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Evidence for involvement of nitric oxide and GABA_B receptors in MK-801- stimulated release of glutamate in rat prefrontal cortex

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Abstract

Systemic administration of NMDA receptor antagonists elevates extracellular glutamate within prefrontal cortex. The cognitive and behavioral effects of NMDA receptor blockade have direct relevance to symptoms of schizophrenia, and recent studies demonstrate an important role for nitric oxide and GABA_B receptors in mediating the effects of NMDA receptor blockade on these behaviors. We sought to extend those observations by directly measuring the effects of nitric oxide and GABA_B receptor mechanisms on MK-801-induced glutamate release in the prefrontal cortex. Systemic MK-801 injection (0.3 mg/kg) to male Sprague-Dawley rats significantly increased extracellular glutamate levels in prefrontal cortex, as determined by microdialysis. This effect was blocked by pretreatment with the nitric oxide synthase inhibitor L-NAME (60 mg/kg). Reverse dialysis of the nitric oxide donor SNAP (0.5 – 5 mM) directly into prefrontal cortex mimicked the effect of systemic MK-801, dose-dependently elevating cortical extracellular glutamate. The effect of MK-801 was also blocked by systemic treatment with the GABA_B receptor agonist baclofen (5 mg/kg). In combination, these data suggest increased nitric oxide formation is necessary for NMDA antagonist-induced elevations of extracellular glutamate in the prefrontal cortex. Additionally, the data suggest GABA_B receptor activation can modulate the NMDA antagonist-induced increase in cortical glutamate release.

Keywords

glutamate; nitric oxide; MK-801; GABA; NMDA receptor; schizophrenia

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1. Introduction

Extracellular glutamate in rat prefrontal cortex is dramatically elevated following treatment with the NMDA glutamate receptor antagonists MK-801, phencyclidine (PCP), and ketamine (Bubser et al., 1995;Lorrain et al., 2003;Moghaddam et al., 1997;Moghaddam and Adams, 1998). While the neurochemical underpinnings of this effect have not yet been fully defined, studies in multiple laboratories extending from rodents to humans provide evidence linking this phenomenon to outcome measures of direct relevance to schizophrenia (Krystal et al., 1999;Krystal et al., 2003;Moghaddam et al., 1997). For example, NMDA receptor antagonists disrupt prepulse inhibition of startle (Mansbach and Geyer, 1989;Geyer et al., 1990), an operational measure of sensorimotor gating impaired in schizophrenia patients, and also impair cognitive flexibility measured by the Morris water maze (Morris, 1989;Whishaw and Auer, 1989). Both typical and atypical antipsychotic medications including haloperidol, clozapine, aripiprazole, quetiapine, and remoxipride attenuate the PCP-induced disruption of prepulse inhibition (Fejgin et al., 2007;Martinez et al., 2000;Pietraszek and Ossowska, 1998;Swerdlow et al., 1996;Johansson et al., 1994;Bakshi et al., 1994). Additionally, the atypical antipsychotic medications sertindole, risperidone, lurasidone, and clozapine reverse PCP or MK-801-induced impairment in the Morris water maze (Didriksen et al., 2007;Enomoto et al., 2008). We and others have shown that antipsychotic medications also suppress the MK-801-(Lopez-Gil et al., 2007;Roenker et al., 2011) and PCP- (Abekawa et al., 2006;Abekawa et al., 2007) induced increase in extracellular glutamate in the prefrontal cortex.

Enzymatic production and diffusion of the free radical gas nitric oxide provides one mechanism through which glutamate receptors exert downstream second messenger signaling effects. Increased intracellular calcium entry through glutamate- gated NMDA and AMPA ion channel receptors activate nitric oxide synthase via calmodulin-dependent mechanisms (Bhardwaj et al., 1997;Fedele and Raiteri, 1999;Garthwaite, 1991). Nitric oxide synthase activity is also modulated by metabotropic glutamate receptors, as well as by other neurotransmitters including GABAergic and cholinergic systems (Boix et al., 2011;Fedele and Raiteri, 1999), demonstrating that nitric oxide signaling may be modulated by multiple converging mechanisms. Nitric oxide synthesized in post-synaptic neurons is able to diffuse out to neighboring pre-synaptic terminals, where it may exert effects through activation of guanylyl cyclase and stimulation of cyclic GMP synthesis. Nitric oxide may also be formed pre-synaptically and exert post-synaptic effects, thereby acting as a neurotransmitter (Garthwaite, 1991).

