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Extracellular glutamate release in the prefrontal cortex in rat models with relevance to schizophrenia

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Abstract

Non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists produce psychotic symptoms in humans. In rodents, NMDA antagonists produce hyperlocomotion and stereotypies, as well as increased cortical glutamatergic neurotransmission. Several of the behaviors and cognitive impairment associated with NMDA receptor blockade appear to involve increased glutamatergic neurotransmission in the prefrontal cortex. In the present study, the increase in extracellular glutamate in the prefrontal cortex induced by MK-801 was examined in rats treated with antipsychotic agents, risperidone and paliperidone. In addition, the effects of the nitric oxide synthase inhibitor L-NAME and the GABA_B receptor agonist baclofen were examined on the MK-801-induced glutamate increase in the prefrontal cortex. Furthermore basal and stimulated glutamate release in the prefrontal cortex was assessed in rats exposed prenatally to immune activation. Using in vivo microdialysis, it was determined that treatment with MK-801 (0.3 mg/kg, s.c.) significantly increased extracellular glutamate in the prefrontal cortex. The glutamate response to MK-801 was significantly attenuated in rats treated chronically for 21 days with risperidone (0.01 mg/kg/day) and paliperidone (0.01 mg/kg/day) in the drinking water, or in rats treated acutely with L-NAME (60 mg/kg, i.p.) or baclofen (5mg/kg, i.p.). Prenatal immune activation was achieved by treatment of pregnant rats on day 14 of gestation with polyinosinic:polycytidylic acid (poly I:C). Microdialysis was performed in male offspring on postnatal day 56, and it was determined the MK-801-induced increase in extracellular glutamate in the prefrontal cortex was significantly diminished in poly I:C-exposed rats. In addition, basal extracellular glutamate in the prefrontal cortex of poly I:C-exposed male rats were significantly greater than that in control animals. The elevated basal extracellular glutamate in the prefrontal cortex of poly I:C offspring was reduced to that of controls by
paliperidone and to a lesser extent risperidone. These data support the view that increased
eextracellular glutamate in the prefrontal cortex in response to NMDA receptor blockade involves
an nitergic and GABAergic mechanism and can be prevented by chronic antipsychotic drug
treatment. It can be speculated further that the relative insensitivity of poly I:C exposed rats to
NMDA receptor blockade may be indicative of a state of NMDA receptor hypofunction induced
by prenatal immune activation.
# Table of Contents

Abstract iii  
Table of Contents vi  
List of figures vii  
Introduction 1  
Hypothesis and Specific Aims 6  
Chapter 1 7  
Introduction 8  
Materials and Methods 10  
Results 13  
Discussion 16  
Chapter 2 26  
Introduction 27  
Materials and Methods 29  
Results 31  
Discussion 33  
Future Directions 40  
References 42
List of Figures

Chapter 1

1. Effect of MK-801 on extracellular glutamate in the prefrontal cortex 21
2. Effect of antipsychotic agents on the MK-801-induced extracellular glutamate increase in the prefrontal cortex 22
3. Effect of L-NAME on the MK-801-induced increase in extracellular glutamate in the prefrontal cortex 23
4. Effect of SNAP infusion on extracellular glutamate in the prefrontal cortex 24
5. Effect of baclofen on the MK-801-induced extracellular glutamate increase in the prefrontal cortex 25

Chapter 2

1. Effect of prenatal immune activation on the MK-801-induced extracellular glutamate efflux in the prefrontal cortex 37
2. Effect of prenatal immune activation on basal extracellular glutamate in the prefrontal cortex 38
3. Effect of antipsychotics on elevated basal extracellular glutamate in the prefrontal cortex of poly I:C-treated offspring 39
Introduction

Schizophrenia is a multiple symptom psychiatric disorder that affects approximately 1% of the population worldwide. The etiology of the disorder is believed to be a combination of genetic factors, as well as environmental factors, including maternal infection. Symptoms typically emerge during late adolescence and early adulthood. Symptoms include the common positive symptoms of psychosis, hallucinations, and delusions, as well as the negative symptoms of social withdrawal and impoverished thinking. Cognitive deficits of impaired learning, memory, and attention are some of the hardest symptoms to treat. Schizophrenia also includes comorbid conditions of depression, anxiety, and substance abuse. Most antipsychotic agents treat the positive symptoms. However, more emphasis is being placed on improving the debilitating cognitive deficits.

Although many neurotransmitter systems have been implicated in the pathophysiology of schizophrenia, including dopamine, acetylcholine, serotonin, glutamate, and GABA, the prevailing hypothesis invokes primarily dopamine abnormalities in the etiology of the disorder. Chlorpromazine, one of the first antipsychotics, was first developed in 1950 as part of an anesthetic cocktail and then was discovered to have antipsychotic properties. Subsequently, it was shown to be an antagonist for the dopamine D₂ receptor. Conversely, dopamine releasing drugs, e.g. amphetamine, can induce positive symptoms similar to schizophrenia (Lieberman et al., 1987). The dopamine hypothesis of schizophrenia posits that dopamine hyperactivity in subcortical regions is associated with positive symptoms of the disorder; current antipsychotic agents block the dopamine D₂ receptor. More recently the dopamine hypothesis has been expanded to include the view that there may be dopamine hypoactivity in the prefrontal cortex that is associated with the negative symptoms (Abi-Dargham and Moore, 2003).
Recently, attention also has been given to glutamate neurotransmission. However, the first findings related to glutamate emerged in the 1950s. Luby and co-workers observed that PCP, a dissociative anesthetic, produced a schizophrenia-like psychotic state in human subjects (Luby et al., 1959). Thirty years later, Lodge and colleagues suggested that blockade of the N-methyl-D-aspartate (NMDA) glutamate receptors was the primary mechanism by which PCP disrupts brain function (Lodge and Anis, 1982; Lodge et al., 1987). It was also discovered that other drugs that block NMDA receptors induce acute psychotic symptoms, and this led to the hypothesis that dysfunction of the NMDA glutamate receptor may underlie the pathophysiology of schizophrenia. These drugs, e.g. PCP, ketamine, and MK-801, are non-competitive antagonists of the NMDA glutamate receptor which bind and block the ion channel. These antagonists have been shown to induce not only the positive symptoms, but also the negative symptoms and cognitive deficits associated with schizophrenia in healthy individuals (Krystal et al., 1994; Malhotra et al., 1996). NMDA antagonists also exacerbate the positive and negative symptoms when taken by schizophrenic patients (Lahti et al., 1995; Malhotra et al., 1997). Thus, schizophrenia is believed to include a state of NMDA receptor hypofunction. In accordance with this view, postmortem studies have shown decreased expression of NMDA receptor subunits in the prefrontal cortex of schizophrenia subjects (Beneyto and Meador-Woodruff, 2008).

