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Neurodevelopmental Animal Models Reveal the Convergent Role of Neurotransmitter Systems, Inflammation, and Oxidative Stress as Biomarkers of Schizophrenia: Implications for Novel Drug Development

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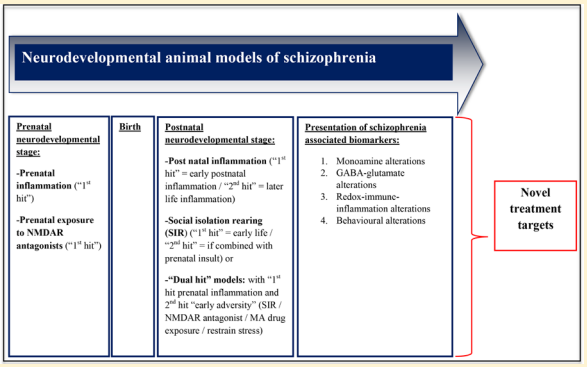
ABSTRACT: Schizophrenia is a life altering disease with a complex etiology and pathophysiology, and although antipsychotics are valuable in treating the disorder, certain symptoms and/or sufferers remain resistant to treatment. Our poor understanding of the underlying neuropathological mechanisms of schizophrenia hinders the discovery and development of improved pharmacological treatment, so that filling these gaps is of utmost importance for an improved outcome. A vast amount of clinical data has strongly implicated the role of inflammation and oxidative insults in the pathophysiology of schizophrenia. Preclinical studies using animal models are fundamental in our understanding of disease development and pathology as well as the discovery and development of novel treatment options. In particular, social isolation rearing (SIR) and pre- or postnatal inflammation (PPNI) have shown great promise in mimicking the biobehavioral manifestations of schizophrenia. Furthermore, the "dual-hit" hypothesis of schizophrenia states that a first adverse event such as genetic predisposition or a prenatal insult renders an individual susceptible to develop the disease, while a second insult (e.g., postnatal inflammation, environmental adversity, or drug abuse) may be necessary to precipitate the full-blown syndrome. Animal models that emphasize the "dual-hit" hypothesis therefore provide valuable insight into understanding disease progression. In this Review, we will discuss SIR, PPNI, as well as possible "dual-hit" animal models within the context of the redox-immune-inflammatory hypothesis of schizophrenia, correlating such changes with the recognized monoamine and behavioral alterations of schizophrenia. Finally, based on these models, we will review new therapeutic options, especially those targeting immune-inflammatory and redox pathways.



KEYWORDS: *Redox-immune-inflammatory, social isolation rearing, inflammation models, "dual-hit" models, schizophrenia, monoamines*

1. INTRODUCTION

Schizophrenia literally translates as "shattered mind". It is among the world's top 10 causes of long-term disability, affecting about 24 million people worldwide.[1] Furthermore, the majority of schizophrenia patients do not receive treatment, which contributes to the chronicity of the illness, while 20% of patients experience a relapse despite being on antipsychotic medication.[1] Moreover, the main hurdle in treating schizophrenia is nonadherence to antipsychotic medication, with 50% of patients being noncompliant, that escalates after the onset of the disorder.[2] All these factors emphasize an urgent need for new treatment strategies.

Various hypotheses have been suggested to explain the underlying biological mechanisms of schizophrenia, such as the first dopamine (DA) hypothesis,[3] alterations in γ-aminobutyric acid (GABA) and glutamate function,[4] as well as oxidative stress[5] and inflammatory insults.[6] In terms of the etiology of schizophrenia, a strong link to genetic susceptibility has been suggested, and remains a contentious research issue.[7] Adverse events experienced in early life, whether physiological (poor nutrition, obstetric complications, and compromised fetal blood supply), pharmacological (maternal drug abuse and early use of psychoactive drugs), or psychological (maternal loss and separation, social isolation, and sexual and physical abuse), play a critical role in the development of schizophrenia.[8] Moreover, Weinberger[9] first formulated the "neurodevelopmental hypothesis of schizophrenia" stating that abnormalities of early brain development increases the risk for subsequent emergence of clinical symptoms. A number of clinical studies have confirmed the presence of inflammation in patients

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suffering from schizophrenia[10,11] with both prenatal and postnatal inflammation suggested to mediate a host of neurodevelopmental changes in the brain that have been regarded as prodromal events in the development of schizophrenia later in life.[12]

In line with the above-mentioned evidence, and with the aim of identifying potential new targets for drug treatment,[13] researchers have identified translational developmental animal models specific to schizophrenia, focusing on epidemiological risk factors (e.g., prenatal viral insult and social isolation rearing (SIR)). Recently, these models have provided much vaunted information pertaining to the neurobiology and treatment of schizophrenia and in particular have emphasized the causal role of inflammation in this illness as well as revealing new opportunities for treatment.

Prenatal and postnatal infection (PPNI) has received a great deal of interest in the past decade as a causative factor in the development of schizophrenia, as noted above, which has turned the focus to an immune-inflammatory basis (reviewed in ref 14). Studies have employed several methods to induce infection in animal models, including the influenza virus,[15] the synthetic viral polyriboinosinic-polyribocytidilic acid (Poly I:C),[16] the bacterial endotoxin lipopolysaccharide (LPS),[17,18] individual cytokine administration,[19] or local inflammation using turpentine.[20] Of these methods, Poly I:C and LPS seem to be the most widely used methods in studies of PPNI. Poly I:C is a synthetic analogue of double-stranded RNA that stimulates the production and release of a variety of pro-inflammatory cytokines after injection through binding to the toll-like receptor (TLR)-3.[21] LPS, on the other hand, is found in the cell membrane of Gram-negative bacteria (e.g., *Escherichia coli*) and thus mimics the immunological effects of Gram-negative infections.[20] LPS binds to TLR-4 on macrophages and other immune cells to trigger a signal transduction cascade leading to the activation of transcription factors such as nuclear factor kappa β NF-$\kappa\beta$, as well as genes coding for pro- and anti-inflammatory mediators such as cytokines, chemokines, and proteins of the complement system.[22] These models have also demonstrated behavioral and neurochemical equivalence with schizophrenia as well as reversal of these manifestations with antipsychotic treatment (see ref 6 for review). Because of the popularity of these methods, they will be the most covered in this Review. However, certain sections also contain referrals to results obtained using other models as well.

Postweaning SIR is regarded as a translational model of schizophrenia, and has been reviewed extensively.[23−25] Within a colony of rats, a strict social structure is at hand, allowing them to develop a hierarchy that plays a critical role in development and especially neurodevelopment.[13] This postnatal developmental model explores the effects of environmental insults via social deprivation on the developing brain after birth.[26] The SIR model presents with numerous behavioral and neurochemical manifestations akin to schizophrenia and that can be reversed using a wide range of antipsychotics,[13,23] thus confirming its validity for schizophrenia. Importantly, SIR has also revealed the presence of a pro-inflammatory state thus aligning itself with the earlier mentioned PPNI inflammatory models.[27,28] However, the aforementioned PPNI model and SIR are not necessarily exclusive. Indeed, the "dual-hit" hypothesis has been developed to describe increasing evidence that an environmental insult in an already (prenatally or postnatally) immune-compromised individual may translate into an even greater risk for developing schizophrenia.[29]

More heuristic models have explored the use of pharmaceuticals to induce schizophrenia-like behaviors in rodents. These models are aimed at understanding the developmental neuropathology of the disease, such as neonatal *N*-methyl-D-aspartate (NMDA) receptor (NMDA-R) antagonist administration (reviewed in ref 25). The glutamate hypofunction model of schizophrenia was first proposed over two decades ago, based upon the observation that phencyclidine (PCP), ketamine, and similarly acting psychotomimetic compounds (dizocilpine (MK-801)) induced their unique behavioral effects by blocking neurotransmission at NMDA glutamate receptors.[30] The ability of PCP or ketamine to induce schizophrenia-like psychosis has become well established in schizophrenia research,[31] although its value in the current Review will be its contribution toward the "dual-hit" hypothesis.

Earlier it was noted that schizophrenia may be subsequent to maternal drug abuse, or drug exposure. This propagated the idea that exposure to psychotogenic drugs such as methamphetamine (MA) may be useful to model schizophrenia in animals. This was first conceptualized after World War II when Japanese psychiatrists observed overlapping symptoms between the clinical manifestation of MA abuse and schizophrenia.[32] Considering the earlier mentioned "dual-hit" hypothesis,[29] since MA abuse occurs mostly in adolescents and in adolescents with a troubled childhood, combined MA plus SIR or PPNI models may reveal much on the interplay between MA as an environmentally relevant adverse event that may worsen the outcome in individuals who have suffered an early life (psychosocial or inflammatory) insult. However, a MA model of schizophrenia has not received a great deal of attention in recent years, and as such valuable data regarding this model is limited. In this paper, we will review the behavioral and neurochemical impact of MA administration in rodents as part of the "dual-hit" hypothesis, while also highlighting how this method may provide valuable insights into the psychopathology of schizophrenia.

While most of the above-mentioned animal models of schizophrenia have been extensively reviewed,[13,23,33] this Review will focus specifically on the role of inflammation and oxidative stress as biomarkers of schizophrenia, as presented in these models, and to discuss the relevance of these processes as novel drug targets. Moreover, despite the increasing evidence and support for a redox-immune-inflammatory basis for schizophrenia, it is imperative that altered neurotransmission be integrated into this theory, especially given the broad pharmacological and neuroimaging evidence that DA remains necessary and sufficient to explain the biobehavioral changes that characterize the illness.[34]

2. NEUROTRANSMITTER THEORIES OF SCHIZOPHRENIA

2.1. Monoamine Alterations in Schizophrenia. The oldest and most established hypothesis of schizophrenia proposes that DA hyperactivity in the striatum is responsible for positive symptom expression, while DA hypoactivity in the frontal cortex mediates the cognitive and negative symptoms observed in schizophrenia patients.[35] Post-mortem studies have indeed confirmed this hypothesis with findings of frontal cortical hypo-DAergia and striatal hyper-DAergia described in schizophrenia.[36] However, based on recent clinical findings of DA involvement in schizophrenia patients, it is speculated that

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multiple "'hits'" (i.e., adverse environment, infection, chronic substance abuse, etc.) interact in order to induce dysregulated DA transmission typical of the disorder.[37] Moreover, the effectiveness of especially conventional or typical antipsychotics, which block D_2 receptors, in ameliorating psychotic symptoms is also supportive of the hyper-DAergic hypothesis.[38] Furthermore, a clinical study has observed decreased plasma 3,4-dihydroxyphenylacetic acid (DOPAC) levels and decreased DA metabolic activity in relapsed patients with schizophrenia compared to first-episode patients, as well as decreased DA levels and increased DOPAC and DA transporters (DAT) in patients *after* risperidone treatment.[39] Interesting is that recent neuroimaging studies have indicated that DA transmission is altered even before psychotic symptoms are manifested.[40] Although the DA hypothesis has wide influence, a recent imaging study failed to find direct evidence for DAergic alterations in cortical and other extrastriatal regions in schizophrenia, thus warranting controlled studies in drug-naive patients to verify these findings.[41] However, the fact that D_2 receptor antagonist antipsychotics are ineffective in reversing cognitive and negative symptoms is an important shortcoming of the hyper-DAergic hypothesis of schizophrenia,[42] emphasizing that further research is needed. Although dysregulated DA is insufficient to explain the full complexity of schizophrenia, it offers a direct relationship to symptom severity and to treatment effectiveness.

The serotonin (5-HT) hypothesis of schizophrenia found its place following the evidence that a partial or full agonist at 5-HT receptors, such as lysergic acid diethylamine (LSD), produce hallucinations in patients, prompting the idea that 5-HT transmission might be increased in schizophrenia patients.[43] Subsequent cerebrospinal fluid, genetic and neuroimaging studies have confirmed this hypothesis with evidence of increased central 5-HT-ergic transmission in schizophrenia patients,[44,45] while increased striatal but diminished frontal cortical 5-HT uptake sites have also been described.[46] Furthermore, a post-mortem study observed decreased frontal cortical 5-HT_{2A} and increased 5-HT_{1A} receptor density in schizophrenia patients,[47] while other studies observed higher metabolic 5-HT activity in first-episode compared to relapsed schizophrenia patients.[39] Although positive symptoms of schizophrenia may be associated with an increase in 5-HT transmission in the striatum,[45] clinical studies have failed to describe the clinical efficacy of selective $5HT_{2A/C}$ antagonists in the treatment of schizophrenia-related psychosis.[48] On the other hand, novel antipsychotic agents that function as 5-$HT_{2A/C}$ antagonists (such as clozapine) appear to be superior to first generation "typical" antipsychotics (often referred to as neuroleptics) for treating negative symptoms and treatment-resistant schizophrenia,[49] thus clearly implying a causal role for 5-HT in at least the negative and cognitive symptoms of the disorder. Moreover, some antipsychotics, such as ziprasidone, are partial 5-HT_{1A} agonists that would similarly abrogate excessive 5-HT activity.[50] Activation of 5-HT_{1A} receptors also inhibits NMDA receptor-mediated currents in the prefrontal cortex to subsequently modulate the effect of glutamate on subcortical/striatal release of DA (see review by Schwartz et al.[51]). This emphasizes the role of 5-HT in indirectly controlling emotional and cognitive processes mediated by DA.[52,53] A recent review also observed that DAergic and 5-HTergic projections in the prefrontal cortex form a framework of complex neuronal interactions with glutamatergic projections that support cognitive function.[54] Importantly, these findings link the 5-HT and DA hypotheses with the GABA-glutamate hypothesis (discussed in subsection 2.2). Serotonin also exerts an inhibitory effect on frontal cortical DA and NA release,[55] and it has been suggested that frontal cortical hypo-DAergia in schizophrenia, and associated negative and cognitive symptoms, is reversed following blockade of $5HT_{2A/C}$ receptors.[50] A clinical study also observed a relationship between 5-HT_{1A} agonist binding in the frontal cortex (with ziprasidone) and the degree of improvement in negative symptoms.[56] Interestingly, alterations of 5-HT transporters are generally not associated with schizophrenia.[57] Although there are some controversies, the studies mentioned above generally support the role of hyper-5-HTergic activity in the pathophysiology of schizophrenia, especially in the frontal cortical brain area.

A decade ago Yamamoto and Hornykiewicz posited evidence that hyperactivity and hypoactivity of noradrenaline (NA) transmission are associated with the positive and negative symptoms of schizophrenia, respectively.[58] Increased NA transmission has also been associated with the anxiety symptoms of schizophrenia,[59] and a recent study also observed higher NA turnover rate in undifferentiated patients in comparison to paranoid schizophrenia patients.[39] However, the role of NA in schizophrenia pathophysiology is not clearly understood and further studies are needed to conclude the presence of any brain-region-specific alterations. It could be argued, however, that the hyper-5-HTergic activity in the frontal cortex, noted above, inhibits NA release resulting in negative and cognitive symptoms.

