PCP).<sup>31,32</sup> This connection became possible with the demonstration by Lodge's laboratory that ketamine and PCP were noncompetitive antagonists at NMDARs.<sup>33</sup> However, the link between NMDAR antagonism and their psychotomimetic effects was not clear because the dissociative anesthetics interacted with multiple receptors and channels.<sup>34</sup> Building upon their basic studies, linking the PCP binding site to NMDA receptors,<sup>35</sup> Javitt and Zukin<sup>36</sup> in their highly cited (~1500 times) review reported that PCP abuse causes the full range of psychiatric and neurocognitive symptoms associated with schizophrenia at plasma concentrations causing NMDAR blockade and proposed explicitly that NMDAR dysfunction contributed to the pathophysiology of schizophrenia.

One important reservation about the Javitt and Zukin<sup>36</sup> proposal was that the psychotic disorder brought upon by illicit abuse of these drugs might result from an underlying vulnerability in the abusers.<sup>37</sup> To counter this concern and the uncontrolled nature of self-administration of PCP by substance abusers including co-use of other drugs, Krystal et al.<sup>38</sup> carried out a landmark study (cited 1020 times) in which normal volunteers were infused with subanesthetic doses of ketamine. The doses used did not affect performance on the Mini-Mental Status Exam, precluding delirium as an explanation for the findings. The subjects developed the full range of symptoms associated with schizophrenia including positive and negative symptoms and impairments in vigilance, verbal fluency, and the Wisconsin Card Sort. Physiologic and neuroendocrine alterations were also noted. Subsequent studies reinforced

the evidence for similarities to schizophrenia including selective cognitive deficits,<sup>39</sup> augmented amphetamine-induced dopamine release,<sup>40</sup> poor facial emotion recognition,<sup>41</sup> and impaired auditory-sensory gating.<sup>42</sup> Prepulse inhibition of the startle reflex was actually augmented by ketamine in normal volunteers, whereas it is disrupted in schizophrenia.<sup>42</sup> Lahti et al.<sup>43</sup> showed that pharmacologically stabilized patients with schizophrenia experienced a return of their unique symptoms when administered ketamine.

In early translational studies in experimental animals, Tamminga et al.<sup>44</sup> showed that metapit blocked the increased glucose metabolism in rat brain caused by PCP and speculated that this effect might be informative about the pathophysiology of schizophrenia through its action at NMDAR. Carlsson and his colleagues followed a line of inquiry on the mechanisms mediating the hyperactivity caused by dizocilpine (MK-801), a potent ligand at the PCP site in the NMDAR, and implicated 5-HT<sub>2A</sub> receptors, which they suggested could be a target for antipsychotic drug development.<sup>45</sup> However, since the hyperactivity induced by MK-801 is not reversed by antipsychotic drugs, its relevance to psychosis is unclear.

Olney's laboratory focused on the cytotoxic effects of the dissociative anesthetics on corticolimbic neurons in rats.<sup>46</sup> A single dose of MK-801 causes acute vacuole formation in the majority of pyramidal neurons in layers II and IV of the posterior cingulate and retrosplenial cortex. These pathologic changes are partially reversed by muscarinic receptor antagonists, 5-HT<sub>2A</sub> receptor agonists, and antipsychotics that affect negative symptoms. This vulnerability to NMDAR antagonists exhibits a similar developmental profile as the human risk for psychosis with ketamine, which typically has its onset after puberty.<sup>47</sup> Limitations of the model, however, include the fact that careful studies have not demonstrated significant loss of cortical neurons in schizophrenia<sup>48</sup> postulated by the model and that the antipsychotic protection against the lesions correlates poorly with their clinical efficacy.<sup>49,50</sup>