PCP, ketamine, MK-801, and other NMDA receptor antagonists have been employed as a pharmacological model to study schizophrenia. In rodents, these drugs produce behavioral abnormalities, as well as altered neurotransmission. Several investigations have reported MK-801 and NMDA receptor antagonists produce hyperlocomotion, as well as stereotypies in rodents (Bubser et al., 1992; Bubser et al., 1995; Contreras et al., 1986; Schmidt et al., 1992). In addition, numerous behavioral tests have been employed to measure cognition and learning in
rodents, as these are some the most difficult symptoms to improve by antipsychotic treatment in humans. Impairment in spatial learning and working memory, measured by Morris water maze or discrete trial delayed alteration task respectively, have been reported following PCP (Wass et al., 2006b; Adams and Moghaddam, 1998). Furthermore, PCP administration results in deficits in active avoidance learning and radial arm maze performance, additional measurements of spatial learning (Kesner et al., 1983; Wass et al., 2008). Of importance, the disruptive effect of ketamine on spatial delayed alternation performance was reversed by antipsychotics, haloperidol and raclopride (Verma and Moghaddam, 1996). In addition to behavioral impairment, several neurochemical alterations have been reported. Adams and Moghaddam (1998) observed an increase in dopamine, serotonin (5-HT), and glutamate efflux in the prefrontal cortex of rats following NMDA receptor blockade (Adams and Moghaddam, 1998; Martin et al., 1998). The increase in cortical glutamate release following NMDA receptor blockade is believed to model NMDA receptor hypofunction. Therefore, drugs that prevent the increase in glutamate release as a result of NMDA hypofunction have potential to be effective antipsychotic therapies. In accordance, Lopez-Gil and colleagues have reported the increase in glutamate and serotonin efflux produced by MK-801 can be prevented by the antipsychotic clozapine, while haloperidol only blocked the induced glutamate efflux (Lopez-Gil et al., 2007).

In addition to the acute pharmacological model of schizophrenia presented by NMDA antagonists, there are also neurodevelopmental animal models that may more closely mimic the pathophysiology of the disease. The basis for these models is the higher incidence of schizophrenia following maternal infection that is believed to affect brain development of the fetus. One method currently used is the neonatal ventral hippocampal lesion (NVHL) model that alters regions of the hippocampus that project into the prefrontal cortex. Lesions are induced by
bilateral injections of ibotenic acid into the ventral hippocampus of neonates that result in behavioral and pharmacological abnormalities in adolescents and adults. Disruptions in latent inhibition, prepulse inhibition, and working memory have been reported in lesioned rats (Grecksch et al., 1999; Lipska et al., 1995; Lipska et al., 2002). Furthermore, the antipsychotic agents, clozapine, olanzapine, and risperidone reverse impaired prepulse inhibition in NVHL rats (Le Pen and Moreau, 2002). In addition, Al-Amin and co-workers have shown the MK-801-induced hyperlocomotion emerged only in adult lesioned rats (Al-Amin et al., 2000; Al-Amin et al., 2001). Collectively these studies are suggestive of an animal model with relevance to schizophrenia.

Another method currently used is the viral mimic polyinosinic:polycytidylic acid (poly I:C) delivered to pregnant rats or mice. Poly I:C is a synthetic double-stranded RNA which mimics viral infection and elicits cytokine response, fever, and anorexia in the pregnant dams (Kimura et al., 1994; Fortier et al., 2004). Offspring of rats or mice exposed prenatally to immune activation by poly I:C have been reported to have behavioral, cognitive, and pharmacological dysfunctions with relevance to schizophrenia. Meyer and colleagues reported that offspring of mice receiving poly I:C had impaired sensory motor gating, decreased latent inhibition, and increased locomotor response to amphetamine (Meyer et al., 2005). Abnormalities of neurons in the hippocampus of poly I:C-exposed offspring have been documented, as well, supporting altered brain development in this model (Zuckerman et al., 2003). Interestingly, in a study by Zuckerman and co-workers the offspring of poly I:C dams showed normal latent inhibition at postnatal day 35 (juvenile) but had decreased latent inhibition as adults (3 months) (Zuckerman et al., 2003). This corresponds with the etiology of schizophrenia emerging during late adolescents and early adulthood. Furthermore, haloperidol
and clozapine treatment attenuated the decreased latent inhibition in the adult offspring exposed to poly I:C. Thus, poly I:C animals could provide a neurodevelopmental animal model that more closely mimics schizophrenia.

Although considerable attention has been given to the behavioral abnormalities evident in rats exposed to prenatal immune activation, there are few reports that have examined glutamatergic neurotransmission in these animals. Glutamate neurotransmission was recently analyzed in the hippocampus of adult mice exposed to poly I:C neonatally. Ibi and colleagues reported elevated extracellular glutamate in the hippocampus of poly I:C-exposed adults compared to control, and this was reduced in the presence of tetrodotoxin. In addition, poly I:C-treated mice had reduced K+ evoked glutamate release (Ibi et al., 2009). These results are suggestive of abnormalities in neuronal glutamate release in mice exposed to prenatal immune activation.
Specific Aims

Currently, it is unknown whether rats exposed to prenatal immune activation exhibit abnormalities in glutamatergic transmissions in the prefrontal cortex that mimic the acute effects elicited by NMDA antagonists. Moreover, little is known about the neurochemical substrates that mediate/modulate the increased glutamatergic transmission produced by NMDA antagonists.

The specific aims are:

1. To determine whether the MK-801-induced glutamate release is suppressed by antipsychotic agents and modulated by GABA<sub>B</sub> receptor activation or the activity of nitric oxide synthase. *It is hypothesized that NMDA antagonists increase extracellular glutamate in the prefrontal cortex as a result of reduced activation of GABA<sub>B</sub> receptors and an activation of nitric oxide signaling.*

2. To ascertain whether glutamatergic neurotransmission is dysregulated in rats exposed to prenatal immune activation and whether this dysregulation is normalized by antipsychotic agents. *It is hypothesized that prenatal immune activation results in a state of NMDA hypofunction that is reflected in elevated glutamate release that is normalized by antipsychotic agents.*
Chapter 1
1. Introduction

MK-801 is a non-competitive NMDA receptor antagonist which induces psychotic symptoms in humans and produces hyperlocomotion and stereotypies, as well as increased cortical glutamatergic neurotransmission in rodents (Bubser et al., 1995; Contreras et al., 1986; Adams and Moghaddam, 1998; Moghaddam et al., 1997). Several behaviors and cognitive impairments associated with NMDA receptor blockade appear to involve increased glutamatergic transmission in the prefrontal cortex (Adams and Moghaddam, 1998; Verma and Moghaddam, 1996; Moghaddam et al., 1997).