2.2. GABA-Glutamate Alterations in Schizophrenia. As noted earlier, the hypo-glutamatergic hypothesis of schizophrenia was pivotal in its support that NMDA receptor antagonists induce behavioral alterations similar to symptoms observed in schizophrenia.[30] Despite its emphasis on glutamate, the dysfunctional glutamate transmission hypothesis of schizophrenia integrates GABA transmission and in particular the glutamate−GABA−glutamate feedback loops as the basis for psychopathology. In fact, GABA−glutamate interactions are directly responsible for modulating ascending 5-HTergic, NAergic, and DAergic pathways that ultimately drive the numerous symptoms of schizophrenia.[60]

The glutamate−GABA−glutamate neurocircuitry of schizophrenia could be interpreted as explained below and illustrated in Figure 1 (reviewed in ref 51). The cortical brainstem glutamate projections are known as the corticocortical glutamatergic pathways and project from cortical pyramidal neurons in the prefrontal cortex to brainstem neurotransmitter centers to regulate neurotransmitter release, or descend from the prefrontal cortex to innervate the striatum and the nucleus accumbens. Thalamocortical glutamate pathways in turn project from the thalamus to innervate pyramidal neurons in the cortex. In schizophrenia, the cortical brainstem glutamate projection is compromised by suboptimal NMDA receptor activation. Normally a fully functioning primary prefrontal cortical glutamate neuron fires upon a GABA interneuron, but as a consequence of a poorly functioning NMDA receptor the GABA interneuron no longer fires adequately (hypo- or under-functioning GABA). This leads to a disinhibition of a secondary cortical glutamate neuron resulting in excessive glutamate-mediated activation of the mesolimbic DA pathway and the positive symptoms of schizophrenia. If the onset of negative symptoms is considered, again we have a dysfunctional NMDA receptor on cortical GABA interneurons culminating in the absence of sufficient GABA tone again resulting in an overactive

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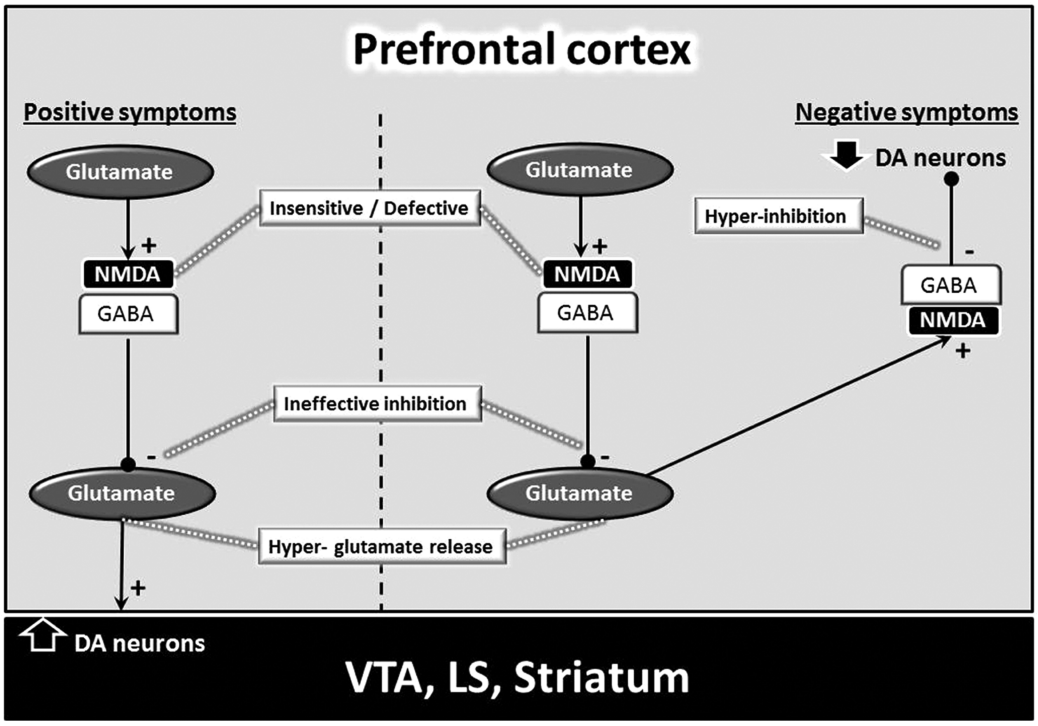

Figure 1. Simplified diagram depicting the neurocircuits involved in positive (left panel) and negative (right panel) symptoms observed in schizophrenia that arise due to neurodevelopmental abnormalities (Adapted from refs 51 and 40). DA activity in the midbrain is controlled by primary glutamate neurons in the PFC that activate NMDA receptors on inhibitory GABA interneurons. These GABA interneurons in turn synapse with secondary cortical glutamate neurons responsible for downstream neurotransmitter (DA, NA, 5HT) release in the VTA, LS, and striatum (left panel). In schizophrenia, PFC hypoglutamatergia will lead to excessive DA release in the VTA, LS, and striatum via the ineffective inhibition of the glutamate−GABA−glutamate loop, ultimately responsible for the positive symptoms (left panel). Regarding the negative symptoms of schizophrenia (right panel), as with the defect in the cortical glutamate−GABA−glutamate loop described for positive symptom development, the secondary glutamate neuron is again hyperactive (right panel). However, in this scenario, the secondary glutamate neuron impinges on another NMDA-GABA interneuron that connects with DA neurons in the PFC where the high glutamate tone will increases the release of GABA. The ensuing increase in GABA inhibits PFC DA release resulting in lowered DA mesocortical activity, eventually causing hypofrontality and negative symptoms (right panel). Abbreviations: dopamine (DA); prefrontal cortex (PFC); *N*-methyl-D-aspartate (NMDA); γ-amino-butyric acid (GABA), noradrenaline (NA); serotonin (5-HT); ventral tegmentum area (VTA); limbic system (LS).

secondary glutamate neuron. However, this secondary glutamatergic neuron impinges upon another GABA mediated interneuron responsible for regulating striatal DA release. This GABA interneuron is now overstimulated by glutamate to release more GABA that in turn inhibits the firing of the midbrain DA mesocortical pathway. The result is an underactive frontal cortical DA projection resulting in hypofrontality and negative symptoms (reviewed in refs 51 and 61); see Figure 1 for further explanation.

In line with the above hypothesis, numerous postmortem schizophrenia studies have observed deficits in glutamate systems in the temporal cortex, medial temporal lobe, and striatal regions (reviewed in ref 40), along with decreased glutamate uptake sites[62] and increased NMDA receptors in the same brain regions.[63] However, a recent clinical study indicated elevated GABA and glutamate levels in the medial prefrontal cortex of unmedicated patients, with no alterations in medicated schizophrenia patients, suggesting possible normalization of GABA and glutamate levels after antipsychotic treatment.[64] Moreover, deficits in GABA-parvalbumin (PV) containing neurons in the frontal cortex and hippocampus[65] and GABAergic uptake sites in the hippocampus have been observed in schizophrenia patients.[66] Proton magnetic resonance spectroscopy (^{1}H-MRS) is a technique that allows the assessment of the glutamatergic index as a measure of glutamate function in the brain (reviewed in refs 61 and 67). Interesting is that recent studies using ^{1}H-MRS in schizophrenia observed that unmedicated and medication-free patients present with elevated glutamatergic levels in the prefrontal cortex and basal ganglia (especially the striatum). A possible relationship was also observed between elevated glutamate and glutamine in the hippocampus and decreased hippocampal volume as compared to healthy controls (reviewed in refs 61 and 67). These studies are in contrast to the hypoglutamatergic hypothesis explained above. However, it could be debated that a hyperglutamatergic state brought about by the activation of neurotoxic oxidative stress processes (implicated in schizophrenia) underlie this observation (explained in subsection 3.2). It could also be theorized that both states (hyper- and hypoglutamate) exist, but at different glutamate neurons, where cortical hypoglutamatergia leads to hyperglutamatergia at a secondary interneuron (as explained above and illustrated in Figure 1). The presence of dysfunctional (hyper- or hypo-) glutamategic neurocircuitry has also been linked to numerous genetic alterations observed in schizophrenia patients, such as ionotropic glutamate receptor genes (GRIN1, GRIN2A, GRIN2B, and GRIK3), metabotropic glutamate receptor genes (GRM3), the G72/G30 locus, d-amino acid oxidase (DAO) and G72 (DAOA) genes, as well

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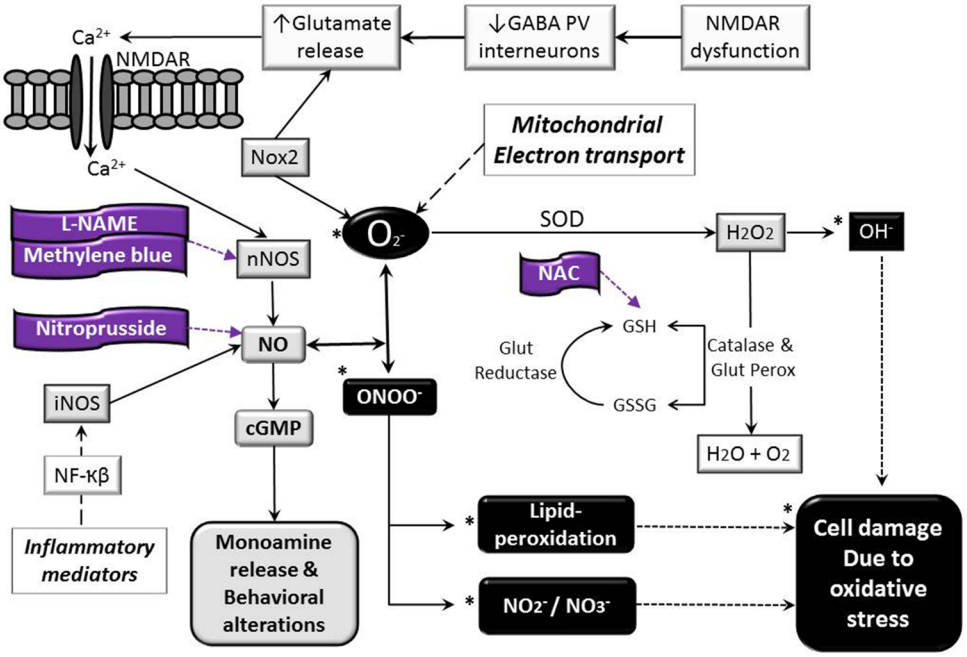

Figure 2. Activation of iNOS and nNOS and subsequent biological effects leading to changes in cellular redox status, monoamine release, and behavioral modulation (adapted from ref 149). Glutamate-directed activation of the NO-cGMP pathway via nNOS (Ca^{2+} dependent) will modulate monoamine release.[147] However, this pathway can also lead to oxidative stress and cell damage if excessive glutamate-mediated NO synthesis combines with O_2^- from aerobic metabolism via (Nox2)[149] to form $ONOO^-$. Also depicted is the effect of inflammatory mediators via NF-$\kappa\beta$, that promote Ca^{2+}-independent activation of iNOS leading to neurotoxic levels of NO being released thereby promoting the formation of cell-damaging reactive oxygen and nitrogen species. These pro-oxidative mechanisms can be abrogated by endogenous antioxidant systems such as SOD and GSH that act as a sink to quench excessive H_2O_2 and/or O_2^-. Furthermore, NMDAR dysfunction, observed in schizophrenia patients, leads to downregulation of GABA PV interneurons, which in turn causes an increase in glutamate release that over stimulates the NMDAR to increase oxidative stress mediators. Also depicted in this figure are L-NAME and methylene blue as nNOS inhibitors; nitroprusside as a NO donor; and NAC as a glutathione precursor, which will suppress activity or act as stimulants in the pathway. Abbreviations: nicotinamide adenosine dinucleotide phosphate (NADPH)-oxidase 2 (Nox2); superoxide (O_2^-); peroxynitrite ($ONOO^-$); cyclic guanosine monophosphate (cGMP); glutathione (GSH); reduced glutathione (GSSG); glutathione peroxidase (glut perox); inducible nitric oxide synthase (iNOS); nuclear factor kappa-beta (NF-$\kappa\beta$); neuronal nitric oxide synthase (nNOS); nitric oxide (NO); NMDA receptor (NMDAR); γ-aminobutyric acid parvalbumin (GABA PV); superoxide dismutase (SOD); L-NG-nitroarginine methyl ester (L-NAME); *N*-acetyl cysteine (NAC).

GABAergic genes (GAD1 and GABRB2) (reviewed in refs 68 and 69).

Another marker of glutamatergic neuronal function is *N*-acetyl aspartate (NAA), which is a precursor for the biosynthesis of *N*-acetylaspartylglutamate (NAAG), a neuropeptide with actions at NMDA and metabotropic glutamate receptors.[70] Decreased NAA has been observed in the temporal cortex, striatum, and hippocampus of schizophrenia patients,[65] which could be interpreted as decreased cortico-striatal glutamatergic function. Further support for glutamate dysfunction is provided by evidence for disturbances in the primary downstream messenger of the NMDA receptor, namely, the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) cascade.[71] The enzyme nitric oxide synthase (NOS) generates NO via two isoforms, namely, neuronal NOS (nNOS) which is Ca^{2+} dependent and inducible NOS (iNOS) which is Ca^{2+} independent (see Figure 2) (reviewed in ref 72). Neuronal NOS is activated following stimulation of the NMDA receptor, while the expression of iNOS forms part of the immune-inflammatory cascade, illustrated in Figure 2 (reviewed in refs 71 and 72). Indeed, patients with schizophrenia present with numerous NO-related alterations that will be discussed in subsection 3.2.

3. IMMUNE-INFLAMMATORY-REDOX ALTERATIONS IN SCHIZOPHRENIA

3.1. Mitochondrial Alterations. The mitochondria are cell organelles responsible for many vital processes in the growth and functioning of an organism, such as energy production, intracellular calcium buffering, generation of reactive oxygen species (ROS) and apoptosis.[73] When the mitochondria of a specific cell does not function optimally, it can lead to changes in neuronal functions and plasticity, giving way to behavioral and cognitive abnormalities such as those observed in schizophrenia.[74] In fact, factors involved in cellular oxidative stress are known to evoke monoaminergic changes that may underlie various psychiatric manifestations.[75] In clinical studies, an array of observations has been made regarding the role of mitochondrial dysfunction in the development of schizophrenia, including deoxyribonucleic acid (DNA) mutations, functional abnormalities, and faulty adenosine triphosphate (ATP) production and storage.[76] Moreover, when abnormalities in mitochondrial function exist, ROS production, such as an increase in superoxide levels (indicated in Figure 2), increases, resulting in an amplified risk for oxidative damage to lipids, proteins, and DNA.[76] Thus, a close relation exists between mitochondrial dysfunction and oxidative stress that is observed in schizophrenia, and discussed below.

