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# REGIONAL AND TEMPORAL DIFFERENTIAL REGULATION OF THE N-METHYL-D-ASPARTATE RECEPTOR BY PHENCYCLIDINE DURING DEVELOPMENT

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# REGIONAL AND TEMPORAL DIFFERENTIAL REGULATION OF THE N-METHYL-D-ASPARTATE RECEPTOR BY PHENCYCLIDINE DURING DEVELOPMENT

by
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For my family, especially my parents, Phil and Bobbi.
Thanks for all your love and support throughout the years.

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# REGIONAL AND TEMPORAL DIFFERENTIAL REGULATION OF THE N-METHYL-D-ASPARTATE RECEPTOR BY PHENCYCLIDINE DURING DEVELOPMENT

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Disruptions in glutamatergic neurotransmission may play a role in the pathogenesis of schizophrenia. The purpose of this study was to determine phencyclidine (PCP)-induced changes in the NMDA receptor subunit composition and the relationship of these changes to the deficits in pre-pulse inhibition (PPI) caused by PCP treatment. Postnatal rats were treated with atypical or typical antipsychotics or selective dopamine or serotonin receptor antagonists prior to acute or sub-chronic PCP. This study provides evidence that two distinct mechanisms underlie effects of acute and sub-chronic PCP on NMDA receptor subunit up-regulation. Furthermore, we discovered that D1, D2, and 5-$HT_{2A}$ receptors play a pivotal role in sub-chronic PCP-induced up-regulation of NR1 and NR2A. Finally, we were able to correlate changes in NMDA receptor subunits to the behavioral effects of PCP in this animal model of schizophrenia.

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# INTRODUCTION

Phencyclidine [1-(1-phenylcyclohexyl) piperidine hydrochloride or PCP] was originally developed in the 1950s for use as a surgical anesthetic (Johnson and Jones, 1990). Despite its effectiveness at producing a state of dissociative anesthesia with little cardio-respiratory depression, clinical use was abandoned because patients reported post-surgical hallucinations, delirium and disoriented behavior (Morris et al., 2005). Due to its hallucinogenic effects and availability, PCP appeared on the drug of abuse scene in the mid-1960's known as "angel dust", "hog" or the PeaCe Pill (Johnson and Jones, 1990; Morris et al., 2005). However, illicit use of PCP fell out favor because of its psychotomimetic properties (Johnson and Jones, 1990). Ketamine, a structurally related arylcycloalkylamine, is a shorter acting dissociative anesthetic that is still used clinically in veterinary and pediatric anesthesia (Hirsch et al., 1997). Ketamine replaced PCP as an anesthetic in humans and is mainly administered to children because evidence suggests that administration to post-pubescent humans results in the formation of adverse effects similar to those of PCP (Hirsch et al., 1997). It is also abused illegally and is known on the street as "special K" or "vitamin K" [Morris et al., 2005; National Institute on Drug Abuse (NIDA)]. Users report dream-like states and hallucinations; however, high doses can result in respiratory depression, delirium and amnesia (NIDA).

PCP elicits its major actions as a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist ($K_i$ of 50-100 nM) (Anis et al., 1983). PCP acts as an open channel blocker of the NMDA receptor, binding within the channel pore in a voltage and use dependent manner (Honey et al., 1985; MacDonald et al., 1987; Johnson and Jones, 1990). The NMDA receptor is a member of the ionotropic glutamate family of receptors, which also includes AMPA and kainate receptors. It is composed of multiple subunits including NR1, NR2A-D and NR3A/B. The NR1 subunit forms a heteromeric complex with one of the four NR2 subunits. The NMDA receptor is unique among the glutamate receptors in that it requires binding of glutamate to the NR2 subunit and the co-agonist glycine binding to the NR1 subunit as well as alleviation of the voltage-dependent $Mg^{2+}$

block in order for the receptor to be open and functionally active. NMDA receptors appear to play an important role in long-term depression (LTD), long-term potentiation (LTP) and synaptic plasticity (Cooper, Bloom and Roth, 2003). However, over activation of NMDA receptors can lead to damage that will eventually kill neurons in a process known as excitotoxicity (Cooper, Bloom and Roth, 2003).

PCP possesses a complex and broad pharmacology, affecting various neurotransmitter systems and ion channels (Lodge and Johnson, 1990; Johnson and Jones, 1990; Morris et al., 2005). PCP blockade of other ion channels includes voltage-dependent sodium and potassium channels (French-Mullen and Rogawski, 1989; Vincent et al., 1983) as well as the nicotinic acetylcholine (ACh) receptor (Oswald et al., 1984). It has also been reported that PCP inhibits acetyl cholinesterase (AChE) and butyryl cholinesterase (Maayani et al., 1974), resulting in higher levels of ACh. PCP also inhibits the σ binding site (Largent et al., 1984), norepinephrine (NE) reuptake (Taube et al., 1975; Garey and Heath, 1976), and serotonin (5-HT) reuptake (Smith et al., 1976). PCP blockade of the NMDA receptor can also lead to the release of 5-HT in the prefrontal cortex and hippocampus (Martin et al., 1998) and the subsequent activation of $5\text{-HT}_{2A}$ receptors on glutamatergic neurons in the cortex, resulting in the increased release of glutamate (Adams and Moghaddam, 2001). In addition, PCP blocks dopamine (DA) reuptake (Steinpreis and Salamore, 1993; Garey and Heath, 1976), facilitates its release (Verma and Moghaddam, 1996) and affects the synthesis and metabolism of DA (Johnson and Jones, 1990). DA antagonists are able to antagonize behavioral effects produced by PCP such as cognitive tasks involving working memory and hyperlocomotor activity (Castellani and Adams, 1981; Verma and Moghaddam, 1996). These varying actions of PCP are less potent than its actions on the NMDA receptor; therefore, it is likely that the psychotomimetic actions of PCP are mostly due to its blockade of the NMDA receptor (Johnson and Jones, 1990; Morris et al., 2005), but inhibition of monoamine transport may also play a role.

PCP intoxication in humans has been shown to mimic both the positive and negative symptoms of schizophrenia as well as exacerbate psychosis in schizophrenics

(Luby et al., 1962; Javitt and Zukin, 1991; Steinpress, 1996). Schizophrenia is a complex neuropsychiatric disorder whose etiology remains unknown (Weinberger, 2000). It afflicts around 1% of the population worldwide and shows strong genetic tendencies, with symptoms first presenting in early adulthood (Lewis and Lieberman, 2000; Bromet and Fennig, 1999). The disease is characterized by the presence of both positive (paranoia, hallucinations, delusions) and negative symptoms (emotional withdrawal, anhedonia, depression) as well as cognitive impairments, such as memory and attention deficits (McGlashan, 1996).

The psychotomimetic properties of PCP led researchers to examine the effects of PCP in animals (Luby et al., 1962; Javitt and Zukin, 1991). PCP administration to rats results in increased locomotor activity, stereotypy, ataxia, circling, head weaving, walking backwards and deficits in pre-pulse inhibition (PPI) of acoustic startle (Steinpreis, 1996; Castellani and Adams, 1981; Braff and Geyer, 1990; Martinez et al., 2000). These behavioral phenomena are thought to be relevant models of the symptoms of schizophrenia [hyperlocomotor activity and sensitization is thought to be a model of the positive symptoms while deficits in PPI of acoustic startle are correlated to the negative symptoms of schizophrenia (Wang et al., 2001)] and may also serve as investigatory tools in the possible mechanisms of the pathophysiology of the disease (Adams and Moghaddam, 1998; Castellani and Adams, 1981).

Dopamine antagonists have been shown to inhibit PCP-induced locomotor activity, turning, and ataxia (Tsutsumi, 1995). More specifically, dopamine D1 and D2 receptors may play a role in acute PCP-induced hyperlocomotion, while only D2 receptors regulate chronic PCP-induced hyperlocomotor activity (Phillips et al., 2001). Chronic administration of PCP to adult rats results in locomotor sensitization (Hanania et al., 1999; Johnson et al., 1998). Locomotor sensitization induced by chronic PCP can be inhibited by pretreatment with the atypical antipsychotic clozapine (Johnson et al., 1998), which possesses affinity for dopamine D2 and D4 receptors, 5-$HT_2$, 5-$HT_3$, 5-$HT_6$ and 5-$HT_7$ receptors, α1 noradrenergic receptors, muscarinic ACh receptors and histamine receptors (Lieberman 1993). Atropine, a selective muscarinic antagonist, was able to

partially inhibit chronic PCP-induced locomotor sensitization; however, ketanserin, a 5-$HT_{2A/2C}$ receptor antagonist, had no effect (Phillips et al., 2001). These results suggest a regulatory role for acetylcholine and dopamine in the manifestations of PCP-induced hyperlocomotor activity in adult rats.

Schizophrenics have an impaired ability to filter external sensory information, a process known as sensorimotor gating (Bunney et al., 2000). Deficits in sensorimotor gating are thought to contribute to thought disorder and cognitive fragmentation characteristic of the disease (Braff and Geyer, 1990). This led researchers to study the pre-pulse inhibition (PPI) of acoustic startle response in animals as a model of sensorimotor gating. PPI is a measure of the reduction of the startle response when a smaller non-startling acoustic stimulus (pre-pulse) is presented 80-120 ms prior to the startling stimulus (pulse) (Swerdlow et al., 1994). Studies show that acute PCP treatment produces an inhibition in PPI in adult rats, similar to that seen in schizophrenic patients (Martinez et al., 2000). Typical antipsychotics, such as haloperidol, are not able to reverse deficits in PPI caused by acute PCP treatment in adult rats (Geyer et al, 2001) or in pre-pubertal rats (Martinez et al., 2002), but can reverse the effects of DA agonists, e.g. apomorphine. Atypical antipsychotics, including clozapine, olanzapine, and quetiapine, are effective at alleviating acute PCP inhibition of PPI in adult rats (Geyer et al., 2001; Ballmaier et al., 2001; Martinez et al., 2002), but not in pups (PN16-19) or pre-pubertal (PN45) rats (Martinez et al., 2002).

Originally, a dopamine theory of schizophrenia was postulated and widely accepted as an explanation for the pathophysiology of the disease. This 'hyper-dopaminergic state' was based on evidence that the dopamine agonist amphetamine was capable of mimicking positive symptoms similar to those of schizophrenics (Carlsson, 1988). In further support of the dopamine hypothesis of schizophrenia, all of the early "typical" antipsychotics like haloperidol had clinical potency related to their affinity for dopamine D2 receptors and were able to effectively alleviate positive symptoms of the disease (Bunney et al., 2000; Moghaddam, 1994). However, drugs such as haloperidol are not effective at alleviating the negative symptoms of schizophrenia. Introduction of

newer antipsychotics, termed atypical antipsychotics (clozapine, olanzapine, risperidone), that are effective at treating both positive and negative symptoms, suggested that dopamine may not be the only player in schizophrenia, since atypical antipsychotics possess affinity for dopamine, serotonin, histamine, α1 noradrenergic, and muscarinic ACh receptors (Bondolfi et al., 1998).

In order to understand the mechanism whereby PCP mimics the negative symptoms of schizophrenia, researchers began to investigate disruptions in glutamate neurotransmission in schizophrenics. A hypo-glutamatergic state was hypothesized based on the discovery of low glutamate concentrations in the cerebrospinal fluid of schizophrenics (Kim et al., 1980). Furthermore, administration of PCP or other noncompetitive NMDA antagonists, such as MK-801 (dizoclipine), in the adult rat resulted in neurotoxicity and structural damage in brain regions similar to those in which damage is present in schizophrenics (Olney et al., 1989; Olney and Farber, 1995b). It has been reported that high dose of PCP and MK-801 cause structural damage, such as neuronal vacuolization in the adult rat retrosplenial and posterior cingulate cortex (Olney et al., 1989). Also, repeated administration of either MK-801 or PCP in adult rats has been reported to cause neurodegeneration (silver positive) in the anterior cingulate, parietal, temporal, piriform, and entorhinal cortices, hippocampus and amygdala (Olney and Farber, 1995b). Structural damage to the cingulate cortex, hippocampus, parahippocampal gyrus and entorhinal cortex has been observed in postmortem analysis of schizophrenic brains (Kovelman and Scheibel, 1984; Bogerts 1993).

The similarities between PCP-induced neurotoxicity in animals and its ability to mimic symptoms of schizophrenia led Olney to propose the NMDA hypofunction theory of schizophrenia (Olney and Farber, 1995a). Briefly, activation of NMDA receptors on GABAergic neurons in the basal ganglia results in an inhibition of excitatory neurotransmission to the cortex. However, PCP blockade of these NMDA receptors on GABAergic neurons was postulated to result in a disinhibition of neurotransmission from the thalamus to the cortex, producing an increase in excitation in the cortex and

ultimately excitotoxic cell death, which may be responsible for alterations in behavior caused by PCP treatment (Figure 1) (Olney and Farber, 1995a).

Based on Olney's hypothesis, the effects of acute and chronic PCP or MK-801 (dizoclipine, noncompetitive NMDA receptor antagonist) treatment has been extensively studied in the adult rat as an animal model of the disease (Hanania et al., 1999; Jentsch et al., 1997; Johnson et al., 1998; Moghaddam et al., 1997; Phillips et al., 2001). However, the etiology of schizophrenia has recently been described as a neurodevelopmental disorder (Weinberger 1987). Neurodegeneration during early stages of development has been shown following PCP or MK-801 treatment in the cortex, hippocampus, and striatum, all of which are regions of the brain implicated in schizophrenia (Ikonomidou et al., 1999). In addition, acute PCP treatment on postnatal day (PN) 7, results in neurodegeneration (positive silver staining) in the frontal cortex, striatum, and hippocampus within 9 hours of treatment (Wang and Johnson, 2005). This laboratory has also reported the presence of both non-specific neurodegeneration (positive silver staining) and apoptotic neurons (TUNEL positive) in the cortex, but not the in the striatum or hippocampus, following sub-chronic PCP administration on PN7, 9, and 11 (Wang et al., 2001; Wang and Johnson 2005), thereby suggesting either developmental or pharmacological tolerance in these regions. Also, locomotor sensitization and deficits in PPI of acoustic startle were reported in postnatal (PN24-28) rats treated with PCP on PN7, 9, and 11 (Wang et al., 2001). Pretreatment with olanzapine was able to prevent both behavioral and biochemical indices of postnatal PCP administration, suggesting that this treatment paradigm may model certain aspects of schizophrenia in rats (Wang et al., 2001).

Several studies from this laboratory have investigated the possible mechanisms by which PCP may elicit its neurotoxic effects and produce alterations in behavior in rats. Chronic PCP treatment of adult rats (20 mg/kg x 5 days) resulted in increases in the NMDA receptor subunit NR1 mRNA in the olfactory tubercle, piriform cortex, frontal cortex and anterior striatum (Wang et al., 1999). In addition, this treatment of adult rats also produced locomotor sensitization that was accompanied by an increase in NMDA-

induced release of striatal $^{14}$C-GABA and $^{3}$H-ACh. This was correlated with an increase in NR1 immunoreactivity, suggesting that alterations in NMDA receptor function in the striatum may underlie PCP-induced locomotor sensitization (Hanania et al., 1999). In developing rats, sub-chronic PCP treatment of pups on PN7, 9, and 11 resulted in increased expression of NR1 mRNA in the frontal cortex, striatum, nucleus accumbens and olfactory cortex that was inhibited by pretreatment with the atypical antipsychotic olanzapine (Wang et al., 2001). Wang et al. (2001) confirmed that increases in NR1 protein levels in the frontal cortex of PCP treated pups coincided with increases in NR1 protein that was observed in PCP treated adult rats (Hanania et al., 1999). Up-regulation of NR1 protein in the frontal cortex caused by sub-chronic PCP administration was associated with increases in TUNEL positive cells as well as an increase in the Bax (pro-apoptotic protein) to Bcl-$_{XL}$ (anti-apoptotic protein) ratio, both of which were inhibited by olanzapine pretreatment (Wang et al., 2001). Based on these observations, this laboratory hypothesized that PCP administration in the postnatal rat results in up-regulation of the NMDA receptor subunits and that this may result in PCP-induced neurotoxicity. This hypothesis is consistent with PCP-induced neurotoxicity involving increased superoxide formation and increased NF-κB nuclear transport, two mechanisms that would be activated by excessive intracellular $Ca^{2+}$ levels (McInnis et al., 2002), possibly secondary to increased NMDA receptor function.