Several studies provide evidence supporting a role for nitric oxide in PCP-induced disruption of prepulse inhibition (Klamer et al., 2004a;Palsson et al., 2010;Klamer et al., 2005). The nitric oxide synthase inhibitors N(omega)-propyl-L-arginine (Klamer et al., 2004b) and Nω-Nitro-L-arginine methyl ester (L-NAME) (Klamer et al., 2001) prevent disruption of prepulse inhibition elicited by PCP. Furthermore, L-NAME also attenuates the PCP-induced disruption in Morris water maze performance (Wass et al., 2008). Nitric oxide exerts second messenger effects primarily through activation of guanylyl cyclase resulting in increased cGMP; PCP increases cGMP in prefrontal cortex, and this effect is also prevented by L-NAME pretreatment (Fejgin et al., 2008). Fejgin and colleagues have hypothesized that L-NAME disrupts PCP behavior through an inhibition of glutamate- stimulated nitric oxide formation. This supposition is based on several reports demonstrating a stimulatory effect of glutamate to enhance nitric oxide formation. Collectively, these findings support the involvement of glutamate- stimulated nitric oxide, mediated via a non-NMDA glutamate receptor mechanism, in cognitive impairments following NMDA receptor antagonism.

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). The observation that the GABA_B agonist baclofen decreased nitric oxide formation in prefrontal cortex provided a link between GABA_B receptors and nitric oxide (Fejgin et al., 2009). Hence, it has been proposed that GABA_B receptor activation opposes PCP-induced glutamate release and the subsequent non-NMDA glutamate receptor mediated stimulation of nitric oxide formation, thereby diminishing PCP- evoked behaviors.

Collectively, these findings suggest the model illustrated in Figure 1. Under normal conditions, stimulation of NMDA receptors on GABAergic interneurons in the thalamus or hippocampus provides tonic inhibition of glutamatergic pyramidal neurons projecting to prefrontal cortex. NMDA receptor blockade results in a disinhibition of cortical glutamate release, thereby activating non-NMDA (AMPA/kainite) glutamate receptors expressed on cortical pyramidal neurons. Increased calcium influx through these receptors activates nitric oxide synthase within the cortical pyramidal neuron. Nitric oxide also diffuses retrogradely to the glutamate axon terminals projecting from hippocampus and thalamus, where it functions to sustain and enhance glutamate efflux through elevations of cGMP levels. In contrast, stimulation of cortical GABA_B receptors inhibits nitric oxide formation, limiting glutamate release. Glutamate – nitric oxide interactions are therefore bi-directional in this model, with glutamate acting through non-NMDA glutamate receptors to stimulate nitric oxide production, and nitric oxide subsequently stimulating elevated glutamate release.

While the available data support this proposed model, to our knowledge direct measurement of extracellular glutamate to test the model has not been performed. The aim of the present study was to determine the effects of the nitric oxide synthase inhibitor L-NAME, the nitric oxide donor (S)-Nitroso-N-acetylpenicillamine (SNAP), and the GABA_B receptor agonist baclofen on MK-801-stimulated extracellular glutamate in rat prefrontal cortex in order to directly test this model. Consistent with the proposed model, we identified effects of L-NAME, SNAP, and baclofen on MK-801 stimulated extracellular glutamate as described below.

2. Materials and Methods

2.1. Animal Procedures

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) 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 (N ω -Nitro-L-arginine methyl ester), and ($\pm$)-baclofen were purchased from Sigma-Aldrich (St. Louis, MO). (S)-Nitroso-N-acetylpenicillamine (SNAP) was purchased from Tocris Bioscience (Ellisville, MO). 2-(2-Ethoxyethoxy)ethanol (Transcutol), used as a solvent to dissolve baclofen, was purchased from Gattefosse SA (St Priest Cedex France). 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 in a concentration range between 0.5 – 5 mM. Baclofen was dissolved in Transcutol:saline (1:2 v/v) and administered at a dose of 5 mg/kg, i.p. The MK-801 dose was selected based upon previous studies demonstrating measurable extracellular glutamate change at this MK-801 dose (Ito et al., 2006; Roenker et al., 2011). The L-NAME dose was

selected because it is on the upper edge of the dose-response curve for rat dosages demonstrating effects upon both nitric oxide synthase activity and brain monoamine neurochemistry (Yamada et al., 1995). The baclofen dose was selected based upon a prior study demonstrating this dose reversed dizocilpine-induced reduction in prepulse inhibition of startle in the rat (Bortolato et al., 2004). The SNAP dose range was selected based upon prior studies demonstrating concentration-dependent neurochemical effects of this dose range when administered by microdialysis in freely moving rats (Watts et al., 2004).

2.3. In vivo microdialysis

Rats were implanted with a stainless steel guide cannula under ketamine/xylazine (70/6 mg/kg i.p.) anesthesia 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 microdialysis probes were concentric and constructed as previously described (Yamamoto and Pehek, 1990). The coordinates for the tip of the probe were AP, 3.2 mm; LM, 0.8 mm from bregma; and DV, -3.5 mm from the surface of the brain, according to the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 1986). The active portion of the membrane (SpectraPor, 13,000 molecular weight cutoff; 216 μ m outside diameter) for the prefrontal cortex was 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 1.0 μ l/min overnight. On the following morning the flow rate was adjusted to 2.0 μ l/min and, 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. Basal values for extracellular glutamate in the prefrontal cortex were 3.8 $\pm$ 0.2 ng/20 μ l.