#### *Clinical Trials to Increase NMDAR Function*

Intervention studies to test the hypothesis that enhancement of NMDAR function at the GMS would attenuate symptoms of schizophrenia were initiated quite early. In 1989, Deutsch's group carried out an open label study in which they examined the efficacy of glycine (10.8 g/day) in chronically psychotic patients as an adjunct to conventional antipsychotic treatment<sup>51</sup> and reported inconclusive results. In a subsequent open label study, they examined the effects of milacimide, a prodrug for glycine, and found no significant therapeutic effects. Javitt and his colleagues initiated a series of placebo-controlled clinical trials with glycine in patients stabilized on antipsychotics.<sup>52-54</sup> Recognizing the poor penetration

of glycine through the blood-brain barrier, they studied daily doses ranging from (0.4–0.8 g/kg/day). The high-dose intervention increased plasma levels of glycine by 6-fold, resulted in significant reductions in negative symptoms, the very symptoms resistant to antipsychotic treatment, and improved cognition.

At the same time, Goff et al.<sup>55</sup> examined the effects of D-cycloserine (DCS), a drug used to treat tuberculosis, which is a partial agonist at the GMS and crosses the blood-brain barrier better than glycine. Preclinical studies indicated that DCS had cognitive-enhancing effects.<sup>56</sup> In patients with chronic schizophrenia stabilized on antipsychotics, DCS exhibited a U-shaped dose response curve with the optimal dose, 50 mg/day, significantly reducing negative symptoms and improving performance on a cognitive task. A subsequent placebo-controlled, parallel clinical trial with DCS replicated the finding of a significant reduction in negative symptoms but not positive symptoms.<sup>57</sup> In contrast, the addition of DCS to clozapine resulted in a significant exacerbation of negative symptoms,<sup>58</sup> a counterintuitive finding consistent with clozapine causing full occupancy at the GMS<sup>59</sup>. A functional imaging study in schizophrenic patients receiving antipsychotics demonstrated that the addition of DCS significantly enhanced performance on a memory task and increased activation of the left temporal superior gyrus required for performance, and this effect correlated inversely with negative symptom change.<sup>60</sup> The therapeutic utility of DCS was limited by the fact that its efficacy decreased with increasing duration of treatment.<sup>61</sup>

Over the last 15 years, 26 placebo-controlled double-blind trials have been carried out on agents that directly or indirectly (sarcosine) act at the GMS in patients with chronic schizophrenia who were receiving antipsychotic medications. These include glycine, D-serine, alanine, and sarcosine. The effects on negative and depressive symptoms are substantial (effect size = 0.4) and highly significant, whereas the effects on cognition, positive symptoms, and general psychopathology are more modest but still significant.<sup>62</sup> Because of poor penetration through the blood-brain barrier, metabolism and modest intrinsic potency, these natural products are unlikely to be useful treatments, but they have provided proof of principle that enhancing NMDAR function can significantly reduce symptoms in schizophrenia, especially those least responsive to existing antipsychotic medications.

As noted above, GlyT1 maintains subsaturating levels of glycine at synaptic NMDARs.<sup>24</sup> Thus, pharmacologic inhibition of GlyT1 could enhance NMDAR function, thereby reducing negative symptoms and cognitive impairments in schizophrenia. Bergeron et al.<sup>24</sup> first demonstrated NMDAR-enhancing effect of GlyT1 inhibition with NFPS (*N*-[3-([1,1-Biphenyl]-4-yloxy)-3-(4-fluorophenyl)propyl]-*N*-methylglycine), the prototypic GlyT1 inhibitor that was developed by the biotech,

Trophix Pharm. GlyT1 is now major target for drug development for schizophrenia treatment.<sup>63</sup>