The overall purpose of the present study was to characterize PCP-induced regulation of the NMDA receptor subunits (NR1, NR2A, and NR2B) in the frontal cortex and striatum following both acute (PN7) and sub-chronic (PN7, 9, and 11) PCP treatment in order to gain insight into the mechanism of neurotoxicity and behavioral alterations caused by postnatal PCP administration. The following hypotheses were addressed:

1) Acute and sub-chronic PCP treatment induces differential regulation of the NMDA receptor subunits protein expression in the frontal cortex and striatum.

2) In order to determine the potential relationship between NMDA receptor subunit regulation and the detrimental effects of PCP in an animal model of schizophrenia, the effects of atypical and typical antipsychotics as well as selective DA

and 5-HT receptor antagonists on PCP-induced changes in the NMDA receptor subunits protein expression in the frontal cortex and striatum were examined. We hypothesize that both typical and atypical antipsychotic pretreatment will inhibit acute and sub-chronic PCP-induced changes in the concentration of the NMDA receptor subunits in the frontal cortex and striatum. We postulate that antagonism at DA D2 and 5-$HT_{2A}$ receptors by the antischizophrenic drugs is the mechanism whereby these agents attenuate PCP's effects.

3) In order to relate the changes in NMDA receptor subunit expression to the behavioral effects induced by PCP administration, we hypothesize the atypical antipsychotic olanzapine and the typical antipsychotic haloperidol are differentially effective at preventing the deficit in PPI of acoustic startle observed in pre-pubertal rats following sub-chronic PCP treatment.

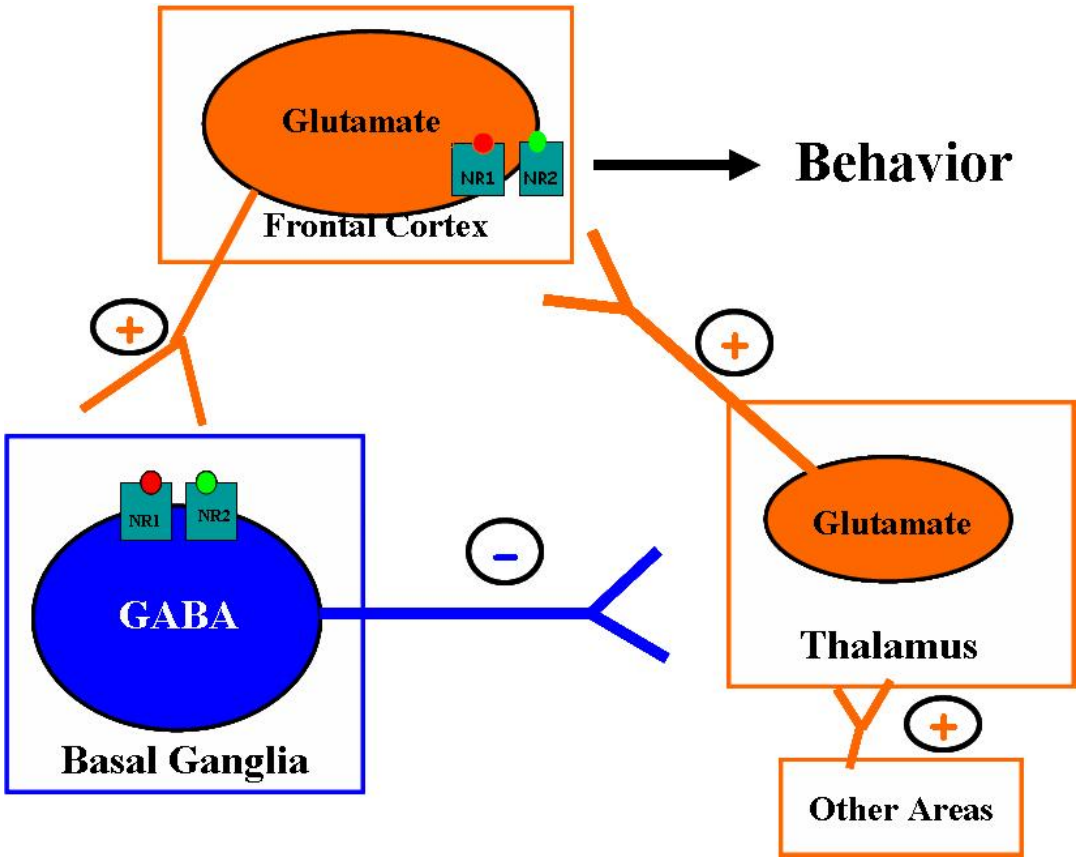


**Figure 1. NMDA hypofunction theory of schizophrenia**

Activation of NMDA receptors on GABAergic neurons in the basal ganglia results in an inhibition of the excitatory input from the thalamus to the frontal cortex leading to normal behavior. However, PCP blockade of the NMDA receptors in the basal ganglia results in a disinhibition of neurotransmission from the thalamus to the frontal cortex. This may produce an increase in excitation to the cortex and ultimately excitotoxic cell death in this region, which may be responsible for the increased behavior that is observed following PCP treatment (adapted from Olney and Farber, 1995a).

# MATERIALS AND METHODS

## Animals

Timed day 14 pregnant female Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). The dams were housed individually with a regular 12h light-dark cycle (lights on 0700, off at 1900) with food and water ad libitum. Following parturition, pups from four dams were combined and randomly cross-fostered to one of the four lactating dams. Each litter consisted of ten to twelve pups.

## Drugs

Phencyclidine (PCP) was acquired from the National Institute on Drug Abuse (NIDA, Rockville, MD). Risperidone was obtained as a solution from Janssen Pharmaceutica (Titusville, NJ); haloperidol was obtained as a solution from GensiaSicor Pharmaceuticals (Irvine, CA); olanzapine was a generous gift from Eli Lilly and Company (Indianapolis, IN); M100907 was obtained from Hoeschst Marion Roussel (Cincinatti, OH); SCH23390 was acquired from RBI (Research Biochemicals International, Natick, MA); sulpiride was purchased from Sigma-Aldrich (St. Louis, MO). PCP was dissolved in 0.9% NaCl. Risperidone and haloperidol were diluted in 0.9% NaCl. Olanzapine was dissolved in 0.1 N HCl and titrated to pH 7.0 with 0.1 N NaOH and finally diluted with 0.9% NaCl. M100907 was dissolved in 1% Tween 80 and 0.9% NaCl. SCH23390 was dissolved in 0.9% NaCl. Sulpiride was dissolved in water and 5% ethanol and adjusted to a pH of 7.0 with 0.1 N NaOH and finally diluted with 0.9% NaCl. The corresponding vehicle controls (1 ml/kg) were injected as appropriate. Doses were chosen based on prior experiments that addressed PCP-induced regulation of the NMDA receptor (Wang et al., 2001) or those assessing PCP-induced locomotor activity and PCP-induced inhibition of PPI of acoustic startle (Phillips et al., 2001; Wang et al., 2001).

**Experimental design**

For biochemical experiments, male and female rat pups were treated on either PN7 (acute) or on PN 7, 9, and 11 (sub-chronic) with 10 mg/kg subcutaneous (s.c.) PCP or saline/vehicle. Olanzapine (2 mg/kg s.c.), risperidone (0.25 mg/kg s.c.), haloperidol (0.25 mg/kg s.c.), sulpiride (100 mg/kg s.c.), SCH23390 (0.5 mg/kg s.c.) or M100907 (1 mg/kg s.c.) were administered 30 minutes prior to PCP or saline/vehicle administration on PN7 (acute) or on PN 7, 9, 11 (sub-chronic). In the acute studies, pups were sacrificed by decapitation on PN7 at 0, 4, 8, or 24 hrs following saline (control, time=0 hours), PCP/vehicle, antagonist/vehicle, or antagonist/PCP treatment. In the sub-chronic studies, pups were sacrificed by decapitation 24 hrs (PN12) following the last injection of the aforementioned drug regimens on PN7, 9 and 11. For both acute and sub-chronic biochemical studies, the frontal cortex and striatum were dissected and used for Western blot analysis.

For the behavioral experiments, female pups were treated on PN7, 9, and 11 (sub-chronic) with 10 mg/kg PCP or saline/vehicle. Olanzapine (1 or 2 mg/kg s.c.) or haloperidol (0.25 mg/kg s.c.) were injected 30 minutes prior to PCP or saline/vehicle on PN7, 9, and 11. Animals were first tested for pre-pulse inhibition (PPI) of acoustic startle on PN14-15. Following the first round of testing, the pups were returned to their home cage with dams and then weaned on PN21. On PN24-25, animals were again tested in the PPI paradigm described below.

**Western blot analysis**

Protein extracts were prepared from the frontal cortex or striatal brain tissue as previously described with some modifications (Wang et al., 2001). Briefly, 2 mm sections corresponding to 4.7 to 2.7 mm anterior to Bregma for the frontal cortex and 0.7 mm to -1.3 mm for the striatum (Paxinos and Watson, 1986) were cut with the aid of an aluminum brain mold. Cortical and striatal brain sections were homogenized in 500 μL of lysis buffer with the aid of an automatic tissue grinder (Kontes Pellet Pestle Motor, Kimble / Kontes, Vineland, New Jersey). The lysis buffer consisted of 10 mM HEPES (pH 7.4), 1 mM EDTA, 2 mM EGTA, and 500 μM DTT (dithiothreitol). Just prior to

use, protease inhibitor cocktail [4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), pepstatin A, E-64, bestatin, leupeptin, and aprotinin without metal chelators (Sigma-Aldrich, St Louis, MO)] at a concentration of 10 μL/mL was added to the lysis buffer. The homogenate was then centrifuged at 1000 x g at 4$^{o}$C for 10 minutes to pellet the nuclear protein fraction (P1). The supernatant (S1) was collected and centrifuged at 20,000 x g at 4$^{o}$C for 30 minutes to pellet the membrane bound protein fraction (P2), which was resuspended in boiling 1% SDS (sodium dodecyl sulfate) in order to solubilize the membrane bound protein fraction. To isolate endoplasmic reticulum (ER) protein, the S2 fraction was centrifuged at 100,000 x g at 4$^{o}$C for 20 min. The pellet (P3) containing the ER fraction was then resuspended in lysis buffer. Total protein concentrations were determined using the BCA (bicinchoninic acid) protein assay© [colorimetric method-O.D. determined at an absorbance of 595 nm] (Pierce Chemical, Rockford, IL). Equal amounts of protein (10 μg) were separated on 10% Bis-Tris gels (Invitrogen, NY) using SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) with a Tris-glycine running buffer system, pH 7.4.

Following electrophoresis (110 V for 2 hours), proteins were transferred to polyvinylidene difluoride (PVDF) membranes (0.2 μm) in a Mini Electrotransfer Unit (Bio-Rad, Hercules, CA) overnight. The membrane was blocked in 5% milk (Carnation Instant Nonfat dry milk, Nestle USA, Wilkes-Barre, PA) for 2 hours, followed by incubation with the primary antibody in 1% milk for 2 hours. Following three 30 minute washes in TBS+1 % Tween 20 (TBST), the membrane was incubated with either horseradish peroxidase conjugated anti-mouse (for all monoclonal antibodies) (Chemicon, Temecula, CA) or anti-rabbit (for all polyclonal antibodies) (Santa Cruz Biotechnology, Santa Cruz, CA) secondary antibody (1:2000) for 1.5 hours. The membrane was then washed three times for 30 min. each with TBST. Analysis was carried out using the enhanced chemiluminescence (ECL) plus Western blotting detection reagents (Amersham Biosciences, Piscataway, NJ). The bands corresponding to the various proteins of interest were scanned and densitometrically analyzed by using an automatic imaging analysis system (Alpha Innotech Corporation, San Leandro, CA).

These quantitative analyses were normalized to β-actin for membrane bound proteins or protein disulfide isomerase (PDI), an ER housekeeping protein, for ER proteins [after stripping (Reblot mild, Chemicon International, Temecula, CA)].

**Primary antibodies**

An anti-NR1 subunit antibody (PharMingen, San Diego, CA) was used for Western blots at a concentration of 1:1000 (monoclonal). The polyclonal antibody for NMDA receptor subunit 2A (Santa Cruz Biotechnology, Santa Cruz, CA) was used at a concentration of 1:500 (polyclonal). The monoclonal anti-NR2B subunit antibody was obtained from Chemicon International (Temecula, CA) and used at a concentration of 1:1000. The anti-PSD95 antibody (monoclonal) was used at a concentration of 1:2000 (monoclonal) and purchased from Upstate Biotechnology (Lake Placid, NY). The anti-protein disulfide isomerase (PDI) (Affinity Bioreagents Inc. Golden, CO) antibody was a gift from the laboratory of Dr. Geoffrey Swanson (UTMB Galveston). The β-actin antibody (Chemicon, Temecula, CA) was used at a concentration of 1:5000 (monoclonal).

**Pre-pulse inhibition (PPI) of acoustic startle**

Measurement of pre-pulse inhibition of acoustic startle was performed according to previously published procedures with minor modifications (Wang et. al., 2001; Wang et al., 2003). Testing was performed between 0900 and 1600 hours as described below. Female rat pups (PN14-15 or PN24-25) were transferred into the behavior room on the day of testing and allowed to acclimate to the room for 20 minutes. Animals were then placed into one of three startle chambers (SR-Lab, San Diego Instruments, San Diego, CA) with a background noise level of 65 dB. Following a 10 min. acclimation period, rats were exposed to three randomly administered stimuli: no stimulus, a 73 dB pre-pulse 100 ms prior to a 120 dB pulse, or a 120 dB pulse alone with a variable inter-trial interval (5-20 sec) for a total of 63 trials (21 no stimulus, 21 pulse alone, and 21 pre-pulse + pulse). % PPI of acoustic startle was calculated as the [pulse-(pre-pulse + pulse)]/pulse x 100.

**Data/Statistical analysis**

For the biochemical studies, an initial experiment testing only saline vs. PCP-treated animals (N= 5/group) was conducted. Subsequent studies conducted to examine the effect of each antagonist + PCP (N=3-5/group) included additional saline and PCP control animals (N=2/group). Since results from each saline and PCP group tested were similar, the values were pooled from each experiment for each brain region and NMDA receptor subunit studied for a total N=15/group. For the behavioral experiments, % PPI on PN14-15 or PN24-25 was analyzed between the 4 groups (saline, PCP, antagonist alone, antagonist + PCP) with each day of testing considered separately. All values are presented as mean ± SEM. Statistical comparison between groups was performed with one-way analysis of variance (ANOVA) and the appropriate post hoc test. Statistical significance was set at $p<0.05$.

# RESULTS

**Effects of acute PCP administration on NMDA receptor (NMDAR) subunit concentration in the frontal cortex and striatum**

In order to ascertain the effects of acute PCP (10 mg/kg) treatment on the concentration of the NMDAR subunits, membrane bound protein from both the frontal cortex and striatum was extracted on PN 7 at 0, 4, 8 and 24 hrs after PCP treatment. Saline treated animals were sacrificed at t=0 hr on PN7 and served as the control in the biochemical studies of acute PCP effects. Acute PCP treatment produced a 3-fold increase in NR1 and NR2B subunits at 8 hrs that persisted for at least 24 hrs in the frontal cortex as evidenced by Western blot analysis (Figure 2B). In addition, post-synaptic density 95 (PSD-95) protein expression levels in the frontal cortex were increased following 8 hrs of PCP treatment and remained elevated at 24 hrs after treatment (Figure 2B). Figure 2A shows representative Western blots of NR1, NR2B, and PSD-95 from the frontal cortex of acute PCP or saline treated postnatal rats at all time points examined on PN7. There was no effect of acute PCP treatment on NR2A protein levels in the frontal cortex (Figure 2B). Interestingly, acute PCP treatment had no effect on NR1, NR2A or NR2B protein levels in the striatum at any time point examined (Figure 3B). PSD-95 protein levels in the striatum were also not affected by acute PCP treatment (Figure 3B). Figure 3A shows representative Western blots of striatal NR1, NR2B, and PSD-95 from saline or acute PCP treated animals at all time points examined on PN7.