MK-801, PCP, and ketamine have been reported to increase extracellular glutamate in the prefrontal cortex in rats (Bubser et al., 1995; Moghaddam et al., 1997; Moghaddam and Adams, 1998). Lopez-gil and colleagues have shown that the antipsychotic agents haloperidol and clozapine suppress the MK-801-induced increase in extracellular glutamate in the prefrontal cortex (Lopez-Gil et al., 2007). PCP has also been shown to disrupt cognitive flexibility measured by Morris water maze and prepulse inhibition, a measurement of preattentive information processing. The antipsychotic sertindole reversed the PCP-induced impairment in the Morris water maze, while aripiprazole attenuates the PCP-induced disruption of prepulse inhibition (Didriksen et al., 2007; Fejgin et al., 2007).

Several studies have provided evidence in support of the view that nitric oxide is involved in the PCP-induced disruptions of prepulse inhibition (Fejgin et al., 2008; Klamer et al., 2004a; Klamer et al., 2005; Klamer et al., 2004b). Specifically, inhibition of nitric oxide synthase by L-NAME prevents disruption of prepulse inhibition elicited by PCP. L-NAME also attenuates the PCP-induced disruption in the Morris water maze (Wass et al., 2008).
Collectively, these findings support the involvement of nitric oxide in cognitive impairments following NMDA receptor antagonism. Fejgin and colleagues also reported that PCP increases cGMP in the prefrontal cortex, an effect prevented by L-NAME pretreatment (Fejgin et al., 2008). Soluble guanylyl cyclase, the main target for nitric oxide, forms cGMP; therefore an increase in cGMP presumably is the result of an increase in nitric oxide formation. Fejgin and colleagues have speculated that L-NAME disrupts PCP behavior through an inhibition of glutamate stimulated nitric oxide formation. This supposition is based on several reports that have demonstrated a stimulatory effect of glutamate through NMDA receptors to enhance nitric oxide formation.

Recently, Fejgin and co-workers reported that activation of GABA_B receptors by baclofen in the prefrontal cortex prevents the PCP-induced disruption of prepulse inhibition (Fejgin et al., 2009). A link between GABA_B receptors and nitric oxide was suggested by these investigators on the basis of the finding that baclofen decreased nitric oxide formation in the prefrontal cortex (Fejgin et al., 2009). Hence, it has been proposed that GABA_B receptor activation diminishes the PCP-induced glutamate releases and the subsequent stimulation of nitric oxide formation and, ultimately, the behaviors evoked by PCP.

The aim of the present study was to examine effects of GABA_B receptor activation on the MK-801-induced increase in extracellular glutamate in the prefrontal cortex. Specifically, effects of antipsychotic agents, a nitric oxide synthase inhibitor, and a GABA_B receptor agonist on the MK-801-induced increase in extracellular glutamate in the prefrontal cortex were examined.
2. Materials and Methods

2.1 Animal Procedures

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) were used in these studies. The animals were housed two per cage in a temperature- and humidity-controlled room with a 12-h light/dark cycle and allowed food and water ad libitum. Animals undergoing surgery were housed one per cage postoperatively. All procedures were in strict adherence to the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee.

2.2 Drugs and Drug Treatment

(+)–MK-801 hydrogen maleate, L-NAME hydrochloride (Nω-Nitro-L-arginine methyl ester), and (±)-baclofen were purchased from Sigma-Aldrich (St. Louis, MO). (S)-Nitroso-N-acetylpenicillamine (SNAP) was purchased from Tocris Bioscience (Ellisville, MO). MK-801 and L-NAME were dissolved in 0.15 M NaCl and administered at a dose of 0.3 mg/kg s.c. and 60 mg/kg i.p., respectively. SNAP was dissolved in modified Dulbecco's phosphate buffered saline containing 1.2 mM CaCl₂ and 5 mM glucose to a 5 mM concentration. Baclofen was dissolved in Transcutol:saline (1:2 v/v) and administered at a dose of 5 mg/kg, i.p. Risperidone oral solution and paliperidone powder were from Janssen. Rats were treated with risperidone (0.01 mg/kg/day), paliperidone (0.01 mg/kg/day) or vehicle via drinking water beginning on postnatal day 35 up until the day of the experiment (~ PD 56). Risperidone stock solution (0.1 mg/ml) and paliperidone stock solution (0.1 mg/ml) were prepared in DI water. These stock solutions were further diluted 1:10 in DI water. Daily weight and water consumption was monitored for each animal, and necessary adjustments were made for the animal’s water bottle to supply the appropriate dosages.
2.3 In vivo microdialysis procedures

Rats were implanted with a stainless steel guide cannula under ketamine/xylazine (70/6 mg/kg i.p.) anesthesia 48 to 72 h before the insertion of the dialysis probe. On the afternoon before the dialysis experiment, a concentric style dialysis probe was inserted through the guide cannula into the prefrontal cortex. The coordinates for the tip of the probe were AP, 3.2 mm; LM, 0.8 mm; and DV, −3.5 mm from bregma, according to the stereotaxic atlas of Paxinos and Watson (1986). The active portion of the membrane for the prefrontal cortex is 3.0 mm. The probes were connected to an infusion pump set to deliver modified Dulbecco's phosphate buffered saline containing 1.2 mM CaCl$_2$ and 5 mM glucose at a constant rate 2.0 μl/min. After an equilibration period of 1.5 hours, dialysis samples were collected every 30 min. At least 3 baseline samples were obtained prior to drug treatment. Dialysis samples were collected for an additional 3 hours.

2.4 Analysis of glutamate dialysate concentrations

The extracellular concentrations of glutamate were analyzed by HPLC-EC using the method of Donzanti and Yamamoto (1988). A precolumn derivatization was performed by mixing an aliquot of the dialysate sample with an o-pthalaldehyde/β-mercaptoethanol reagent. A stock solution of the derivatization reagent was prepared by dissolving 27 mg of o-pthalaldehyde in 1 ml of 100% methanol, followed by the addition of 9 ml of a 0.1 M sodium tetraborate solution and 5 μl of β-mercaptoethanol. On a daily basis the stock reagent was further diluted 1:3 in 0.1 M sodium tetraborate. The derivatization reagent was added to each dialysate sample (1:2 v/v), and the reaction proceeded for 2 mins before being injected onto an OPA-HS column connected to a BAS amperometric detector set at +700 mV. The mobile phase consisted of: 0.1 M Na$_2$HP0$_4$, 50 mg/L Na$_2$EDTA, 15% CH$_3$OH, pH 6.4, delivered at 0.800 ml/min. Peak heights were quantified using a Hewlett-Packard integrator.
2.5 Statistical Analysis

Values for glutamate were converted to a percent of mean baseline values and analyzed by two-way repeated measures analysis of variance (ANOVA) (Sigma Stat, Jandel Scientific). ANOVAs were constructed using three baseline values and all values following drug administration. Subsequent multiple comparisons between treatment groups were conducted by post hoc analysis with Student-Newman-Keuls test. Treatment differences were considered statistically significant at p < 0.05.
3. Results

Effect of MK-801 on extracellular glutamate in the prefrontal cortex

The NMDA receptor antagonist MK-801 (0.3 mg/kg, s.c.) significantly increased extracellular glutamate in the prefrontal cortex (Figure 1). MK-801 increased extracellular glutamate to 250% of the baseline values, and glutamate remained elevated for at least 3hr following drug administration. Vehicle administration had no significant effect on extracellular glutamate in the prefrontal cortex. Analysis of the data revealed a significant main effect of treatment \[ F (1, 15) = 17.25, p<0.001 \], effect of time \[ F (8, 110) = 6.79, p<0.001 \] and treatment \( \times \) time interaction \[ F (8, 110) = 7.06, p<0.001 \].