### Postmortem Studies

Early postmortem studies to explore the potential role of the NMDAR in the pathophysiology of schizophrenia were constrained by the limited availability of brain tissue, indirect methods of analysis such as ligand-binding techniques, and superficial understanding of the glutamatergic synapse. Nevertheless, Kornhuber et al.<sup>64</sup> reported nonsignificant elevation (except putamen) of [<sup>3</sup>H]-MK-801 binding, a surrogate for the NMDAR channel, in corticolimbic regions in a small number of subjects. Kerwin et al.<sup>65</sup> used quantitative autoradiography in the hippocampal formation to demonstrate significant reductions in [<sup>3</sup>H]-kainate binding in subfields (CA3-4 mossy fiber terminals, dentate gyrus) without alterations in NMDARs. The finding was confirmed using *in situ* hybridization autoradiography for the transcript for the “KA/AMPA-R” in a small number of cases.<sup>66</sup> Using synaptosomes prepared from frozen postmortem brain tissue from subjects with schizophrenia and controls, Sherman et al.<sup>72</sup> found reduced kainate and NMDA evoked release of glutamate, consistent with the receptor studies, and increased NMDA evoked release in homogenates prepared from frozen *postmortem* brain from subjects with schizophrenia and suitable controls. However, in an extensive study of glutamate receptor subtypes using *in situ* hybridization for the transcripts encoding the 14 subunits and ligand-binding autoradiography, Beneyto et al.<sup>68</sup> found sparse changes in schizophrenia (as compared with more robust and widespread changes in bipolar disorder) with an isolated decrease of GluR5 in the perirhinal cortex uncorroborated by [<sup>3</sup>H]-kainate binding. The focus of research on synaptic pathology has now shifted to the NMDAR complex associated proteins that affect signal transduction.<sup>69</sup>

Tsai et al.<sup>70</sup> measured glutamatergic related neurochemicals and enzymes in *postmortem* brains from subjects with schizophrenia on or off antipsychotics prior to death and from controls. Although 8 brain regions were assayed, significant differences were restricted to the hippocampus and prefrontal cortex (PFC) where results were consistent with reduced glutamate and reduced catabolism of N-acetylaspartylglutamate (NAAG), an endogenous NMDAR antagonist. Using the acute hippocampal slice, Gruze et al.<sup>71</sup> showed that the NMDARs on the GABAergic interneurons were several fold more sensitive to NMDAR antagonists including NAAG than those on the pyramidal neurons and proposed a computational model that plausibly accounted for the cognitive symptoms of schizophrenia as a consequence of reduced GABAergic inhibition. The levels of kynurenic acid, another endogenous NMDAR antagonist, are also elevated in brain and in the CSF in schizophrenia.<sup>72,73</sup>



These findings provided one mechanism for explaining NMDAR hypofunction in schizophrenia: elevated levels of endogenous NMDAR antagonists.

### *The NMDAR as Part of a Circuit*

While several investigators speculated about the neurocircuitry affected by NMDAR hypofunction,<sup>7,46</sup> Moghaddam et al.<sup>6</sup> used *in vivo* dialysis to measure the effects of systemic ketamine on glutamate and dopamine release in the PFC. She demonstrated that sub-anesthetic doses of ketamine increased extracellular glutamate in rat PFC, whereas anesthetic doses did not increase glutamate release. Furthermore, low-dose ketamine stimulated a robust release of endogenous dopamine in the PFC but not in the striatum. They further showed that a Group II mGluR agonist reversed the behavioral effects of PCP and the augmented cortical dopamine efflux.<sup>74</sup> This increased release of dopamine in the PFC is driven by stimulation of both AMPA receptors on the dopaminergic cell bodies in the ventral *tegmentum* and AMPA receptors in the cortex.<sup>83</sup> But why the increase in glutamate release? In the hippocampal CA1 region, Grunze et al.<sup>71</sup> found that GABAergic interneurons were 10-fold more sensitive to NMDAR antagonists than the pyramidal neurons. Subsequent studies in acute slice preparations of the olfactory bulb<sup>84</sup> and of the posterior cingulate and retrosplenial cortex<sup>77</sup> showed that NMDAR provides excitatory drive on GABAergic interneurons so that NMDAR antagonists disinhibit glutamate release.