**Pharmacological antagonism of the effects of acute PCP administration on the NMDAR subunits in the frontal cortex and striatum**

To examine the possible mechanism involved in acute PCP-induced up-regulation of NR1 and NR2B subunits 24 hrs following treatment in the frontal cortex, postnatal animals were treated with the atypical antipsychotics olanzapine (2 mg/kg) or risperidone (0.25 mg/kg) or the typical antipsychotic haloperidol (0.25 mg/kg) 30 min prior to PCP treatment on PN7. As mentioned previously, acute PCP treatment had no effect on the levels of either NR1 or NR2B protein expression 4 hrs after treatment in the frontal

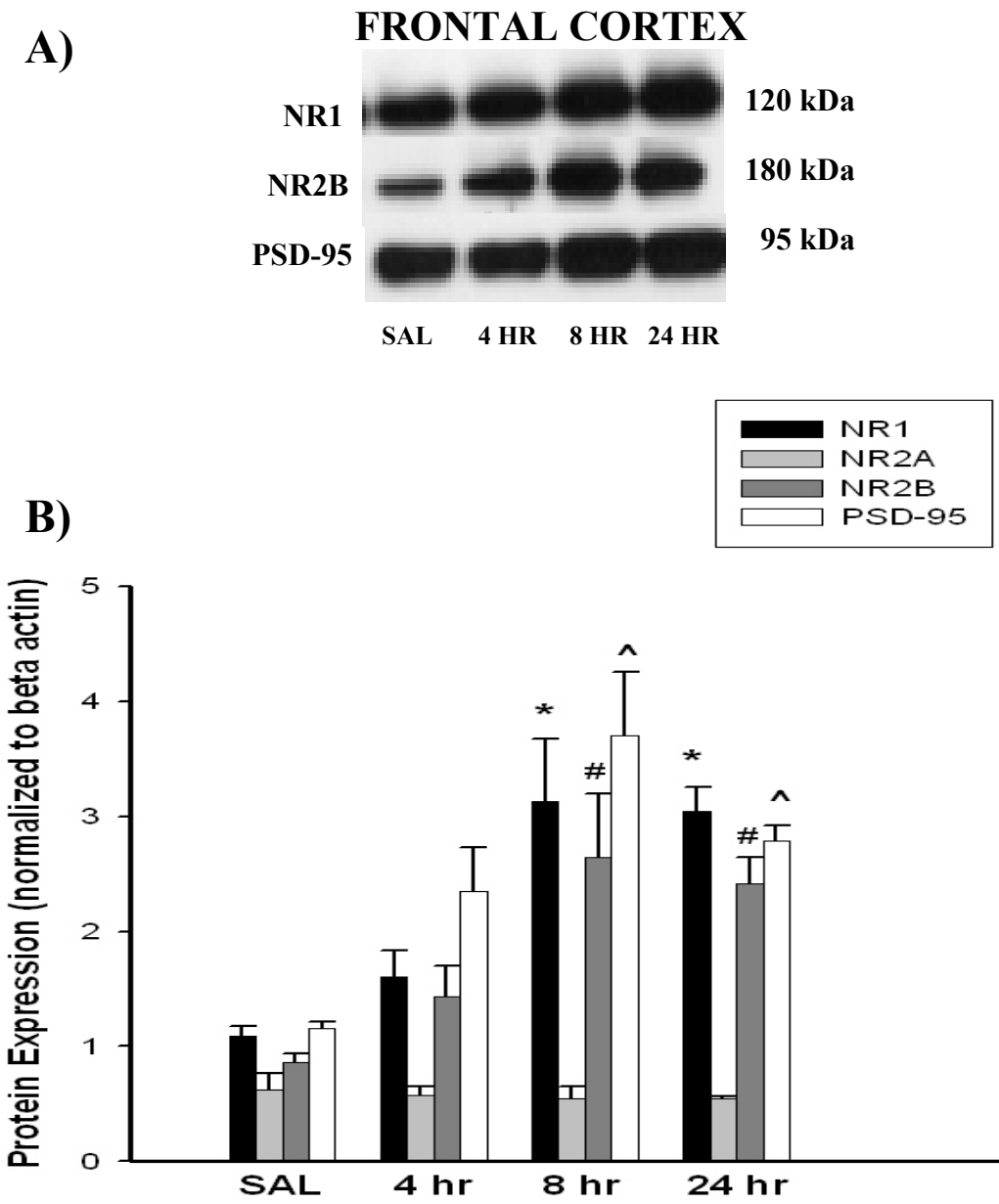


**Figure 2.** (A) Representative Western blots from the frontal cortex of acute PCP (10 mg/kg) or saline treated animals at all time points examined on PN7. (B) Quantitative analysis of the effects of acute PCP treatment on NR1, NR2A, NR2B and PSD-95 membrane bound protein levels in the frontal cortex (N=4/group). * $p<0.05$ vs. SAL NR1 (one-way ANOVA with Bonferroni's post hoc test) ^ $p<0.05$ vs. SAL NR2B (one-way ANOVA with Bonferroni's post hoc test) # $p<0.05$ vs. SAL PSD-95 (one-way ANOVA with Bonferroni's post hoc test)

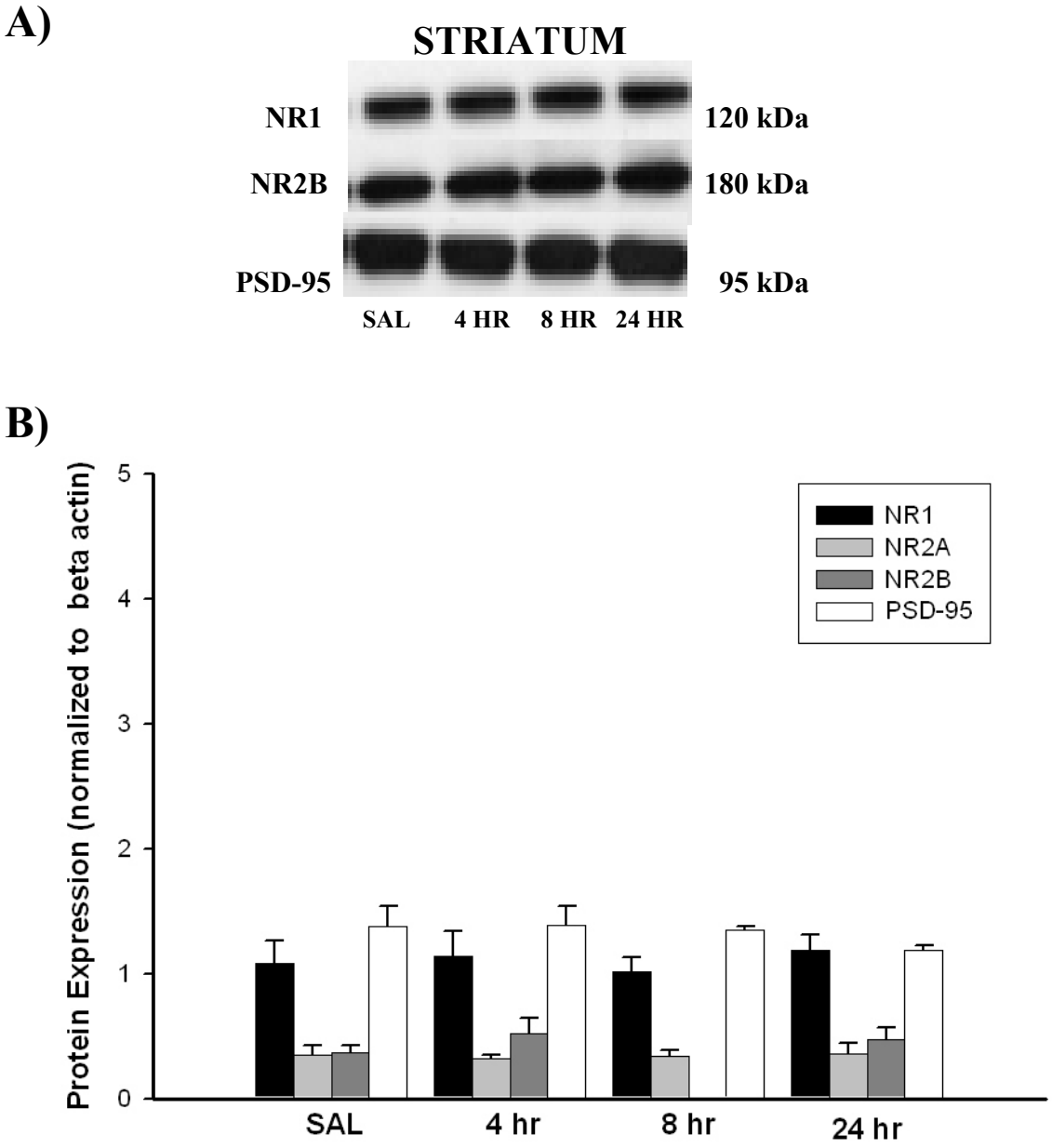


**Figure 3.** (A) Representative Western blot from the striatum of acute PCP (10 mg/kg) or saline treated animals at all time points examined on PN7. (B) Quantitative analysis of the lack of effects of acute PCP treatment on NR1, NR2A, NR2B and PSD-95 membrane bound protein levels in the striatum (N=4/group)

cortex (Figure 2B); therefore, antagonist studies were conducted at this time point as well to serve as a control for any effects they may possess. Pretreatment with olanzapine, risperidone or haloperidol did not alter PCP's effects at 4 hrs post-treatment (Table 1a). None of the antipsychotics studied had an effect on their own at 4 hrs following treatment (Table 1b). Importantly, pretreatment with either an atypical or typical antipsychotic was unable to inhibit the observed up-regulation of NR1 (Figure 4) or NR2B (Figure 5) that was evident 24 hrs following acute PCP treatment in the frontal cortex. Olanzapine, risperidone, or haloperidol treatment alone had no effect on the concentration of NR1 or NR2B in the frontal cortex at 24 hrs following treatment on PN7 (Table 2).

Since olanzapine, risperidone, and haloperidol possess varying affinity for dopamine and serotonin receptors, selective antagonists for D1, D2, and 5-$HT_{2A}$ receptors were implemented in these studies. Sulpiride (selective D2 receptor antagonist, 100 mg/kg), SCH23390 (selective D1 receptor antagonist, 0.5 mg/kg), and M100907 (selective 5-$HT_{2A}$ receptor antagonist, 1 mg/kg) were administered 30 min prior to acute PCP treatment on PN7. The effects of the antagonists were examined at 4 and 24 hrs following acute PCP administration. Pretreatment with the selective antagonists did not alter the lack of effect that was observed from acute PCP on NR1 or NR2B at 4 hrs following treatment (Table 1a). Sulpiride, SCH23390, or M100907 treatment alone had no effect on cortical NR1 or NR2B protein expression at 4 hrs (Table 1b) or 24 hrs (Table 2) following treatment on PN7. More importantly, blockade of 5-$HT_{2A}$, D1, and D2 receptors did not prevent acute PCP-induced up-regulation of NR1 protein levels observed 24 hrs following treatment in the frontal cortex as evidenced by the lack of blockade from pretreatment with M100907, SCH23390 or sulpiride (Figure 4). Furthermore, sulpiride, SCH23390 or M100907 pretreatment was unable to inhibit the PCP-induced increase in NR2B protein levels in the frontal cortex following 24 hrs of treatment (Figure 5).

As mentioned previously, acute PCP treatment had no significant effect on NR1 or NR2B protein expression levels in the striatum at either 4 or 24 hrs following

treatment (Figure 3B). The effects of pretreatment with the antipsychotics or selective antagonists on NR1 and NR2B protein levels in the striatum were also investigated following acute PCP treatment. Treatment with olanzapine, haloperidol, risperidone, sulpiride, SCH23390, or M100907 did not significantly alter expression of NR1 or NR2B protein levels in the striatum prior to PCP on PN7 at 4 hrs following treatment (Table 3a) or when administered alone (Table 3b). Similarly, treatment with either atypical or typical antipsychotics or selective D1, D2, and 5-$HT_{2A}$ receptor antagonists prior to PCP did not effect striatal NR1 or NR2B protein expression at 24 hrs following treatment (Table 4a) or when administered alone (Table 4b).

In summary, acute PCP increases cortical expression of NR1 and NR2B by 3-4-fold within 8 hrs and this increase is stable for at least 24 hrs. This increase is accompanied by a similar increase in expression of PSD-95. The effects of PCP were not antagonized by either multifunctional antischizophrenic drugs or antagonists selective for D1, D2, or 5-$HT_{2A}$ receptors. Interestingly, these effects of PCP were selective for the cortex in that PCP had no effect on NR1, NR2A or NR2B in the striatum of these rats.

| Table 1a. Effect of acute PCP administration on NMDA receptor subunit protein expression in the frontal cortex (normalized to β-actin) | | |
|---|---|---|
| | 4 hr | |
| | NR1 | NR2B |
| Saline (N=15) | 1.17±0.06 | 0.97±0.06 |
| PCP (N=15) | 1.55±0.15 | 1.41±0.13 |
| Olanzapine+PCP (N=3) | 0.54±0.05 | 0.87±0.13 |
| Risperidone+PCP (N=3) | 1.39±0.11 | 1.39±0.34 |
| Haloperidol+PCP (N=3) | 1.20±0.24 | 1.14±0.31 |
| Sulpiride+PCP (N=3) | 1.30±0.24 | Not tested |
| SCH23390+PCP (N=3) | 1.35±0.24 | 1.09±0.09 |
| M100907+PCP (N=3) | 1.17±0.52 | 1.05±0.12 |
| Data presented as mean ± SEM | | |

| Table 1b. Effect of acute antagonist alone administration on NMDA receptor subunit protein expression in the frontal cortex (normalized to β-actin) | | |
|---|---|---|
| | 4 hr | |
| | NR1 | NR2B |
| Olanzapine (N=3) | 1.24±0.41 | 0.98±0.15 |
| Risperidone (N=3) | 1.29±0.06 | 0.95±0.01 |
| Haloperidol (N=3) | 1.30±0.25 | 1.36±0.49 |
| Sulpiride (N=3) | 1.32±0.44 | Not tested |
| SCH23390 (N=3) | 1.77±0.04 | 0.96±0.31 |
| M100907 (N=3) | 1.60±0.75 | 0.43±0.09 |
| Data presented as mean ± SEM | | |

