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LONG-TERM BEHAVIORAL AND NEURODEGENERATIVE EFFECTS OF PERINATAL PHENCYCLIDINE ADMINISTRATION: IMPLICATIONS FOR SCHIZOPHRENIA

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Abstract—Both acute and chronic administration of *N*-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine and dizocilpine have been proposed to mimic some of the symptoms of schizophrenia. The purposes of the present study were first, to characterize the long-term behavioral and neurodegenerative effects of subchronic administration of phencyclidine to perinatal rats and second, to determine whether pretreatment with olanzapine could attenuate these effects. On postnatal days 7, 9 and 11 rat pups were pretreated with either vehicle or olanzapine prior to administration of either saline or phencyclidine (10 mg/kg). Some pups were killed on postnatal day 12 for biochemical determinations and others were tested on postnatal days 24–28 for prepulse inhibition of acoustic startle, on postnatal day 42 for phencyclidine-induced locomotor activity and between postnatal days 33 and 70 for acquisition of a delayed spatial learning task. Phencyclidine treatment resulted in a substantial increase in fragmented DNA in the frontal and olfactory cortices consistent with neurodegeneration by an apoptotic mechanism. An increase in the NMDA receptor NR1 subunit mRNA was also observed in the cortex. Gel shift assays showed that phencyclidine also increased the nuclear translocation of nuclear factor-κB proteins in the prefrontal cortex. In tissue from the frontal cortex, western blot analysis revealed that phencyclidine treatment increased Bax and decreased Bcl-X_L proteins. Later in development, it was observed that perinatal phencyclidine treatment significantly retarded baseline prepulse inhibition of acoustic startle measured shortly after weaning. In 42-day-old rats, it was found that challenge with 2 mg/kg phencyclidine increased locomotor activity to a significantly greater extent in the rats that had been pretreated with phencyclidine. Similarly, perinatal phencyclidine treatment significantly delayed the acquisition of a delayed spatial alternation task. Each of the aforementioned changes (except for the spatial learning task, which was not tested) was significantly inhibited by olanzapine pretreatment, an antipsychotic drug known to be effective against both positive and negative symptoms of schizophrenia. Further, olanzapine treatment for 12 days following the administration of phencyclidine was also able to reverse the phencyclidine-induced deficit in baseline prepulse inhibition.

Together these data suggest that perinatal administration of phencyclidine results in long-term behavioral changes that may be mechanistically related to the apoptotic neurodegeneration observed in the frontal cortex. It is postulated that these deficits may model the hypofrontality observed in schizophrenia and that this model may be helpful in designing appropriate pharmacotherapy. © 2001 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: *N*-methyl-D-aspartate receptor, frontal cortex, apoptosis, olanzapine, prepulse inhibition, delayed spatial alternation.

The original observation of Luby et al. (1959) that phencyclidine (PCP) administration in humans mimicked many of the signs and symptoms of schizophrenia has been extended in many ways. Javitt and Zukin (1991) emphasized that PCP mimics both positive and negative signs of schizophrenia in man. Also, ketamine reinstates unique individual psychoses in remitted schizophrenic patients (Lahti et al., 1995a,b). While acute ketamine exposure increases cortical blood flow in normal humans and schizophrenics (Lahti et al., 1995a,b; Vollenweider et al., 1997), there is evidence that long-term use of PCP is associated with reduced blood flow and glucose utilization (Cosgrove and Newell, 1991; Hertzmann et al., 1990). The latter effect is similar to what is observed in schizophrenic patients (Lahti et al., 1995a,b; Weinberger et al., 1986). The well known abilities of ketamine and PCP to mimic many of the symptoms of schizophrenia

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Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; ANOVA, analysis of variance; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetra-acetate; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; GFAP, glial fibrillary acidic protein; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(4-ethanesulfonic acid); MK-801, dizocilpine; NF-κB, nuclear factor-κB; NMDA, *N*-methyl-D-aspartate; PBS, phosphate-buffered saline; PCP, phencyclidine; PMSF, phenylmethylsulfonyl fluoride; PN, postnatal day; PPI, prepulse inhibition; ROS, reactive oxygen species; SDS, sodium dodecylsulfate; TUNEL, terminal deoxynucleotidyl dUTP transferase nick-end labeling.

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and to block the *N*-methyl-D-aspartate (NMDA) receptor (Anis et al., 1983) have given rise to several variations of a glutamatergic hypofunction model of schizophrenia (Carlsson and Carlsson, 1990; Deutsch et al., 1989; Olney and Farber, 1995; Olney et al., 1989). The models of Deutsch and coworkers and the Carlssons focused on the relationship between glutamatergic and dopaminergic neurons in an effort to accommodate the postulated role of dopamine in schizophrenia, while that of Olney et al. (1989, 1991) related the neurotoxic effects of NMDA antagonists such as PCP and MK-801 to the structural deficits found in schizophrenia (Goldstein et al., 1999; Pearlson and Marsh, 1999). More recently, it has been demonstrated that mice having only 5% of the normal levels of the obligatory NR1 subunit of the NMDA receptor display a behavioral phenotype characterized by increased locomotor activity and deficits in social and sexual interactions (Mohn et al., 1999). Although schizophrenia is known not to be associated with dramatic loss of NMDA receptors, the observation that these behavioral deficits could be ameliorated with antipsychotic drugs further supports the general hypothesis that 'NMDAergic' hypofunction may underlie certain features of schizophrenia. It is possible that the neuroanatomical deficits associated with schizophrenia combine to produce a state that closely resembles hypofunction of NMDA-mediated neurotransmission.

Microscopic examination of post-mortem brain tissue has led other investigators to postulate that the loss of neurons and/or a developmental deficit, particularly in subregions of the frontal cortex, underlies many core symptoms of schizophrenia (Benes, 1995; Benes et al., 1991; Goldman-Rakic, 1999; Lewis, 1997). It has been suggested that in at least some cases of schizophrenia, the primary pathological insult may occur in the pre- or perinatal period (Benes et al., 1991; Murray et al., 1992; Pilowski et al., 1993), though the functional consequences of this insult do not become evident until after puberty, a time at which the affected neural networks reach maturity (Weinberger, 1987). Apoptosis, or programmed cell death, could play a role in either neuronal loss or aberrant neural development. Obviously, such a multifaceted disease will be difficult to model completely. Repetitive PCP use results in persistent symptoms of schizophrenia (Carlin et al., 1979; Cosgrove and Newell, 1991; Lewis and Hordan, 1986). Repetitive PCP administration in adult rats has been reported to produce behavioral, cellular and biochemical deficits sensitive to antipsychotics (Jentsch et al., 1997; Johnson et al., 1998). While these and other reports have demonstrated enduring changes in brain function following chronic PCP or ketamine, it is unlikely that the behavioral and underlying neuronal plasticity observed in adult animals completely mimics the changes in the developing brain initiated by a perinatal insult.