Effect of antipsychotic agents on the MK-801-induced glutamate increase in the prefrontal cortex

To determine the effects of antipsychotic agents on the MK-801-induced increase in extracellular glutamate in the prefrontal cortex, risperidone (0.01 mg/kg/day) and paliperidone (0.01 mg/kg/day) were delivered via drinking water chronically for 21 days. Treatment with either risperidone or paliperidone resulted in a suppression of the MK-801-induced increase in extracellular glutamate in the prefrontal cortex (Figure 2). The ANOVA indicated a significant effect of treatment \[ F(2, 35) = 3.512, p=0.04 \], effect of time \[ F(8, 258) = 4.4, p<0.001 \], and treatment \( \times \) time interaction \[ F(16, 258) = 1.78, p=0.03 \].

Effect of Nitric Oxide on the MK-801-induced glutamate release in the prefrontal cortex

Nitric oxide contribution to the MK-801-induced increase in extracellular glutamate was evaluated using a nitric oxide synthase inhibitor. L-NAME (Nω-Nitro-L-arginine methyl ester) inhibits two isoforms of nitric oxide synthase, iNOS and nNOS. L-NAME (60 mg/kg, i.p.) was
injected 1hr prior to the administration of MK-801. In rats treated with L-NAME, the MK-801-induced increase in glutamate in the prefrontal cortex was markedly diminished (Figure 3). Analysis of the data revealed a significant main effect of treatment \[ F(3,35) = 5.66, p=0.003 \] and treatment x time interaction \[ F(24, 265) = 3.01, p<0.001 \], but no significant effect of time \[ F(8, 265) = 0.87, p=.54 \]. Post hoc analysis revealed that extracellular glutamate in the L-NAME + MK-80-treated rats was significantly \( p<0.05 \) less than that in MK-801-treated controls. L-NAME or vehicle treatment alone had no significant effect on extracellular glutamate.

The role of nitric oxide in modulating glutamate release in the prefrontal cortex was further evaluated using the nitric oxide donor, (S)-Nitroso-N-acetylpenicillamine (SNAP). SNAP (5mM) was infused through the probe directly into the prefrontal cortex for 30 min. Infusion of SNAP significantly increased glutamate to 250\% of baseline values beginning immediately with drug infusion. Extracellular glutamate remained elevated for at least 2 hr after termination of drug infusion (Figure 4). The ANOVA indicated a significant effect of treatment \[ F(1,19) = 8.93, p=0.007 \], effect of time \[ F(9, 144) = 13.54, p<0.001 \], and treatment x time interaction \[ F(9, 144) = 3.904, p<0.001 \]. Vehicle administration had no significant effect on extracellular glutamate in the prefrontal cortex.

Effect of GABA_B receptor activation on the MK-801-induced glutamate increase in the prefrontal cortex

The GABA_B agonist baclofen (5 mg/kg, i.p.) was administered 30 min prior the injection of MK-801 (0.3 mg/kg, s.c.). In baclofen treated animals, the MK-801-induced increase in extracellular glutamate was completely absent (Figure 5). Analysis of the data revealed a significant main effect of treatment \[ F(1, 15) = 7.77, p<0.001 \], and treatment x time interaction
[F (27, 235) = 4.95, p<0.001], but no significant effect of time [F (9, 235) = 1.06, p=0.40].

Baclofen administration alone and vehicle had no significant effect on extracellular glutamate in the prefrontal cortex.
4. Discussion

The purpose of the present study was to examine glutamatergic transmission in the prefrontal cortex following NMDA receptor blockade. The involvement of nitergic and GABAergic mechanisms on the MK-801-induced glutamate release was also examined. The major findings in this study were: 1). MK-801 increased the extracellular concentration of glutamate in the prefrontal cortex, and this response was attenuated in rats treated with the antipsychotics risperidone and paliperidone. 2). inhibition of nitric oxide synthase with L-NAME attenuated the MK-801-induced glutamate release. 3). the nitric oxide donor SNAP significantly increased cortical extracellular glutamate. 4). GABA B receptor activation by baclofen suppressed the increase in glutamate following MK-801 administration.

The effect of MK-801 to increase extracellular glutamate in the prefrontal cortex of rats in the present study is in accord with previous reports that have shown NMDA receptor blockade achieved by PCP or ketamine also increased extracellular glutamate in the prefrontal cortex of rats (Adams and Moghaddam, 1998; Moghaddam et al., 1997). Lorrain and colleagues also observed that systemic administration of ketamine increased glutamate in the prefrontal cortex; however direct infusion of ketamine through the microdialysis probe into the prefrontal cortex failed to elicit an increase (Lorrain et al., 2003). These results suggest that the MK-801-induced increase in extracellular glutamate in the prefrontal cortex involves a neuronal circuit residing outside of the prefrontal cortex. Previous work has established that the increased extracellular glutamate produced by MK-801 is largely dependent on neuronal activity. Although basal extracellular glutamate has been shown to be unaltered by tetrodotoxin (TTX), the MK-801-induced increase in glutamate was shown to be markedly attenuated by TTX (Lopez-Gil et al.,
These findings also are consistent with prior work (Lorrain et al., 2003; Timmerman and Westerink, 1997; Ceglia et al., 2004).

The effect of NMDA antagonists, such as PCP and MK-801, to increase extracellular glutamate in the prefrontal cortex is thought to underlie the cognitive impairments produced by these drugs. Indeed, activation of group II metabotropic glutamate receptors has been shown not only to reduce PCP-induced glutamate release in the prefrontal cortex, but also to minimize the behavioral disruption produced by this NMDA antagonist (Moghaddam and Adams, 1998; Baker et al., 2008).

The acute and chronic administration of clinically effective antipsychotic agents also has been demonstrated to attenuate the stimulatory effect of NMDA antagonists on cortical glutamate release and the behavioral activation produced by these agents. Abekawa and co-workers have demonstrated that the acute or chronic administration of clozapine suppresses the PCP-induced glutamate release in the prefrontal cortex (Abekawa et al., 2006; Abekawa et al., 2007). Consistent with these reports Lopez-Gil and colleagues have reported that the intracortical administration of clozapine or haloperidol also suppresses the MK-801-induced glutamate release in this brain region (Lopez-Gil et al., 2007). The results of the present study in which chronic treatment with risperidone or paliperidone markedly attenuated the MK-801-induced increase in extracellular glutamate in the prefrontal cortex are in accord with these earlier reports.