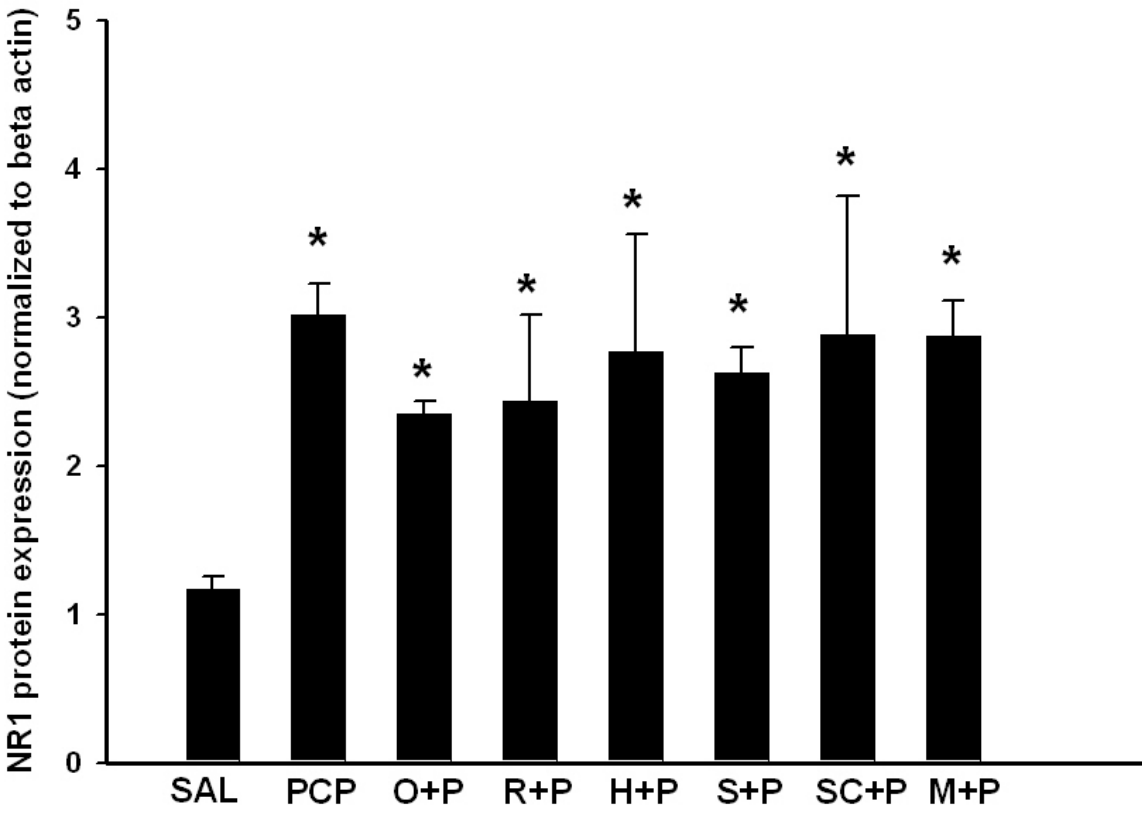


**Figure 4.** Antagonist pretreatment has no effect on acute PCP-induced up-regulation of NR1 protein levels in the frontal cortex following 24 hours of PCP (10 mg/kg, N=15) treatment. * $p<0.05$ vs. SAL NR1 (one-way ANOVA with Bonferroni's post hoc test) O=olanzapine (2 mg/kg, N=3) R=risperidone (0.25 mg/kg, N=3) H=haloperidol (0.25 mg/kg, N=3) S=sulpiride (100 mg/kg, N=3) SC=SCH23390 (0.5 mg/kg, N=3) M=M100907 (1 mg/kg, N=3)

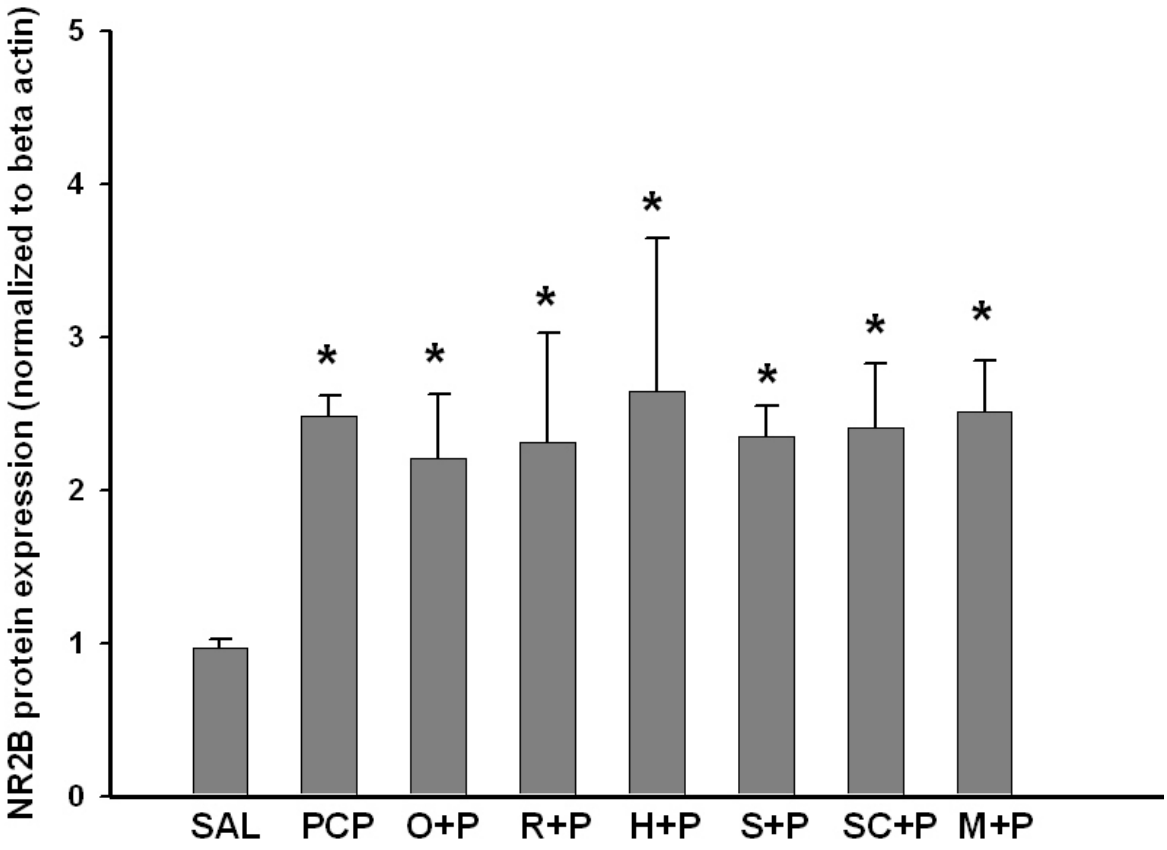


**Figure 5.** Antagonist pretreatment was unable to inhibit acute PCP-induced up-regulation of NR2B protein in the frontal cortex after 24 hours of PCP (10 mg/kg) treatment (N=15). * $p<0.05$ vs. SAL NR2B (one-way ANOVA with Bonferroni's post hoc test) O=olanzapine (2 mg/kg, N=3) R=risperidone (0.25 mg/kg, N=3) H=haloperidol (0.25 mg/kg, N=3) S=sulpiride (100 mg/kg, N=3) SC=SCH23390 (0.5 mg/kg, N=3) M=M100907 (1 mg/kg, N=3)

| Table 2. Lack of effect of acute antagonist alone administration on NMDA receptor subunit protein expression in the frontal cortex (normalized to β-actin) | | |
|---|---|---|
| | 24 hr | |
| | NR1 | NR2B |
| Olanzapine (N=3) | 0.84±0.12 | 0.87±0.15 |
| Risperidone (N=3) | 1.22±0.14 | 0.82±0.02 |
| Haloperidol (N=3) | 0.81±0.09 | 1.08±0.23 |
| Sulpiride (N=3) | 1.71±0.31 | 1.05±0.17 |
| SCH23390 (N=3) | 1.20±0.34 | 1.54±0.29 |
| M100907 (N=3) | 1.63±0.50 | 1.22±0.24 |
| Data presented as mean ± SEM | | |

| Table 3a. Effect of acute PCP administration on NMDA receptor subunit protein expression in the striatum (normalized to β-actin) | | |
|---|---|---|
| | 4 hr | |
| | NR1 | NR2B |
| Saline N=15 | 1.06±0.09 | 0.56±0.55 |
| PCP N=15 | 1.32±0.13 | 0.59±0.06 |
| Olanzapine+PCP N=3 | 1.30±.45 | 0.81±0.21 |
| Risperidone+PCP N=3 | 1.06±0.14 | Not tested |
| Haloperidol+PCP N=3 | 1.11±0.14 | 0.61±0.15 |
| Sulpiride+PCP N=3 | 1.13±0.34 | 0.46±0.19 |
| SCH23390+PCP N=3 | 1.25±0.48 | 0.70±0.23 |
| M100907+PCP N=3 | 0.80±0.30 | 0.50±0.18 |
| Data presented as mean ± SEM | | |

| Table 3b. Effect of acute antagonist alone administration on NMDA receptor subunit protein expression in the striatum (normalized to β-actin) | | |
|---|---|---|
| | 4 hr | |
| | NR1 | NR2B |
| Olanzapine (N=3) | 0.66 ±0.17 | 0.62±0.03 |
| Risperidone (N=3) | 1.18±0.13 | Not tested |
| Haloperidol (N=3) | 1.21±0.17 | 0.68±0.18 |
| Sulpiride (N=3) | 0.94±0.14 | 0.43±0.09 |
| SCH23390 (N=3) | 1.17±0.04 | 0.51±0.15 |
| M100907 (N=3) | 0.93±0.13 | 0.68±0.26 |
| Data presented as mean ± SEM | | |

**Table 4a. Effect of acute PCP administration on NMDA receptor subunit protein expression in the striatum (normalized to β-actin)**

| | 24 hr | |
|---|---|---|
| | **NR1** | **NR2B** |
| **Saline N=15** | **1.06 ± 0.09** | **0.56 ± 0.06** |
| **PCP N=15** | **1.12 ± 0.08** | **0.55 ± 0.06** |
| **Olanzapine+PCP N=3** | **0.70 ± 0.12** | **0.57 ± 0.09** |
| **Risperidone+PCP N=3** | **1.10 ± 0.13** | **Not tested** |
| **Haloperidol+PCP N=3** | **1.11 ± 0.16** | **0.43 ± 0.05** |
| **Sulpiride+PCP N=3** | **0.93 ± 0.36** | **0.59 ± 0.14** |
| **SCH23390+PCP N=3** | **1.23 ± 0.26** | **0.44 ± 0.12** |
| **M100907+PCP N=3** | **0.89 ± 0.28** | **0.54 ± 0.17** |

**Data presented as mean ± SEM**

**Table 4b. Effect of acute antagonist alone administration on NMDA receptor subunit protein expression in the striatum (normalized to β-actin)**

| | 24 hr | |
|---|---|---|
| | **NR1** | **NR2B** |
| **Olanzapine (N=3)** | **1.25 ±0.16** | **0.44±0.04** |
| **Risperidone (N=3)** | **0.74±0.22** | **Not tested** |
| **Haloperidol (N=3)** | **1.26±0.15** | **0.51±0.17** |
| **Sulpiride (N=3)** | **1.06±0.05** | **0.59±0.14** |
| **SCH23390 (N=3)** | **0.55±0.20** | **0.54±0.24** |
| **M100907 (N=3)** | **1.22±0.77** | **0.57±0.18** |

**Data presented as mean ± SEM**

**Effects of sub-chronic PCP administration on NMDAR subunit concentration in the frontal cortex and striatum**

This laboratory has previously reported that sub-chronic PCP administration produced an increase in NR1 mRNA in the frontal cortex and striatum as evidenced by *in situ* hybridization as well as an increase in total cytoplasmic NR1 protein levels in the frontal cortex of rat pups (Wang et al., 2001). To extend this investigation of the effects of sub-chronic PCP administration, the concentration of the membrane bound protein for the NMDAR subunits (NR1, NR2A, and NR2B) in both the frontal cortex and striatum were determined 24 hrs following treatment with either saline or PCP (10 mg/kg) on PN7, 9, and 11. Cortical and striatal membrane bound protein extracts were then subjected to Western blot analysis. Figure 6A and 6C show representative Western blots of NR1 and NR2A subunits from the frontal cortex and striatum, respectively. Sub-chronic PCP treatment caused a 3-fold increase in NR1 and a 10-fold increase in NR2A protein levels in the frontal cortex with no effect on NR2B protein expression (Figure 6B). However, sub-chronic PCP administration resulted in no significant effect on cortical PSD-95 protein levels (Figure 7B). In contrast, sub-chronic PCP treatment resulted in a down-regulation of NR1 protein expression in the striatum with no effect on either NR2A or NR2B (Figure 6D).

**Pharmacological antagonism of the effects of sub-chronic PCP administration on the NMDAR subunits in the frontal cortex and striatum**

To analyze the actions of atypical and typical antipsychotics on PCP-induced concentration of the NMDAR subunits, animals were pretreated with olanzapine (2 mg/kg), risperidone (0.25 mg/kg), or haloperidol (0.25 mg/kg) 30 min prior to PCP on PN7, 9, and 11. Each of the three antipsychotics investigated was able to inhibit PCP-induced up-regulation of the NR1 subunit in the frontal cortex (Figure 8), while having no effect on their own (Table 5). Selective D1, D2, and 5-$HT_{2A}$ receptor antagonists were then utilized to determine the neurotransmitter receptors that may be responsible for the effects of olanzapine, risperidone and haloperidol. Sulpiride (selective D2 receptor antagonist, 100 mg/kg), SCH23390 (selective D1 receptor antagonist, 0.5 mg/kg) and M100907 (selective 5-$HT_{2A}$ receptor antagonist) were all able to effectively prevent the

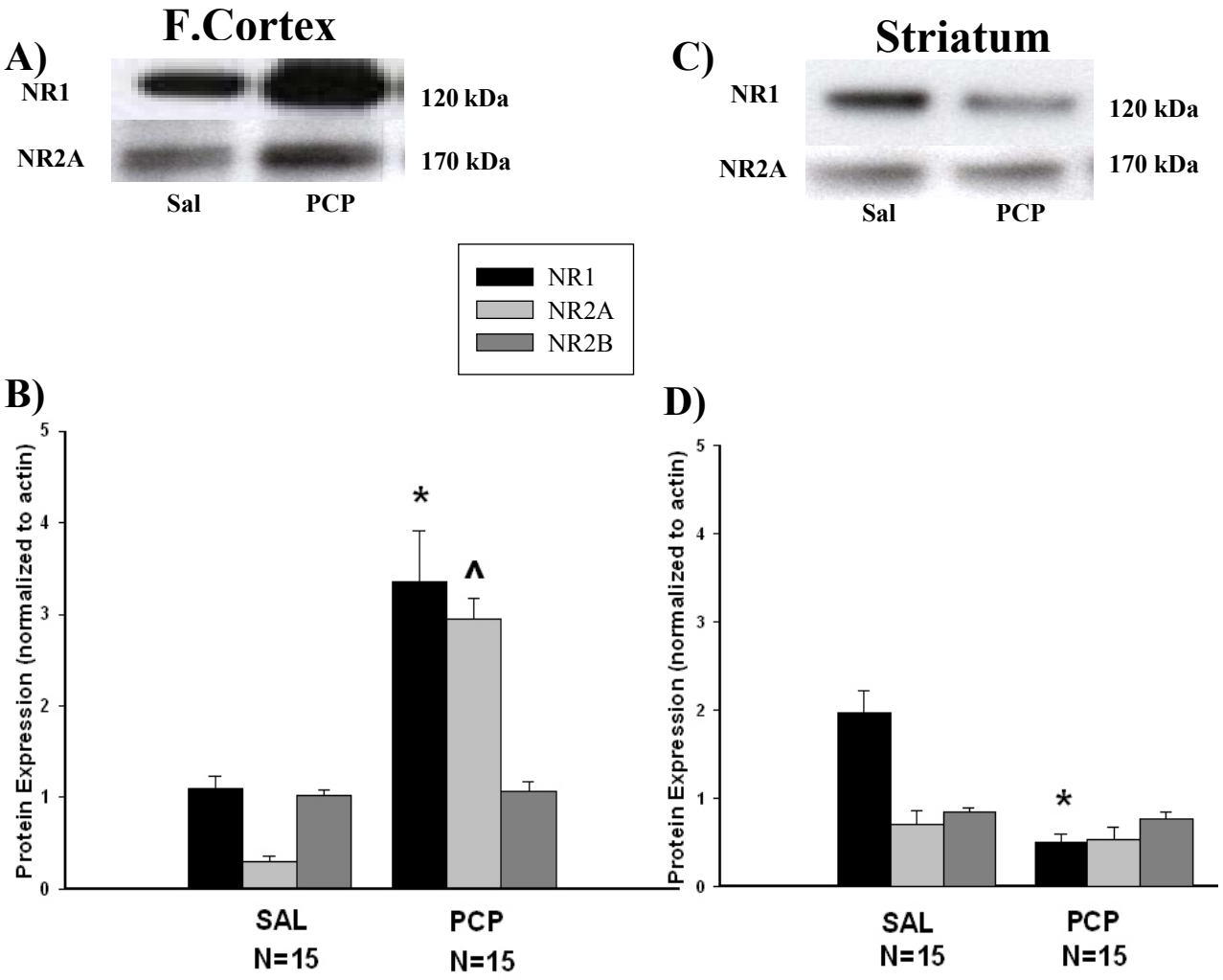


**Figure 6. A)** Representative Western blot from the frontal cortex showing NR1 and NR2A membrane protein up-regulation following sub-chronic PCP (10 mg/kg) administration. **B)** Quantitative analysis of PCP's effects on NMDAR subunit proteins in the frontal cortex. *p<0.05 vs. SAL NR1 (one-way ANOVA with Bonferroni's post hoc test) ^p<0.05 vs. SAL NR2A (one-way ANOVA with Bonferroni's post hoc test). **C)** Representative Western blot from the striatum showing PCP-induced NR1 down-regulation. **D)** Quantitative analysis of sub-chronic PCP-induced down-regulation of NR1 protein in the striatum. *p<0.05 vs. saline(one-way ANOVA with Bonferroni's post hoc test)