For this reason, we assessed the potential behavioral effects, as well as some potential biochemical mechanisms that might underlie the apoptotic effect of PCP treatment in perinatal rats. Our working hypothesis was that apoptosis induced by PCP treatment during a critical period of development would result in long-lasting deficits in function that are directly or indirectly under cortical control. In order to assess the potential relevance of the postulated changes to schizophrenia, we administered the atypical antipsychotic drug, olanzapine, either before or after the PCP regimen. An additional rationale for this treatment was that if olanzapine were able to prevent the neurotoxic, neurochemical and behavioral effects of PCP, the causal relationship between these dependent variables would be strengthened.

EXPERIMENTAL PROCEDURES

Experimental design

Timed pregnant female Sprague–Dawley rats were obtained at day 14 of pregnancy from Charles River. They were housed individually with a regular 12-h light–dark cycle (lights on at 07.00 h, off at 19.00 h); food and water were always available. Within 12 h of parturition, the female pups from six or seven dams were combined and then randomly assigned to one of four litters of 8–10 pups each that were then randomly cross-fostered to four lactating dams. On postnatal days (PN) 7, 9 and 11, the pups were treated with PCP and/or olanzapine as described below. On PN 12, 24 h after drug treatment, half of each litter was killed and various brain regions were processed for biochemical or histochemical determinations. The remaining pups were kept in the same litters and weaned at 21 days of age. On PN 24–28, the rats were tested for baseline prepulse inhibition (PPI) of acoustic startle. In other experiments, 42-day-old rats were placed in locomotor activity cages and assessed for their response to PCP challenge. In all experimental procedures, all efforts were made to minimize animal suffering. The minimal number of animals necessary to produce reliable scientific results was used. There are no feasible alternatives to the use of live animals in this study.

Drugs and treatment paradigm

In these experiments, rat pups were treated in one of four ways. The pups were pretreated with either vehicle or 2 mg/kg (s.c.) olanzapine 30 min prior to the administration of either saline or PCP (10 mg/kg, s.c.). Thus, the four treatment groups were vehicle/saline, olanzapine/saline, vehicle/PCP and olanzapine/PCP. In one experiment, pups were treated with PCP (10 mg/kg, s.c.) or saline as above on PN 7, 9 and 11 and then half of each group was treated with olanzapine (2 mg/kg, s.c.) or vehicle twice each day on PN 12–23 prior to testing in the PPI paradigm on PN 24 and PN 25.

PCP was acquired from the National Institute on Drug Abuse (Rockville, MD, USA). Olanzapine was a gift from Eli Lilly and Company (Indianapolis, IN, USA). PCP was dissolved in 0.9% NaCl. Olanzapine was dissolved in minimal 0.1 N HCl and titrated to pH 7 with 0.1 N NaOH and finally diluted with 0.9% NaCl. Corresponding vehicle (1 ml/kg) was injected as a control for PCP or olanzapine.

Assessment of neurotoxicity

Nucleosomal DNA fragmentation is characteristic of apoptotic nuclei (Gavriele et al., 1992; Rabacchi et al., 1994). Cut DNA was assayed using a method referred to as TUNEL (terminal dUTP transferase nick-end labeling) essentially as previously described (Johnson et al., 1998). Deoxynucleotidyl transferase (TdT), a template-independent polymerase, was used to incorporate biotinylated nucleotides at sites of DNA breaks. The signal was amplified by avidin–biotin–peroxidase, enabling conventional histochemical identification by light microscopy. In brief, the brain sections were treated with proteinase K to dissociate proteins from DNA and the sections were

then washed in phosphate-buffered saline (PBS). Endogenous peroxidase was inactivated by covering the sections with H_2O_2. The sections were rinsed with PBS solution and immersed in TdT buffer (30 mM Tris, pH 7.2, 140 mM sodium cacodylate, 1 mM cobalt chloride). The reaction mixture was then replaced by TdT (0.3 U/μl; Boehringer Mannheim, Indianapolis, IN, USA) and biotinylated dUTP (0.2 nM/10 U TdT; Boehringer Mannheim) in TdT buffer, then incubated in a humid atmosphere at 37°C for 60 min. The reaction was terminated by transferring the sections to buffer (300 mM NaCl, 30 mM sodium citrate) for 15 min at room temperature. The sections were rinsed with PBS, covered with 2% bovine serum albumin (BSA) for 10 min at room temperature, and rinsed in PBS solution. The sections were covered with biotin–avidin (1:50 in PBS solution; Vectastain ABC kit; Vector, Burlingame, CA, USA), incubated for 30 min at 37°C, and immersed in 0.05 M Tris–HCl. The reaction product was visualized with 3,3′-diaminobenzidine (Sigma, St. Louis, MO, USA). For negative controls, TdT was omitted from the reaction mixture. (As a positive control, the brain sections were treated with 1 N HCl for 20 min prior to the terminal transferase.) Photographs of the TUNEL-stained sections (1.4 mm^2) from representative regions of the dorsolateral frontal cortex, piriform cortex, anterior cingulate cortex, hippocampus and cerebellum were made and the number of TUNEL-positive cells were counted by three observers, two of whom were blind to the treatment. The mean counts from the three observers were used to calculate the mean and S.E.M. from each brain region from the four animals in the four treatment groups.

Astrocytosis was qualitatively evaluated using light microscopic immunocytochemistry to visualize cells expressing glial fibrillary acidic protein (GFAP). Rabbit polyclonal antibody (Dakopatts, Copenhagen, Denmark) to GFAP was used (1:300 dilution). Sections adjacent to those used in the TUNEL assay were taken and the indirect immunofluorescence technique was used to visualize immunoreactivities. The sections were permeabilized with a solution of PBS/0.5% BSA/0.3% Triton X-100, and incubated with the primary antibody at 4°C overnight. Bound antibodies were revealed with rhodamine-conjugated sheep anti-rabbit IgG (diluted 1:40; Boehringer) secondary antibody (diluted in PBS/0.5% BSA solution). The sections were examined with an Olympus light microscope equipped with epifluorescence.

In situ hybridization

A generic oligonucleotide probe complementary to the mRNA encoding all the splice variants of the NR1 subunit of the NMDA glutamate receptor was selected on the basis of cloned cDNA sequences. The sequence of the probe used for *in situ* hybridization was: 5′-TTCCTCCTCCTCCTCACTGTT-CACCTTGAATCGGCCAAAGGGACT (corresponding to amino acid residues 566–580). It was 3′-end labeled by incubation with [^{35}S]deoxy-ATP (New England Nuclear, Boston, MA, USA) and terminal deoxynucleotidyl transferase (Boehringer) to attain specific activities of about $5–8\times10^8$ c.p.m./μg. The specificity of the probe has been previously described (Monyer et al., 1992).

Coronal sections (10 μm) through the forebrain were cut with a cryostat, rinsed in PBS, fixed in 4% paraformaldehyde, and processed for *in situ* hybridization as described previously (Bartanusz et al., 1993). After an overnight hybridization at 41°C, slides were washed successively in 4×, 1× and 0.1× sodium saline citrate, quickly dehydrated in ethanol (70%) and air-dried. Autoradiography was achieved using Kodak NTB3 emulsion and slides were exposed for 3 weeks at 4°C. Adding an excess amount (50-fold) of the unlabeled probe served as a negative control.