Although the exact mechanism through which risperidone and paliperidone suppress the stimulatory effect of MK-801 on glutamate release is unknown, it can be speculated that actions on 5-HT2 receptors are involved. It is well documented that risperidone and paliperidone exhibit
high affinity for 5-HT2 receptors (van Beijsterveldt et al., 1994; Stockmeier et al., 1993). Importantly, acute blockade of 5-HT2A receptors has been shown to antagonize the behavioral and neurochemical effects produced by NMDA antagonists (Abekawa et al., 2007; Gleason and Shannon, 1997). In addition, the potency of drugs to suppress NMDA antagonist-induced hyperlocomotion correlates with their affinity for 5-HT2 receptors (Millan et al., 1999; Maurel-Remy et al., 1995). Down regulation of 5-HT2 receptors produced by the chronic administration of clozapine is also associated with a suppression of the PCP-induced glutamate release in the prefrontal cortex (Abekawa et al., 2007). The therapeutic effects of these 5-HT2 antagonist type antipsychotic agents may be due, in part, to the removal of a facilitating effect of 5-HT2 receptors on glutamatergic neurotransmission in the prefrontal cortex (Aghajanian and Marek, 2000).

Another potential mediator of the actions of NMDA antagonists is nitric oxide. Previous research has shown that inhibition of the production of nitric oxide reverses the behavior deficits, including prepulse inhibition, working memory, and spatial learning, associated with NMDA receptor blockade (Wass et al., 2006b; Fejgin et al., 2008; Klamer et al., 2004a; Wass et al., 2006a). Fejgin and colleagues also observed an increase in cGMP levels in the prefrontal cortex following PCP administration that could be prevented by pretreatment with L-NAME, an inhibitor of nitric oxide synthase (Fejgin et al., 2008). cGMP is formed by soluble guanylyl cyclase, which is the main target for nitric oxide; therefore an increase in nitric oxide would lead to an increase in cGMP. Moreover, sodium nitroprusside, a nitric oxide donor, delivered into the frontal cortex of anaesthetized rats results in an increased extracellular concentration of cGMP (Laitinen et al., 1994). Recently, Palsson and co-workers directly measured nitric oxide levels in the prefrontal cortex using microsensors and observed an increase following PCP administration
that was also blocked by L-NAME (Palsson et al., 2009). Fejgin and colleagues have suggested that the behavioral effects of NMDA antagonists are mediated by an increased release of glutamate, activation of NMDA and non-NMDA receptors, and a subsequent increased formation of nitric oxide.

Previous work has demonstrated that activation of NMDA receptors results in production of nitric oxide and leads to long term potentiation (LTP) and synaptic plasticity (Dawson and Dawson, 1996). LTP and synaptic plasticity are involved in learning and memory. Neuronal nitric oxide synthase (nNOS) has been found to be coupled to NMDA receptors, and activation of NMDA receptors leads to Ca$^{2+}$ influx with subsequent nNOS activation. This cascade results in nitric oxide/cGMP formation, as well as LTP (Garthwaite and Boulton, 1995; Brenman and Bredt, 1997; Fedele and Raiteri, 1999).

However, in the present study, inhibition of the production of nitric oxide by L-NAME prevented the increase in glutamate in the prefrontal cortex following MK-801 administration. Secondly, an infusion of SNAP a nitric oxide donor directly into the prefrontal cortex induced an immediate increase in extracellular glutamate. This is consistent with the earlier report that SNAP increased extracellular glutamate in the hippocampus (Watts et al., 2005). Thus, the present finding suggests that nitric oxide not only mediates the cognitive impairments produced by NMDA antagonists, but also mediates the increased release of glutamate produced by MK-801.

Although the neuronal circuit by which NMDA antagonists increase extracellular glutamate in the prefrontal cortex is not entirely understood, it has been postulated that NMDA receptor blockade removes a stimulatory input to inhibitory GABAAergic interneurons, thereby
disinhibiting cortical pyramidal neurons in the prefrontal cortex. The GABA interneurons that modulate the prefrontal cortex are believed to be located in the thalamus and/or the hippocampus (Sharp et al., 2001; Lewis and Moghaddam, 2006). In support of the loss of GABAergic control following NMDA antagonist treatment, Yonezawa and colleagues reported a decrease in GABA release in the prefrontal cortex following local administration of PCP and MK-801 (Yonezawa et al., 1998). Fejgin and co-workers demonstrated that treatment with baclofen, a GABA_B receptor agonist, prevented the PCP disruption of prepulse inhibition (Fejgin et al., 2009). In the same study administration of the nitric oxide synthase inhibitor L-NAME also prevented the deficits in prepulse inhibition. Interestingly, co-administration of baclofen and L-NAME had a synergistic effect with raising prepulse inhibition levels above that of controls. Further suggestive of an interaction between GABA and nitric oxide in these behavioral effects of PCP, baclofen was shown to decrease nitric oxide in the prefrontal cortex (Fejgin et al., 2009). Indeed, GABA has been reported to exert a modulatory control on the production of NO/cGMP (Pepicelli et al., 2004). In the present study baclofen, attenuated the MK-801-stimulated release of glutamate in the prefrontal cortex. Collectively, these data support the view that MK-801 decreases GABAergic transmission, thereby removing an inhibitory control of nitric oxide production and resulting subsequently in an increased nitergic stimulation of glutamate release in the prefrontal cortex.

In summary, MK-801 increases extracellular glutamate in the prefrontal cortex and risperidone and paliperidone suppress the efflux. Inhibition of nitric oxide synthase blocks the MK-801-induced glutamate increase, while a nitric oxide donor increases glutamate in the prefrontal cortex. Finally, GABA_B receptor activation attenuates the MK-801-stimulated increase of extracellular glutamate in the prefrontal cortex.
Figure 1. **Effect of MK-801 on extracellular glutamate in the prefrontal cortex**

Rats were treated with an injection of vehicle or MK-801 (0.3 mg/kg, s.c.) at time 0. Extracellular glutamate was measured in the prefrontal cortex for an additional 3 hrs following injection. The values represent the percent of mean baseline values ± S.E of 7-10 rats/group. * indicates values that are significant (p<0.05) compared to values for saline treated animals.
Fig 2. Effect of antipsychotic agents on the MK-801-induced extracellular glutamate increase in the prefrontal cortex