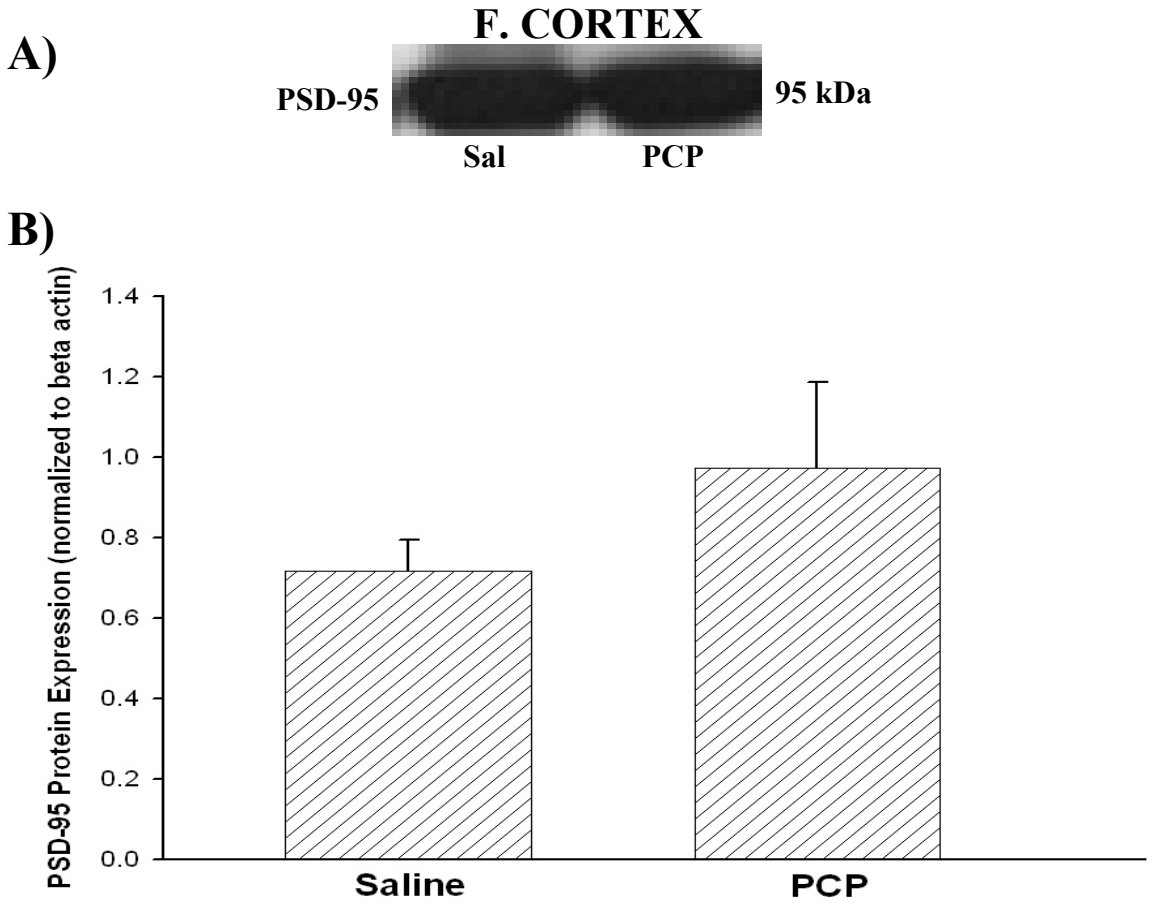


**Figure 7.** Sub-chronic PCP treatment (10 mg/kg, N=4) has no effect on the levels of membrane bound post-synaptic density 95 (PSD-95) protein in the frontal cortex. (A) Representative Western blot of cortical protein from saline and sub-chronic PCP treated animals. (B) Quantitative analysis of protein expression from saline and sub-chronic PCP treatment on PSD-95 protein levels in the frontal cortex.

up-regulation of NR1 protein levels in the frontal cortex caused by sub-chronic PCP administration (Figure 8). None of the selective antagonists studied produced an effect on their own (Table 5).

The expression of NR2A protein levels in the frontal cortex was also increased following sub-chronic PCP administration (Figure 6B); therefore, the effects of the various antipsychotics and antagonists on PCP-induced up-regulation of NR2A protein levels were examined. Pretreatment with either olanzapine, risperidone, or haloperidol was also able to completely block the increase in NR2A protein levels caused by sub-chronic PCP treatment (Figure 9) with no effect on their own (Table 5). Further, inactivation of 5-$HT_{2A}$, D1, and D2 receptors by M100907, SCH23390, and sulpiride, respectively, also prevented the up-regulation of NR2A in the frontal cortex induced by sub-chronic PCP administration (Figure 9). Administration of the selective antagonists alone produced no effect on NR2A levels in the frontal cortex (Table 5).

Unlike the up-regulation of NR1 observed in the cortex, Western blot analysis of membrane bound protein showed that levels of striatal NR1 protein were significantly decreased following sub-chronic PCP treatment (Figure 6D). Pretreatment with the anti-schizophrenic drugs olanzapine or haloperidol, but not risperidone, prevented the down-regulation of NR1 protein expression in the striatum caused by sub-chronic PCP administration (Figure 10). Administration of these drugs alone had no effect on NR1 or NR2A protein levels in the striatum (Table 6a). Unexpectedly, pretreatment with the selective D2, D1, or 5-$HT_{2A}$ receptor antagonists (sulpiride, SCH23390, and M100907, respectively) were unable to prevent the decrease in striatal NR1 levels caused by sub-chronic PCP treatment (Figure 10) while having no effect on their own (Table 6a).

As mentioned previously, sub-chronic PCP treatment did not alter the levels of NR2A protein in the striatum (Figure 6D). Treatment with either the aforementioned antipsychotics or selective antagonists alone (Table 6a) or prior to PCP on PN7, 9, and 11 did not significantly alter NR2A protein expression in the striatum (Table 6b).

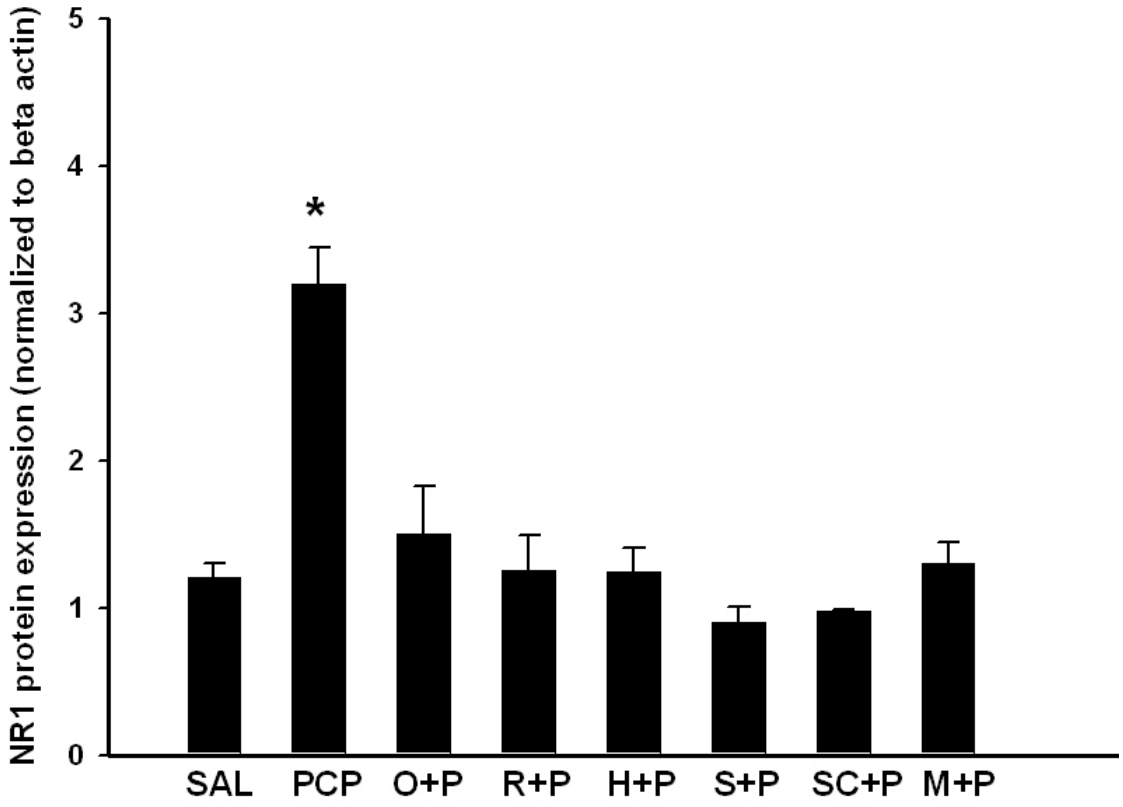


**Figure 8.** Administration of each of the antagonists studied was able to prevent sub-chronic PCP-induced (10 mg/kg) up-regulation of NR1 protein in the frontal cortex. * $p<0.05$ vs. SAL NR1 (one-way ANOVA with Bonferroni's post hoc test) O=olanzapine (2 mg/kg, N=6) R=risperidone (0.25 mg/kg, N=4) H=haloperidol (0.25 mg/kg, N=4) S=sulpiride (100 mg/kg, N=4) SC=SCH23390 (0.5 mg/kg, N=6) M=M100907 (1 mg/kg, N=6)

**Table 5. Effect of sub-chronic antagonist alone administration on NMDA receptor subunit protein expression in the frontal cortex (normalized to β-actin)**

| | NR1 | NR2A |
|---|---|---|
| **Olanzapine (N=6)** | **1.40 ±0.24** | **0.59±0.11** |
| **Risperidone (N=4)** | **1.07±0.21** | **0.77±0.12** |
| **Haloperidol (N=4)** | **1.07±0.12** | **0.99±0.20** |
| **Sulpiride (N=4)** | **0.93±0.12** | **0.78±0.09** |
| **SCH23390 (N=6)** | **0.97±0.45** | **0.85±0.05** |
| **M100907 (N=6)** | **1.42±0.04** | **0.44±0.20** |
| **Data presented as mean ± SEM** | | |

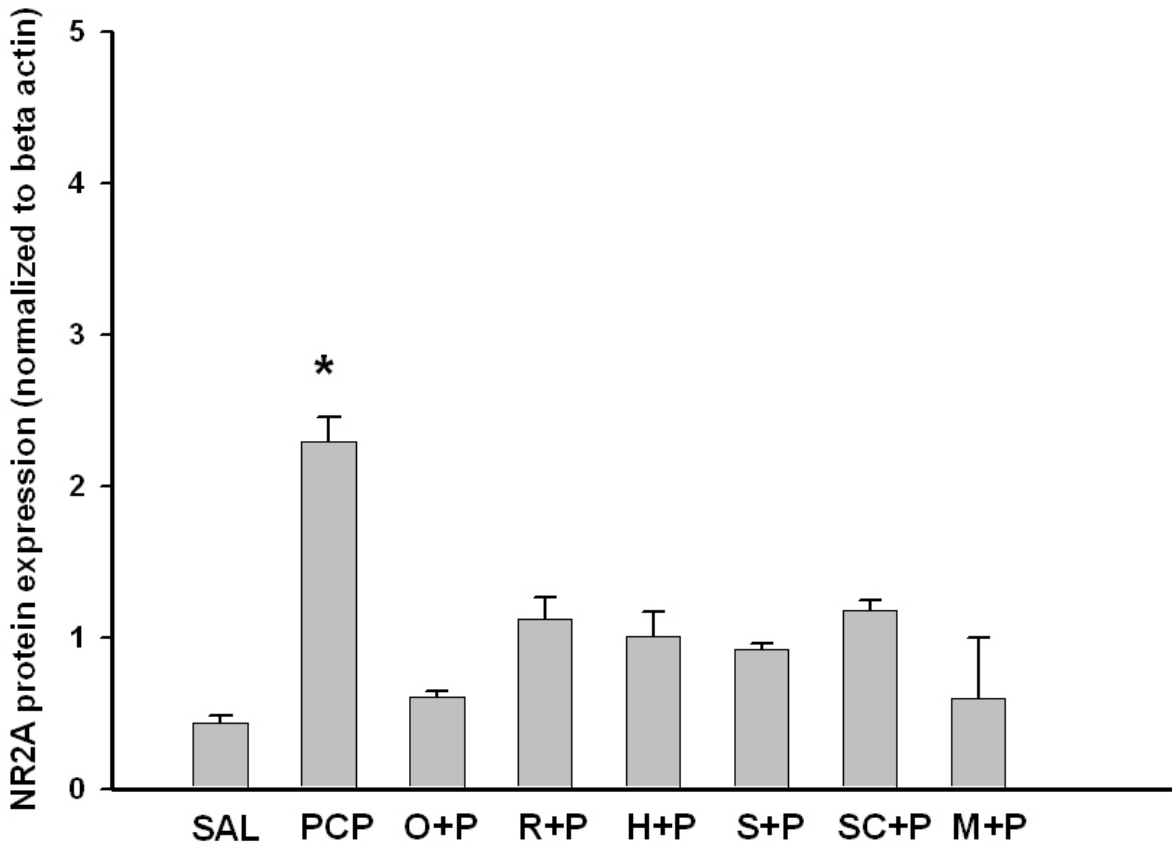


**Figure 9.** Administration of each of the antagonists studied was also able to prevent sub-chronic PCP-induced (10 mg/kg) up-regulation of NR2A protein in the frontal cortex. * $p<0.05$ vs. SAL NR1 (one-way ANOVA with Bonferroni's post hoc test) O=olanzapine (2 mg/kg, n=3) R=risperidone (0.25 mg/kg, N=3) H=haloperidol (0.25 mg/kg, N=6) S=sulpiride (100 mg/kg, N=3) SC=SCH23390 (0.5 mg/kg, N=6) M=M100907 (1 mg/kg, N=5)