Analysis of the *in situ* hybridization autoradiographs was facilitated by using hematoxylin–eosin-counterstained sections. Quantitation of these autoradiographs was accomplished using a custom-made image analysis system as previously described (Wang et al., 1999). In brief, the density of the cluster of silver grains associated with individual neurons placed within a circle of fixed diameter (20 μm) was measured in the piriform cortex, dorsolateral frontal cortex, anterior striatum, hippocampus and cerebellum. The selection of regions for measurement was done as previously described (Wang et al., 1999). Before each session of measurement, the value of gray threshold was adjusted to highlight only the silver grains from background illumination. Therefore, all images were analyzed with a constant gray value threshold. The area of the circle whose intensity was above threshold was measured, and this was considered to be an indication of labeling density per cell (an index of the levels of mRNA per cell). Background labeling was determined on each section by measuring densities of areas adjacent. These background values were then subtracted from individual cell labeling density. This measurement was used to compare the level of labeling among cells from sections of PCP-treated and control rats processed in the same experiment and exposed simultaneously for the same time. A minimum of seven cells was analyzed in at least eight different sections for each group, with four animals in each.

Electrophoretic mobility shift assay

Nuclear extracts were prepared from dorsal frontal cortex brain tissue according to published methods (Dignam et al., 1983; Osborn et al., 1989) with some modifications. In brief, a 2-mm section corresponding to 4.7–2.7 mm anterior to bregma (Paxinos and Watson, 1986) was cut with the help of an aluminum brain mold. The dorsal region (above +4.5 mm dorsoventral) of this section was homogenized in 3.5 μl of buffer A/mg tissue weight with approximately 15 strokes of a 1-ml manual Wheaton Tenbroeck tissue grinder. Buffer A consisted of 10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 μg/ml antipain, 2 μg/ml chymostatin, 2 μg/ml pepstatin, and 2 μg/ml leupeptin (pH 7.8). The lysate was microcentrifuged (8000 r.p.m., 2 min) to collect nuclei. Nuclear proteins were extracted by suspending the nuclei in extraction buffer B (20 mM HEPES, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 2 μg/ml antipain, 2 μg/ml chymostatin, 2 μg/ml pepstatin, and 2 μg/ml leupeptin; pH 7.9) for 20 min (4°C). The nuclei were subjected to centrifugation and the supernatant was divided into aliquots.

Double-stranded DNA containing the sequence corresponding to the classical nuclear factor-κB (NF-κB) consensus site (5′-AGTTGAGGGGACTTTCCCAGGC-3′, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was end-labeled with [γ-^{32}P]ATP using T4 kinase (Life Technology). Unincorporated nucleotides were removed using two Sephadex G-50 columns (Amersham Pharmacia Biotech). Binding reactions were carried out in Tris–HCl, 60 mM KCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, 2 μg of poly(dI-dC), 15 μg of nuclear extract, and 0.5 ng of ^{32}P-labeled oligonucleotide probe (50 000 c.p.m.). Reactions were incubated for 20 min at room temperature. Binding reactions were subjected to non-denaturing polyacrylamide electrophoresis through 4% gels in a 1×Tris–EDTA buffer system. Gels were dried and subjected to autoradiography.

Western blot analysis

The dorsal frontal cortex was dissected as described above and the section was sonicated in sodium dodecylsulfate (SDS). After centrifugation samples were measured for protein concentration with BCA® Protein Reagent (Pierce, Rockford, IL, USA). Equal amounts of total protein (10–30 μg) were loaded on each lane and run on SDS-polyacrylamide gel with a Tris–glycine running buffer system and then transferred to a polyvinylidene difluoride membrane (0.2 μm) in a Mini Electrotransfer Unit (Bio-Rad, Hercules, CA, USA). The blots were probed with an anti-NMDAR1 (1:300, monoclonal, PharMingen) antibody, an anti-Bcl-X_L (1:1000, polyclonal, Santa Cruz) antibody, anti-Bax (1:1000, polyclonal, Santa Cruz) antibody and/or an anti-actin (1:3000, monoclonal, housekeeping protein, Amersham Pharmacia Biotech). Immunoblot analysis was performed with horseradish peroxidase-conjugated anti-rabbit or anti-