2.3.1. Analysis of glutamate dialysate concentrations—Extracellular glutamate concentrations were analyzed by HPLC-EC using procedures similar to those described by Donzanti and Yamamoto (Donzanti and Yamamoto, 1988), as we have previously described (Wallace et al., 2001). The HPLC consisted of an OPA-HS column connected to a BAS amperometric detector set at +700 mV. The mobile phase consisted of: 0.1 M Na_2HPO_4 , 50 mg/L Na_2EDTA , 15% CH_3OH , pH 6.4, delivered at 0.800 ml/min, with 20 microliters of dialysate injected onto the HPLC. Peak heights were quantified using a Hewlett-Packard integrator.

2.4. Statistical Analysis

Values for glutamate were converted to a percent of mean baseline values prior to all analyses. ANOVAs were constructed using three baseline values and all values following drug administration. MK-801 and SNAP effects on glutamate release were analyzed by two-way repeated measures analysis of variance (ANOVA) (Sigma Stat, Jandel Scientific). Subsequent multiple comparisons between treatment groups were conducted by post hoc analysis with Student-Newman-Keuls test. Effects of L-NAME and baclofen on glutamate release following MK-801 administration were analyzed by three-way repeated measures ANOVA using the MIXED procedure of SAS System for Windows (SAS Institute, Cary, NC). Pre-treatment (Vehicle vs. L-NAME or Baclofen) and post-treatment (Vehicle vs. MK-801) were used as main factors, time as the repeated measure and percent glutamate as the dependent factor. Where significant interactions with Time were identified, further analyses of simple effects were conducted (slice ANOVA) using the MIXED procedure and slice option. Treatment effects within each 30-minute interval were subsequently adjusted together for multiple planned comparisons by False Discovery Rate (error rate = 0.05). For all analyses, treatment differences were considered statistically significant at $p < 0.05$.

3. Results

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

As previously reported (Lopez-Gil et al., 2007; Lopez-Gil et al., 2009; Pietraszek et al., 2009; Roenker et al., 2011), the NMDA receptor antagonist MK-801 (0.3 mg/kg, s.c.) significantly increased extracellular glutamate in prefrontal cortex (Figure 2). MK-801 increased extracellular glutamate to 250% of baseline values, and glutamate remained elevated for more than 3hr following drug administration. Vehicle administration had no significant effect on extracellular glutamate. Analysis of the data revealed a significant main effect of treatment [$F(1, 15) = 17.25, p < 0.001$], time [$F(8, 110) = 6.79, p < 0.001$] and treatment $\times$ time interaction [$F(8, 110) = 7.06, p < 0.001$].

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

We evaluated whether nitric oxide activity is necessary for MK-801-stimulated extracellular glutamate using the nitric oxide synthase inhibitor L-NAME (N ω -Nitro-L-arginine methyl ester), which inhibits two isoforms of nitric oxide synthase, iNOS and nNOS. L-NAME (60 mg/kg, i.p.) was injected 1hr prior to MK-801-administration. L-NAME pretreatment markedly diminished the MK-801-induced increase in prefrontal cortical glutamate (Figure 3). Data analysis identified a significant main effect of post-treatment [$F(1, 34.5) = 5.08, p = 0.031$], and interactions of post-treatment $\times$ time [$F(8, 265) = 2.77, p = 0.0059$], and pre-treatment $\times$ post-treatment $\times$ time [$F(8, 265) = 2.35, p = 0.019$] but no significant effects of pre-treatment, time, pre-treatment $\times$ time or pre-treatment $\times$ post-treatment interaction. Subsequent slice ANOVAs identified that rats treated with vehicle + MK-801 had significantly enhanced extracellular glutamate levels compared to vehicle + vehicle rats at all time points following MK-801 injection ($p < 0.05$). Administration of L-NAME prior to MK-801 significantly lowered extracellular glutamate levels compared to vehicle + MK-801 treated animals ($p < 0.05$). L-NAME + MK-801 treated rats were not significantly different from vehicle + vehicle rats. L-NAME treatment alone had no significant effect on extracellular glutamate.

We next evaluated whether nitric oxide activity was sufficient in modulating glutamate release in prefrontal cortex using the nitric oxide donor, (S)-Nitroso-N-acetylpenicillamine (SNAP). SNAP (0.5–5 mM) was infused through the probe directly into the prefrontal cortex for 30 min. SNAP produced a dose-dependent increase in the extracellular concentration of glutamate (Figure 4). Extracellular glutamate was increased significantly to approximately 400% of baseline values following reverse dialysis with 5 mM or 1.5 mM SNAP; an increase in glutamate to approximately 275% of baseline values was produced by 0.5 mM SNAP. ANOVA indicated a significant effect of drug [$F(3, 30) = 5.36, p = 0.004$] and time [$F(9, 230) = 12.28, p < 0.001$] and a significant drug $\times$ time interaction [$F(27, 230) = 2.42, p < 0.001$]. Vehicle administration had no significant effect on extracellular glutamate levels.