Rats were treated with risperidone (0.01 mg/kg/day), paliperidone (0.01 mg/kg/day), or vehicle via drinking water chronically for 21 days prior to microdialysis beginning on PD 35. On the day of dialysis, rats were treated with an injection of MK-801 (0.3 mg/kg, s.c.) at time 0. Extracellular glutamate was measured in the prefrontal cortex for an additional 3 hr following injection. The values for extracellular glutamate were converted to a percent of mean baseline values ± S.E of 10-16 rats/group. * represents values that are significant (p<0.05) compared to values at corresponding time for control rats. # indicates values that differ significantly (p<0.05) compared to control rats.
Figure 3. Effect of L-NAME on the MK-801-induced increase in extracellular glutamate in the prefrontal cortex

Rats were treated with vehicle or L-NAME (60 mg/kg, i.p.) 1 hr prior to injection of MK-801 (0.3 mg/kg, s.c.), or vehicle at time 0. Extracellular glutamate was measured in the prefrontal cortex 3 hr following injection. The values represent the percent of mean baseline values ± S.E of 6-8 rats/group * indicates values for the L-NAME + MK-801 animals that differ significantly (p<0.05) from those of the saline + MK-801 animals.
Figure 4. Effect of SNAP infusion on extracellular glutamate in the prefrontal cortex

Rats were treated with vehicle or SNAP (5mM) infusion through the dialysis probe directly into the prefrontal cortex for 30 min at time 0. Extracellular glutamate was measured in the prefrontal cortex for an additional 2.5 hr following infusion. The values for extracellular glutamate were converted to a percent of mean baseline values ± S.E of 8-13 rats/group. * represents values that are significant (p<0.05) compared to values at corresponding time for control rats.
Figure 5. Effect of baclofen on the MK-801-induced extracellular glutamate increase in the prefrontal cortex

Rats were treated with vehicle or baclofen (5 mg/kg, i.p.) 30 min prior to injection of MK-801 (0.3 mg/kg, s.c.), or vehicle at time 0. Extracellular glutamate was measured in the prefrontal cortex 3 hr following injection. The values represent the percent of mean baseline values ± S.E of 5-12 rats/group * indicates values for the baclofen + MK-801 animals that differ significantly (p<0.05) from those of the saline + MK-801 animals.
Chapter 2
1. Introduction

Environmental factors play a role in combination with genetic factors in the pathology of schizophrenia. Epidemiological studies suggest that maternal infection is one such environmental factor that increases the risk for schizophrenia in offspring. Exposure to infection, presumably resulting in exposure to an immune response in utero, is believed to alter critical periods of central nervous system development that may result in pathophysiology similar to schizophrenia. Therefore, exposure of pregnant dams to agents that provoke an immune response, including poly I:C, has been employed to generate animals with relevance to schizophrenia. Poly I:C is a synthetic double-stranded RNA which mimics viral infection and elicits an immune response, including cytokine induction, fever, stress hormones, and malnutrition in the pregnant dams (Kimura et al., 1994; Fortier et al., 2004; Meyer et al., 2006). Offspring exposed to prenatal immune activation have been shown to exhibit behavioral and cognitive abnormalities, as well as abnormal pharmacological responses. Ozawa and colleagues reported adult offspring of poly I:C dams had deficits in sensory-motor gating and cognitive impairments. Interestingly, the offspring of poly I:C had decreased prepulse inhibition and methamphetamine-induced hyperlocomotion as adults compared to controls; however no differences were observed as juveniles (Ozawa et al., 2006).

The ability of antipsychotic agents to attenuate the behavioral and cognitive deficits observed in offspring exposed to prenatal immune activation has also been examined. Meyer and co-workers demonstrated chronic clozapine prevents the deficits in prepulse inhibition and latent inhibition in offspring exposed to poly I:C, while haloperidol only attenuates the latent inhibition (Meyer et al., 2008). Interestingly, Romero and colleagues observed chronic haloperidol treatment prevented prepulse inhibition deficits in offspring exposed to LPS, a bacterial toxin.
also used to induce an immune response for prenatal immune models (Romero et al., 2007). These findings also support the concept that offspring exposed to prenatal immune activation may provide an animal model with deficits that are relevant to schizophrenia.

Recently, Ibi et al. (2009) examined glutamate neurotransmission in the hippocampus of adult mice following neonatal poly I:C treatment. The poly I:C-exposed adults had elevated basal extracellular glutamate concentrations in the hippocampus compared to control animals. The elevated extracellular glutamate was reduced in the presence of tetrodotoxin which suggests abnormalities in neuronal glutamate release in animals exposed to prenatal immune activation. In support of this view is the additional finding that K⁺-evoked release of glutamate in poly I:C-exposed mice was markedly reduced relative to that in control animals (Ibi et al., 2009).

The aim of the present study was to examine glutamate neurotransmission in the prefrontal cortex of offspring exposed to poly I:C, as well as evaluate the potential of two antipsychotic agents, risperidone and paliperidone, to regulate glutamate neurotransmission in the prefrontal cortex of offspring from poly I:C-treated dams.
2. Materials and Methods

2.1 Animal Procedures

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) were used in these studies. The animals were housed two per cage in a temperature- and humidity-controlled room with a 12-h light/dark cycle and allowed food and water ad libitum. Animals undergoing surgery were housed one per cage postoperatively. All procedures were in strict adherence to the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee.

2.2 Generation of rats for prenatal immune activation

Female Sprague-Dawley rats, 3-5 months of age were mated overnight. The following day (gestational day 0) females were separated and singly housed. On gestational day 14 dams were weighed and pregnant dams (weight gain > 40 grams) were injected with poly I:C (8 mg/kg, i.p.) or saline (1 ml/kg, i.p.). Day 14 of gestation in rats corresponds to day 52 of gestation in humans (http://www.translatingtime.net/). On postnatal day 1, litters were culled to eight. Pups were weaned on postnatal day 21 and housed by sex and litter. Offspring remained undisturbed with cage mates until the day of experimental testing. No more than 2 rats per litter were used in each experimental group to avoid litter effect confounds. Only male offspring from poly I:C and vehicle treated dams were used for these studies. Animals underwent experiments between postnatal days 54-58.

2.3 Drugs and Drug Treatment

(+)-MK-801 hydrogen maleate and polyinosinic:polycytidylic acid (poly I:C) were purchased from Sigma-Aldrich (St. Louis, MO). Poly I:C was stored at -20° C in a dessicator and was
made fresh on day of injections. The drugs were dissolved in 0.15 M NaCl. MK-801 was administered at a dose of 0.3 mg/kg s.c. for in vivo microdialysis. Risperidone oral solution and paliperidone powder were provided by Janssen Research Foundation. Rats were treated with risperidone (0.01 mg/kg/day), paliperidone (0.01 mg/kg/day) or vehicle via drinking water beginning on postnatal day 35 up until the day of the experiment (PD 56-58). Risperidone stock solution (0.1 mg/ml) and paliperidone stock solution (0.1 mg/ml) were prepared in distilled water. Stock solutions were further diluted 1:10 in distilled water. Daily weight and water consumption was monitored for each animal, and necessary adjustments were made for the animal’s water bottle to supply the appropriate dosages.