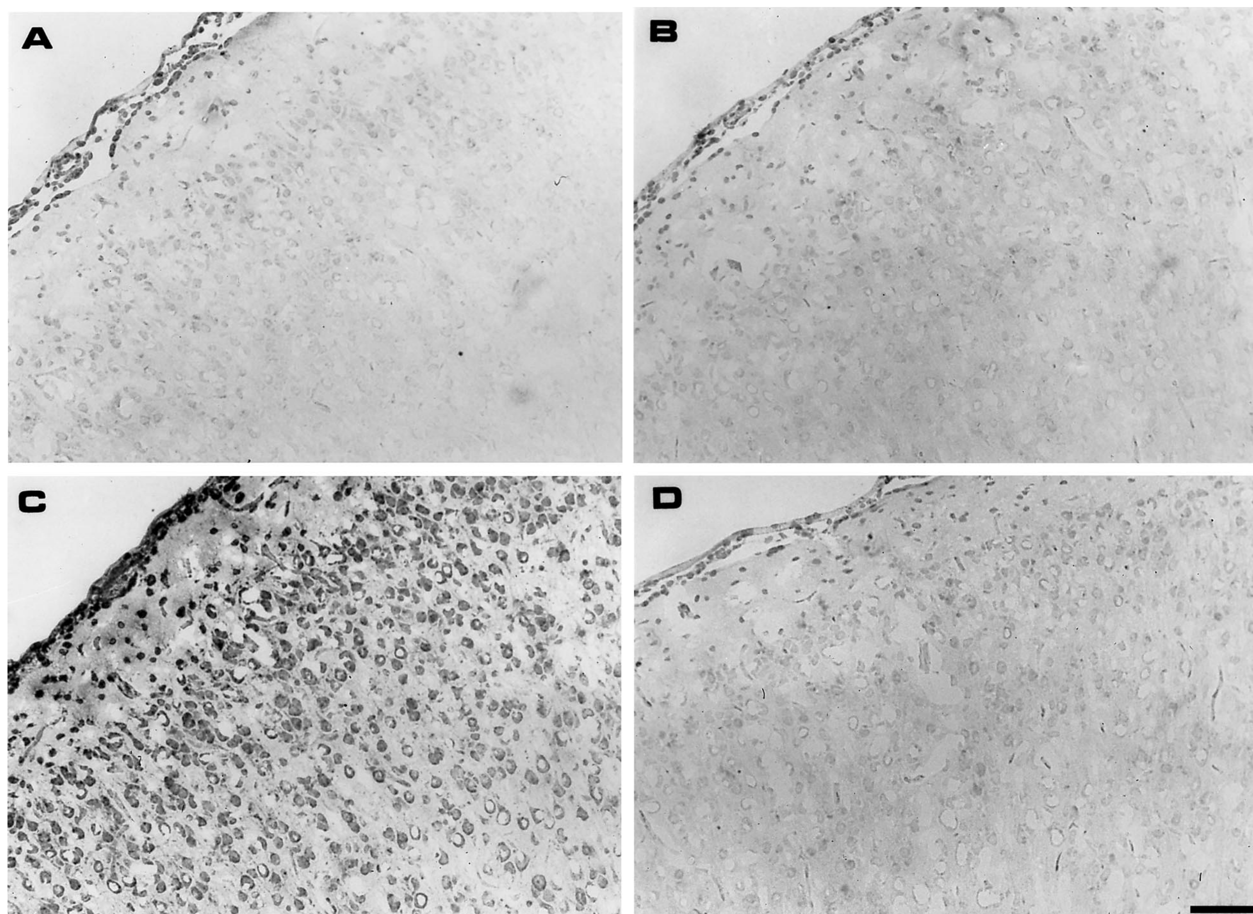

Fig. 1. Effect of PCP and olanzapine alone or as a pretreatment on TUNEL labeling of cells in the frontal cortex. Examples of TUNEL labeling of cells in the frontal cortex from vehicle/saline- (A), olanzapine/saline- (B), vehicle/PCP- (C), or olanzapine/PCP- (D) treated rats are shown. Rats were processed for the TUNEL assay 24 h following the last injection (on PN 12). TUNEL-positive meningeal cells can be seen in all groups. TUNEL-positive cells are remarkably more numerous in the vehicle/PCP (C) group. Scale bar = 100 μm.

mouse IgG using the enhanced chemiluminescence western blotting detection reagents (Amersham). The Bcl-X_L/Bax and NR1/actin ratios were analyzed by the Lynx 5000 imagine analysis system.

Prepulse inhibition

Measurement of PPI of acoustic startle was accomplished essentially according a previously outlined procedure (Pietraszek and Ossowska, 1998). Testing was performed between 9.00 and 15.00 h using two startle chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA) that were placed in a sound-attenuated room with a 60-dB ambient noise level. After an acclimation period of 5 min, each rat was exposed to two stimulus types: a 120-dB 40-ms pulse or a pulse preceded 100 ms earlier by a 20-ms noise burst of 68 dB (prepulse), with a variable inter-trial interval for a total of 40 trials (20 prepulse trials and 20 pulse alone trials). In a later set of experiments, these data were verified and extended in a paradigm that also measured the response to no stimulus (baseline) and to prepulse alone. The percentage PPI was calculated as the percentage of inhibition of the startle amplitude evoked by the pulse alone: [pulse−(prepulse+pulse)/pulse]×100.

Delayed spatial alternation

This task was conducted in a T-maze that was constructed from black Plexiglas (long arm = 50 cm long, choice arms = 40 cm each, all arms = 15 cm wide with 30 cm walls). A between-trial holding box (25×15 cm) was located at the end of the long arm. The terminal end of each choice arm contained a food cup, shielded from sight by a 3 cm high barrier. Two groups of female rats were trained in the maze task. One group was treated with 10 mg/kg PCP and the other group was treated with saline on PN 7, 9, and 11, as described for the other experiments. Following the perinatal injections, the pups remained with the dams until weaning and then were pair-housed with a rat that had received the same treatment. They were weighed and handled at least twice a week and had free access to food and water.

Habituation to the maze began on PN 28 and continued for 5 days until PN 32. During habituation, each rat was placed in the maze with all doors open and allowed to explore for 5 min. Each food cup contained a 45-mg Bio Serv rodent food pellet (Frenchtown, NJ, USA). Between the testing of each rat, the floor of the maze was wiped with an alcohol solution to attenuate olfactory cues. Additionally, the choice points were wiped with alcohol solution between trials. On PN 34, acquisition training began. During acquisition training, rats were mildly food-restricted in order to facilitate food pellet consumption. Each acquisition session consisted of 10 trials with an inter-trial interval of 10 s. On the first trial, the rat was rewarded with a food pellet for entry into either choice arm. After eating the pellet, the rat was returned to the holding box where it was confined until the next trial 10 s later. On the second and subsequent trials, the rat was rewarded with a food pellet for entry into the arm opposite the one that previously contained the food

pellet. The number of correct and incorrect responses was recorded. Acquisition continued daily until PN 49. From PN 50 to PN 70, rats received 10 acquisition sessions. On PN 70, the average number of correct trials over this 10-session period was assessed. Acquisition was defined as an average of 7.5 correct trials per session over a period of 10 consecutive training sessions.

Locomotor activity assessment

Rats were placed in locomotor activity boxes and allowed to habituate for 75 min before receiving a challenge dose of saline or 2 mg/kg PCP (i.p.). Locomotor activity was assessed for an additional 100 min. Locomotor activity was measured via an open-field activity system (San Diego Instruments) comprised of four individual Plexiglas enclosures (40×40×40 cm) consisting of a 4×4 photobeam matrix to measure central and peripheral activity. The number of horizontal (central+peripheral activity) photobeam interruptions was summed over the 100 min following challenge and differences between the four groups were analyzed as described below.

Statistical analysis

The data for all experiments are presented as the mean values ± S.E.M. With the exception of the delayed spatial alternation task, significant differences between treatment groups were assessed by one-way analysis of variance (ANOVA) and an appropriate post-hoc test with the help of SigmaStat Statistical Software. $P < 0.05$ was necessary to reject the null hypothesis. For the purposes of analyzing the delayed spatial alternation data, the mean number of correct trials for each rat over each successive three-session interval between PN 34 and PN 49 for a total of five intervals was calculated. In addition, the mean number of correct trials during the 10-session assessment period

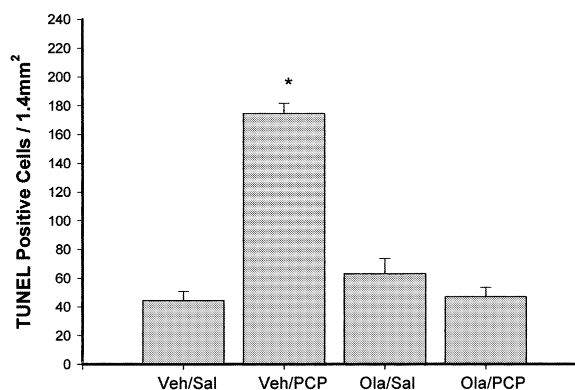

Fig. 2. The number of TUNEL-positive cells per 1.4 mm^2 in the frontal cortex. TUNEL-positive cells are significantly more numerous in the vehicle/PCP group. $*P < 0.05$.