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

We next investigated the role of GABA_B receptor activation on MK-801-stimulated glutamate release. The GABA_B agonist baclofen (5 mg/kg, i.p.) was administered 30 min prior to MK-801 (0.3 mg/kg, s.c.) injection (Figure 5). Data analysis revealed a significant main effect of post-treatment [$F(1, 31.8) = 6.70, p = 0.014$] and interactions of post-treatment $\times$ time [$F(9, 260) = 4.90, p < 0.0001$], pre-treatment $\times$ post-treatment [$F(1, 31.8) = 5.75, p = 0.023$], and pre-treatment $\times$ post-treatment $\times$ time [$F(9, 260) = 2.81, p = 0.0036$]. Pre-treatment, time and pre-treatment $\times$ time interactions were not significant. Slice ANOVAs indicated that glutamate levels of animals treated with vehicle + MK-801 were significantly elevated compared to vehicle + vehicle animals at all time points one hour following

MK-801 administration ($p < 0.05$). Extracellular glutamate levels of baclofen-treated rats were significantly attenuated from vehicle-treated rats following MK-801 administration ($p < 0.05$) and were not significantly different from vehicle + vehicle treated animals. Baclofen administration alone had no significant effect on extracellular glutamate levels.

4. Discussion

The purpose of the present study was to directly measure the involvement of nitergic and GABAergic mechanisms on MK-801-induced glutamate release in the prefrontal cortex. The major findings were: 1). MK-801 increased the extracellular concentration of glutamate in the prefrontal cortex. 2). inhibition of nitric oxide synthase with L-NAME attenuated 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 consistent with previous reports that have shown NMDA receptor blockade achieved by PCP, MK-801 or ketamine increases extracellular glutamate in the prefrontal cortex of rats (Roenker et al., 2011; Abekawa et al., 2003; Lorrain et al., 2003; Lopez-Gil et al., 2007; Pietraszek et al., 2009; Moghaddam et al., 1997; Adams and Moghaddam, 2001). Lorrain and colleagues observed that systemic ketamine administration 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 demonstrate that MK-801-induced elevation in extracellular glutamate in 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., 2007). These findings also are consistent with prior work (Timmerman and Westerink, 1997; Ceglia et al., 2004; Lorrain et al., 2003).

Evidence suggests that nitric oxide is a potential mediator of the actions of NMDA antagonists. Previous research has shown that inhibition of nitric oxide production reverses the behavior deficits, including prepulse inhibition, working memory, and spatial learning, associated with NMDA receptor blockade (Fejgin et al., 2008; Klamer et al., 2001; Klamer et al., 2004b; Wass et al., 2006a; Wass et al., 2006b). PCP administration increases cGMP levels in the prefrontal cortex, and this effect could be prevented by pretreatment with the nitric oxide synthase inhibitor L-NAME (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 increases extracellular cGMP concentration (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 (Palsson et al., 2010) 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 increase in nitric oxide formation.

Here we have demonstrated that inhibition of nitric oxide production by L-NAME prevented the increase in glutamate in the prefrontal cortex following MK-801 administration. Secondly, infusion of the nitric oxide donor SNAP directly into 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 findings support the view that increased nitric oxide formation is necessary for NMDA antagonist-induced elevations of extracellular glutamate in the prefrontal cortex. Additionally, the data suggest enhanced nitric oxide availability is in itself sufficient to increase extracellular glutamate in this brain region. In view of the well-documented role of glutamate receptor activation in promoting nitric oxide formation and the present findings supportive of a role of nitric oxide in generating MK-801 stimulated glutamate release, it would appear that glutamate – nitric oxide interactions are bi-directional.

Inasmuch as L-NAME is a non-selective nitric oxide synthase inhibitor with inhibitory effects upon both iNOS and nNOS, the current data do not identify whether the observed effects are mediated via neuronal (nNOS) or cytokine-inducible (iNOS) isoenzymes. The current data also do not indicate whether the different time course profiles of glutamate elevation following MK-801 administration vs. following SNAP administration result from differences between effects upon the NMDA receptor versus effects upon nitric oxide synthase, or merely reflect pharmacokinetic differences between MK-801 and SNAP. The slower increase and more prolonged time course of glutamate elevation following MK-801 administration suggest the possibility that a cascade of effects in other neural systems may be requisite intermediaries between NMDA receptor blockade and effects upon nitric oxide synthase driving the rise in extracellular glutamate.