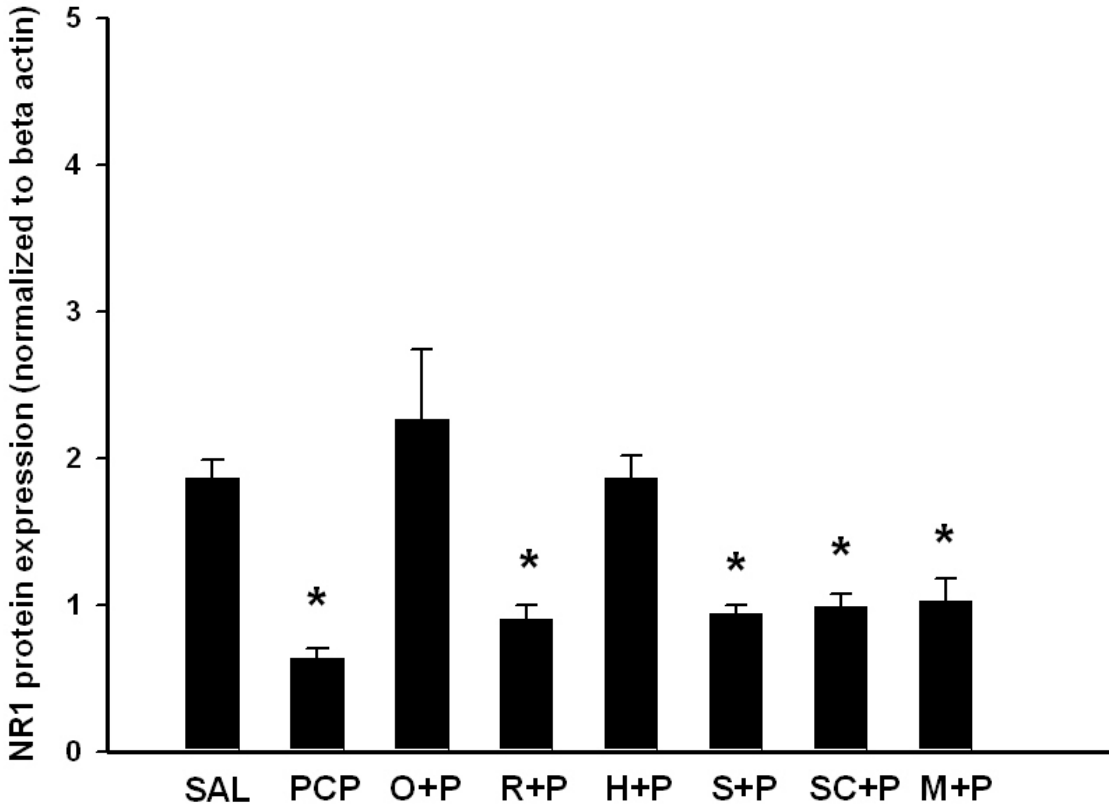


**Figure 10.** Pretreatment with olanzapine (O) (2 mg/kg, N=3) or haloperidol (H) (0.25 mg/kg, N=4) was able to inhibit sub-chronic PCP-induced down-regulation of NR1 protein in the striatum. * $p<0.05$ vs. saline (one-way ANOVA with Bonferroni's post hoc test) R=risperidone (0.25 mg/kg, N=3) S=sulpiride (100 mg/kg, N=4) SC=SCH23390 (0.5 mg/kg, N=6) M=M100907 (1 mg/kg, N=3)

**Table 6a. Effect of sub-chronic antagonist alone administration on NMDA receptor subunit protein expression in the striatum (normalized to β-actin)**

| | NR1 | NR2A |
|---|---|---|
| Olanzapine (N=3) | 2.26 ±0.46 | 0.85±0.07 |
| Risperidone (N=3) | 1.57±0.18 | 1.07±0.16 |
| Haloperidol (N=4) | 1.88±0.14 | 0.64±0.03 |
| Sulpiride (N=4) | 1.35±0.19 | 0.90±0.09 |
| SCH23390 (N=6) | 1.31±0.13 | 0.97±0.06 |
| M100907 (N=3) | 1.58±0.28 | 0.74±0.15 |
| Data presented as mean ± SEM | | |

**Table 6b. Effect of sub-chronic PCP administration on NMDA receptor subunit protein expression in the striatum (normalized to β-actin)**

| | Sub-chronic PCP |
|---|---|
| | NR2A |
| Saline N=15 | 0.82 ± 0.08 |
| PCP N=15 | 0.67 ± 0.08 |
| Olanzapine+PCP N=6 | 0.88 ± 0.08 |
| Risperidone+PCP N=4 | 0.98 ± 0.08 |
| Haloperidol+PCP N=5 | 0.70 ± 0.11 |
| Sulpiride+PCP N=5 | 0.78 ± 0.13 |
| SCH23390+PCP N=3 | 1.04 ± 0.18 |
| M100907+PCP N=6 | 0.73 ± 0.23 |
| Data presented as mean ± SEM | |

**Mechanism of PCP-induced regulation of NMDA NR1 subunit in the frontal cortex**

In order to gain insight into the mechanism by which PCP may regulate the expression of NMDAR subunits, cortical NR1 endoplasmic reticulum (ER) protein was extracted from saline and acute PCP or sub-chronic PCP treated animals and subjected to Western analysis. Relative to protein disulfide isomerase (PDI), an ER house-keeping protein, acute PCP treatment on PN7 at 24 hours following administration produced a 3-fold decrease in NR1 protein levels in the ER fraction of the frontal cortex (Figure 11B). This effect is opposite to the effect of acute PCP observed in the membrane fraction. However, sub-chronic PCP treatment produced a 2.5 fold increase in cortical NR1 ER protein levels measured 24 hours following the last of the three injections (Figure 11B). This increase roughly parallels the increase previously observed in the membrane fraction.

**Effects of sub-chronic PCP administration on pre-pulse inhibition (PPI) of acoustic startle**

Since the PPI paradigm is thought to model sensorimotor gating deficits observed in schizophrenics, it was hypothesized that sub-chronic PCP administration to postnatal rats would result in deficits in baseline PPI of acoustic startle. PCP treatment significantly reduced PPI from 22% to 9% measured on PN14-15 (Figure 12). However, treatment on PN7, 9, and 11 with the atypical antipsychotic olanzapine at 2 mg/kg also resulted in a significant decrease in PPI of acoustic startle measured on PN14-15 (Figure 12). Based on this observation, the dose of olanzapine was lowered to 1 mg/kg. Animals tested on PN14-15 that were treated with olanzapine (1 mg/kg) or haloperidol (0.25 mg/kg ) 30 min prior to PCP on PN7, 9, and 11 showed a partial inhibition of PCP-induced deficits in PPI of acoustic startle (Figure 13). Inhibition of baseline PPI caused by sub-chronic PCP administration that was initially measured on PN14-15 was still evident 13-14 days after PCP administration (PN24-25) (Figure 14). Furthermore, pretreatment with olanzapine (1 mg/kg) on PN7, 9, and 11 was able to partially inhibit sub-chronic PCP-induced inhibition of PPI in animals tested on PN24-25 (Figure 14). Pretreatment with haloperidol (0.25 mg/kg) on PN7, 9, and 11 was able to inhibit sub-

chronic PCP-induced inhibition of PPI in animals tested on PN24-25 (Figure 14). While not statistically significant from control, it appears that this dose of haloperidol (0.25 mg/kg) when administered alone on PN7, 9, and 11 may be behaviorally toxic to the animals on either day of testing (Figure 13 and Figure 14). Therefore, these experiments will be repeated with a lower non-toxic dose of haloperidol.

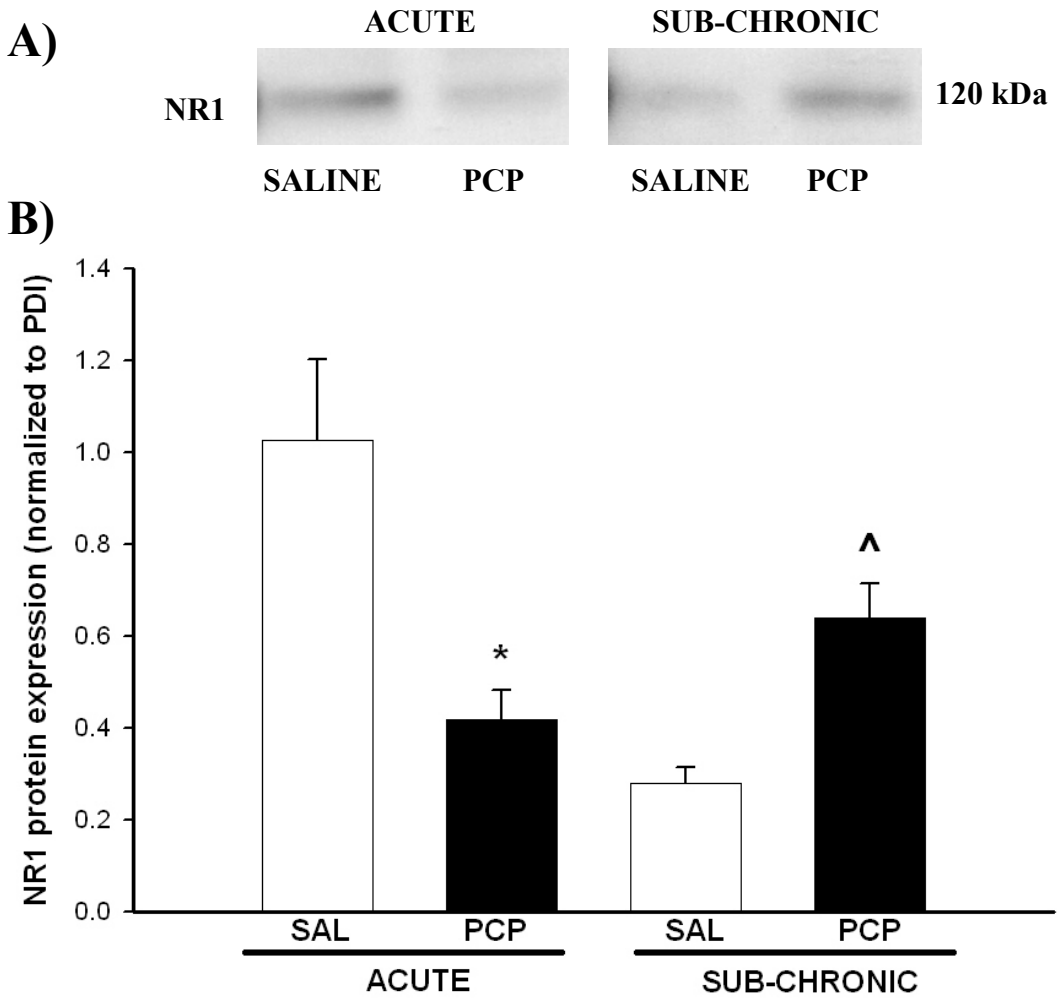


**Figure 11.** A) Representative Western blot of NR1 endoplasmic reticulum protein from the frontal cortex of either acute or sub-chronic saline or PCP-treated animals. B) Quantitative analysis of PCP's temporal effects on endoplasmic reticulum protein levels of NR1 in the frontal cortex following both acute and sub-chronic PCP treatment (10 mg/kg) (N=3/group). *$p<0.05$ vs. acute saline (Student's t-test) ^$p<0.05$ vs. sub-chronic saline (Student's t-test)

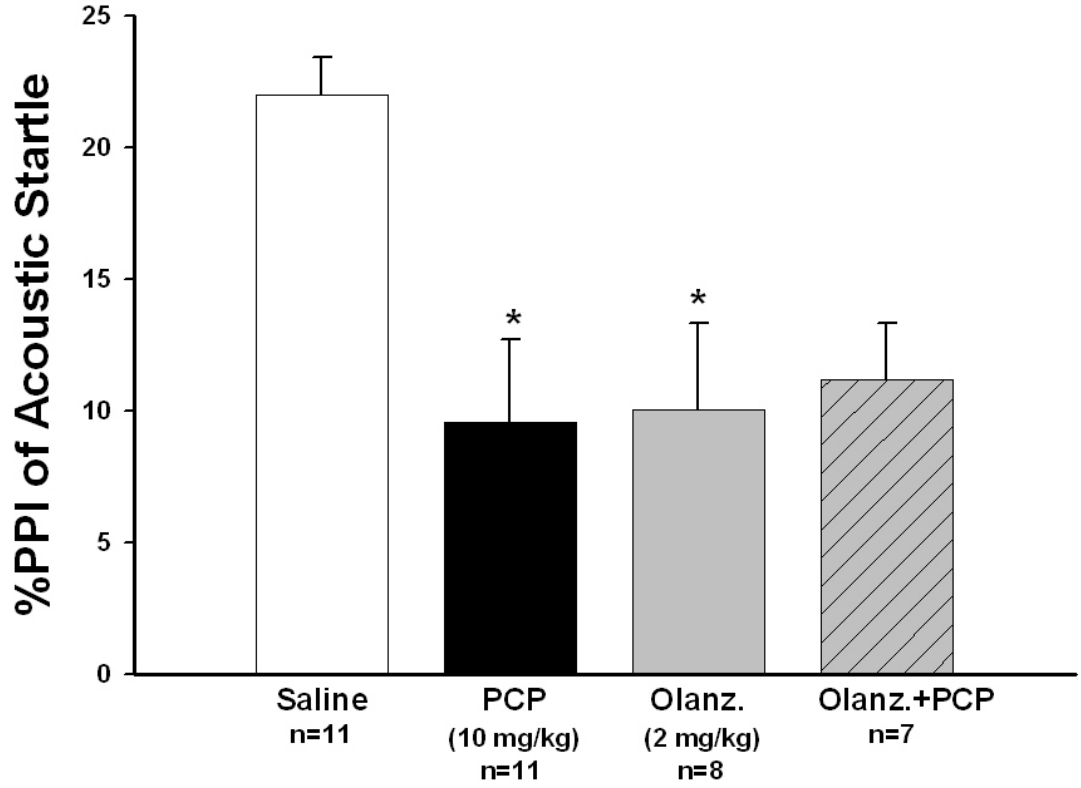


**Figure 12.** Effects of sub-chronic PCP (10 mg/kg) and olanzapine (2 mg/kg) treatment to postnatal rats on the inhibition of baseline PPI of acoustic startle. Sub-chronic PCP produced a reproducible deficit in PPI of acoustic startle 3-4 days (PN14-15) after treatment to postnatal rats. Sub-chronic treatment with olanzapine alone produced a significant deficit in PPI as well. *$p<0.05$ vs. saline (one-way ANOVA with Bonferroni's post hoc test)

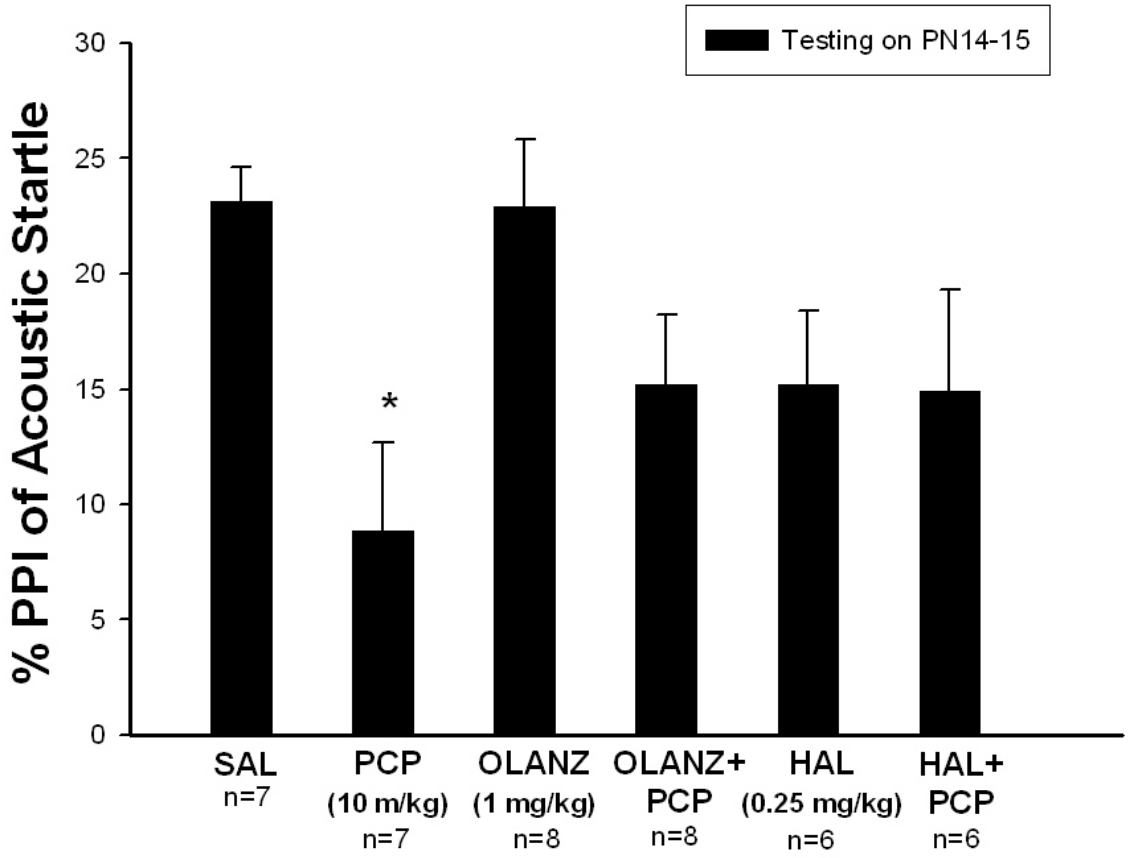


**Figure 13.** Effects of olanzapine (1 mg/kg) or haloperidol (0.25 mg/kg) pretreatment on the development of deficits in PPI of acoustic startle caused by postnatal sub-chronic PCP administration (tested on PN14-15). Both olanzapine and haloperidol pretreatment were able to inhibit sub-chronic PCP-induced deficits in PPI on PN14-15. *$p<0.05$ vs. saline (one-way ANOVA with Dunnett's post hoc test).