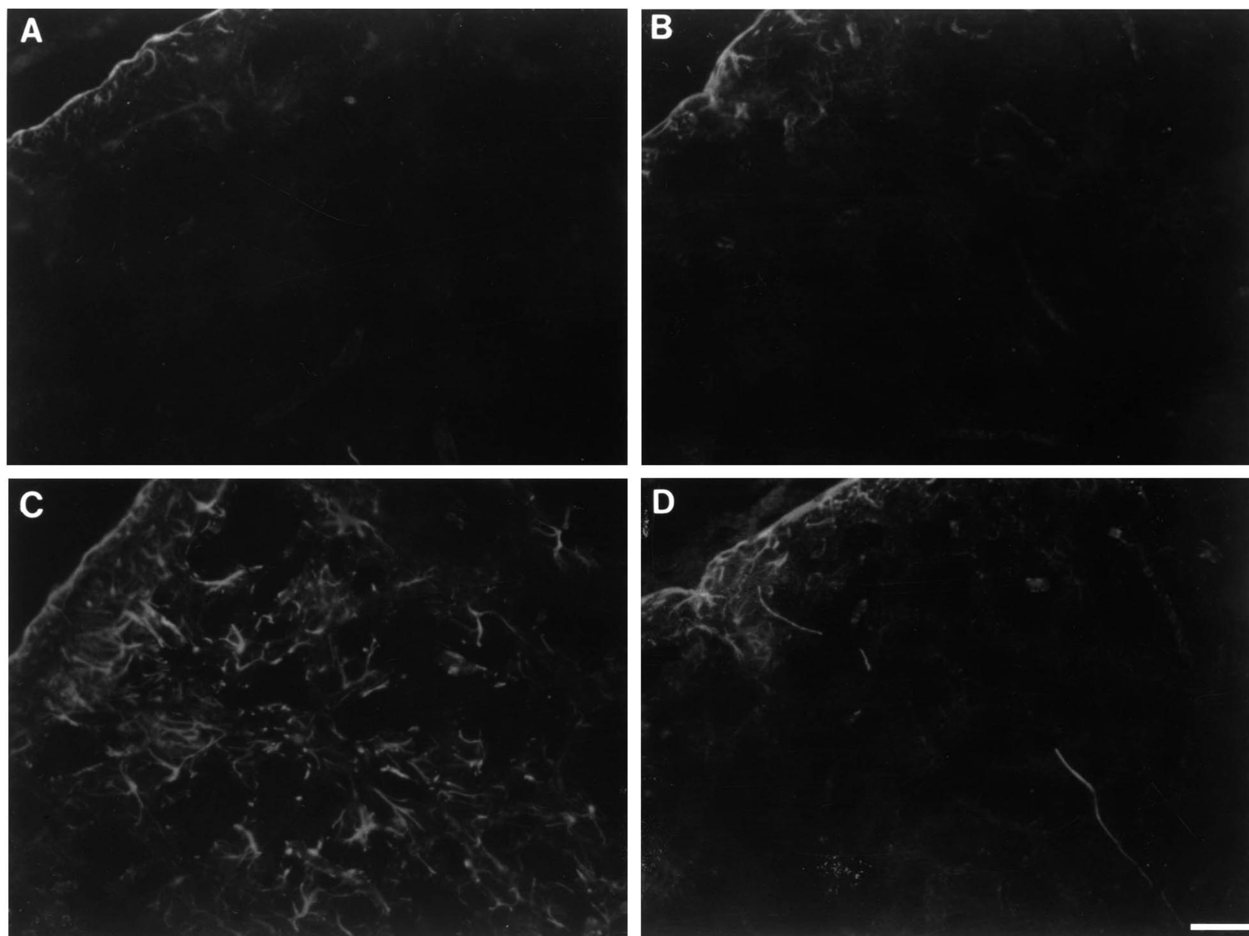

Fig. 3. Effect of PCP and olanzapine alone or as a pretreatment on GFAP immunoreactivity in cells in the frontal cortex. GFAP immunoreactivity in the dorsal frontal cortex of vehicle/saline- (A), olanzapine/saline- (B), vehicle/PCP- (C), and olanzapine/PCP- (D) treated rats is depicted. A dense layer of labeling of astrocytes is seen next to the meningeal cells in all pictures. The density of GFAP-positive astrocytes is dramatically increased by chronic PCP treatment (C). Scale bar = 90 μm.

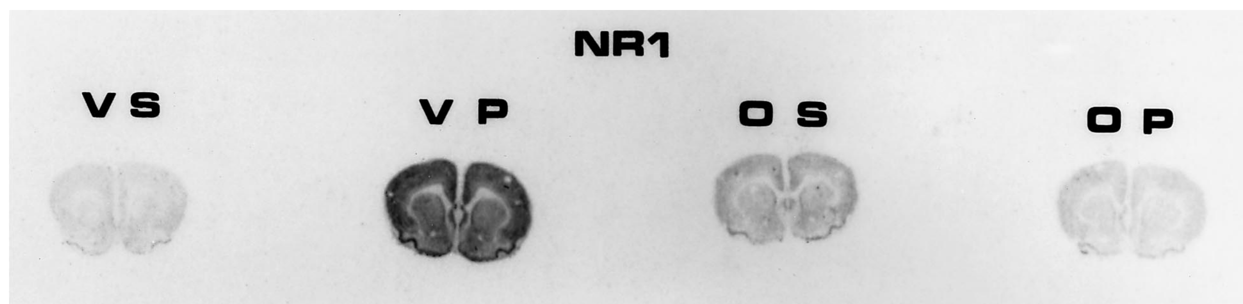

Fig. 4. *In situ* hybridization of NR1 subunit mRNA in rats pretreated with either vehicle (V) or 2 mg/kg olanzapine (O), prior to treatment with either saline (S) or 10 mg/kg PCP (P), on PN 7, 9, 11 (s.c.). The rats were killed on PN 12. The effect of chronic PCP was evident in the frontal cortex, striatum, nucleus accumbens and olfactory cortex, but was not observed in the hippocampus or cerebellum.

(PN 50–70) was calculated, resulting in six acquisition scores for each rat. The means (±S.E.M.) of these scores were calculated separately for each treatment group and were subjected to a split-plot ANOVA with group as the between-subjects factor and session interval as the within-subjects factor. Tukey LSD post-hoc tests ($\alpha = 0.05$) were used to specify differences between the groups.

RESULTS

Effects of PCP on measures of neurotoxicity

On PN 12, sections from several brain areas were processed for TUNEL. No significant increase in the number of TUNEL-positive cells was observed following PCP treatment in the anterior cingulate cortex, hippocampus or cerebellum. However, there was a significant increase following PCP in both the piriform and dorsolateral frontal cortices. Figure 1 shows representative sections through the dorsolateral frontal cortex from each of the four treatment groups. The TUNEL-positive cells seen in the meningeal layer in this region in all four panels serve as a positive control for the TUNEL assay as it is well known that these cells have a normally high rate of apoptosis and renewal. It is evident that PCP-treated rats (panel C) have many darkly stained, TUNEL-positive cells throughout layer II and layer III. A few TUNEL-positive cells can also be seen in layer I, or the molecular layer. Very condensed nuclei and crescent-shaped nuclei indicative of apoptosis can be seen throughout this section. It is obvious that the number of TUNEL-positive cells in the vehicle/PCP-treated rat far exceeds those in either the vehicle/saline- (panel A), olanzapine/saline- (panel B) or olanzapine/PCP- (panel D) treated rats. This observation was verified by counting TUNEL-positive cells in representative sections from each rat (Fig. 2). Here it can be seen that PCP treatment caused an approximate 3.5-fold increase in the number of TUNEL-positive cells relative to vehicle/saline-treated controls (df = 3, $F = 62.8$, $P <$ 0.05). It is also evident that olanzapine pretreatment prevented this increase, while having no significant effect of its own.