Although the neuronal circuit by which NMDA antagonists increase extracellular glutamate in the prefrontal cortex has not been explicitly defined, it has been postulated that NMDA receptor blockade removes a stimulatory input to inhibitory GABAergic interneurons, thereby disinhibiting glutamatergic efferents to cortical pyramidal neurons. While GABA interneurons modulating glutamatergic efferents to prefrontal cortex are believed to be located in the thalamus and/or the hippocampus (Sharp et al., 2001; Lewis and Moghaddam, 2006), evidence for a prefrontal cortical localization is supported by data from Yonezawa and colleagues, who observed decreased GABA release in the prefrontal cortex following local administration of PCP and MK-801 (Yonezawa et al., 1998). Moreover, Fejgin et al. (Fejgin et al., 2009) have demonstrated that direct infusion of baclofen into prefrontal cortex prevented PCP disruption of prepulse inhibition. These data suggest GABA receptors, specifically those of the GABA_B subtype, localized within the prefrontal cortex provide further regulation of cortical glutamate release.

Additional evidence suggests an interaction between GABA_B and nitergic systems. Whereas treatment with baclofen, a GABA_B receptor agonist, prevented PCP disruption of prepulse inhibition, co-administration of baclofen and L-NAME had a synergistic effect, raising prepulse inhibition levels above that of controls (Fejgin et al., 2009). Further suggestive of an interaction between GABA and nitric oxide in these behavioral effects of PCP, baclofen decreases nitric oxide in the prefrontal cortex (Fejgin et al., 2009), consistent with earlier observations of GABAergic modulation of NO/cGMP production (Pepicelli et al., 2004). Here we have demonstrated that baclofen, as well as L-NAME, attenuate MK-801-stimulated glutamate release in prefrontal cortex. This suggests that the ability of baclofen and L-NAME to mitigate behavioral disruptions produced by NMDA receptor antagonists involve suppression of stimulated glutamate release.

Collectively, these data provide additional support for the model of MK-801 stimulated glutamate release illustrated in Figure 1. While the data described above suggest nitric oxide has local effects within prefrontal cortex stimulating and sustaining cortical glutamate release, and that GABA_B receptors inhibit prefrontal glutamate release, other details of this model remain only partially defined. For example, the relative contributions of thalamic and/or hippocampal GABA interneurons (Sharp et al., 2001; Lewis and Moghaddam, 2006) vs.

cortical GABAergic interneurons (Yonezawa et al., 1998) regulating glutamatergic transmission in prefrontal cortex have not been clearly determined. Future studies determining the effects of direct cortical infusion of GABA_B agonists or nitric oxide synthase inhibitors upon MK-801-mediated effects on extracellular glutamate could refine our understanding of the local circuits mediating these effects. Additionally, the relationship of serotonin and mGluR2/3 receptors to glutamatergic and GABAergic cells within the prefrontal cortex remains undefined. Finally, the role of glial and other non-neuronal modulation of this system is not known. Further study is needed to more clearly define mechanisms regulating NMDA antagonist-stimulated glutamate release in the prefrontal cortex, and to translate this understanding into improvements in prevention and treatment for schizophrenia patients.

Acknowledgments

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Reference List

- Abekawa T, Honda M, Ito K, Koyama T. Effects of NRA0045, a novel potent antagonist at dopamine D4, 5-HT_{2A}, and alpha1 adrenaline receptors, and NRA0160, a selective D4 receptor antagonist, on phencyclidine-induced behavior and glutamate release in rats. *Psychopharmacology (Berl)*. 2003; 169:247–256. [PubMed: 12898123]
- Abekawa T, Ito K, Koyama T. Role of the simultaneous enhancement of NMDA and dopamine D1 receptor-mediated neurotransmission in the effects of clozapine on phencyclidine-induced acute increases in glutamate levels in the rat medial prefrontal cortex. *Naunyn Schmiedebergs Arch Pharmacol*. 2006; 374:177–193. [PubMed: 17103144]
- Abekawa T, Ito K, Koyama T. Different effects of a single and repeated administration of clozapine on phencyclidine-induced hyperlocomotion and glutamate releases in the rat medial prefrontal cortex at short- and long-term withdrawal from this antipsychotic. *Naunyn Schmiedebergs Arch Pharmacol*. 2007; 375:261–271. [PubMed: 17458542]
- Adams BW, Moghaddam B. Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. *Biol Psychiatry*. 2001; 50:750–757. [PubMed: 11720693]
- Bakshi VP, Swerdlow NR, Geyer MA. Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. *J Pharmacol Exp Ther*. 1994; 271:787–794. [PubMed: 7965797]
- Bhardwaj A, Northington FJ, Ichord RN, Hanley DF, Traystman RJ, Koehler RC. Characterization of ionotropic glutamate receptor-mediated nitric oxide production in vivo in rats. *Stroke*. 1997; 28:850–856. [PubMed: 9099207]
- Boix J, Llansola M, Cabrera-Pastor A, Felipe V. Metabotropic glutamate receptor 5 modulates the nitric oxide-cGMP pathway in cerebellum in vivo through activation of AMPA receptors. *Neurochem Int*. 2011; 58:599–604. [PubMed: 21300123]
- Bortolato M, Frau R, Aru GN, Orru M, Gessa GL. Baclofen reverses the reduction in prepulse inhibition of the acoustic startle response induced by dizocilpine, but not by apomorphine. *Psychopharmacology (Berl)*. 2004; 171:322–330. [PubMed: 13680072]
- Bubser M, Tzschenkte T, Hauber W. Behavioural and neurochemical interactions of the AMPA antagonist GYKI 52466 and the non-competitive NMDA antagonist dizocilpine in rats. *J Neural Transm Gen Sect*. 1995; 101:115–126. [PubMed: 8695042]
- Ceglia I, Carli M, Baviera M, Renoldi G, Calcagno E, Invernizzi RW. The 5-HT receptor antagonist M100,907 prevents extracellular glutamate rising in response to NMDA receptor blockade in the mPFC. *J Neurochem*. 2004; 91:189–199. [PubMed: 15379899]
- Dedriksen M, Skarsfeldt T, Arnt J. Reversal of PCP-induced learning and memory deficits in the Morris' water maze by sertindole and other antipsychotics. *Psychopharmacology (Berl)*. 2007; 193:225–233. [PubMed: 17406859]