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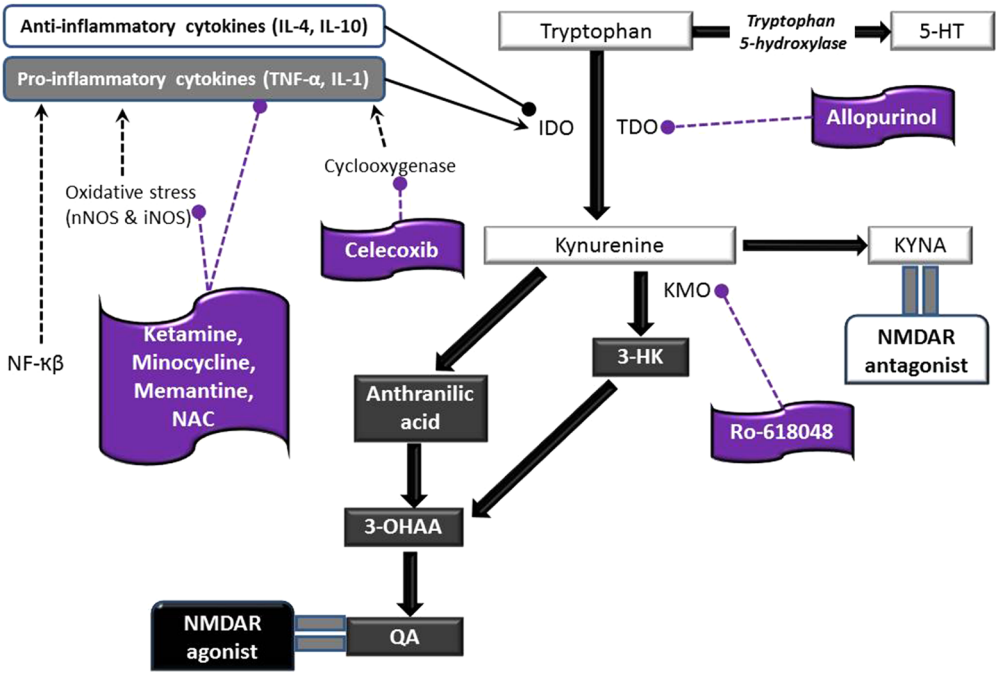

Figure 3. Simplified diagram of the kynurenine pathway, indicating the metabolism of tryptohan to either 5-HT or kynurenine via the principle enzymes, tryptophan 5-hydroxylase, IDO and TDO respectively. IDO and TDO are activated by proinflammatory cytokines or inhibited by anti-inflammatory cytokines (Adapted from refs 106 and 107). The metabolism of kynurenine then either follows a neuroprotective pathway with the synthesis of KYNA or a neurotoxic pathway with the synthesis of QA. Increased activity of the tryptophan-kynurenine synthesis (by TDO/IDO) will also diminish the availability of tryptophan for 5-HT synthesis via tryptophan-5-hydroxylase, with resulting effects on mood and behavior. Oxidative stress, such as activation of nNOS, iNOS, NF-$\kappa\beta$ (illustrated in Figure 2), and cyclooxygenase (COX) may all contribute to the synthesis of pro-inflammatory cytokines, which in turn contributes to the activation of IDO. Also depicted in this figure are ketamine, minocycline, memantine, and NAC as antioxidants and anti-inflammatory agents; celecoxib as an inhibitor of COX2; allopurinol as a TDO inhibitor; and RO-61848 as a KMO inhibitor, offering pharmacological tools with which to modulate these pathways. Abbreviations: tryptophan 2,3-dioxygenase (TDO); indoleamine-2,3-dioxygenase (IDO); kynurenine 3-monoxygenase (KMO); kynurenic acid (KYNA); 3-hydroxykynurenine (3-HK); quinolinic acid (QA); interleukin (IL)-1,10 and -4; 3-hydroxy anthranilic acid (3-OHAA); *N*-methyl-D-aspartate receptor (NMDAR); serotonin (5-HT); tumor necrosis factor (TNF)-α.

3.2. Redox Alterations. Oxidative and nitrosative stress occur when there is an imbalance between the overproduction of ROS and reactive nitrogen species (RNS) on the one hand and a deficiency of enzymatic (superoxide dismutase (SOD) and catalase) and nonenzymatic antioxidants (e.g., glutathione (GSH)) on the other, as illustrated in Figure 2.[77] The brain is particularly vulnerable to oxidative stress due to its high content of polyunsaturated fatty acids, high oxygen consumption, and low levels of antioxidant enzymes.[78] Moreover, significant evidence has accumulated that schizophrenia is associated with oxidative stress.[5] Glutathione provides endogenous protection against ROS and RNS,[79] while reduced GSH levels have been observed in the postmortem prefrontal cortex of patients with schizophrenia.[80,81] Another widely used marker of oxidative stress is the measurement of lipid peroxidation and 4-hydroxynonenal (4-HNE), a major product of lipid peroxidation, which has been found to be significantly increased in postmortem anterior cingulate brain sections of schizophrenia patients.[82] Furthermore, postmortem studies have described elevated levels of nNOS, responsible for the production of NO (see Figure 2), in the brain tissue of subjects with schizophrenia.[83] Although NO has important neuromodulatory effects when released via constitutive NOS (e.g., nNOS[84]), excessive release (either via over activation of NMDA receptors or induction of iNOS) will evoke nitrosative stress, especially in the presence of high ambient levels of superoxide (see Figure 2). Other studies observed increased NOS activity in the blood platelets of drug-naive schizophrenia patients, increased NOS proteins in the cerebellum and increased numbers of NOS-expressing brain stem neurons in schizophrenia (reviewed in ref 72). This revelation has fuelled exciting new research into the clinical utility of NO modulators in the treatment of schizophrenia (see subsection 3.2 for further discussion).

Research over the last two decades has also begun to link oxidative stress and deficits in GABA-expressing PV-interneuron signaling in the neocortex and hippocampus with schizophrenia pathophysiology (reviewed in ref 25). NMDA receptor dysfunction, as observed in schizophrenia patients, would result in the downregulation of GABA containing fast-spiking PV neurotransmission,[25] excessive glutamate release, and overt NMDA receptor activation, culminating in altered redox balance and subsequent oxidative stress (see Figure 2) (reviewed in ref 85). Plasma protein carbonyl levels is also a reliable biomarker of oxidative stress and has been found to be elevated in schizophrenia patients.[86] Interesting is that recent research has observed a deficit in total antioxidant status that is inversely associated with some cognitive abnormalities observed in schizophrenia patients.[87] In line with these findings, plasma SOD activity has been found to be negatively correlated with positive symptoms in first-episode schizophrenia patients.[88] Other studies in first-episode schizophrenia patients also found decreased levels of total antioxidant status, catalase, glutathione peroxidation, as well as a positive association between GSH levels and executive function.[89]

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Having described the evidence of redox dysfunction in schizophrenia, it is worth noting that said oxidative status in schizophrenia may also in part be influenced by certain environmental factors, especially cigarette smoking. The prevalence of cigarette smoking in patients with schizophrenia is significantly higher than that compared to the general population (reviewed in ref 90). Cigarette smoking contributes greatly to oxidative stress, especially if one considers that every gram of tar found in cigarette smoke contains 10^{15} free radical molecules, while it also provokes the release of ROS by inflammatory cells in the lungs.[91] Furthermore, cigarette smoking can also compromise endogenous antioxidant systems (reviewed in ref 92), rendering an organism susceptible to oxidative damage by free radicals. Schizophrenia also places tremendous stress on the psychosocial well-being of the patient, while stress in itself is a powerful pro-oxidant in both humans[93] and animals.[94]

The above-mentioned findings provide a clear picture of oxidative stress in schizophrenia and have led to the clinical utility of antioxidants as adjunctive treatment in schizophrenia, as will be discussed in due course (subsection 5.2). Importantly, evidence for oxidative stress is also clearly evident in the neurodevelopmental models under discussion (subsections 4.1.3, 4.2.3, and 4.3.3).

3.3. Immune-Inflammatory Pathways. In line with the genetic predisposition of schizophrenia, it has recently been observed that patients and their first-degree relatives present with elevated proinflammatory cytokines and decreased anti-inflammatory cytokines,[10] indicative of a proinflammatory state. First-episode schizophrenia patients also present with elevations in proinflammatory cytokines as well as a positive correlation between IL-6 (a proinflammatory cytokine) and the duration of illness.[11] In their 2008 meta-analysis, Potvin and colleagues provide a clear demonstration of the central role for cytokine profiles in schizophrenia.[95] Regarding the prominent place for the neurodevelopmental anomalies in schizophrenia, and indeed as applied in the SIR model, it is noteworthy that cytokines play a prominent role in the neurodevelopmental hypothesis due to their integral role in brain growth during fetal periods and thereafter (reviewed in ref 96). Indeed, preclinical studies have documented that circulating cytokines following a pathogen infection may enter the amniotic fluid and alter cytokine expression in the developing fetal brain.[97,98] Furthermore, the anti-inflammatory cytokine IL-10 was found to be significantly decreased in the late stages of schizophrenia while IL-6 was significantly increased in the early stages of schizophrenia.[99] Elevated CSF levels of another proinflammatory cytokine, IL-1, have been suggested as a marker of acute psychotic relapse in schizophrenia patients.[100] Important to note here is that the inflammatory abnormalities observed in schizophrenia do not seem to relate to antipsychotic treatment.[100] Moreover, a positive correlation between the severity of cognitive deficits and IL-1β, IL-6, and TNF-α levels has provided further evidence of the causal role of proinflammatory cytokines in schizophrenia pathology.[101] Furthermore, microglia activation is central to the release of proinflammatory cytokines and is a key event in the activation of the innate immune system in the brain.[102] Such a response has been implicated in the pathophysiology of schizophrenia[103] as well as during the acute phase of paranoid schizophrenia.[104] Previous postmortem studies have also observed activated microglia in the fronto-temporal regions of schizophrenia patients (reviewed in ref 105).

A very important link between the inflammatory cascades mentioned above and schizophrenia symptomology is tryptophan metabolism via the kynurenine pathway[106] (Figure 3). Tryptophan is responsible for the synthesis of 5-HT via tryptophan-5-hydroxylase or kynurenine via tryptophan 2,3-dioxygenase (TDO) or indoleamine-2,3-dioxygenase (IDO) conversion, respectively.[106] The metabolism of kynurenine can then lead to the formation of 3-hydroxy-kynurenine (3HK), 3-hydroxy-anthranilic acid (3-OHAA), anthranilic acid, and quinolinic acid (QA) respectively, which have neurotoxic effects, or to kynurenic acid (KYNA) which has neuroprotective properties (Figure 3).[107] These actions on neuronal integrity are due to the opposing actions of QA and KYNA on NMDA receptor activity. QA is a NMDA receptor agonist while KYNA has NMDA receptor antagonistic properties.[108] These opposing effects of KYNA and QA on NMDA receptors also link to the glutamate hypotheses discussed in subsection 2.2, implying that a dysfunctional neurocircuitry of glutamate activity in schizophrenia can be engendered by an initial inflammatory insult with the kynurenine pathway being the comediator. Furthermore, increased KYNA (NMDA receptor antagonist) or QA (NMDA receptor agonist) levels could be implicated in a hypoglutamatergic or a hyperglutamatergic state respectively, as observed in schizophrenia.[61,67] Postmortem schizophrenia studies have observed elevated levels of tryptophan, 3-OHAA, kynurenine and QA in various brain regions.[109] Previous studies have observed increased KYNA levels in the CSF of patients with schizophrenia[110] and in postmortem brain tissue of schizophrenia patients who had received antipsychotic treatment.[111] However, another study observed a significant reduction in plasma KYNA levels and a decrease in the neuroprotective ratio (a measure of the relationship between KYNA and kynurenine levels) in treatment-free schizophrenia patients.[112] These findings could indicate that antipsychotic treatment significantly improves the KYNA deficit observed in treatment-free schizophrenia patients. Moreover, schizophrenia patients also presented with increased levels of tryptophan in the CSF and plasma, which was independent of antipsychotic treatment.[113,114] The above-mentioned evidence could point toward a decrease in 5-HT synthesis, but with concomitantly increased neurotoxic metabolites synthesized from tryptophan (Figure 3), ultimately leading to deregulated neuro-circuitries and neuro-degeneration, as observed in schizophrenia.[115] These studies emphasize the importance of drug-free clinical studies to identify potential biomarkers, although data points toward a deficit in the neuroprotective KYNA metabolite, but which is amenable to treatment with an antipsychotic. Such a decrease in KYNA levels can also be linked to the hypo-glutamatergic state of schizophrenia (discussed in subsection 2.2).

Important enzymes in the kynurenine pathway that have been studied in psychiatric research are IDO and kynurenine 3-monoxygenase (KMO), IDO being responsible for the catabolism of tryptophan to kynurenine while KMO is responsible for the catabolism of kynurenine to 3HK (Figure 3).[116] It has been observed that IDO is generally inhibited by anti-inflammatory cytokines (such as IL-10) or activated by proinflammatory cytokines (such as IL-1 and TNF-α), which in turn reduces peripheral tryptophan availability for the synthesis of 5-HT, and increases the synthesis of the neurotoxic metabolites of kynurenine, viz. 3-OHAA and QA (see Figure 3).[108] Recent evidence suggests that an alternate mechanism, namely a reduction in KMO activity in the periphery and/or in

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the brain, may underlie the abnormalities observed in the kynurenine pathway in schizophrenia.[117,118]

4. NEURODEVELOPMENTAL ANIMAL MODELS OF SCHIZOPHRENIA: BIOLOGICAL AND BEHAVIORAL EQUIVALANCE

The neurodevelopmental hypothesis of schizophrenia was first formulated by Weinberger nearly three decades ago.[9] This hypothesis was based on (a) structural brain abnormalities observed at the onset of schizophrenia with no evidence for neurodegeneration in post-mortem studies; (b) the frequent occurrence at a young age of cognitive and motor abnormalities in patients who went on to develop schizophrenia later on; and (c) the idea that adult-onset disorders could have their origins in development, supported by studies in primates that showed that neonatal lesions can have delayed effects on behavior. Moreover, the abnormal developmental trajectory of the schizophrenia brain, which may be initiated and directed by environmental factors, appears to be established during gestation, long before clinical symptoms of the disease appear in early adult life.[119]

The neurodevelopmental hypothesis therefore suggests that brain development can be adversely affected at a critical time of life (pre- or postnatal), particularly through early life exposures to stress that may provoke the onset of psychosis in later adolescence or adulthood,[120,121,24] along with associated neurochemical and redox-immune-inflammatory changes as discussed above. Thus, in order to further evaluate the key neurodevelopmental factors in the pathogenesis of schizophrenia, and to highlight potential new targets for novel drug treatment, analogous neurodevelopmental animal models of schizophrenia are needed,[24] such as the SIR model, inflammation models and "dual-hit" models. This review will now discuss each of these models with regards to their effect on monoamines, GABA-glutamate pathways, redox-immune-inflammatory activation and behavioral alterations, respectively.