Following PCP treatment, the number of cells expressing GFAP immunoreactivity also appeared to be increased in the dorsolateral frontal cortex (Fig. 3). In control as well as in the three treatment groups, GFAP-labeled astrocytes were primarily localized adjacent to the pia mater. However, after PCP treatment, there appeared to be a migration of astrocytes into deeper layers of the cortex that corresponds roughly to the same areas in which PCP-induced apoptosis was observed. Again, olanzapine pretreatment appeared to prevent the effect of PCP treatment, while having no effect of its own.

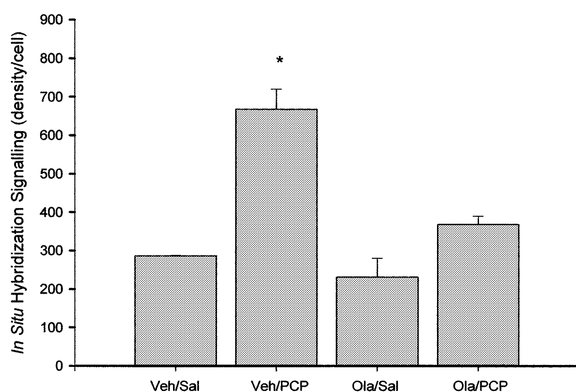

Fig. 5. Quantitation of *in situ* hybridization signal for NR1 mRNA in frontal cortex. These data are taken from two proximal sections from each animal in each group. *$P < 0.05$ vs. Veh/Sal.

Regulation of the NMDA receptor

As we have observed previously in adult rats 3 days

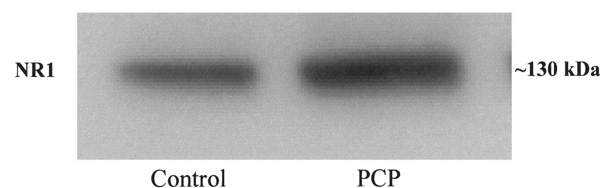

Fig. 6. Representative western blot of NR1 protein taken from the dorsal frontal cortex of rat pups treated with either saline or PCP on PN 7, 9 and 11. Total cytoplasmic protein was extracted and subjected to polyacrylamide gel electrophoresis (30 µg of protein per lane) and then probed with anti-NR1. By comparing to molecular weight standards (Santa Cruz), a band of ~130 kDa was identified as NR1 according to labeling by anti-NR1 antibody.

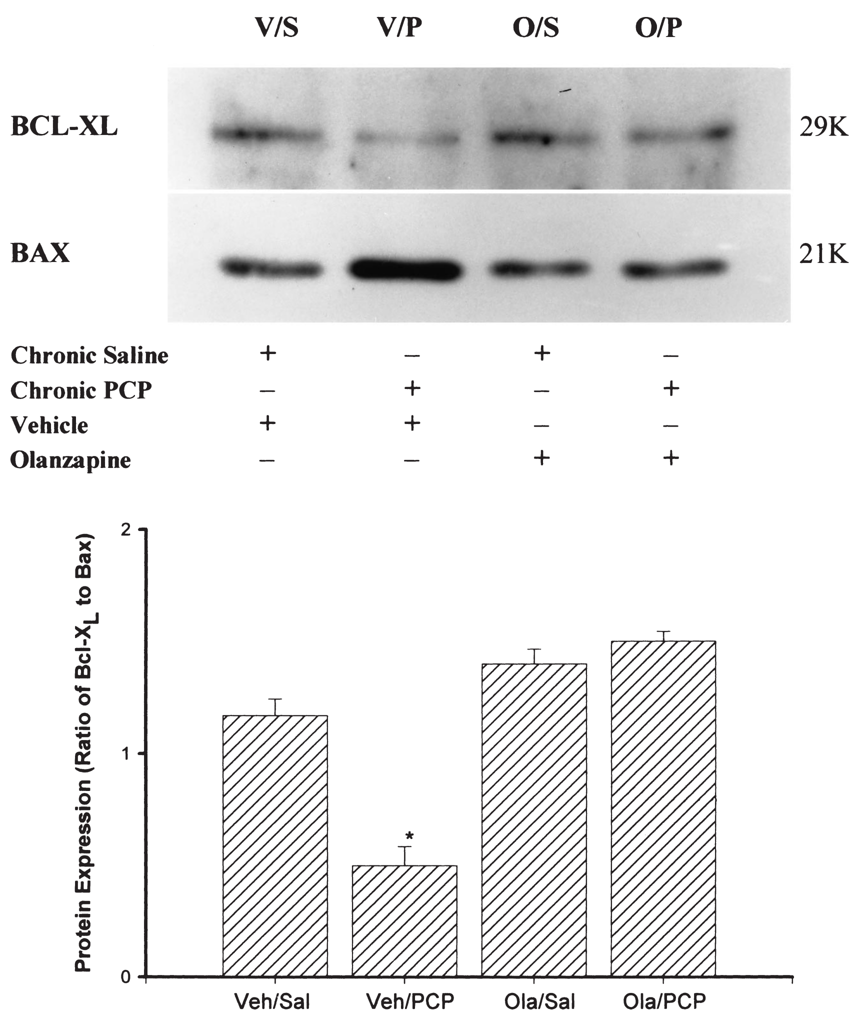

Fig. 7. Western blot analysis of Bcl-X_L and Bax expression in dorsal frontal cortex. Total cytoplasmic protein was extracted and subjected to polyacrylamide gel electrophoresis (15 μg of protein per lane) and then probed with anti-Bcl-X_L (upper) and anti-Bax (lower). By comparing to molecular weight standards (Santa Cruz), a band of ~29 kDa was identified as Bcl-X_L and another band of ~21 kDa was identified as Bax according to their labeling by anti-Bcl-X_L and anti-Bax antibodies, respectively. Below are the densitometry data from three independent experiments. *$P<0.05$ as compared to Veh/Sal.

following five daily doses of PCP (Wang et al., 1999), subchronic treatment of neonates resulted in a marked up-regulation of NR1 mRNA throughout the anterior forebrain (Fig. 4). No such changes were observed in more posterior sections taken through either the hippocampus or cerebellum. Olanzapine had no effect alone, but was able to completely prevent NR1 mRNA up-regulation. Quantitation of this effect in the dorsolateral frontal cortex revealed that PCP treatment more than doubled the level of mRNA (df = 3, $F=26.5$, $P<0.05$, Fig. 5).