2.4 Statistical Analysis

For microdialysis experiments in which the response to MK-801 was determined, values for extracellular glutamate were converted to a percent of mean baseline values and analyzed using a two-way repeated-measures analysis of variance (ANOVA) (Sigma Stat, Jandel Scientific). ANOVAs were constructed using three baseline values and all values following drug administration. Subsequent multiple comparisons between treatment groups were conducted by post hoc analysis with Student-Newman-Keuls test. Student’s t test was used to analyze absolute values (ng/20µl) for baseline glutamate concentrations in poly I:C and vehicle rats. A two-way ANOVA was used to analyze the effects of pretreatment (Risp. 0.01 and Pali. 0.01) on absolute values (ng/20µl) for baseline glutamate concentrations between poly I:C and vehicle rats. Treatment differences were considered statistically significant at P<0.05.
3. Results

Effect of prenatal immune activation on the MK-801-induced glutamate efflux in the prefrontal cortex

MK-801 increased extracellular glutamate by approximately 75% for a period of 3 hr in control animals, and the response was significantly (p<0.001) blunted in the prefrontal cortex of offspring from poly I:C-treated dams (Figure 1). Analysis of the data revealed a significant effect of treatment [ F(1, 26) = 11.63, p=0.002], effect of time [ F(8, 193) = 3.54, p<0.001], and treatment x time interaction [ F(8, 193) = 3.67, p<0.001].

Effect of prenatal immune activation on basal extracellular glutamate in the prefrontal cortex

Basal concentrations (ng/20µl) of extracellular glutamate in dialysis sample of the prefrontal cortex were examined in the offspring of dams exposed to poly I:C and saline (Figure 2). Offspring in the poly I:C-treated group had significantly (p<0.05) elevated basal extracellular glutamate values of 7.0 ± 0.8 ng/20µl when compared to the values, 4.7 ± 0.7 ng/20µl, of control animals.

Effect of antipsychotics on elevated basal extracellular glutamate in the prefrontal cortex of poly I:C-treated offspring

To determine the effects of antipsychotic agents on basal extracellular glutamate in the prefrontal cortex, risperidone (0.01 mg/kg/day) and paliperidone (0.01 mg/kg/day) were delivered via drinking water chronically for 21 days to the offspring of saline and poly I:C-treated dams. Chronic exposure to risperidone or paliperidone did not significantly alter basal extracellular concentration of glutamate in the prefrontal cortex of control animals. In contrast, treatment with paliperidone significantly (p<0.05) reduced basal extracellular glutamate in offspring exposed to poly I:C (Figure 3). Treatment with risperidone produced a trend towards a
reduction of basal glutamate in poly I:C-exposed animals, but this effect was not statistically significant. Basal concentration of extracellular glutamate in the prefrontal cortex of poly I:C-exposed rats was significantly (p<0.05) greater than that of control animals. Two way ANOVA revealed a significant effect of pretreatment [F(1, 71) = 6.84, p=0.011], but no significant effect of treatment [F(2,71) = 2.13, p=0.126] or interaction of pretreatment x treatment [F(2,71) = 2.59, p=0.082].
4. Discussion

The purpose of the present study was to assess the effect of prenatal immune activation on glutamate neurotransmission in the prefrontal cortex. The major findings of this study were: 1) offspring in the poly I:C-treated group had a blunted glutamate response to MK-801 in the prefrontal cortex. 2) poly I:C-exposed offspring had elevated basal extracellular glutamate in the prefrontal cortex. 3) the elevated basal extracellular glutamate in the prefrontal cortex in offspring of poly I:C-treated dams were reduced following paliperidone treatment and, to a lesser extent, with risperidone.

It is well documented that NMDA antagonists, e.g. MK-801, increase extracellular glutamate in the prefrontal cortex. In the present study, the MK-801-induced increase in extracellular glutamate was markedly reduced in the prefrontal cortex of offspring exposed to poly I:C. It is noteworthy that Lena and colleagues also observed a blunted glutamate response to MK-801 in the prefrontal cortex of rats exposed to methylazoxymethanol (MAM) (Lena et al., 2007). MAM is another agent used to induce developmental abnormalities in animal models with relevance to schizophrenia.

Increased extracellular glutamate in the prefrontal cortex following the administration of NMDA antagonists is thought to result from the blockade of NMDA receptors on inhibitory GABAergic neurons projecting to glutamatergic neurons in the prefrontal cortex and a subsequent disinhibition of cortical glutamate release (Moghaddam et al., 1997; Krystal et al., 2003). The lack of a glutamate response to MK-801 in poly I:C exposed rats may be due to a relative state of NMDA receptor hypofunction in these rats rendering them less sensitive to further NMDA receptor blockade. Alternatively, dysfunction may exist down-stream in this
NMDA-GABA-glutamate circuit in which inhibitory GABAergic tone may already be diminished. A potentially diminished inhibitory GABAergic input to cortical glutamatergic neurons may also account for the elevated basal concentrations of extracellular glutamate in the poly I:C animals.

It would seem unlikely that the elevated basal extracellular concentration of glutamate presents a ceiling effect for increased extracellular glutamate, since local infusion of a nitric oxide donor or high $K^+$ evokes an increase in extracellular glutamate that is greater than the elevated basal concentrations observed in poly I:C-exposed animals (Roenker, Gudelsky, Richtand, unpublished observations; Ibi et al., 2009).

The present results are consistent with the recent report of Ibi et al (2009) in which the extracellular concentration of glutamate in the dorsal hippocampus was examined in rats exposed prenatally to immune activation. Ibi and colleagues reported that the basal extracellular concentrations of glutamate in the hippocampus of poly I:C-exposed rats were greater than that in control animals. In addition, $K^+$-evoked release of glutamate was significantly less in poly I:C-exposed animals than in controls (Ibi et al., 2009). Thus, elevated basal extracellular glutamate and blunted stimulated glutamate release in rats exposed to prenatal immune activation is evident in both the hippocampus and prefrontal cortex.

Further demonstration of abnormalities in glutamatergic transmission in the prefrontal cortex and hippocampus has been provided by Bitanihirwe and co-workers who report that tissue concentrations of glutamate in the prefrontal cortex and hippocampus of poly I:C-exposed mice are lower than those of control animals (Bitanihirwe et al., 2010). Although the relationship between tissue concentrations of glutamate and extracellular concentrations of glutamate is not
clear, it is noteworthy that reduced tissue concentrations of glutamate and increased extracellular concentrations of glutamate are evident in both the hippocampus and prefrontal cortex (Ibi et al., 2009; present study).