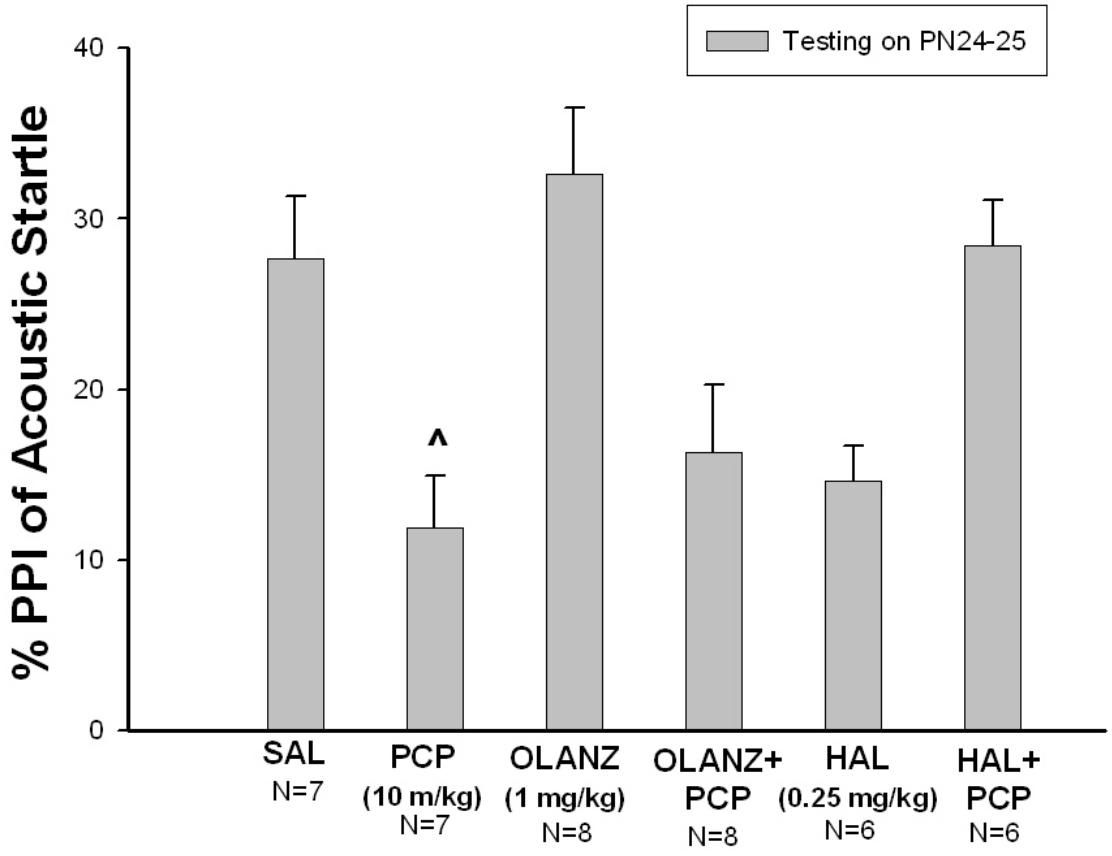


**Figure 14.** Inhibition of baseline PPI of acoustic startle caused by sub-chronic PCP administration that was observed on PN14-15 was still evident 13-14 days after treatment (PN24-25). Both olanzapine and haloperidol pretreatment on PN7, 9, and 11 was able to inhibit sub-chronic PCP-induced deficits in PPI tested on PN24-25. *$p<0.05$ vs. saline (one-way ANOVA with Dunnett's post hoc test).

# DISCUSSION

In addition to the psychotomimetic symptoms produced by PCP, several parallels exist between PCP administration and schizophrenia. PCP has been reported to increase cerebral metabolic activity in several limbic brain regions including the entorhinal cortex, retrosplenial and anterior cingulate cortex, the subiculum and the nucleus accumbens (Allen and Iverson, 1990; Hargreaves et al., 1993; Weissman et al., 1989). Ketamine has also been shown to increase cerebral blood flow in the anterior cingulate cortex (Lahti et al., 1995). In addition, acute PCP administration was reported to cause neurotoxicity in the posterior cingulate/retrosplenial cortex (Olney et al., 1989). However, chronic administration of MK-801 or PCP caused neurodegeneration in additional brain regions including the anterior cingulate, parietal, temporal, piriform, and entorhinal cortices, hippocampus, and amygdala (Olney and Farber, 1995b). These brain regions in which PCP and other noncompetitive NMDA antagonists alter cerebral blood flow and glucose utilization as well as produce neurotoxicity are some of the same brain regions that are thought to be affected in schizophrenia (Strous and Javitt, 1996). Specifically, postmortem analysis of schizophrenic brains revealed neuronal vacuolization and structural abnormalities in the hippocampus, parahippocampal gyrus, entorhinal cortex, amygdala, cingulate cortex and septum (Kovelman and Scheibel, 1984; Bogerts 1993). Based on Olney's studies and the similarities between schizophrenia and PCP administration, Olney hypothesized the NMDA receptor hypofunction theory of schizophrenia (Olney and Farber, 1995a).

In accordance with Olney's hypothesis, the effects of acute and chronic PCP or MK-801 treatment has been extensively studied in the adult rat as an animal model of the disease (Hanania et al. 1999; Jentsch et al. 1997; Johnson et al. 1998; Moghaddam et al. 1997; Phillips et al. 2001). However, it was postulated that since symptoms of schizophrenia do not appear until early adulthood that the etiology of the disease may be developmental in nature (Weinberger, 1987). This led researchers to investigate the neurotoxic effects of acute PCP or MK-801 during stages of development (Ikonomidou et

al., 1999; Wang and Johnson, 2005 in press). This laboratory has also reported the presence of neurotoxicity in the frontal cortex, but not the striatum following sub-chronic PCP administration to postnatal rats (Wang et al., 2001; Wang and Johnson 2005, in press). The atypical antipsychotic olanzapine was able to prevent both behavioral and biochemical indices of PCP administration, suggesting this treatment paradigm as a suitable animal model of schizophrenia (Wang et al., 2001).

In order to determine the possible mechanism by which PCP may elicit its neurotoxic effects and produce alterations in behavior in postnatal rats, this laboratory began to investigate the effects of postnatal PCP administration on the concentration of the NMDA receptor subunits. Sub-chronic PCP treatment to pups on PN7, 9, and 11 resulted in increased expression of NR1 mRNA in the frontal cortex, striatum, nucleus accumbens and olfactory cortex, which was inhibited by pretreatment with the atypical antipsychotic olanzapine (Wang et al., 2001). Wang et al. (2001) confirmed that the increase in NR1 mRNA assessed by *in situ* hybridization resulted in an increase in NR1 protein expression in the frontal cortex. Up-regulation of the NMDA receptor subunits may be due to either increased trafficking of assembled NR1/NR2 receptors from the endoplasmic reticulum to the cell surface or an increase in new receptor protein synthesis.

**In the frontal cortex, acute PCP treatment induces increased trafficking of the NMDAR**

This present study was conducted in order to determine the possible mechanism of PCP-induced regulation of the NR1 subunit and to extend this investigation to include the most prominent NR2 subtypes. Acute PCP treatment on PN7 resulted in increased levels of NMDA subunits NR1 and NR2B membrane protein expression in the frontal cortex at 8 and 24 hours following PCP. In addition, acute PCP administration produced an increase in cortical membrane protein levels of post-synaptic density 95 (PSD-95), a member of the NMDA receptor post-synaptic density complex, which paralleled the observed increase in NR1 and NR2B in the frontal cortex. PSD-95 is thought to be responsible for anchoring the assembled NR1/NR2 complex in the membrane through its interactions with the C-terminus of the NR2 subunit (Wenthold et al., 2003).

Furthermore, analysis of the cortical endoplasmic reticulum (ER) fraction demonstrated a decrease in NR1 protein following acute PCP administration, consistent with an increase in trafficking of NR1 subunits from the ER to the membrane.

**Possible mechanisms of PCP-induced NMDAR trafficking**

The NR1 subunit contains eight splice variants, one in the N terminus and two in the C terminus that possess distinguishing features. The splice variants differ in regard to their regional pattern of expression, their regulation by phosphorylation, polyamines or protons, their electrophysiological properties, and their affinity for cytoskeletal proteins (Nestler, Hyman and Malenka, 2001). The C terminal splice variant composition affects trafficking of the NR1 subunit. It has been suggested that the C2' cassette present in four of the NR1 splice variants possesses a PDZ binding domain (STVV) (Wenthold et al., 2003). In addition, the NR1 subunit contains an ER retention signal (RXR) in the C1 cassette that must be masked for the polypeptide to be released from the ER (Wenthold et al., 2003). It is reasonable to predict that following acute PCP administration, binding of PSD-95 to the PDZ binding domain of the C2' cassette of the NR1 subunit may mask the RXR motif in the C1 cassette, resulting in the exit of the NR1 subunit from the ER and insertion in the membrane (Standley et al., 2000); therefore, the robust increase in cortical membrane levels of PSD-95 caused by acute PCP treatment could be due to its persistent binding to the C2' cassette of the NR1 subunit in conjunction with its presence in the post-synaptic density complex. This suggests that PSD-95 interacts early in the secretory pathways functioning as an export protein that overrides the ER retention signal for the NR1 subunit in addition to serving to anchor the functional, assembled receptor complex in the synapse by binding to the C-terminus of the NR2 subunit (Wenthold et al., 2003).

Activation of the NMDA receptor results in the influx of $Ca^{2+}$ which in turn activates the calcium-dependent neutral cysteine protease, calpain. Excessive activation of calpain results in the enzymatic cleavage of cytoskeletal proteins, loss of structural integrity and disturbances in axonal transport (Yamashima, 2004). NMDA treatment of organotypic hippocampal cultures produced activation of calpain and the subsequent cleavage of PSD-95 (Lu et al., 2000). Truncation of PSD-95 could then lead to an unstablilized NR2 subunit in the membrane and cause the internalization and down-regulation of the assembled NMDA receptor (Dong et al., 2004). However, blockade of the NMDA receptor by acute PCP treatment would prevent the increase in intracellular $Ca^{2+}$ and the subsequent activation of calpain. This in turn would protect PSD-95 from cleavage by calpain and ultimately prevent the removal of the receptor from the membrane. Therefore, increased trafficking of NMDA receptors from the ER to the membrane in conjunction with intact PSD-95 following acute PCP administration would result in the observed up-regulation of the receptor in the frontal cortex.

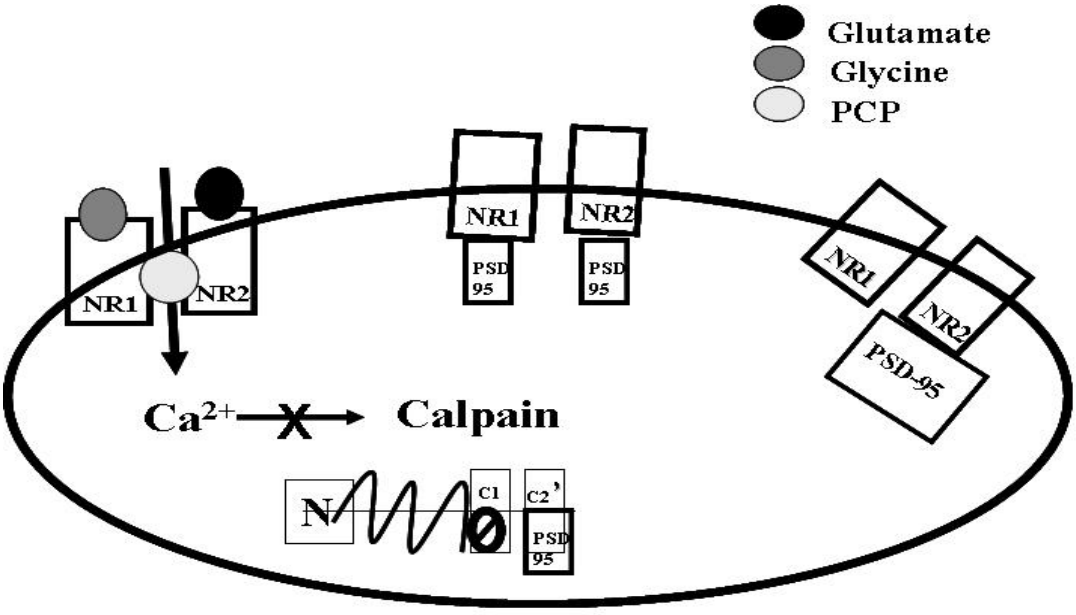


**Figure 15. Possible mechanism of acute PCP-induced trafficking of the NMDA receptor in the frontal cortex**

PCP blockade of the NMDA receptor results in the efflux of glutamate in the frontal cortex (Adams and Moghaddam, 2001) leading to the subsequent activation of either AMPA/KA receptors or metabotropic glutamate receptors (mGluRs). Investigation of the interactions between mGluRs and NMDA receptors suggests that stimulation of mGluRs potentiates NMDA receptor function (Aniksztejn et al., 1991; Bleakman et al., 1992; Harvey and Collingridge, 1993). Modulation of NMDA receptor function by mGluRs is thought to be limited to Group I, both mGluR1 (Lan et al., 2001) and mGluR5 (Pisani et al., 2001) have been implicated. Group I mGluRs are G-protein coupled receptors that are positively coupled to phospholipase C (PLC) resulting in the formation of diacylglycerol (DAG) and activation of protein kinase C (PKC), leading to the release of $Ca^{2+}$ from intracellular stores (Conn and Pin, 1997). Potentiation of NMDA receptor function by mGluR5 is dependent on G-protein second messenger signaling cascades, which includes PKC and Src kinase (Benquet et al., 2002). PKC phosphorylation of sites near the RXR motif (ER retention signal) of the NMDA receptor NR1 subunit, masks the ER retention signal resulting in the trafficking of the subunit to the membrane (Scott et al., 2003).