An essential aspect of a translational animal model is the behavioral changes evident in the model and how these correlate with that of the human disorder. Some of the relevant behavioral paradigms used in translational animal models of schizophrenia replicate the psychological presentation of cognitive dysfunction, as well as positive and negative symptoms of schizophrenia.[122,24,120] These behaviors can be assessed in animals using a specific array of behavioral tests, which include:

- social behavior (interactive, self-directed and aggression) relating to asociality symptoms,
- increased locomotor activity relating to hyper-activity or anxiety symptoms, as well as decreased locomotor activity possibly relating to the negative symptoms,[123]
- sensorimotor gating (prepulse inhibition (PPI)) relating to the inability to gate intero- and exteroceptive stimuli,
- novel object recognition test (nORT) that assesses cognitive memory,
- spatial learning memory, as assessed in the Morris water maze test,
- spatial learning and working memory, as assessed in the food alternating dry maze matching-to-position paradigm, Y-maze, T-maze and win-shift task in a radial eight arm-maze,
- depressive symptoms, as assessed in the forced swim test (FST) or sucrose consumption test,
- cognitive deficits, as assessed in the latent inhibition test (refers to retarded conditioning to a stimulus that has repeatedly been presented without reinforcement),
- anxiety-like behaviors, as assessed in the elevated plus maze (EPM) test,
- altered water homeostasis, as assessed using the schedule-induced polydipsia test (relating to excessive nonphysiologic drinking observed in schizophrenia), and
- contexual and conditioned associative learning deficits, as assessed in the conditioned emotion response task (relating to deficits in fear-motivated associative learning as observed in schizophrenia).

4.1. Social Isolation Rearing (SIR).

4.1.1. Effect on Monoamines. SIR decreases DA, DOPAC, and homovanillic acid (HVA) levels in the prefrontal cortex.[28,124,125] However, this is contradictory to other SIR studies[126,127] that have observed increased DA levels in this brain region. Previous studies have observed elevated DA, DOPAC and HVA levels in the striatum and nucleus accumbens (NAcc) following SIR,[28,125,127] no change in striatal DA levels,[124] and increased DA turnover in the amgydala and NAcc.[128,129] Moreover, a recent study observed that SIR in juvenile postnatal day (PND) 77 and adult (PND 181) rats increased DA release as well as increased DAT in the NAcc.[130] Increased DA release has also been observed in the prefrontal cortex of SIR rats after systemic administration of the atypical antipsychotics clozapine and olanzapine, but not the typical antipsychotic, haloperidol.[131] A similar differential response following clozapine and haloperidol was also observed in SIR rats with respect to frontal cortical D_1 receptor binding.[132] The latter two studies provide a possible explanation how atypical but not typical antipsychotics reverse the frontal cortical DA deficits observed in schizophrenia, thereby explaining the former presumed superior efficacy in treating negative symptoms.[133] Other results regarding DA receptor density in the SIR model include down-regulation of D_2 in the striatum,[129] and no change in D_2 receptor density in the mesolimbic,[134] hippocampal and prefrontal cortex or amygdala areas.[135] The general evidence discussed here suggests increased DA (and related metabolites) levels in the NAcc and striatum areas following SIR, whereas evidence in prefrontal cortical areas is varied and often contradictory. Inconsistencies also prevail with regard to DA receptor alterations in this model, also noted in clinical studies.[37] These conflicting findings in animal studies and the fact that imaging studies in clinical studies failed to show frontal cortical hypo-DAergia and striatal hyper-DAergia, emphasizes the need for more preclinical and drug-free clinical studies.

The inconsistencies observed using the SIR model could possibly be explained by the differences in SIR protocols used. A different SIR protocol, especially the commencement and duration of isolation, could significantly affect the release of several stress-related hormones, such as glucocorticoids, implicated in most of the stress-induced physiological changes in the brain (reviewed in 136), and in this manner differentially affect DA release. Another consideration is using the same animals for behavioral and neurochemical analysis as opposed to using different cohorts for both. For example, Han et al.[127] used the same animals, while Möller et al.[28] used separate animals for behavioral (social interaction test) and neurochemical analysis, which may underlie their differences in monoamine findings. Previous studies have indeed found that rodents subjected to social aggression (as observed in Han et

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al.[127]) developed increased anxiety, ultimately increasing glucocorticoid release and activation of the DA system,[137] whereas Möller et al.[28] observed significantly decreased social contact and interaction in the SIR rats (discussed in subsection 4.1.4). The housing condition of animals is also very important, as a previous study observed a significant positive correlation between plasma corticosterone levels and DA in the left NAcc of SIR rats housed in grid-floor cages (used by ref 127) compared to SIR rats housed in sawdust cages (used in ref 28).[128] Heidbreder et al. also observed a decreased basal turnover of DA in the medial prefrontal cortex of SIR rats in grid-floor cages compared to SIR rats in sawdust cages.[128] The nature and extent of corticosterone release following the isolation procedure may modulate physiological monoamine levels by altering 5-HT neurotransmission in the brain.[138] Corticosterone also increases NA transporters and DA hydroxylase (the enzyme that catalyzes DA to NA).[136]

Another consideration is the role of oxidative stress and its effect on monoamine release.[75] Different SIR protocols have been found to differently affect oxidative stress parameters (as discussed in section 4.1.3) that could in turn affect DA release in a different manner. Indeed, DA dysfunction has been related to oxidative stress in SIR[27] and schizophrenia.[139] Thus, evaluating oxidative stress and glucocorticoid-induced modulation on neural systems may further clarify the differences observed in monoamine levels in the different SIR protocols of Han et al.[127] and Möller et al.[28] A final note of caution comes from a study by Trabace and colleagues[124] who highlighted differences in monoamine profiles in two rat strains in response to SIR, and who then conclude that strain-related monoamine differences in response to SIR may affect the ability of the model to replicate the biobehavioral changes typical of schizophrenia. Concluding, due consideration for applying the same SIR protocol with consistency across all studies is key to obtaining reproducible results.

With regard to 5-HT levels in the SIR model, the following has been observed: decreased 5-HT and 5-HIAA levels[140] as well as decreased 5-HT/5-HIAA[124] in the prefrontal cortex and increased 5-HT and 5-HIAA levels[140] as well as increased 5-HT turnover[127] in the striatum and NAcc. Conflicting results in other studies observed increased 5-HT levels;[127] and no effect[126] on 5-HT/5-HIAA in the prefrontal cortex and decreased[128] 5-HT turnover in the NAcc of SIR rats. The picture of 5-HT and 5-HIAA levels after SIR is therefore clear with regards to the findings in the striatum (increased 5-HT) and relates to increased 5-HT transmission observed in schizophrenia (section 2.1). However, data are varied when it comes to findings in the prefrontal cortex. Again, the conflicting results found in the prefrontal cortex could be due to different SIR protocols (same vs different animals used for behavioral and neurochemical analysis respectively; different cage conditions) that could possibly affect oxidative stress and glucocorticoid-induced monoamine regulation, as mentioned above. However, hyper-cortical 5-HT levels could be responsible for the inhibition of DA and NA release in the frontal cortex, also explaining the efficacy of 5-$HT_{2A/C}$ antagonists in treating the negative symptoms observed in schizophrenia (as discussed in subsection 2.1).

Studies determining NA levels, metabolism, and function in the SIR model are generally rare. However, some very early studies on the SIR model observed increased NA turnover in the hippocampus, cerebellum, and cortex as well as enhanced hippocampal presynaptic α_2-adrenoceptor function (reviewed in ref 120). Additionally, a recent study in our laboratory observed elevated frontal cortical NA levels, elevated striatal NA and MHPG levels, and decreased frontal cortical MHPG levels following SIR.[140] The elevation in cortico-striatal NA could possibly be linked to the positive and anxiety symptomatology of schizophrenia (reviewed in ref 140). Furthermore, add-on therapy with α-2 adrenoceptor antagonists significantly improves not only the positive symptoms of schizophrenia but also the negative symptoms,[141] emphasizing the role of NA modulation in schizophrenia.

4.1.2. Effect on GABA-Glutamate. Reduced glutamate function has been suggested to underlie the deficits observed in the nORT in SIR animals[142] (explained in subsection 4.1.4). SIR in rats also decreases glutamate and glutamine levels in the hippocampus,[143] increases frontal cortical NMDA receptor binding[132] and reduces glutamate receptors subunits GluR1 and NR1 in the prefrontal cortex.[144] Moreover, SIR decreases GABA PV- immunoreactivity (IR) in the hippocampus and prefrontal cortex.[145,146] The latter observation linked to the downregulation of cortical GABA-PV neurotransmission described in schizophrenia[25] as well as to NMDA receptor dysfunction[31] and excessive glutamate release[147] (see Figure 2 and discussion in subsection 2.2). Altered cortical NMDA receptor density seems to also show a differential response to typical vs atypical antipsychotics.[132] Decreased NAA levels have been observed in the temporal cortex of SIR rats, in line with clinical findings in schizophrenia patients[65] and indicative of decreased glutamate function.

4.1.3. Effect on Redox-Immune-Inflammatory Processes. A study from our own laboratory observed mitochondrial dysfunction in SIR animals, evident by increased striatal ATP, together with decreased ATP in the frontal cortex.[28] These changes were also fully reversed by clozapine.[28] Importantly, Ben-Shachar[74] suggested a vital link between mitochondrial alterations and DA disruptions, and supported by decreased frontal cortical DA levels in our SIR study,[28] thus providing convincing evidence of mitochondrial dysfunction in schizophrenia pathology. Another important consequence of mitochondrial dysfunction and altered mitochondrial electron transport is oxidative stress via increased release of superoxide (see Figure 2).

Nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase 2 (Nox2) generates superoxide and is a major source of ROS (see Figure 2) as well as plays a role in controlling glutamate release in the prefrontal cortex[148] (reviewed in ref 149; see Figure 2). Moreover, elevations in Nox2 are observed in the prefrontal cortex of rats exposed to SIR.[146] Another critical mechanism of oxidative stress is the down-regulation of cortical GABA-PV neurotransmission,[25] which in patients with schizophrenia appears to be attributable to the NMDA receptor dysfunction,[31] leading to excessive glutamate release and subsequent cortical excitation and oxidative stress[147] (see Figure 2 and discussion in subsection 3.2). Interestingly, SIR decreased GABA PV- IR in the hippocampus[145] and in the prefrontal cortex, as noted earlier, while blockade of Nox2 abolishes these effects.[146] SIR also increases SOD activity, decreases oxidized:reduced ratio of GSH, and increases lipid peroxidation in both the striatum and frontal cortex of SIR rats.[27] Of particular significance is that the latter effects are reversed by clozapine treatment.[27] Another study described down-regulated GSH, decreased SOD activity, induced NF-$\kappa\beta$ (a major transcription factor for the expression of genes involved in the inflammatory responses) and up-regulated

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Table 1. Dopamine Alterations in Pre- and Postnatal Studies of Inflammation, in the Striatum, Frontal Cortex, NAcc, and Other Brain Tissue[a]

prenatal immunogens	striatum	frontal cortex	NAcc	other
LPS[78,166,168,172,176−179]	↑↓DA ↓ HVA ↑↓DOPAC	↓ DA ↓DOPAC ↑HVA ↓D2-R function	↑DA	↓DA ↑HVA
Poly I:C[164,177,180,181]	↑↓D2-R function ↑D1-R function ↑TH	↑D2-R function ↑TH	↑D2-R function ↑D1-R function	↑DAT
cytokines[182]		↓D2-R function ↓D1-R function	↓DA ↑DOPAC	
turpentine[183,184]			↑DA ↑DOPAC ↑HVA ↑TH	

[a]Arrows: ↑ = increase, ↓ = decrease, ↔ = no change.

expression of iNOS in prefrontal cortex of rats exposed to SIR,[150] thus linking associated redox changes to the immune-inflammation cascade (see Figure 2). The activation of the (immunological) iNOS cascade is known to follow stressful conditions[94] and to involve the activation of NF-$\kappa\beta$ signaling,[151] therefore affirming how environmental adversity will have immunological consequences. The literature is however conflicting with regards to changes in SOD. Thus, Möller et al.[27] observed increased SOD while Zlatković and Filipović[150] observed decreased SOD. These variations could be due to different SIR protocols and rat strains used (e.g., 8 weeks SIR and Sprague−Dawley rats[27] 3 weeks SIR and Wistar rats[150]). Importantly, 8 weeks of SIR can be considered a chronic stressor, known to deplete SOD and to engender a pro-oxidative state,[152] while 3 weeks of SIR is more a subacute stressor that *increases* SOD release in an attempt to curb excessive production of ROS.[153] As noted earlier, oxidative stress may have profound effects on monoamine release, especially DA (reviewed in ref 154). Increased DA neurotransmission in turn can induce oxidative stress,[155] creating a positive feedback loop with escalating severity.

Elevated proinflammatory cytokines (TNF-α, IFN-γ) and decreased anti-inflammatory (IL-4) and dual-action (IL-6) cytokines has been described in the SIR model, and which are for the most part reversed by clozapine treatment.[28] Furthermore, the SIR model also presents with elevated plasma tryptophan, kynurenine, anthranilic acid, 3-OHAA and QA with reduced KYNA and neuroprotective ratio, with all alterations reversed with clozapine.[156] Considering the inter-relationship between tryptophan and kynurenine synthesis, these changes can be expected to have marked effects on behavior, as will be discussed shortly. The above redox-immune findings raise new hope for using mitochondrial regenerators, such as N-acetyl cysteine (NAC) and methylene blue, in the treatment of schizophrenia, while the central role for inflammation and kynurenine metabolism hints of the possible clinical utility of kynurenine modulators and antioxidants in the treatment of schizophrenia (see subsection 5.2).