To insure that the increase in NR1 mRNA was associated with an increase in NR1 as previously observed in adult rats (Hanania et al., 1999), we performed western analysis of cortical protein extracts taken from saline- and PCP-treated rats on PN12. Figure 6 shows a representative western blot from dorsolateral frontal cortex from one saline- and one PCP-treated rat. Densitometric analysis of NR1 blots (normalized to actin) from four control rats and four PCP-treated rats revealed that PCP significantly increased the expression of NR1 protein in dorsolateral frontal cortex by 46% (0.60 ± 0.06 for controls; 0.88 ± 0.03 for PCP; $P=0.006$).

Regulation of Bax, Bcl-X_L, and NF-κB

Because our previous study of forebrain neurons in primary culture demonstrated that NMDA treatment caused an up-regulation of the pro-apoptotic protein Bax and a decrease in the anti-apoptotic protein Bcl-X_L (Wang et al., 2000a), we again assessed these two proteins in frontal cortex following PCP treatment in this paradigm. PCP caused an increase in Bax and a decrease in Bcl-X_L (Fig. 7). Densitometric quantitation of these two proteins revealed that PCP caused a reduction of the Bcl-X_L/Bax ratio of greater than 50% (df = 3, $F=27.9$, $P<0.05$). Olanzapine alone showed a non-significant trend towards increasing this ratio and was able to completely prevent the PCP-induced decrease.

Our previous *in vitro* study of neuronal cultures also demonstrated that reactive oxygen species (ROS) such as superoxide anion and/or peroxynitrite might play a role

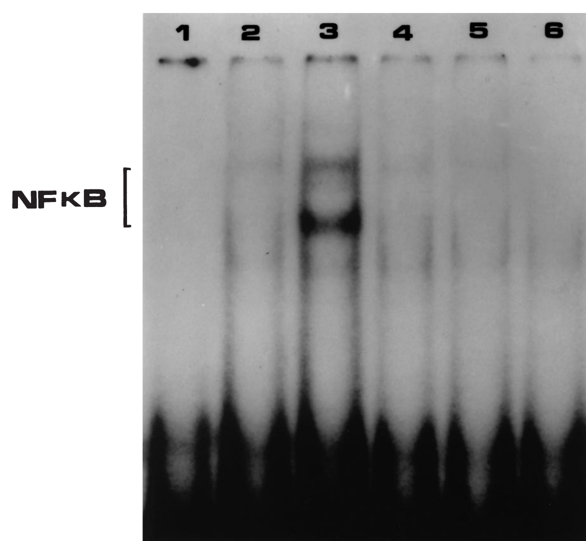

Fig. 8. The effect of perinatal PCP treatment on NF-κB nuclear translocation as assessed by electrophoretic mobility shift assay. Lane 1, hot probe only (NF-κB); lane 2, vehicle/saline; lane 3, vehicle/PCP; lane 4, olanzapine/saline; lane 5, olanzapine/PCP; and lane 6, competition by excess unlabeled probe. These results are representative of the three additional experiments, each carried out with nuclear protein from the frontal cortex of one rat from each of the four treatment groups. In each experiment, olanzapine produced a similar reduction in the apparent PCP-induced nuclear translocation of NF-κB proteins.

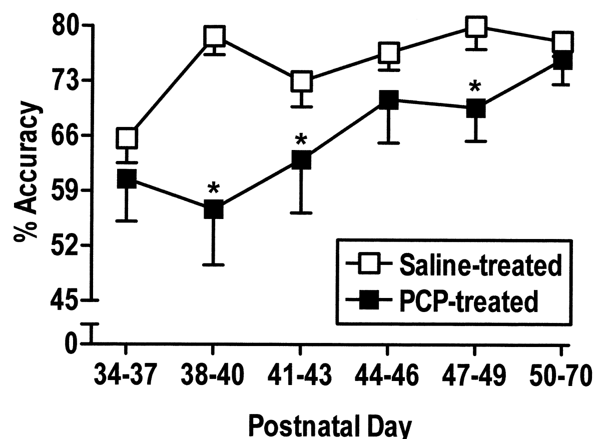

Fig. 9. The effect of perinatal PCP treatment on the acquisition of a delayed spatial alternation task. At the outset of training there was no difference in percent accuracy between the saline- and PCP-treated rats, but the acquisition of this task by the latter group was significantly impaired. $*P < 0.05$.

in mediating the effects of NMDA on Bax and Bcl-X_L expression (Wang et al., 2000a). Therefore, in the current study we examined the effect of PCP treatment of neonatal rats on the nuclear translocation of NF-κB, a transcription factor that is well known to be responsive to the redox status of the cell (O'Neill and Kaltschmidt, 1997; Gius et al., 1999). Nuclear protein extracts from the frontal cortex of control rats (lane 2, Fig. 8) was able to retard the migration of a ^{32}P-labeled oligonucleotide probe corresponding to the NF-κB protein binding domain. Three somewhat faint bands are observable. The binding of only the top two bands is specific in that a 50-fold excess of the unlabeled probe completely inhibited the binding of these proteins, but had no effect on the lower band (lane 6). PCP treatment results in a marked increase in the two proteins with the highest molecular weight (top two bands in lane 3). These bands represent proteins that have affinity for the broad specificity NF-κB binding sequence. Their identity is unknown at this time, but they could be any of the members of the NF-κB family including p65 (relA), p52, p50, p27, c-Rel and RelB. Olanzapine was without effect alone (lane 4), but it was able to prevent the increased nuclear translocation of these NF-κB transcription factors caused by PCP treatment (lane 5).

Enduring behavioral effects of phencyclidine

Cognitive symptoms in schizophrenia include deficits in working memory that may be partially mediated by dysfunction in the prefrontal cortex (Goldman-Rakic and Selemon, 1997). Performance of a delayed spatial alternation task, an animal model that has been used to examine these deficits, is sensitive to disruption by lesions of the prefrontal cortex as well as by dopaminergic manipulations and by administration of high affinity open channel blockers of NMDA receptors (Carter et al., 1995; Feeser and Raskin, 1987; Verma and Moghaddam, 1998). Developmentally, rats begin to be able to perform this task around the time of weaning (Green and Stanton, 1989). The purpose of the present experiment was to measure the speed of acquisition of delayed spatial alternation in rats that were administered PCP perinatally.

Figure 9 shows acquisition of the delayed spatial alternation task. ANOVA revealed a significant interaction ($F(5,90) = 2.6$; $P = 0.03$). Although initial accuracy of the saline- and PCP-treated rats did not differ, accuracy improved faster in the saline-treated group than in the PCP-treated group. Between PN 38 and PN 49, saline-treated rats were significantly more accurate during three of the four three-session intervals. Nevertheless, by PN

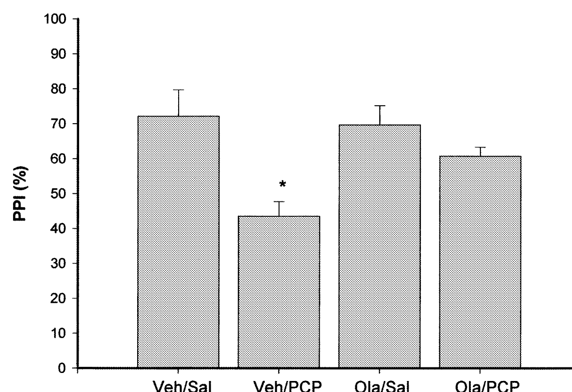

Fig. 10. The effect of olanzapine pretreatment on the inhibition of baseline PPI caused by perinatal PCP treatment. Thirteen to 17 days after chronic PCP administration (PN 24–28), the baseline PPI of acoustic startle was diminished in the vehicle/PCP group compared to the vehicle/saline group. $*P < 0.05$.