The cortical GABAergic interneurons, which are responsible for recurrent inhibition to the pyramidal neurons, were critical for NMDAR hypofunction model as supported by several lines of evidence. Since the first *postmortem* studies of schizophrenia, GABAergic deficits in cortex and limbic system were reported<sup>72</sup> although whether this was an artifact of the agonal events such as a slow death was a concern.<sup>79</sup> Immunocytochemical studies revealed these recurrent inhibitory neurons represented a subset that expressed parvalbumin (PV) and appeared to be selectively vulnerable in schizophrenia through reduced number<sup>80</sup> or expression of PV,<sup>89</sup> which proved to be robust in quantitative measurements of gene expression.<sup>90</sup> Treatment of mice with ketamine reduces the levels of PV and GAD67 through generation of superoxides<sup>83</sup> and diminishes inhibitory postsynaptic currents on the pyramidal neurons.<sup>84</sup>

Another highly replicable pathologic feature of schizophrenia is the dystrophic dendrites with reduced synaptic spines. Using electron microscopy, Uranova described dystrophic dendrites in the frontal and limbic cortices.<sup>85</sup> Roberts et al.<sup>86</sup> reported a 30% reduction in the size of spines in the basal ganglia in schizophrenia. Several studies using Golgi staining in various cortical regions have revealed reduced neuropil, reduced dendritic complexity

of pyramidal neurons, and decreased spine density.<sup>87,88</sup> Etienne and Baudry<sup>89</sup> first speculated that impaired NMDAR function contributed to the synaptic pathology in schizophrenia. Recent studies in which mutant mice that have constitutively reduced NMDAR function have confirmed that NMDAR hypofunction causes reduced dendritic complexity and spine density.<sup>90,91</sup>

### *Genetics*

Family, adoption, and twin studies indicate that schizophrenia has high heritability but it does not follow Mendelian genetics. Schizophrenia appears to be due to complex genetics, whereby multiple, common alleles of modest effect interact with the environment to produce the phenotype.<sup>92</sup> Currently, the results of risk-gene association studies for schizophrenia are currently compiled in running meta-analyses at [www.szgene.org](http://www.szgene.org). Notably, 8 of the top 45 putative risk genes are within 2 degrees of separation from the NMDAR including 3 that determine the availability of D-serine. Furthermore, rare, recurrent genomic copy number variants that substantially increase risk for schizophrenia are very significantly enriched with genes that encode members of the NMDAR complex.<sup>93</sup>

### *Comment*

The dopamine hypothesis has dominated thinking about the pathophysiology and treatment of schizophrenia for nearly 50 years in spite of considerable evidence that it was inconsistent with the pathology of schizophrenia and treatment outcomes. For example, both the micro-pathology involving widespread dendritic and spine dysplasia and the macropathology with cortical atrophy and ventricular enlargement are difficult to reconcile with subcortical dopaminergic dysfunction. The inability of antipsychotic medications aside from clozapine to affect negative symptoms and cognitive impairments indicated that manipulation of dopaminergic function has not affected the core, disabling symptoms of schizophrenia. Karl Popper<sup>94</sup> proposed that the fundamental requirement for scientific advances is the proposition of falsifiable hypotheses. It would seem that the dopamine hypothesis of schizophrenia has not been subject to Popper's principle for decades.

The emergence of the NMDAR hypofunction hypothesis clearly has had a liberating effect on thinking about the pathophysiology and treatment of schizophrenia. First, it evolved out of clinical observations demonstrating that pharmacologic blockade of NMDARs produced the component symptoms—negative symptoms and cognitive impairments—that were neither affected by antipsychotics nor produced by dopaminergic agonists. As these effects were based upon acute pharmacologic challenges, this pointed to a disruption in a neurotransmission that mirrored

the widespread corticolimbic impairments in function observed in brain-imaging studies in schizophrenia. Second, as schizophrenia is a developmental disorder with manifestations prior to the onset of psychosis,<sup>95</sup> it is important to appreciate the crucial role of NMDARs in brain development, especially with regard to dendritic elaboration and spine formation, which are disrupted in the disorder. Third, the NMDAR hypofunction model illuminates potential upstream contributors such as D-serine availability or downstream consequences such as reduced recurrent inhibition to pyramidal neurons, thereby providing context for understanding the role of “unrelated” risk genes such as *NRG1* and the alpha-7 nicotinic receptor.<sup>96</sup> Finally, these studies have led to the proposal of pathologic circuitry in schizophrenia, which while a gross oversimplification nevertheless provides refutable targets for drug development.

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