4.1.4. Behavioral Manifestations of SIR. The SIR model produces a number of deficits in behavioral paradigms convergent with schizophrenia, including sensory-motor gating, object recognition memory in the nORT (reviewed in refs 27, 28, 120, and 130), anxiety-like behavior in the EPM[130] and depressive-like behavior in the FST.[157] In most instances, these deficits are reversed by antipsychotic treatment.[27,28] With regard to social interactive behaviors, previous studies observed a significant decrease in social behavior and an increase in self-directed behavior after SIR that could be reversed with antipsychotic treatment.[28,157] However, Wongwitdecha and Marsden[158] observed a significant increase in aggressive behavior in SIR rats, as did Koike et al.[159] in SIR mice. Another study in female SIR rats also observed that SIR produced marked deficits in social interactive behaviors as well as increased anxiety-like behavior in the open-field test.[144] Important is that increased playful fighting (aggressive behavior) and social contact behaviors has been observed in SIR rats,[127] in contrast to the decreased social interaction observed by Möller and co-workers.[27,28] However, the latter study simultaneously confirmed the presence of other behaviors congruent with schizophrenia, as well as presenting neurochemical data in line with frontal cortical hypo-DAergia and striatal hyper-DAergia.[28] These social interaction studies also emphasize the importance of the type of social interactive behaviors measured during the assessment of sociality. SIR also produces significant hyper-locomotor activity that is reversed by antipsychotic treatment[27,28,120] as well as disrupted latent inhibition in associative learning.[160] Interestingly, SIR also increases the self-administration of ethanol in rats,[161] which is similar to an increased risk for developing ethanol addiction in people exposed to early life stress,[162] and increases polydipsia behaviors (under food restrictions).[163]

4.2. Inflammation Models. *4.2.1. Effect on Monoamines.* Many inflammation-based models have observed alterations in DA function and metabolism. These studies predominantly made use of prenatal immunogen treatment methods, considering that changes in fetal dopaminergic development were observed after maternal immune activation.[164] The studies reviewed here have incorporated varying immunogens with fluctuating results, and as such these outcomes are summarized in Table 1. The table includes effects on DA and DA-receptors (DA-R), as well as other markers, such as tyrosine hydroxylase (TH), the rate-limiting enzyme for DA synthesis,[165] DOPAC, HVA, and DAT. Only a few studies measured DA changes after postnatal immune challenge: one using LPS observed reduced DA in the striatum after treatment,[166] while the other found that direct cytokine administration decreased DA and increased DOPAC levels in the NAcc.[167] Table 1 reveals a great deal of controversy with regards to striatal and NAcc DA levels, as

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determined in the PPNI models. Nevertheless, studies describe a clear deficit in frontal cortical DA and DA metabolite levels, thus congruent with the hypo-DAergic hypothesis of schizophrenia. However, decreased levels of DA in the striatum, as opposed to increased levels as proposed by the DA hypothesis, are paradoxical, although may be explained by a loss of DA neurons following an inflammatory insult.[17] It has also been suggested that disruptions in DA function in different brain regions may fluctuate with the use of specific immunogens.[168] Clearly, further studies are needed to confirm these findings.

Prenatal immune exposure to Poly I:C and LPS significantly increased 5-HT and 5-HIAA in certain brain regions of offspring at PND 80 (adulthood), compared to controls.[169,170] Elevated levels of 5-HIAA was also observed in the hippocampus and frontal cortex of prenatal Poly I:C challenged offspring,[171] while increased 5-HT levels were observed in the frontal cortex,[172] amygdala, hippocampus, and hypothalamus[78] of LPS-treated offspring. This hyper-5-HTergic state in the frontal cortex directly relates to the hypothesis that elevated levels of 5-HT decreases the release of specifically DA in the frontal cortex of patients with schizophrenia, leading to hypo-dopaminergia and hypo-frontality. Postnatal administration of LPS was also found to increase 5-HT and 5-HIAA in the hippocampus.[173] In the striatum no changes in either 5-HT or 5-HIAA levels were observed after prenatal LPS exposure.[172] However, prenatal influenza administration led to a marked reduction in 5-HT in the cerebellum[174] and a significant increase in the density of 5-HT_{2A} receptors (but not 5-HT_{2C}) in the frontal cortex of the offspring.[123] Furthermore, direct administration of either IL-1β or IL-6 enhanced 5-HT turnover in the prefrontal cortex and hippocampus.[175] However, another study that administered cytokines to adult rodents observed that IL-1α administration increased, but IL-2 administration decreased 5-HIAA levels in the NAcc, suggesting diverse effects of different cytokines on 5-HT levels during adulthood.[167] As mentioned earlier, the evidence in PPNI models for 5-HT involvement in the frontal cortex seems to relate directly to the cortical hyper-5-HTergic theory of schizophrenia. However, 5-HTergic alterations induced by PPNI in the NAcc and striatal regions seem to be dependent on the type of inflammation insult and do not relate to the state of hyper-5HTergic transmission observed in schizophrenia patients.

Postnatal administration of LPS to rats significantly increased NA and MHPG in the hippocampus,[173] as well as increased NA-levels in the nucleus of the tractus solitarii (NTS) and the locus coeruleus (LC).[170] Direct IL-1β administration also enhanced NA turnover in the hypothalamus and hippocampus, while IL-2 only had a similar effect in the hypothalamus.[175] Unfortunately no evidence for PPNI induced frontal cortical changes in NA have been described in the literature, although the above-mentioned hippocampal, NTS and LC findings could relate to the hyper- NAergic hypothesis of schizophrenia responsible for the presentation of positive symptoms.

4.2.2. Effect on GABA-Glutamate. Because abnormalities in GABA function have been implicated in the pathology of schizophrenia,[185] it is considered an important biomarker in animal studies of inflammation. Prenatal Poly I:C challenge reduced GABA levels in the hippocampus of female offspring.[169] Furthermore, glutamic acid decarboxylase 67 (GAD67), the enzyme responsible for converting glutamate to GABA and that serves as a marker for GABAergic neurons,[186] was significantly decreased in the hippocampus of offspring exposed to prenatal LPS administration.[187] A reduction in GAD67 was also observed in the prefrontal cortex of prenatal Poly I:C-challenged rodents.[188] In contrast, prenatal LPS and Poly I:C administration significantly increased GAD67 in the hippocampus of the offspring.[186] These contradictory findings could be due to the different gender and age of rats used as well as the different brain regions analyzed in these studies (viz., Boksa[189] and Richetto et al.[188]) while Harvey and Boksa[186] showed that changes in GAD67 levels can also vary between bacterial and viral immune challenges. Prenatal Poly I:C administration in mice also reduced PV interneurons in the medial prefrontal cortex, and up-regulated $GABA_A$ receptor subunit a_2 expression in the ventral hippocampus of offspring.[190] The latter increase in the $GABA_A$ receptor subunit expression was also observed in the ventral dentate gyrus and amygdala of prenatal Poly I:C treated offspring.[191]

It has frequently been reported that prenatal exposure to an immune challenge induces abnormalities in the glutamate system, especially data supportive of the NMDA glutamate hypo-function hypothesis of schizophrenia. Consequently, prenatal Poly I:C exposure decreased levels of glutamate in the hippocampus and the prefrontal cortex of rodents.[169] In contrast, neonatal Poly I:C treatment in mice results in elevated levels of glutamate in the hippocampus, while K^+-induced glutamate release in this brain region was significantly diminished compared to that of saline-treated animals.[192] A possible explanation for this result is that activity-dependent changes in extracellular glutamate levels are inhibited in the hippocampus of immune-challenged rodents, leading to a poorer signal/noise ratio in glutamatergic neurotransmission in adulthood.[192] Importantly, this dysfunction of glutamatergic neurotransmission in the hippocampus may be related to the cognitive deficits seen in schizophrenia.[193] Furthermore, the NMDA glutamate hypo-function hypothesis of schizophrenia predicts that reduced NMDA-subtype glutamate receptor activity in the hippocampus will give way to decreased stimulation of GABAergic inhibitory neurons, leading to elevated glutamate release in the prefrontal cortex.[194] Accordingly, researchers have observed that extracellular basal glutamate are increased in the prefrontal cortex of Poly I:C offspring, and that this effect was successfully reversed after both risperidone and paliperidone treatment.[195] Different studies of prenatal inflammation showed decreased levels of the NMDA- and glutamate-receptor subunits, GluN1,[196,21] NR1,[190] and mGlu2.[123] Two studies incorporating LPS as the prenatal immunogen observed that the NMDA/AMPA ratios[196,197] were significantly decreased in the hippocampus of offspring. These ratios are important measures of glutamate synapses and a decrease in these ratios as mentioned above directly relates to a hypo-glutamaterigc hypothesis of schizophrenia. Prenatal LPS administration has also been found to impair NMDA synaptic currents and long-term potentiation in the hippocampus of offspring.[198] This supports the findings that decreased NMDA receptor expression leads to behavioral alterations akin to that observed in schizophrenia.[199]

4.2.3. Effect on Redox-Immune-Inflammatory Processes. A study using Poly I:C to induce inflammation during gestation observed that ATP production in spleen mitochondria of offspring was significantly reduced.[200] Numerous studies on the offspring of LPS-challenged rats (pre- or postnatal) demonstrate a significant and delayed decrease in hippocampal GSH,[166,198,201−203] as well as an increase in oxidized glutathione (GSSG).[166,198,203] Interestingly, administration of

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Table 2. Cytokines Found to Be Elevated in Pre- and Post Natal Studies of Inflammation, in Different Plasma and Tissue Samples

pre/postnatal insult	maternal plasma	amniotic fluid and/or placenta	fetal/offspring plasma	fetal brain	adult brain (postnatal treatment)	adult plasma (postnatal treatment)
LPS[97,98,166,178,204,205,208,209,214,216−232]	IL-6, IL-1β, TNF-α	IL-6, IL-1β, TNF-α	IL-6, TNF-α, IL-2	IL-6, IL-1β	IL-6, IL-1β, IL-1, TNF-α, IFN-γ, IL-2	IL-6, TNF-α, IFN-γ, IL-10
Poly I:C[97,171,192,207,210,211,233−235]	IL 1β, TNF-α, IL-6	IL-6, IL-1β, TNF-α	IL-6, IL-2, IL-5, TNF-α, IL-3, IL-12, IL-1β, IFN-γ	IL-1β, IL-10, I L-12, IFN-γ, IL-6	IL-6, IL-1β, TNF-α	IL-6
influenza no data						
turpentine[183]	IL-6	IL-6				

clozapine was unable to reverse these alterations in GSH-levels after postnatal treatment.[202] However, considering clozapine has been effective in reducing redox related changes in other postnatal models, such as SIR,[27] these findings are of interest and might point toward the specific period of development when clozapine may offer therapeutic benefit. Numerous postnatal inflammation models also present with increased lipid peroxidation when compared to controls,[202−204] while elevated levels of malondialdehyde (MDA) (a marker of lipid peroxidation) in a postnatal, acute inflammation model has been found to be successfully reduced by earlier administration (30 min before the LPS adiminstration) of risperidone.[204] Still other authors could not reverse these changes with clozapine,[202] illustrating a controversy in the predictive validity of these models. In a prenatal model of inflammation, LPS administration led to increased lipid peroxidation especially in the midbrain of aging offspring.[203]

Postnatal inflammation studies also showed increased levels of iNOS in the hippocampus, striatum and prefrontal cortex following Poly I:C,[202] with similar results noted in the hippocampus[205] and cerebral cortex following LPS challenge.[204] Ribeiro et al.[202] were able to reverse these changes with clozapine. Using this same model, MacDowell et al.[204] successfully used risperidone to reduce levels of oxidative stress. Furthermore, mice exposed to prenatal PolyI:C had decreased GABA-PV-IR in the prefrontal cortex,[190] with neonatal LPS exposure also able to decrease PV-IR interneurons in the hippocampus of adult rats.[206] Following LPS injection during gestation, male fetuses exhibited increased protein carbonyl levels and reduced levels of α-tocopherol.[198]

Cytokine analyses after immunogen treatment have revealed an array of outcomes. Table 2 summarizes the various cytokines that have been found to be increased in different tissues and plasma following a specific immunogen (LPS being the most studied). From this data, IL-6 seems to play a significant role in the PPNI model, while elevated levels of cytokines such as IL-1β, IFN-γ and TNF-α have also been observed frequently. Of these studies, a few have demonstrated successful reversal with antipsychotic treatment, such as clozapine, olanzapine[207] and risperidone.[204,207] Although the data for the different cytokines remain inconsistent between these studies, possibly due to the different immunogens used, various treatment regimens (pre- or postnatal) and varying times of analyses, it is clear that each of these cytokines play an important role in an inflammatory animal model of schizophrenia. Also worth noting is that studies using a prenatal immune challenge with either a systemic endotoxin or viral mimic vs an inducer of local inflammation suggests that maternal infection may affect fetal neurodevelopment through circulating cytokines and/or fever, rather than via direct effects of the infectious agent on the fetus.[20] Moreover, the nature of the infectious agent may also illicit different cytokine responses.[208]

Interestingly, Bison et al.[209] observed increased levels of IL-10 in the plasma of adult rats after a LPS challenge. IL-10, an anti-inflammatory cytokine, may have been released as a physiological protective measure to counteract inflammatory damage. This was also an acute study with LPS administered to adult rats, which could be responsible for the acute activation of the inflammatory response and release of IL-10.[209] Other studies, however, have shown contrasting results, namely, PPNI studies have revealed a *decrease* in IL-10 levels in the fetal central nervous system,[210] placenta,[211] maternal serum,[98,212] and the plasma of adult-rats exposed to an immunogen,[207] pointing to a decreased anti-inflammatory capacity after immunogen treatment. The latter are similar findings to that described in late stage schizophrenia (discussed in subsection 3.3). Furthermore, Sugino et al.[207] showed that olanzapine, clozapine, and risperidone reversed decreased levels of IL-10 after an immune challenge, suggesting therapeutic anti-inflammatory activity. The apparent paradoxical findings with IL-10 indicate how important time of sampling is post PPNI, where earlier sampling will display an immune response that is challenged yet coping, while later analysis may reveal an immune system under severe pressure, with evidence of maladaptation and a developing pro-oxidative state.