The ability of antipsychotic agents to reverse the behavioral abnormalities in offspring exposed to prenatal immune activation has been examined in several studies. Clozapine has been shown to attenuate the cognitive deficit, measured by novel object recognition, in poly I:C-exposed offspring (Ozawa et al., 2006). Meyer and colleagues have reported that haloperidol and clozapine reverse impaired latent inhibition in offspring from poly I:C-treated dams (Meyer et al., 2008).

There are few, if any, reports on the neurochemical effects of antipsychotic in rats exposed to prenatal immune activation. Although neither risperidone or paliperidone altered basal extracellular concentrations of glutamate in control animals, paliperidone reduced the elevated extracellular glutamate in rats exposed to poly I:C. The exact mechanism through which paliperidone reduces basal glutamate in rats exposed prenatally to immune activation is unknown, but it seems reasonable to propose a 5-HT2 receptor dependent process. It is well documented that risperidone and paliperidone exhibit high affinity for 5-HT2 receptors (van Beijsterveldt et al., 1994; Stockmeier et al., 1993). The importance of 5-HT2 antagonist properties of risperidone and paliperidone are of particular relevance in light of the hypothesis that poly I:C-exposed animals exhibit a state of NMDA hypofunction. As with the acute administration of NMDA antagonists to control animals, a state of NMDA hypofunction in poly I:C-exposed rats may be associated with increased cortical glutamatergic neurotransmission. Importantly, acute blockade of 5-HT2A receptors has been shown to antagonize the behavioral and neurochemical effects produced by NMDA antagonists (Abekawa et al., 2007; Gleason and
Shannon, 1997). Furthermore, down regulation of 5-HT2 receptors produced by the chronic administration of clozapine also is associated with a suppression of the PCP-induced glutamate release in the prefrontal cortex (Abekawa et al., 2007). The therapeutic effects of these 5-HT2 antagonist type antipsychotic agents may be due, in part, to the removal of a facilitating effect of 5-HT2 receptors on glutamatergic neurotransmission in the prefrontal cortex (Aghajanian and Marek, 2000).

In summary, offspring from poly I:C-treated dams had a blunted glutamate response to MK-801 in the prefrontal cortex compared to offspring from saline treated dams. Poly I:C-exposed offspring also had elevated basal extracellular glutamate in the prefrontal cortex compared to saline offspring. Finally, paliperidone normalized the elevated basal extracellular glutamate in the prefrontal cortex of poly I:C-treated offspring, while risperidone lowered basal extracellular glutamate to a lesser extent.
Figure 1. Effect of prenatal immune activation on the MK-801-induced extracellular glutamate efflux in the prefrontal cortex

Male offspring (PD 56) from saline and poly I:C treated dams were treated with an injection of MK-801 (0.3 mg/kg, s.c.) at time 0. Extracellular glutamate was measured in the prefrontal cortex for 3 hr following the injection. The values for extracellular glutamate were converted to a percent of mean baseline values ± S.E of 12-15 rats/group. * indicate values that are significant (p<0.05) compared to values for the poly I:C treated animals.
Figure 2. Effect of prenatal immune activation on basal extracellular glutamate in the prefrontal cortex

Data represents basal extracellular glutamate (ng/20µl ± S.E) concentration in the prefrontal cortex of adult male offspring from saline and poly I:C treated dams for 12-15 rats/group. * indicates significance (p<0.05) compared to saline treated animals.
Figure 3. Effect of antipsychotics on elevated basal extracellular glutamate in the prefrontal cortex of poly I:C-treated offspring

Male rats from saline and poly I:C exposed dams were treated with risperidone (0.01 mg/kg/day), paliperidone (0.01 mg/kg/day), or vehicle via drinking water chronically for 21 days prior to microdialysis. Basal extracellular glutamate was measured in the prefrontal cortex (ng/20µl ± S.E). (#) indicates the number of rats per treatment group. * represents values that are significant (p<0.05) compared to values for saline offspring treated with vehicle. # indicates values that differ significantly (p<0.05) compared to poly I:C offspring treated with vehicle.
Future Directions

The results presented in this study have established that MK-801-induced glutamate increase in the prefrontal cortex can be modulated by nitergic and GABAergic mechanisms, as well as by antipsychotic agents. L-NAME used in the present study inhibits two isoforms of nitric oxide synthase, iNOS and nNOS. The possible role of the different isoforms on the MK-801-induced glutamate increase in the prefrontal cortex could be examined. Potential studies could utilize selective iNOS or nNOS inhibitors to better define the role of nitric oxide in the mechanism of MK-801-induced glutamate release.

In addition, glutamate neurotransmission is altered in animals exposed to prenatal immune activation by poly I:C, an animal model with relevance to schizophrenia. Thus, poly I:C-exposed animals may exhibit NMDA receptor hypofunction. In view of the effects of inhibition of nitric oxide synthase and activation of GABA$_B$ receptor on behavioral and neurochemical effects of MK-801, additional studies could be pursued that examine modulation of glutamatergic transmission in poly I:C-exposed animals through nitergic and GABAergic pathways. Future potential studies could explore the effect of nitric oxide synthase inhibition by L-NAME on the elevated basal extracellular glutamate in the prefrontal cortex of poly I:C-exposed offspring. Furthermore, nitric oxide has been demonstrated to be involved in cognitive impairments associated with NMDA receptor blockade (Wass et al., 2008; Fejgin et al., 2008; Klamer et al., 2005). Therefore, treatment with L-NAME could be examined on the behavioral cognitive deficits found in offspring exposed to prenatal immune activation. Inasmuch as nitric oxide appears to promote glutamate release in the prefrontal cortex, assessment of nitric oxide synthase expression and activity in the prefrontal cortex of poly I:C-exposed animals also seems warranted.
Abnormalities in GABAergic transmission has also been discovered in schizophrenic patients. Postmortem studies have reported reduced GABA_B receptor and glutamic acid decarboxylase (GAD67), the enzyme that synthesizes GABA, as well as GABA membrane transporter (GAT1) in the prefrontal cortex of subjects (Ishikawa et al., 2005; Akbarian et al., 1995; Volk et al., 2001). Moreover, Bitanihirwe and colleagues reported that tissue concentrations of GABA in the hippocampus of poly I:C-exposed mice were lower than those of control animals (Bitanihirwe et al., 2010). Future studies could examine GABA_B activation by baclofen on the elevated basal extracellular glutamate in the prefrontal cortex of animals exposed to prenatal immune activation.

Alterations in glutamate, as well as GABA, have been associated with the pathophysiology of schizophrenia and in the psychotic symptoms elicited by NMDA receptor antagonists. Animal models, including poly I:C-exposed rats, that employ developmental insults to evoke abnormal behavior and neurochemistry appear to have relevance to schizophrenia. Elucidation of the mechanisms (e.g. nitergic, GABAergic) underlying abnormalities in glutamatergic neurotransmission in poly I:C-exposed animals may provide input into novel targets for future therapies.
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