- Donzanti BA, Yamamoto BK. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. *Life Sci.* 1988; 43:913–922. [PubMed: 2901021]
- Enomoto T, Ishibashi T, Tokuda K, Ishiyama T, Toma S, Ito A. Lurasidone reverses MK-801-induced impairment of learning and memory in the Morris water maze and radial-arm maze tests in rats. *Behav Brain Res.* 2008; 186:197–207. [PubMed: 17881065]
- Fedele E, Raiteri M. In vivo studies of the cerebral glutamate receptor/NO/cGMP pathway. *Prog Neurobiol.* 1999; 58:89–120. [PubMed: 10321798]
- Fejgin K, Palsson E, Wass C, Finnerty N, Lowry J, Klamer D. Prefrontal GABA(B) receptor activation attenuates phencyclidine-induced impairments of prepulse inhibition: involvement of nitric oxide. *Neuropsychopharmacology.* 2009; 34:1673–1684. [PubMed: 19145229]
- Fejgin K, Palsson E, Wass C, Svensson L, Klamer D. Nitric oxide signaling in the medial prefrontal cortex is involved in the biochemical and behavioral effects of phencyclidine. *Neuropsychopharmacology.* 2008; 33:1874–1883. [PubMed: 17895915]
- Fejgin K, Safonov S, Palsson E, Wass C, Engel JA, Svensson L, Klamer D. The atypical antipsychotic, aripiprazole, blocks phencyclidine-induced disruption of prepulse inhibition in mice. *Psychopharmacology (Berl).* 2007; 191:377–385. [PubMed: 17235612]
- Garthwaite J. Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci.* 1991; 14:60–67. [PubMed: 1708538]
- Geyer MA, Swerdlow NR, Mansbach RS, Braff DL. Startle response models of sensorimotor gating and habituation deficits in schizophrenia. *Brain Res Bull.* 1990; 25:485–498. [PubMed: 2292046]
- Ito K, Abekawa T, Koyama T. Relationship between development of cross-sensitization to MK-801 and delayed increases in glutamate levels in the nucleus accumbens induced by a high dose of methamphetamine. *Psychopharmacology (Berl).* 2006; 187:293–302. [PubMed: 16850286]
- Johansson C, Jackson DM, Svensson L. The atypical antipsychotic, remoxipride, blocks phencyclidine-induced disruption of prepulse inhibition in the rat. *Psychopharmacology (Berl).* 1994; 116:437–442. [PubMed: 7701046]
- Klamer D, Engel JA, Svensson L. The nitric oxide synthase inhibitor, L-NAME, block phencyclidine-induced disruption of prepulse inhibition in mice. *Psychopharmacology (Berl).* 2001; 156:182–186. [PubMed: 11549221]
- Klamer D, Engel JA, Svensson L. Phencyclidine-induced behaviour in mice prevented by methylene blue. *Basic Clin Pharmacol Toxicol.* 2004a; 94:65–72. [PubMed: 14748849]
- Klamer D, Engel JA, Svensson L. The neuronal selective nitric oxide synthase inhibitor, Nomega-propyl-L-arginine, blocks the effects of phencyclidine on prepulse inhibition and locomotor activity in mice. *Eur J Pharmacol.* 2004b; 503:103–107. [PubMed: 15496304]
- Klamer D, Zhang J, Engel JA, Svensson L. Selective interaction of nitric oxide synthase inhibition with phencyclidine: behavioural and NMDA receptor binding studies in the rat. *Behav Brain Res.* 2005; 159:95–103. [PubMed: 15795002]
- Krystal JH, D'Souza DC, Mathalon D, Perry E, Belger A, Hoffman R. NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmacology (Berl).* 2003; 169:215–233. [PubMed: 12955285]
- Krystal JH, D'Souza DC, Petrakis IL, Belger A, Berman RM, Charney DS, Abi-Saab W, Madonick S. NMDA agonists and antagonists as probes of glutamatergic dysfunction and pharmacotherapies in neuropsychiatric disorders. *Harv Rev Psychiatry.* 1999; 7:125–143. [PubMed: 10483932]
- Laitinen JT, Laitinen KS, Tuomisto L, Airaksinen MM. Differential regulation of cyclic GMP levels in the frontal cortex and the cerebellum of anesthetized rats by nitric oxide: an in vivo microdialysis study. *Brain Res.* 1994; 668:117–121. [PubMed: 7704597]
- Lewis DA, Moghaddam B. Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations. *Arch Neurol.* 2006; 63:1372–1376. [PubMed: 17030651]
- Lopez-Gil X, Artigas F, Adell A. Role of different monoamine receptors controlling MK-801-induced release of serotonin and glutamate in the medial prefrontal cortex: relevance for antipsychotic action. *Int J Neuropsychopharmacol.* 2009; 12:487–499. [PubMed: 18752722]