Numerous studies of prenatal inflammation have demonstrated increased microglia or activated microglia.[105,213] However, this body of evidence too is controversial, with opposing findings noted in the literature.[210,214] In response to an unexpected finding of decreased microglia activation after prenatal LPS challenge, Cai and colleagues suggested that prenatal LPS may result in altered immunoreactivity of microglia.[214] In terms of postnatal immune challenges, several studies observed increased microglia activation,[171,182,202,215] which could be successfully reversed with clozapine.[202] LPS was also able to increase cyclooxygenase (COX)-2 (see subsection 5.4) protein and mRNA in male adult rats, which was also successfully prevented by preadministration of risperidone.[204]

In evaluating the kynurenine pathway, it was observed that prenatal Poly I:C resulted in decreased serum levels of the neuroprotective metabolites, kynurenine and KYNA and elevated levels of the neurotoxic metabolite, QA.[236] An interesting finding of this study was that the KYNA/kynurenine ratio (neuroprotective ratio) was unaltered,[236] which is in contrast to findings following SIR (subsection 4.1.3), as well as in patients with schizophrenia (subsection 3.3). This phenomenon could be clarified by a possible physiological attempt to compensate for the increased levels of neuro-degenerative QA at this developmental stage. The authors also argue that an age-dependent shift in the balance of the

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kynurenine pathway could be due to developmentally specific changes in NMDAR function in the Poly I:C model.[236] Another study observed that postnatal Poly I:C led to a marked increase in IL-6, TNF-α and IL-1β, three cytokines known for their ability to induce IDO.[171] Elevated IDO was confirmed in the hippocampus and frontal cortex of postnatal Poly I:C immune-challenged rodents.[171] A number of other studies have also shown increased IDO after immune activation using either Poly I:C or LPS in adult rodents.[219,228] Connor et al. furthermore confirmed increased KMO mRNA, the enzyme responsible for the degradation of kynurenine into 3HK (see Figure 3), in the cortex and hippocampus of rats 24 h after LPS administration.[219]

4.2.4. Behavioral Manifestations of Inflammation Models. Increased locomotor activity was observed in rats that received influenza A at neonatal age,[235] while a number of studies[190,237] have demonstrated reversal of said hyperlocomotor activity with clozapine and risperidone. Similarly, prenatal administration of LPS to rodents led to increased locomotor activity in offspring at different postnatal ages.[238] However, a recent study observed that prenatal infection with Poly I:C reduced locomotor activity in the offspring, without any preceding drug administration,[239] while decreased locomotor activity was also evident after postnatal administration of either IL-2 or the influenza virus to rodents.[19,123] The latter observations are in contrast not only to a number of preclinical studies noted above and elsewhere,[172,240,241] but also with clinical studies where agitation and hyper-activity is observed in certain patients with schizophrenia.[122] However, it should be considered that such a decrease in locomotor activity is a general feature of sickness behavior induced by a pathogen,[242] while hypo-locomotion may also relate to the *negative symptoms* observed in schizophrenia.

Both prenatal and postnatal LPS[103,229] as well as a prenatal influenza[15] or Poly I:C[169] reduce spontaneous social activities in early and late adulthood in rodents. In contrast, the administration of IL-1α to rat pups resulted in increased social interaction in adulthood, which might be due to a cognitive deficit in recognizing an unfamiliar intruder caused by this specific cytokine.[19] Various studies have revealed deficits in PPI after the prenatal administration of either Poly I:C or LPS,[168,172,177,178,190,202,234,243,244] or postnatally following either subcutaneous[192] or direct intrahippocampal administration.[103,223] Additionally, some of these deficits were reversed with clozapine[202,218,243] and haloperidol.[218] In the study by Meyer et al., PPI deficits were only observed in animals that were subjected to an immune insult (Poly I:C) at GD 9 in comparison to GD17.[190] This suggests that infection early in pregnancy renders offspring more vulnerable to develop sensorimotor gating deficits, and is supported by previous results using LPS.[168,178] However, in contrast to this explanation, both Ozawa et al., and Wolff and Bilkey demonstrated PPI deficits when immune activation (Poly I:C) took place at later gestational periods (GD 12−17 and GD 15, respectively).[177,244] These differences in PPI deficits could possibly be explained by the activation of endogenous cytokines as opposed to the immunogen itself. Such a suggestion is supported by a study where turpentine was administered to pregnant rodents, activating an endogenous inflammatory response and resulting in a reduction in PPI in the offspring.[245] Indeed, it has been suggested that cytokines differ with respect to their detrimental effects[246] and that the behavioral alterations following different immunogens could be attributed to their ability to induce different biological processes.[16] In a study using direct cytokine administration (IL-1α,-2,-6, or IFN-γ) to newborn rat pups, interleukin IL-1α was the only cytokine that caused PPI deficits.[19] However, two recent studies show PPI deficits in offspring after administrating IL-6 to pregnant mice on GD 12.5.[211,246] Furthermore, different behavioral outcomes, as observed in the IL-6 study by Tohmi et al.[19] might be dependent on the time point of administration, in this case suggesting that IL-6 only has detrimental effects on sensorimotor gating during gestational periods. Finally, other forms of prenatal inflammation (viz., Borna disease virus, cytomegalovirus, and influenza) also induce PPI deficits in rodent offspring, thereby providing a common link with an important behavioral manifestation of schizophrenia.[15,247,248]

Prenatal inflammation studies with Poly I:C have revealed deficits in latent inhibition that could be reversed with a variety of antipsychotic treatments (haloperidol, clozapine, and risperidone).[237,243,249] Interestingly, abnormally enhanced latent inhibition has also been observed in the Poly I:C model which the authors correlated with severe negative symptoms of schizophrenia.[169]

Impaired memory, as measured in the nORT, was observed in both pre- and postnatal Poly I:C and LPS models[177,193,213,250] and after a prenatal influenza challenge.[15] Previous studies have also observed that the Poly I:C model[190] and the LPS model[201,226] impair memory in the Morris water maze. The dry maze matching-to-position paradigm also showed impaired working memory in Poly I:C offspring compared to controls,[188] as did the Y-maze task in rats receiving Poly I:C at PND 5−7.[202] Moreover, excessive cognitive switching has been observed in the T-maze after prenatal Poly I:C treatment,[237,249,250] but that could be normalized with risperidone treatment.[237]

A few studies also observed increased time spent in the outer sector of the open field test (an indication of anxiety-like behavior) in prenatal and postnatal Poly I:C administration,[192,211] postnatal LPS administration,[121] prenatal influenza,[15] and IL-6 administration.[211] Interestingly, administration of Poly I:C on GD 17 and 15 also led to a significant decrease in sucrose consumption in offspring, indicative of depressive-like symptoms in these rodents.[169,210]

4.3. "Dual-Hit" Animal Models of Schizophrenia. A multifactorial or "dual-hit" conceptualization of schizophrenia suggests that a combination of genetic susceptibility, an environmental stressor or inflammatory processes in early life coupled with a distinct developmental insult can prime an individual for a later event that ultimately leads to the development of schizophrenia wherein the full clinical syndrome may be presented. This association has indeed been observed in animal models and in human studies (reviewed in ref 251). Moreover, a variety of specific adverse environmental conditions, such as biological and psychosocial risk factors, during the antenatal and perinatal periods, early and late childhood, adolescence and early adulthood, along with genetic susceptibility, have been implicated in the etiology of schizophrenia. Numerous studies have also linked later in life infections (such as *Toxoplasma gondii* and retroviruses),[252,253] hormone imbalances (such as cortisol and estrogen),[254] and structural brain changes due to synaptic pruning[189] with a subsequent increase in the incidence of schizophrenia. The above-mentioned later in life insults could be seen as the "2nd hit" while genetic susceptibility or early life environmental insults could be seen as the "1st hit" of the "dual-hit"

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Table 3. Application of "Dual-Hit" Protocols as Neurodevelopmental Models for Schizophrenia and/or Psychosis

application	1st hit	2nd hit	behavior augmented
neonatal NMDAR antagonist exposure + neurodevelopmental insult	neonatal PCP	SIR	PPI deficits and conditioned emotional response freezing[264]
	neonatal MK-801	SIR	no studies done
neurodevelopmental insult + later life drug exposure	SIR	MK-801	increased or no added effect on locomotor activity[265,253]
	SIR	MA	no added effect on PPI or social interaction[256]
	SIR	ketamine	no studies done
	maternal separation	MA	no studies done
prenatal inflammation insult + later life stress exposure	prenatal LPS	restrain stress	increased anxiety behaviors[121]
	prenatal Poly I:C	restrain stress	PPI deficits[181]
	prenatal Poly I:C	unpredictable stress	no studies done
prenatal inflammation insult + later life drug exposure	prenatal LPS/ Poly I:C	amphetamine/ MA/MK-801	increased locomotor activity[20,177,188,190,190,237,236,243]

hypothesis. A recent review also observed that structural, molecular, and functional changes in glial cells (oligodencrocytes, astrocytes, and microglia cells) that follow the above challenges contribute significantly to the neurodevelopment−neurodegeneration hypotheses of schizophrenia.[255]

Based on the above-mentioned "dual-hit" hypothesis, numerous researchers have proposed that by exposing neurodevelopmental animal models to an experimental manipulation that mimics the effects of environmental risk factors associated with schizophrenia, it may be possible to better model some forms of this mental illness. In this "dual-hit" animal model, early life insults (such as maternal immune activation or SIR) or genetic factors are seen as the "1st hit" that disrupt neurodevelopment and, in so doing, produce long-term vulnerability to a "2nd hit" that is responsible for precipitating the onset of schizophrenia-like pathophysiology and its associated behavioral alterations. Such a "2nd hit" is represented by later life adversity such as drug abuse, with for example, NMDAR antagonists (ketamine, PCP etc.) or MA, or exposure to an environmental stressor (e.g., restraint stress or maternal separation in rodents, as well as abuse, infections later in life as noted above, structural brain changes due to synaptic pruning and hormone imbalances in humans etc.).

Thus, combining neurodevelopmental models and environmental insults with biobehavioral testing will allow new ways to model schizophrenia in rodents and assist in developing the next generation of antipsychotic drugs, as will be discussed below. However, it should be mentioned that the structure of a "dual-hit" model will depend on the specific hypothesis of schizophrenia that the researcher aims to mimic (see Table 3). In this regard, maternal separation or SIR could be a "1st hit" if coupled with a later in life environmental insult (such as drug abuse), or be a "2nd hit" if coupled with a prenatal insult (such as prenatal inflammation) as indicated in Table 3. For the purpose of this Review, and in line with the neurodevelopmental hypothesis of schizophrenia, we will consider "dual-hit" animal models to represent the following: combined neurodevelopmental factors (either SIR or PPNI), representing the "1st hit" *plus* postnatal conditions representative of a severe psychosocial stressor, representing the "2nd hit", such as late life infection or drug abuse (e.g., either exposure to NMDAR antagonists or MA). In some cases neonatal or maternal drug exposure (PCP or MK-801) as the "1st hit" combined with SIR as a "2nd hit" will be discussed as well.

4.3.1. Effect on Monoamines. To the best of our knowledge, published studies evaluating the effect of "dual-hit" models on monoamine alterations are limited, clearly pinpointing an urgent need for future studies. One recent study using postweaning SIR and postnatal (PND 35−50) MA exposure observed a trend toward further elevations in frontal cortical (but not striatal) DA levels in SIR-MA animals compared to SIR alone animals, although this increase was not significant and no effect on other brain monoamines was evident.[256] The authors argue that combining potent neurophysiological challenges, such as SIR-MA, may reach a ceiling beyond which further effects are not attainable, possibly explaining the lack of an additive effect, although this warrants further study. Nevertheless, their findings do emphasize a central role for DA in SIR-MA regulation of frontal cortex-driven behaviors (discussed in subsection 4.3.4). Another study used maternal separation in combination with MA administration later in life (PND 60−70) and observed that this combination significantly decreased striatal DAT and TH levels in comparison to MA administration alone.[257] These findings are in contrast to the increased striatal DAT and TH observed after SIR and PPNI (indicated in Table 4). A possible mechanism whereby maternal separation affects DA regulation differently to SIR could be due to the high levels of glucocorticoids present in the immediate postnatal period that assures DAergic transmission in the mesolimbic, mesocortical and nigrostriatal systems, as determined by the ontogenetic state of development at this early lifetime point.[258] In support of this, studies have observed a significant difference between the effect of early postnatal stressors vs postweaning stressors on the hypothalamic-pituitary-adrenocortical and related glucocorticoid synthesis in rats.[259] In this regard, it should be noted that maternal separation is generally associated with late-onset depression and anxiety studies,[257] not schizophrenia per se.

4.3.2. Effect on GABA-Glutamate. A recent study observed that SIR followed by a ketamine challenge significantly reduced prefrontal cortical GABA levels, in comparison to group reared animals also exposed to a ketamine challenge.[260] This observation is in line with the decreased prefrontal cortex GABA levels observed in unmedicated schizophrenia patients, and draws a parallel with the dysfunctional glutamate hypothesis of schizophrenia. In a study where mice were exposed to a single injection of MK-801 on PND 7, prior to SIR (a second hit design), a significant decrease in prefrontal cortical GABA-PV interneuron expression was observed, together with a down-regulation of the GAD67 gene, compared to group reared rats also receiving MK-801.[261] These studies suggest that SIR combined with NMDAR blockade in early life may modulate glutamatergic function and predict glutamatergic

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Table 4. Overview of the SIR, PPNI, and "Dual-Hit" Animal Models of Schizophrenia with Regard to Monoamine, GABA-Glutamate, Redox-Immune-Inflammatory, and Behavioral Alterations, Including Response to Antipsychotics and the Identification of Novel Treatment Strategies[a]

animal models	SIR	PPNI	"Dual-hit" models	targeted treatment strategies[b]
markers				
monoamines	↓DA, DOPAC, HVA (frontal cortex)[28,124,125]	↓DA, DOPAC (frontal cortex)[17]	↑DA trend (frontal cortex)[327]	atypical antipsychotics
	↑DA (frontal cortex)[126,127]	↓DA, ↑DOPAC (frontal cortex)[175]		NAC
	↑DA, DOPAC, HVA, DAT (striatum, NAcc)[28,125,127,130]	↑↓DA; ↑DOPAC (striatum)[166,172,176]		group II metabotropic glutamate receptor agonist (LY354740)
	↔DA (striatum)[124]	↑TH (striatum)[180]	↓DAT ; TH (striatum)[257]	
	↑DA turnover (amygdala, NAcc)[128,129]	↑TH, DA, DOPAC, HVA, DAT (NAcc)[168,183,184]		
	↑↓↔5-HT and 5-HIAA (frontal cortex)[126,127,140]	↑5-HT, 5-HIAA (hippocampus, frontal cortex, hypothalamus)[171−173]		
	↑5-HT and 5-HIAA (striatum, NAcc)[140,127]	↓↑5-HIAA (NAcc)[167]		
	↓5-HT/5HIAA (NAcc)[128]	↓5-HT (cerebellum)[174]		
		↔5-HT, 5-HIAA (striatum)[172]		
	↑NA and turnover (frontal cortex, cerebellum, hippocampus)[120]	↑NA, MHPG (hippocampus)[173]		
	↑NA, ↓MHPG (frontal cortex)[140]	↑NA (NTS, LC)[170]		
	↑NA and MHPG (striatum)[140]	↑NA turnover (hypothalamus, hippocampus)[175]		
GABA-glutamate	↓glutamate function[142]	↓GABA, (hippocampus)[169]	↓GABA (prefrontal cortex)[260]	atypical antipsychotics
	↓NAA (temporal cortex)[65]	↓↑GAD67 (hippocampus, cortex)[187,188]	↓GAD67 (prefrontal cortex, striatum)[181,261]	LY354740
		↑↓glutamate (hippocampus, frontal cortex)[169,192]		
redox-immune-inflammatory pathways				
mitochondrial	↑ATP (striatum)[28]	↓ATP (spleen)[200]	↑↓energy metabolism, redox processes and neurotrophic proteins (NAcc)[263,328,263]	atypical antipsychotics
	↓ATP (frontal cortex)[28]			minocycline
				NAC
oxidative stress	↑Nox2 (frontal cortex)[148]	↑iNOS (hippocampus, striatum, frontal and cerebral cortex)[202,205]	↓GABA PV-IR interneurons (frontal cortex)[261]	sodium nitroprusside
	↑iNOS (frontal cortex)[150]			methylene blue
	↓GABA PV-IR interneurons (hippocampus, frontal cortex)[145]	↓GABA PV-IR interneurons (hippocampus, frontal cortex)[206,164]		L-NAME
				ketamine
				memantine
	↓SOD (frontal cortex)[150]			minocycline
	↑SOD (striatum, frontal cortex)[27]			Ro 61-8048
	↑lipid peroxidation (striatum, frontal cortex)[27]	↑lipid peroxidation (midbrain)[202−204]		NAC
				NAC-amine
				oleanolic acid
	↓GSH/GSSG (striatum, frontal cortex)	↓GSH, ↑GSSG (hippocampus)[201,203,166]		selenium
				mangiferin
		↑protein carbonyl and ↓α-tocopherol[198]		
inflammation	↑NF-κB[150]		no studies done	ketamine
	↑proinflammatory cytokines (TNF-α, IFN-γ) (plasma)[140]	↑ pro-inflammatory cytokines (IL-1β, IFN-γ, TNF-α) (see Table 2)		minocycline