Thus, activation of mGluRs may be a compensatory mechanism in response to acute PCP blockade of the NMDA receptor. This mechanism could involve up-regulation of NR1 and NR2 subunits through increased trafficking and may ultimately lead to a hyperfunctional NMDA receptor and cell death (McInnis et al., 2002). This is consistent with the observation that the time course of up-regulation of NR1 and NR2B in the frontal cortex following acute PCP treatment coincides with neuronal neuro-degeneration (positive silver staining) seen on PN7 (Wang and Johnson, 2005). However, it should be noted that striatal neurodegeneration occurs following acute PCP treatment in the absence of changes in the NMDA receptor subunits or PSD-95.

Since PCP is known to increase extracellular dopamine (DA) and serotonin (5-HT) release, pharmacological antagonism of DA and 5-HT receptors was conducted in order to determine if these monoamines are involved in the mechanism whereby acute PCP treatment results in increased protein expression of NR1 and NR2B in the frontal

cortex, 24 hrs following treatment. Pretreatment with either the atypical antipsychotics olanzapine and risperidone or the typical antipsychotic haloperidol had no effect on the observed up-regulation of either NR1 or NR2B. Furthermore, pretreatment with the selective DA or 5-HT antagonists sulpiride (selective D2 receptor antagonist), SCH23390 (selective D1 receptor antagonist) or M100907 (selective 5-$HT_{2A}$ receptor antagonist) also had no effect on the up-regulation of either NR1 or NR2B suggesting that activation of 5-$HT_{2A}$, D1, or D2 receptors subsequent to 5-HT or DA release by acute PCP treatment is not involved in this process.

Interestingly, it was recently reported that activation of post-synaptic 5-$HT_{1A}$ receptors can modulate the NMDA receptor function (Yuen et al., 2005). Transport of assembled NR1/NR2B receptor complexes to the membrane is dependent on the integrity of the microtubule network, which is disrupted upon 5-$HT_{1A}$ receptor activation and is regulated by the CAMKII and ERK signaling pathways (Yuen et al., 2005). It is of importance to mention that the recently approved atypical antipsychotic, aripiprazole, is a partial 5-$HT_{1A}$ receptor agonist and is effective at alleviating the negative symptoms of schizophrenia as well as improving cognitive deficits (Li et al., 2004). This finding is thought provoking and future studies will be conducted to investigate the effects of aripiprazole on PCP's regulation of the NMDA receptor in order to determine if 5-$HT_{1A}$ receptors are involved in the increased NMDA receptor subunit trafficking that occurs following acute PCP treatment.

Statistical analysis of the effects of olanzapine treatment prior to PCP administration on cortical NR1 protein levels at 4 hrs on PN7 shows no significant difference from control. In addition, cortical NR2B protein levels are not significantly altered following M100907 treatment alone at 4 hrs. However, careful examination of the data suggest that there may be a trend towards a decrease of these subunits (vs. control) for both of these treatment groups. These experiments will be repeated in order to determine the nature of the effect of olanzapine and M100907 on cortical NR1 and NR2B protein expression, respectively, at 4 hrs on PN7.

**Acute PCP does not effect the NMDAR in the striatum**

Acute PCP treatment had no effect on membrane protein levels of NR1, NR2B or NR2A in the striatum at any time point examined on PN7. Furthermore, acute PCP administration does not alter protein expression of PSD-95 in the striatum. PSD-95 interacts with the last four amino acids of the NR2 subunit and is thought to be responsible for anchoring the functional NR1/NR2 receptor complex in the membrane (Wenthold et al., 2003). Yamada et al. (1999) suggests that PSD-95 is an important determinant in the cell surface expression of assembled NR1/2 receptor complexes. Therefore, it is possible that because acute PCP treatment does not alter striatal PSD-95 protein levels, a change in the expression of striatal NR1 and NR2 subunits in the membrane was not detected. Acute PCP-induced neurotoxicity reported in the striatum on PN7 (Wang and Johnson, 2005) may not be due solely to NMDA receptor membrane expression levels, since no change in receptor subunit density was observed. The exact mechanism by which the striatum is susceptible to a single dose of PCP is unknown, but it is possible that the initial insult of receptor blockade and inhibition of function is sufficient to induce neurodegeneration through disruptions in normal intracellular $Ca^{2+}$ levels (Adams et al., 2004; Takadera et al., 1999).

**Sub-chronic PCP treatment results in new NMDAR protein synthesis in the frontal cortex**

Sub-chronic PCP administration on PN7, 9, and 11 resulted in increased levels of membrane protein expression of NR1 and NR2A in the frontal cortex, with no change in NR2B. In addition, analysis of the cortical ER protein fraction revealed increased levels of NR1 following sub-chronic PCP treatment, suggesting that up-regulation of NR1 may be due to increased synthesis of NR1 protein. This is consistent with a previous report showing an increase in NR1 mRNA using the *in situ* hybridization technique (Wang et al., 2001). Olanzapine, risperidone, and haloperidol pretreatment prevented the observed increase in NR1 and NR2A protein levels. Since these antipsychotics possess relatively high affinity for D1, D2, and 5-$HT_{2A}$ receptors, selective antagonists for each receptor type were used to determine the mechanism through which the antipsychotics may act to

inhibit PCP's effects. Unexpectedly, inactivation of D1, D2 and 5-$HT_{2A}$ receptors by SCH23390, sulpiride, and M100907, respectively, each prevented up-regulation of NR1 and NR2A subunits induced by sub-chronic PCP, suggesting that PCP activation of D1, D2 or 5-$HT_{2A}$ receptors may be sufficient to increase receptor density, perhaps by increasing NR1 and NR2A synthesis.

**Possible mechanisms of sub-chronic PCP-induced NMDAR synthesis**

PCP-induced release of DA in the prefrontal cortex (Verma and Moghaddam, 1996) could lead to the activation of D1 receptors resulting in increased levels of cAMP and increased activation of PKA, which would be expected to increase the phosphorylation of the transcription factor cAMP response element binding protein (CREB) (Lau et al., 2004). The NR1 gene contains a CRE (cAMP response element) binding site upstream of its transcription start site (Lau et al., 2004). PCP-induced phosphorylation of CREB by PKA or from activation of the MAP kinase pathway could then lead to increased binding of P-CREB to the NR1 CRE site, resulting in increased transcription of NR1 subunit protein in the frontal cortex. However, preliminary data indicates that the expected increase in cortical levels of P-CREB following both acute and sub-chronic PCP administration is not quite significant (Robeson and Johnson, unpublished observation).

In addition, the NR1 gene contains a binding site for the transcription factor NF-κB (Liu et al., 2004). Liu et al. (2004) reported that binding for the NR1 NF-κB site up-regulates the NR1 promotor and subsequent transcription of the gene through interactions with Sp1/Sp3 factors. Wang et al. (2001) reported that sub-chronic PCP treatment increased nuclear translocation of NF-κB subunits p50 and p65 as evidenced by electrophoretic mobility shift assay (EMSA); this was prevented by olanzapine pretreatment. Therefore, PCP-induced activation of the NF-κB transcription factors may be an alternative explanation for the observed increase in synthesis of NR1 protein.

Likewise, PCP treatment of corticostriatal slice cultures resulted in increased levels of NR1, NR2A, and Bax (pro-apoptotic protein) in the cortex that was accompanied by an increase in histone- associated DNA fragments as measured by an

ELISA (Wang et al., 2005). Co-incubation with antisense oligonucleotides directed towards either NR1 or NR2A prevented the PCP-induced increase in either Bax or an ELISA for DNA histone-associated fragments in the cortex (Wang et al., 2005). These results from organotypic cultures and the aforementioned neurotoxic effects of sub-chronic PCP treatment *in vivo*, support the possibility that up-regulation of NR1 and NR2A subunits in the frontal cortex may be due to an increase in new protein synthesis ultimately resulting in a hyper-functional NMDA receptor that could lead to a hypercalcemic state and the subsequent activation of the apoptotic cascade (McInnis et al., 2002). The loss of these cortical neurons and their subsequent input to the striatum, hippocampus, and other limbic brain regions could underlie the development of either positive or negative symptoms of schizophrenia. Also, inactivation of 5-HT and DA receptors by either olanzapine or risperidone may represent the mechanism by which these antipsychotics are able to prevent damage to cortical neurons caused by sub-chronic PCP administration.

**Sub-chronic PCP induces differential regulation of the NMDAR subunits in the striatum-mechanism of protection from neurotoxicity?**

Although sub-chronic PCP in the postnatal rat resulted in increased levels of NR1 mRNA expression in the striatum as assessed by *in situ* hybridization (Wang et al., 2001), this same treatment paradigm in this study produced a decrease in NR1 protein levels in the striatum and had no effect on either NR2A or NR2B. The reason for this apparent discrepancy is unknown. It has been reported that sub-chronic PCP treatment results in a regionally selective neurotoxic effect in the frontal cortex (TUNEL positive and positive silver staining), while the striatum appears to be protected from the damaging effects of PCP as assessed 24 hrs following the last injection (Wang et al., 2001; Wang and Johnson, 2005). Down-regulation of the NR1 subunit in conjunction with no change in the NR2 subunit expression levels in the striatum may lead to fewer functional NMDA receptors and thus be a mechanism whereby the striatum is protected from sub-chronic PCP-induced neurotoxicity. In addition, PCP treatment of corticostriatal slice cultures resulted in increased NR1 protein in the striatum which was blocked by administration of

antisense oligonucleotides directed towards NR1 while no effect on NR2A, NR2B or Bax protein levels were observed, suggesting that up-regulation of both NR1 and NR2 subunits may be necessary for PCP-induced neurotoxicity to occur (Wang et al., 2005). The apparent discrepancy between the effects of PCP on the regulation of NR1 in the striatum *in vivo* and *in vitro* may be due to the complex interactions of the multiple neurotransmitter and neuromodulator systems between brain regions that exist in the whole animal that are lacking in the corticostriatal slice culture.

**Pharmacological analysis of sub-chronic PCP-induced down-regulation of NR1 in the striatum**

Pretreatment with olanzapine or haloperidol was able to prevent the down-regulation of striatal NR1 polypeptide that was caused by sub-chronic PCP administration. However, pretreatment with SCH23390 (selective D1 antagonist), sulpiride (selective D2 antagonist), or M100907 (selective 5-$HT_{2A}$ antagonist), did not prevent the down-regulation of NR1 in the striatum. The actions of olanzapine could require simultaneous blockade of DA and 5-HT receptors, if so, co-administration of sulpiride and M100907 would be expected to prevent PCP-induced down-regulation of NR1 protein expression. In addition to affinity for DA and 5-HT receptors, olanzapine possesses high affinity for muscarinic ACh receptors ($K_i$ = 1.89 nM), H1 histamine receptors ($K_i$=7.14 nM), as well as α1 noradrenergic receptors ($K_i$=19 nM) (Arnt and Skarsfeldt, 1998; Raggi et al., 2004); therefore, it is possible that in the striatum, an area rich in muscarinic ACh receptors, olanzapine's effect of blocking down-regulation of NR1 caused by sub-chronic PCP administration to postnatal rats could also involve an action at these receptors.

Haloperidol is a typical antipsychotic that possesses high affinity for D2 and σ receptors (Su, 1982; Largent et al., 1984; Tam and Cook, 1984). It has been postulated that σ receptors play a role in the pathophysiology of schizophrenia through their regulation of the glutamatergic system via direct interactions with the NMDA receptor which could then interfere with dopamine transmission in limbic brain regions (Debonnel and de Montigny, 1996). It has been shown that haloperidol is able to attenuate the σ

agonist-induced potentiation of the NMDA response (Monnet et al., 1992); therefore, it is reasonable to suggest that haloperidol's ability to alleviate the down-regulation of NR1 in the striatum is not due solely to its antagonistic actions at the D2 receptor, but could also involve its inhibitory effects on the σ receptor.

**Sub-chronic PCP treatment in a behavior model of schizophrenia**

In order better understand the role for PCP-induced regulation of NMDA receptors in this model of schizophrenia, the effect of haloperidol and olanzapine on PCP-induced inhibition of PPI of acoustic startle was measured in postnatal rats treated with PCP on PN7, 9, and 11. PCP-induced blockade of PPI of acoustic startle, a measurement of sensorimotor gating, is used as a model of the negative symptoms of schizophrenia (Braff and Geyer, 1990). Sub-chronic PCP administration resulted in a significant inhibition of PPI of acoustic startle in animals first tested on PN14-15 and then again when measured on PN24-25. Pretreatment with 2 mg/kg olanzapine alone resulted in an inhibition of PPI on PN14-15 similar to PCP treatment; therefore, the experiment was repeated and the dose of olanzapine was lowered to 1 mg/kg. Pretreatment with 1 mg/kg olanzapine as well as 0.25 mg/kg haloperidol prevented the inhibition of PPI caused by sub-chronic PCP treatment in animals tested on PN14-15. Furthermore, sub-chronic PCP resulted in a significant inhibition of PPI of acoustic startle in rats tested on PN24-25, which was also prevented by pretreatment with both olanzapine and haloperidol. Although this blockade is not complete, this appears to be the first report of the ability of the typical antipsychotic haloperidol to significantly affect sub-chronic PCP-induced deficits of PPI in pre-pubescent rats.

The ability of olanzapine and haloperidol to prevent this behavioral deficit from developing, as well as their blockade of PCP-induced up-regulation of NR1 and NR2 subunits in the frontal cortex, suggests that these antipsychotics induce a biochemical compensatory mechanism to overcome PCP's deleterious behavioral effects. Clozapine, olanzapine, and M100907, but not haloperidol, have been reported to prevent the blockade of NMDA responses in the mPFC caused by acute PCP administration (Arvanov and Wang, 1998; Wang and Liang, 1998). Furthermore, the atypical

antipsychotic clozapine has been shown to inhibit sub-chronic PCP-induced hypersensitive responses to NMDA (Arvanov and Wang, 1999). It is possible that the ability of olanzapine to inhibit biochemical (neurotoxicity, receptor up-regulation) and behavioral (deficits in PPI) indices of sub-chronic PCP administration stems from its inactivation of 5-$HT_{2A}$ receptors, which in turn results in inhibition of PCP-induced hyperfunctional NMDA receptors. The novel observation of haloperidol inhibition of PCP-induced deficits in PPI as well as its ability to prevent up-regulation of the NMDA receptor subunits in the frontal cortex may be related to its ability to produce hyposensitive NMDA receptors (Jardemark et al., 2000). Therefore, if PCP-induced neurotoxicity and the subsequent loss of these cortical neurons results in a hypo-glutamatergic state, then the ability of antipsychotics to prevent this loss of NMDAergic tone through DA and 5-HT receptors may underlie the mechanism in which these agents are effective at alleviating symptoms of the disease as well as provide insight into the possible pathophysiology of schizophrenia (Jardemark et al., 2000).

In summary, this study provides evidence that two distinct mechanisms of receptor trafficking and new protein synthesis are likely involved in the differences in NMDA receptor subunit up-regulation that exist between postnatal acute and sub-chronic PCP administration in the frontal cortex. Furthermore, we discovered that D1, D2, and 5-$HT_{2A}$ receptors play a pivotal role in sub-chronic PCP-induced up-regulation of NR1 and NR2A. Finally, we were able to correlate changes in receptor subunit concentration to the behavioral effects of PCP in an animal model of schizophrenia. Further investigation of the functionality of the NMDA receptor and the role of DA and 5-HT receptors in this process could provide insight into the interactions between molecular/cellular mechanisms and neurotransmitter systems that may be involved in the detrimental behavioral effects of PCP administration. In conclusion, this knowledge provides insight into the possible similarities between schizophrenia and this developmental model of the disease. This line of research may also lead to the development of more effective therapeutics.

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# VITA

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## Publications

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