- Lopez-Gil X, Babot Z, Amargos-Bosch M, Sunol C, Artigas F, Adell A. Clozapine and haloperidol differently suppress the MK-801-increased glutamatergic and serotonergic transmission in the medial prefrontal cortex of the rat. *Neuropsychopharmacology*. 2007; 32:2087–2097. [PubMed: 17356574]
- Lorrain DS, Baccei CS, Bristow LJ, Anderson JJ, Varney MA. Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. *Neuroscience*. 2003; 117:697–706. [PubMed: 12617973]
- Mansbach RS, Geyer MA. Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. *Neuropsychopharmacology*. 1989; 2:299–308. [PubMed: 2692589]
- Martinez ZA, Oostwegel J, Geyer MA, Ellison GD, Swerdlow NR. "Early" and "late" effects of sustained haloperidol on apomorphine- and phencyclidine-induced sensorimotor gating deficits. *Neuropsychopharmacology*. 2000; 23:517–527. [PubMed: 11027917]
- Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci*. 1997; 17:2921–2927. [PubMed: 9092613]
- Moghaddam B, Adams BW. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science*. 1998; 281:1349–1352. [PubMed: 9721099]
- Morris RG. Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. *J Neurosci*. 1989; 9:3040–3057. [PubMed: 2552039]
- Palsson E, Finnerty N, Fejgin K, Klamer D, Wass C, Svensson L, Lowry J. Increased cortical nitric oxide release after phencyclidine administration. *Synapse*. 2009; 63:1083–1088. [PubMed: 19642219]
- Palsson E, Lowry J, Klamer D. Information processing deficits and nitric oxide signalling in the phencyclidine model of schizophrenia. *Psychopharmacology (Berl)*. 2010; 212:643–651. [PubMed: 20802999]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. San Diego: Academic Press; 1986.
- Pepicelli O, Brescia A, Gherzi E, Raiteri M, Fedele E. GABA(A), but not NMDA, receptors modulate in vivo NO-mediated cGMP synthesis in the rat cerebral cortex. *Neuropharmacology*. 2004; 46:480–489. [PubMed: 14975671]
- Pietraszek M, Michaluk J, Romanska I, Wasik A, Golembiowska K, Antkiewicz-Michaluk L. 1-Methyl-1,2,3,4-tetrahydroisoquinoline antagonizes a rise in brain dopamine metabolism, glutamate release in frontal cortex and locomotor hyperactivity produced by MK-801 but not the disruptions of prepulse inhibition, and impairment of working memory in rat. *Neurotox Res*. 2009; 16:390–407. [PubMed: 19649683]
- Pietraszek M, Ossowska K. Chronic treatment with haloperidol diminishes the phencyclidine-induced sensorimotor gating deficit in rats. *Naunyn Schmiedeberg Arch Pharmacol*. 1998; 357:466–471. [PubMed: 9606034]
- Roenker N, Gudelsky GA, Ahlbrand RL, Bronson SL, Kern JR, Waterman H, Richtand NM. Effect of paliperidone and risperidone on extracellular glutamate in the prefrontal cortex of rats exposed to prenatal immune activation or MK-801. *Neurosci Lett*. 2011; 500:167–171. [PubMed: 21699956]
- Sharp FR, Tomitaka M, Bernaudin M, Tomitaka S. Psychosis: pathological activation of limbic thalamocortical circuits by psychomimetics and schizophrenia? *Trends Neurosci*. 2001; 24:330–334. [PubMed: 11356504]
- Swerdlow NR, Bakshi V, Geyer MA. Seroquel restores sensorimotor gating in phencyclidine-treated rats. *J Pharmacol Exp Ther*. 1996; 279:1290–1299. [PubMed: 8968353]
- Timmerman W, Westerink BH. Brain microdialysis of GABA and glutamate: what does it signify? *Synapse*. 1997; 27:242–261. [PubMed: 9329159]
- Wallace TL, Vorhees CV, Gudelsky GA. Effects of lubeluzole on the methamphetamine-induced increase in extracellular glutamate and the long-term depletion of striatal dopamine. *Synapse*. 2001; 40:95–101. [PubMed: 11252020]