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Table 4. continued

animal models	SIR	PPNI	"Dual-hit" models	targeted treatment strategies[b]
redox-immune-inflammatory pathways				
				NAC
	↓anti-inflammatory cytokines (IL-4, IL-6) (plasma)[140]	↓anti-inflammatory cytokines (IL-10, IL-6) (CNS, serum, plasma)[98,207,210,211]		mangiferin
				γ-mangostin
				celcoxib
		↑IL-10 (plasma)[209]		asprin
		↑COX2 protein (plasma)[204]		estradiol
kynurenine pathway	↑tryptophan, kynurenine, anthranilic acid, 3-OHAA and QA (plasma)[156]	↑QA (serum)[236]	no studies done	ketamine
	↓KYNA and neuroprotective ratio (plasma)[156]	↓KYNA and kynurenine (serum)[236]		Ro 61-8048
		↔neuroprotective ratio[236]		allopurinol
		↑IDO and KMO (hippocampus, frontal cortex)[171,219,228]		NAC
behavior	↓sensory motor-gating (PPI)[28,120]	↓sensory motor-gating (PPI)[172,177,192,234]	↓↔sensory motor-gating (PPI)[256,264]	antipsychotics
	↓recognition memory (nORT)[28,130]	↓recognition and learning memory (nORT, morris water maze)[15,177,192]	↓ conditioned emotional response freezing behavior[264]	ketamine
				memantine
	↓mobility (FST)[157]	↓working memory (Y-maze, T-maze, dry maze matching-position)[202,237,249]		minocycline
				allopurinol
	↓social interaction[27,28]	↓social interaction[15,169,215,229]		NAC
	↑self-directed behaviors[27]	↑social interaction[19]		oleanolic acid
				sodium nitroprusside
	↑aggressive and social contact behaviors[127,158]			
				methylene blue
	↓latent inhibition[160]	↓latent inhibition[237,243,249]		L-NAME
		↑latent inhibition[169]		
	↑anxiety (EPM, OFT)[130]	↑anxiety (OFT, EPM)[121,192,211]	↑anxiety (OFT, EPM)[121]	celecoxib
	↑locomotor activity[27,28,120]	↑↓locomotor activity[123,235,239]		estradiol
	↑ethanol self-administration[161] and polydipsia[163]	↓sucrose consumption[169,210]	↔↑locomotor activity[256,265]	LY354740

[a]Arrows: ↑ = increase, ↓ = decrease, ↔ = no change. [b]See text for specific references.

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and GABAergic alterations later in life. These findings relate directly to observations in clinical studies. Moreover, a reduction in GAD67 levels was also observed in the cortex and striatum of Poly I:C offspring subsequently challenged with restraint stress.[181] Another study in mice that combined prenatal Poly I:C exposure with unpredictable stress from PND 30−40 observed a significant decrease in GABA-PV interneurons in the ventral dentate gyrus of the hippocampal formation, whereas none of the stressors alone had an effect on this marker.[262] Furthermore, prenatal Poly I:C exposure also induced a blunted response (absence of elevated extracellular glutamate) to MK-801 administration in the offspring, supporting the hypothesis that NMDA receptor function is decreased in immune challenged animals.[195]

4.3.3. Effect on Redox-Immune-Inflammatory Processes. A recent study observed that maternal separation in rats caused a reduction of cytoskeletal proteins in the shell of the NAcc and altered cytoskeletal, signaling, energy metabolism and redox proteins in the core of the NAcc.[263] Furthermore, when the authors combined maternal separation with MA exposure, they observed more altered proteins involved in energy metabolism, redox regulatory processes, and neurotrophic proteins in the NAcc, compared to maternal separation or MA exposure alone.[263] These observations support the theoretical rationale for added impact following early life adversity plus drug abuse and their subsequent impact on oxidative stress. In terms of postnatal immune challenges, another study also observed that LPS exposure was only able to increase microglia in offspring obtained from dams that were exposed to stress during gestation.[221]

4.3.4. Behavioral Manifestation of "Dual-Hit" Animal Models. In line with the "dual-hit" hypothesis, a recent study observed that acute neonatal PCP administration combined with SIR produced significant PPI deficits, compared to PCP administration alone.[264] Furthermore, conditioned emotional response freezing behavior (which examines contextual and conditioned associative learning) was significantly reduced by SIR but an even greater effect occurred when combined with neonatal PCP treatment.[264] With regards to locomotor activity, it was observed that SIR combined with subchronic MK-801 exposure (for the last week of SIR) significantly enhanced locomotor activity compared to SIR alone.[265] However, other studies observed that repeated postweaning MK-801 injections *during* the 8 weeks of SIR produced no additive or synergistic effects on locomotor activity, suggesting that these two perturbations may work through different mechanisms.[266,267] Similarly, SIR-induced drinking behavior was *not* bolstered following the addition of MK-801 to the SIR paradigm, suggesting that SIR induces a predisposition to polydipsia without the need for another stressor, or second "hit".[163] Moreover, postweaning SIR and MA exposure from PND35−50 also failed to show an exacerbation of SIR-associated deficits in social behaviors and sensorimotor gating.[256] It could however be argued that some of these environmental stressors/"hits" are equally potent in inducing behavioral alterations and that an additive effect may have been abrogated by a ceiling effect or by specific autoregulatory processes that are brought "on line" following severe adverse challenges. For example, where chronic MA reduces central monoamine levels,[268] the combination of MA+SIR may actually reverse the effect of the MA or have no effect on specific monoamines (see subsection 4.1.1). Strauss and colleagues advocate that redox related processes mediate a reactive protective mechanism that prevents an additive effect.[256]

Prenatal LPS administration followed by restraint stress in adulthood led to heightened anxiety-like behavior in the open field test and the EPM when compared to prenatal LPS treatment alone.[121] Prenatal challenge with LPS or Poly I:C also increases hyper-locomotor activity after postnatal amphetamine,[20,188,190,237,243] MA[177] or MK-801 administration[190,236] in adulthood, compared to LPS or Poly I:C alone. Furthermore, a recent study in juvenile animals receiving prenatal Poly I:C administration only experienced PPI deficits after subsequent restraint stress.[181]

The above evidence indicates that the "dual-hit" models present with more robust behavioral alterations akin to schizophrenia (such as PPI deficits, hyperlocomotor activity, deficits in contextual and associated learning, and anxiety-like behaviors) than a "single-stressor" neurodevelopmental model (see Table 3). However, some conflicting results have been observed in the SIR + MA and SIR + MK-801 models that warrants further study. Moreover, the validity of an apparent ceiling effect when utilizing combined stressors needs further clarification and the mechanisms delineated.

5. TARGETING IMMUNE-INFLAMMATORY-REDOX PATHWAYS IN SCHIZOPHRENIA: NOVEL TREATMENTS

Two previous antipsychotic clinical trials (e.g., CATIE, CUtLASS) highlighted the restrictions of current pharmacological therapies for schizophrenia, and the critical need for novel targets in drug research and development (reviewed in ref 269). Moreover, animal models (SIR, PPNI, and "dual-hit"), as reviewed thus far, may be extremely useful in identifying novel treatment targets. A number of putative targets relating to immune-inflammatory responses, oxidative stress, NO, COX, estrogen regulation, and glutamate receptor activation, have been revealed using these models and have formed the basis for novel drug design. Some of these ideas will now be discussed.

5.1. Compounds Targeting Immune-Inflammatory Responses. The NMDAR antagonist, ketamine, induces biobehavioral alterations[25,270] and inflammation[271] in rodents akin to that of schizophrenia. However, in contrast to the pro-inflammatory effects observed with ketamine, a recent review discussed its possible anti-inflammatory properties in preclinical studies, noting especially that ketamine administration decreases TNF-α and IL-6 levels and improves the IL-6/IL-10 ratio in rats after an acute insult with LPS or *E. coli*[272,273] as well as suppression of NO.[273] Moreover, ketamine is able to improve synaptogenesis by acting on NMDA and AMPA receptors, and probably through activation of mammalian target of rapamycin (mTOR), which in turn triggers the translation of synaptic proteins required for neuronal plasticity (reviewed in ref 273). Additionally, ketamine reduces inflammation, either via an inhibitory effect on NF-$\kappa\beta$ (see Figure 2) or by preventing IDO activation in the kynurenine pathway (see Figure 3) and decreasing the synthesis of neurodegenerative QA (reviewed in ref 273). With evidence indicating significant deficits in synaptic plasticity[274] and oxidative stress in schizophrenia, and that ketamine exerts rapid antidepressant effects within hours,[275] ketamine may be of value in treating the negative, depressive-like symptoms and inflammation associated with schizophrenia, although further studies are needed. However, a recent case report indicated that another NMDAR antagonist, memantine, significantly improved the negative

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symptoms of schizophrenia when used as add-on therapy.[276] Adjunctive memantine together with clozapine also significantly improved not only the negative symptoms but also positive symptoms in refractory schizophrenia patients.[277] The authors argue that this improvement could be related to memantine's NMDAR antagonistic mechanisms, preventing a toxic influx of Ca^{2+} into neuronal cells and as such abrogating oxidative stress (see Figure 2).[276,277]

Another possible anti-inflammatory adjunctive treatment that has been evaluated in schizophrenia is minocycline. Minocycline is an antibiotic that has anti-inflammatory and antioxidant properties by inhibiting TNF-α release and iNOS activation, respectively.[278] An extensive review of minocycline noted that it decreases the release of cytokines (IL-1β, IL-6, and TNF-α), cytochrome *c* and apoptotic factors; it also decreases mitochondrial membrane permeability and inhibits COX-2-induced NO release, among other properties, possibly explaining its neuroprotective properties (see Figure 3).[279] Randomized double-blind placebo-controlled clinical trials have indeed observed that the addition of minocycline to antipsychotic treatment early in the course of schizophrenia predominantly improves negative symptoms.[280,281] This supports evidence that the negative symptoms of schizophrenia is positively correlated with oxidative stress and a proinflammatory state (reviewed in ref 282). Furthermore, other interesting preclinical studies have observed that minocycline treatment reverses MK-801-induced cognitive abnormalities (PPI and memory deficits), an effect it shares with haloperidol, therefore demonstrating possible antipsychotic properties.[280] Minocycline treatment also reverses LPS induced deficits in PPI[234] as well as MA induced deficits in recognition memory.[283]

Altered kynurenine metabolism plays a prominent role in schizophrenia pathology. It is therefore of interest that IL-1β induced deficits in neurogenesis in rats is reversed following cotreatment with the KMO inhibitor, Ro 61-8048.[116] Ro 61-8048 is known to increase KYNA levels and holds promise as a treatment to abrogate neurotoxicity and oxidative stress in schizophrenia. Ro 61-8048 is also associated with an increase in the neuroprotective balance (Figure 3).[156,284] Another recent study observed that allopurinol, a TDO inhibitor (Figure 3), significantly reversed immobility in the FST and increased hepatic kynurenine levels in a chronic stress animal model of depression.[285] These studies indicate that targeted inhibition and possible activation of the kynurenine pathway to increase KYNA production may provide new therapeutic approaches to reverse inflammatory-induced reduction in neurogenesis in schizophrenia. However, some studies have demonstrated elevated levels of KYNA in schizophrenia.[110] An important consideration here is that the NMDA receptor antagonist activity of KYNA could assist in driving a hypoglutamatergic state that is proposed to underlie schizophrenia, as discussed in subsection 2.2. Thus, correction of KYNA levels could also significantly improve glutamate dysfunction in schizophrenia. Further studies evaluating such therapy are warranted.

5.2. Compounds Targeting Oxidative Stress. The impact of oxidative stress in the pathophysiology of schizophrenia has been discussed throughout this Review, which brings us to the possible clinical utility of antioxidant treatment. Indeed, previous studies have observed that NAC, a well-known antioxidant and glutamatergic modulator (reviewed in ref 286) improves negative symptomatology in schizophrenia patients when used as an adjunctive treatment.[287,288] Preclinical studies have also observed that NAC treatment successfully reverses LPS-induced decline in the GSH/GSSG ratio in male fetuses and prevents these oxidative insults when given as pretreatment.[198] More interesting is that NAC was found to augment selected behavioral, neurochemical, mitochondrial, redox, and anti-inflammatory responses to clozapine[28] and to reverse cortico-striatal monoamine alterations induced by SIR.[140] NAC also reversed memory impairment in the Morris water maze in offspring following maternal exposure to LPS.[201] In rodents, MA induced hyper-locomotor activity was also reversed with NAC treatment.[289] Furthermore, MA induced elevations in lipid peroxidation and protein carbonyls as well as decreased GSH levels and glutathione peroxidase activity, were reversed by the administration of NAC-amide, a more lipid soluble form of NAC that aids brain penetration.[290]

Clinical studies have observed that the combination of the antioxidants, omega-3 fatty acids with vitamins E and C can significantly reduce negative symptoms in schizophrenia.[291] However, combined omega-3 fatty acids and α-lipoic acid was ineffective in preventing relapse in patients who had responded well to antipsychotic treatment after a single episode of psychosis.[292] Other recent reviews of clinical trials concur that dietary supplementation of patients with schizophrenia with omega-3 fatty acids does not improve the symptomatology of these patients.[293,294] However, it is worth mentioning that a recent study did show that omega-3 fatty acids reduces the progression of at-risk individuals to the full-blown manifestation of psychosis.[295] Consequently, the value of omega-3 fatty acids as a preventive measure in schizophrenia needs further study. Some earlier clinical work also hinted at the possible benefit of natural antioxidants.[296] However, this goal has not always been reachable. In fact, attempting to predict therapeutic effects of antioxidants is complex, especially comparing animal to human studies. Antioxidants such as α-lipoic acid, NAC, and others may perform differently depending on the cellular milieu, acting either as an antioxidant or as a pro-oxidant depending on the redox status of the organism.[297,298] Moreover, such redox status very likely relates to illness severity and its progression over time that in turn will predetermine how an antioxidant will perform and at what dose. Thus, conditions such as the applied dose of the antioxidant as well as illness state/severity will have a role in determining the clinical outcome. Preclinical studies, however, have observed that oleanolic acid (an antioxidant and anti-inflammatory agent) normalizes MK-801-induced behavioral (PPI and memory deficits) alterations,[299] thus suggestive of some benefits of antioxidant treatment in schizophrenia. Another antioxidant, selenium, has also been proven to reverse MA induced elevation of 3-nitrotyrosine (3-NT) in the rat striatum,[300] while pretreatment of rats with the plant extract, mangiferin (MGF), decreased ketamine-induced elevations in brain IL-6 levels and lipid peroxidation.[301] MGF is a natural xanthone and by virtue of its antioxidant and anti-inflammatory properties has been speculated to be neuroprotective.[301] Another xanthone, γ-mangostin, also increased expression of 5-$HT_{2A/C}$ receptors and has anti-inflammatory properties in vitro.[302] However, no other schizophrenia related studies have been undertaken and further research into this promising area of therapeutics is warranted.

5.3. Compounds Targeting Nitric Oxide. NO contributes significantly to oxidative stress (Figure 2) as well as modulates DA release, and has gained growing intereste in the pathophysiology of schizophrenia.[71] Clinical studies have begun to reveal the potential of NO-modulators, such as methylene blue and sodium nitroprusside (SNP), in the treatment of

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schizophrenia. SNP is a NO donor (Figure 2), and is used clinically to treat hypertension. However, recent preclinical[303,304] and a clinical study[305] have observed its novelty in treating schizophrenia where psychosis-like behavior and c-fos expression in the brain of PCP treated rats[303] as well as amphetamine-induced PPI deficits in rats,[304] were reversed with SNP infusion. Moreover, SNP infusion rapidly improved positive and negative symptoms in patients with schizophrenia within hours.[305] The authors speculate that the antipsychotic mechanism of SNP might be mediated via NMDA-NO-cGMP signaling pathways (see Figure 2), exerting an inhibitory effect on DAT, which in turn would correct frontal cortical hypo-DAergia. Through feedback loops, SNP may also correct the hyper-DAergic levels in striatal regions (see Figure 1).[305] On the other hand, the NOS-guanylyl cyclase inhibitor, methylene blue, has demonstrated therapeutic efficacy in schizophrenia.[306] Via its inhibition of the NO-cGMP pathway, methylene blue modulates the glutamateric system and could, as an extension of its action, modulate DA and 5-HT release (reviewed in ref 307). Preclinical studies have indeed observed that methylene blue increases 5-HT and DA levels via nNOS inhibition in the rat ventral hippocampus, which may underlie its anxiolytic and antidepressant mechanisms, indicating its clinical utility in possibly treating the negative symptoms of schizophrenia (reviewed in ref 307). Deutsch and colleagues observed a statistically significant decrease in the severity of psychopathology in schizophrenia patients following methylene blue treatment that worsened after discontinuation of the drug.[306] In addition, preclinical studies have showed that methylene blue reverses PCP-induced behavioral (PPI and hyperlocomotor activity) alterations in mice,[308] while another animal study observed that L-nitroarginine methyl ester (L-NAME), also a nNOS inhibitor, reversed PCP induced PPI deficits[309] (see Figure 2). Work in our own laboratory has demonstrated the structural characteristics of various methylene blue analogues, confirming that their antidepressant properties reside in the positively charged dimethylamino group.[310,311] This property enables these compounds to modulate the mitochondrial electron transport chain, affording them with potent antioxidant and neuroprotective properties.

5.4. Compounds Targeting Cyclooxygenase (COX). As early as the late 1970s, evidence has accumulated in support of the role of arachidonic acid and its metabolites in the etiology of schizophrenia.[312] When evaluating the redox-immune-inflammatory pathways in schizophrenia, the prostanoid class of fatty acid derivatives (including prostaglandins, thromboxanes, and prostacyclins) produced from arachidonic acid in the COX pathway needs serious consideration. The immunological imbalance observed in schizophrenia, as discussed throughout this Review, has previously been linked to disturbances in the COX pathway, including increased prostaglandin E(2) (PGE(2)) production as well as a possible increase in COX expression.[313] Interestingly, adjunctive treatment with celecoxib, an anti-inflammatory agent and selective COX-2 inhibitor (Figure 3), has proved to be valuable in improving symptomology in schizophrenia patients.[314] However, COX-2 expression is not upregulated in the hippocampus of schizophrenia patients, suggesting that celecoxib may affect the pathophysiology of schizophrenia through COX-2-independent actions rather than by inhibiting the activity of up-regulated COX-2 protein.[315] Moreover, preclinical evidence indicates that celecoxib treatment prevents hyper-locomotor activity in rats exposed to prenatal Poly I:C combined with later life MK-801 administration.[236] The nonselective COX inhibitor, aspirin, has also gained some interest recently, demonstrating in clinical studies that it too can significantly improve schizophrenia symptomatology.[316] However, no preclinical evidence in support of this finding is currently available.

5.5. Other. The early life neurodevelopmental aspect of schizophrenia and its presentation during puberty and predominantly in male subjects raises a question as to the role of estrogen. The estrogen theory posits a protective effect for estrogen in schizophrenia, with periods of protection occurring when estrogen levels are high, for example, during puberty and pregnancy.[317] Treatment studies using estrogen or related hormones have shown some benefit in treating schizophrenia and related cognitive impairments.[318,319] Further, there is evidence in multiple systems that estrogens exhibit neuroprotective effects and can interact with immune processes to reduce CNS inflammation and to activate t-helper cell-2 response (reviewed in ref 320). Although there is some evidence that estrogens may have proinflammatory effects as well (reviewed in ref 321), further exploration of its benefits and clinical application have been limited by worrying side effects such as breast and uterine cancer.[319] Preclinical studies have observed that estradiol treatment has a protective effect against MK-801 induced PPI disruptions,[322] as well as against PCP induced cognitive deficits in the nORT.[323] Interestingly, a recent study observed some sex specific neurochemical and behavioral alteration in SIR rats,[324] the findings of which could, in part, indicate that sex hormones represent a novel drug target in schizophrenia.

Another interesting treatment option evaluated in schizophrenia research is LY354740, a group II metabotropic glutamate receptor agonist, which has been shown to reverse ketamine-induced increases in NA levels in the hippocampus of rats.[325] Furthermore, LY354740 also blocked the behavioral effects induced by PCP and MK-801 as well as the increased glutamate release elicited by PCP and ketamine in rodents (reviewed in ref 326). These studies indicate that LY354740 could correct the glutamate dysfunction in schizophrenia, and possibly also decrease glutamate-NMDA associated oxidative stress and immune-inflammatory pathways (see Figure 2). However, no clinical studies have yet been undertaken.

6. FINAL SUMMARY AND CONCLUSIONS

To date, new antipsychotics with adjunctive mechanisms other than an action on monoamine regulation remains disappointing, while many patients suffering from schizophrenia are nonresponsive to current treatments. Furthermore, the diagnosis of schizophrenia mainly relies on behavioral symptomatology observed in patients, with no laboratory or biological tests available to confirm and/or extend the diagnosis. In this Review, we have evaluated the neurodevelopmental theories of schizophrenia, focusing specifically on SIR, PPNI, and "dual-hit" animal models of schizophrenia in an attempt to reveal new biomarkers for diagnosis and/or to shed new light on novel treatment targets as well as targets for possible adjunctive treatment strategies, summarized in Table 4.

Monoamine alterations observed in these models are outlined in the first row of Table 4. The SIR and PPNI models present with general hypocortical and hyperstriatal DA activity as well as increased NAcc activity, with some opposing studies noted. Opposing (increased or decreased) 5-HTergic activity is also evident in the same brain regions in both models,

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while a general hyper-NAergic activity is evident in the hippocampus, hypothalamus, striatum, cortex, and cerebellum, also in both SIR and PPNI models. Overall, atypical antipsychotics are the most effective in reversing the above-mentioned monoamine alterations in these models. However, new treatment options such as NAC and LY354740 were also effective in reversing monoamine alterations in the SIR and ketamine models, respectively (Table 4).

Evaluating GABA−glutamate alterations (Table 4), a decrease in GABA and GAD67 (one study observed an increase in GAD67) was observed in the PPNI models, but appears to be more pronounced in the "dual-hit" models. Glutamate function was decreased in the SIR models but decreased or increased in the PPNI models, emphasizing some opposing findings in the PPNI models. Decreased NAA was observed in the SIR model (not accessed elsewhere), which is in line with clinical findings. Again, atypical antipsychotics appear to be the mostly effective in reversing some of these alterations. LY354740 also reversed PCP induced increases in glutamate release (Table 4).

With regards to the redox-immune-inflammatory pathways (Table 4), alterations in ATP levels have been observed in the SIR model (increased in the striatum and decreased in the frontal cortex), in the PPNI model (decreased in the spleen), as well as in the "dual-hit" model (altered energy metabolism, redox processes, and neurotrophic proteins). SIR and the PPNI models are associated with a general increase in Nox2 and iNOS, lipid peroxidation, pro-inflammatory cytokines and the neurotoxic kynurenine metabolites, along with a general decrease in PV-IR interneurons, GSH, anti-inflammatory cytokines (although one PPNI study observed an increase in IL-10), and the neuroprotective kynurenine metabolites. In addition, SIR results in an increase and decrease in SOD while PPNI is associated with an increase in protein carbonyl and a decrease in α-tocopherol. Some differences that have been observed between these models include a decrease in the neuroprotective ratio following SIR while no change in respect of this parameter was observed in the PPNI models. Some new treatment strategies that may be identified from these redox-immune-inflammatory studies, apart from atypical antipsychotics, include: minocycline, NAC, SNP, methylene blue, L-NAME, ketamine, memantine, the KMO inhibitor (Ro 618048), allopurinol, NAC-amide, oleanolic acid, selenium, mangiferin, γ-mangostin, celecoxib, asprin, estradiol (Table 4).

In the behavioral alterations section (Table 4), SIR, PPNI and "dual-hit" models (except for one study), generally engender a deficit in PPI and an increase in anxiety-like behavior and locomotor activity (except for one PPNI study that observed decreased locomotor activity), making this the most valid and reliable behavioral changes across all three models. Furthermore, deficits in recognition memory, social interaction and latent inhibition (except for one PPNI study that observed increased latent inhibition), were observed in the SIR and PPNI models. Behavioral alterations that have to-date only been observed in SIR include a decrease in mobility in the FST, increased self-directed, aggressive-like and social contact behavior, increased ethanol self-administration and polydipsia. Behavioral alterations noted in PPNI models include decreased working memory and sucrose consumption, while decreased conditioned emotional response freezing behavior has been observed in the "dual-hit" model. These behavioral differences are generally only due to the lack of consistent behavioral tests performed across all the models, emphasizing that further studies are needed in this regard. Overall, it is well-known that antipsychotics are effective in reversing most of these behavioral alterations in at least the SIR and PPNI models, confirming their predictive validity. New treatment strategies that also reversed some of these behavioral alterations include: ketamine, memantine, minocycline, allopurinol, NAC, oleanolic acid, SNP, methylene blue, L-NAME, celecoxib, estradiol, and LY354740 (Table 4). However, further work in this regard is recommended.

It should, however, be noted as a general shortcoming that numerous conflicting results are evident in the SIR, PPNI, and "dual-hit" models throughout this Review, which emphasizes the need for further research in order to standardize these models as translational resources in the evaluation of schizophrenia. Thus, the translation of findings taken from these animal models to clinical practice needs careful consideration. A recent review that evaluated various adjunctive treatments found that aspirin, estrogens and NAC were the only treatment strategies that showed significant efficacy on symptom severity in schizophrenia patients.[316] Indeed, these findings are very much in line with most of the findings discussed in this review, indicating that, while translational animal models are essential in drug discovery, investigators need to be cognisant of their limitations. In fact, many such models can effectively reproduce only certain symptoms of the human illness, may respond to nonspecific treatments, or show variable biomarkers responses. Given these restrictions, this Review has shown that future use of different established schizophrenia animal models *in combination* might provide a more robust "dual-hit" approach in translating pathological findings in humans and for improving the search for new drug treatments. Earlier we discussed the dilemma of attempting to predict the therapeutic effects of antioxidants by inferring animal data are immediately transferrable to human studies. Indeed, illness severity, comorbidities and dose of the drug in question have a definitive role in determining outcome within a given clinical scenario.

The neurodevelopmental animal models (SIR and PPNI models) discussed in this Review have proven themselves to be of signifcant value in schizophrenia research. Furthermore, a "dual-hit" hypothesis built on the latter paradigms, followed by a specific later life insult or drug abuse, has provided a growing body of evidence in support of this theory as well as neurobiological data in respect of its construct and predictive validity. Future studies should therefore incorporate SIR or PPNI with the "2nd hit" more representative of a specific clinical condition, for example, SIR combined with MA to study the link between early life adversity and drug abuse (as illustrated in Table 3). Schizophrenia research is needful of a "new champion", and given the data discussed here redox and inflammatory markers, especially mitochondrial and kynurenine pathways, may provide the melting point for the next generation of antipsychotic drugs. The goal of this Review is to mobilize ideas in order to discover and develop more beneficial and effective treatments for this life altering disease. Although it is too soon to predict the outcome, the novel treatments discussed here show great promise and more studies focusing on their underlying biological mechanisms and effects on behavior are warranted. To this end, "dual-hit" paradigms designed around neurodevelopmental models and the immune-inflammatory redox hypothesis of schizophrenia are likely to provide a wide array of new therapeutic options. Furthermore, these models in turn will provide the thrust for the search for

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much needed biomarkers of the disease that will benefit diagnosis and treatment of schizophrenia.

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Author Contributions

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