- Wass C, Archer T, Palsson E, Fejgin K, Alexandersson A, Klamer D, Engel JA, Svensson L. Phencyclidine affects memory in a nitric oxide-dependent manner: working and reference memory. *Behav Brain Res.* 2006a; 174:49–55. [PubMed: 16935357]
- Wass C, Archer T, Palsson E, Fejgin K, Klamer D, Engel JA, Svensson L. Effects of phencyclidine on spatial learning and memory: nitric oxide-dependent mechanisms. *Behav Brain Res.* 2006b; 171:147–153. [PubMed: 16677724]
- Wass C, Svensson L, Fejgin K, Palsson E, Archer T, Engel JA, Klamer D. Nitric oxide synthase inhibition attenuates phencyclidine-induced disruption of cognitive flexibility. *Pharmacol Biochem Behav.* 2008; 89:352–359. [PubMed: 18321564]
- Watts J, Fowler L, Whitton PS, Pearce B. Release of arginine, glutamate and glutamine in the hippocampus of freely moving rats: Involvement of nitric oxide. *Brain Res Bull.* 2005; 65:521–528. [PubMed: 15862924]
- Watts J, Segieth J, Pearce B, Whitton PS. Regulatory role of nitric oxide over extracellular taurine in the hippocampus of freely moving rats. *Neurosci Lett.* 2004; 357:179–182. [PubMed: 15003279]
- Whishaw IQ, Auer RN. Immediate and long-lasting effects of MK-801 on motor activity, spatial navigation in a swimming pool and EEG in the rat. *Psychopharmacology (Berl).* 1989; 98:500–507. [PubMed: 2505290]
- Yamada K, Noda Y, Nakayama S, Komori Y, Sugihara H, Hasegawa T, Nabeshima T. Role of nitric oxide in learning and memory and in monoamine metabolism in the rat brain. *Br J Pharmacol.* 1995; 115:852–858. [PubMed: 8548187]
- Yamamoto BK, Pehek EA. A neurochemical heterogeneity of the rat striatum as measured by in vivo electrochemistry and microdialysis. *Brain Res.* 1990; 506:236–242. [PubMed: 2137360]
- Yonezawa Y, Kuroki T, Kawahara T, Tashiro N, Uchimura H. Involvement of gamma-aminobutyric acid neurotransmission in phencyclidine-induced dopamine release in the medial prefrontal cortex. *Eur J Pharmacol.* 1998; 341:45–56. [PubMed: 9489855]

- MK-801 induces increased extracellular glutamate in prefrontal cortex
- MK-801-induced glutamate release is attenuated by nitric oxide inhibition
- Extracellular glutamate in prefrontal cortex is elevated by increased nitric oxide
- MK-801-induced glutamate release is attenuated by GABA_B receptor activation

Hippocampus/Thalamus

Prefrontal Cortex

Figure 1. Model of NMDA receptor mediated glutamate release

A.) NMDA receptors on GABAergic neurons in hippocampus and thalamus provide inhibitory control of glutamatergic efferents to prefrontal cortex. Blockade of these NMDA receptors disinhibits glutamatergic outflow, increasing glutamate release in prefrontal cortex. B.) Stimulation of non-NMDA (AMPA/ kainite) receptors on dendrites of glutamatergic neurons allows calcium entry, stimulating nitric oxide synthase (NOS). C.) nitric oxide diffusion sustains increased glutamate release within prefrontal cortex. D.) GABA_B receptor stimulation inhibits glutamate release within prefrontal cortex. E.) Serotonin 5-HT_{2A} and mGluR2/3 receptors also play important roles in regulation of extracellular glutamate, however their precise localization within this model remains incompletely defined.

Figure 2. 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 $\pm$ S.E of 7–10 rats/group. * indicates values that are significant ($p < 0.05$) compared to values for saline treated animals.

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 $\pm$ S.E of 6–8 rats/group (* p <0.05 L-NAME + MK-801 compared to vehicle + MK-801, + p <0.05 Vehicle + MK-801 compared to vehicle + vehicle).

Figure 4. Effect of SNAP infusion on extracellular glutamate in the prefrontal cortex

Rats were treated with vehicle or SNAP (0.5 – 5mM) infusion through the dialysis probe directly into the prefrontal cortex for 30 min at time 0, as indicated by the horizontal, hatched bar. 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 $\pm$ S.E of 5–11 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 $\pm$ S.E of 5–12 rats/group (* $p < 0.05$ baclofen + MK-801 compared to vehicle + MK-801, + $p < 0.05$ vehicle + MK-801 compared to vehicle + vehicle).