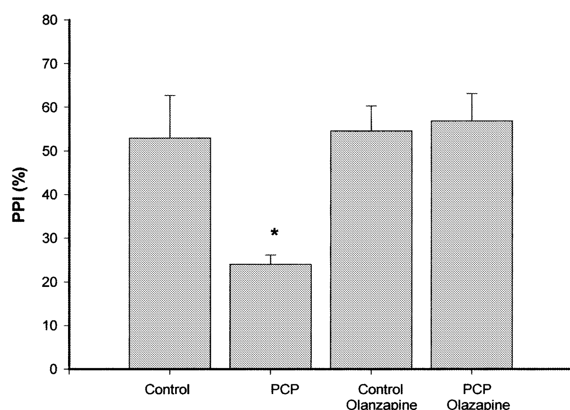

Fig. 11. The effect of olanzapine post-treatment on the inhibition of baseline PPI caused by perinatal PCP treatment on PN 7, 9 and 11. Rats were treated with olanzapine (10 mg/kg) once per day on PN 13–23 and were tested between PN 24 and 26. Baseline PPI of acoustic startle was diminished in the PCP group compared to the control group. $*P<0.05$.

70, both groups had acquired the task with approximately equal accuracies.

Given that the PPI paradigm is thought to model the sensorimotor gating deficits seen in schizophrenia, it was postulated that PCP treatment of neonates would result in a long-lasting deficit in baseline PPI of acoustic startle. Treatment with PCP, olanzapine, and a combination of PCP and olanzapine did not have any effect on startle amplitude ($F(5,31)=0.31$; $P=0.90$). However, as can be seen in Fig. 10, PCP treatment significantly reduced PPI from 72% to 42% (df = 3, $F=6.05$, $P<0.05$). The effect on basal PPI observed here, though quite large, is smaller than that observed previously following acute administration of PCP or other NMDA antagonists (Bakshi et al., 1999). Pretreatment with olanzapine prevented this effect on PPI, but it had no effect alone.

A second experiment was conducted approximately 6 months after those shown in Fig. 10 to determine whether olanzapine treatment following the administration of PCP was able reverse the effect of PCP observed above. In this experiment, several additional controls were run. Here, we observed no effect of any treatment on either startle due to the prepulse alone ($P=0.75$), the pulse alone ($P=0.90$) or under 'no stimulus' conditions ($P=0.55$). Figure 11 shows the results of this experiment which used twice daily olanzapine treatment on PN 12–23 following PCP treatment on PN 7, 9 and 11. PCP treatment significantly reduced the effect of prepulse warning from about 52% inhibition to 23% inhibition. Olanzapine pretreatment blocked this effect of PCP as before (data not shown). Further, olanzapine post-treatment was able to reverse the effect of PCP (df = 5, $F=5.18$, $P=0.002$).

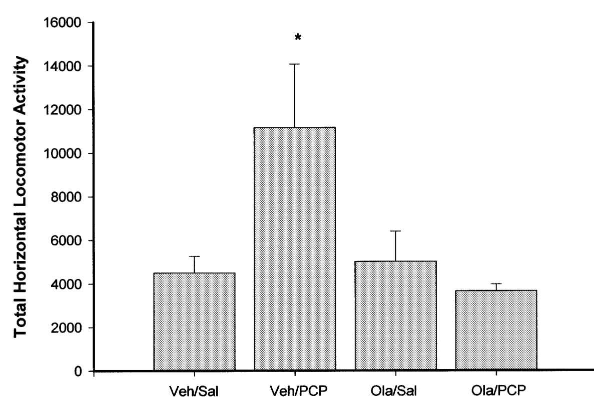

Fig. 12. The effect of perinatal treatment with saline or PCP (10 mg/kg) on total (75 min) horizontal locomotor activity produced by administration of 2 mg/kg PCP on PN 42. The locomotor activity in the vehicle/PCP group was significantly different from the activity of the vehicle/saline (control) group, while neither the olanzapine/saline nor the olanzapine/PCP groups were different from control. $*P<0.05$.

Chronic treatment of adult rats with PCP has previously been demonstrated to result in a sensitized locomotor response to PCP challenge (Hanania et al., 1999; Johnson et al., 1998; Xu and Domino, 1994). Therefore, we proposed that PCP treatment of neonates would also alter the response to PCP challenge. Figure 12 shows that when PN 42 rats were challenged with 2 mg/kg PCP (31 days after subchronic treatment), they exhibited a sensitized locomotor response (approximately 2.5-fold greater than saline-treated controls; df = 3, $F=5.18$, $P<0.05$). As observed in other experiments conducted in this study, olanzapine pretreatment prevented the development of sensitization and had no effect of its own.

DISCUSSION

There are several major findings of this study. First, perinatal PCP exposure results in long-lasting deficits in behavioral measures of sensorimotor gating, cognitive function and locomotor sensitization to acute PCP in adult animals. Second, perinatal PCP treatment results in evidence of enhanced cortical apoptosis including increased TUNEL staining, migration of GFAP-labeled astrocytes, reduced Bcl-X_L/Bax ratio, and increased nuclear translocation of NF-κB. Third, perinatal PCP causes an up-regulation of the obligatory NR1 subunit of the NMDA receptor in the anterior forebrain, including the frontal cortex. Fourth, blockade of these effects by the atypical antipsychotic drug, olanzapine, suggests that this treatment protocol may result in behavioral and biochemical alterations that may model important aspects of the development of schizophrenia. Finally, olanzapine treatment immediately following PCP treatment also was able to reverse the effect of PCP on baseline PPI, suggesting that this drug is able to compensate for the behavioral deficits caused by this treatment paradigm.

PCP-induced neurotoxicity and underlying mechanisms

A recent report showed that perinatal administration of NMDA antagonists including PCP results in the widespread appearance of apparently apoptotic neurons (Ikonomidou et al., 1999). The magnitude of the effect of MK-801 (0.5 mg/kg, t.i.d.) depended on the brain region and the time of treatment, with a peak apoptotic

effect of about 25% of the total number of neurons in certain brain areas on PN 8. Neither the mechanism underlying this effect nor the behavioral consequences of such a treatment were described.

We chose to administer 10 mg/kg PCP once daily on PN 7, 9 and 11 in hopes of producing a significant, but less widespread and milder insult. Ikonomidou et al. (1999) found that 10 mg/kg PCP (t.i.d.) essentially mimicked the effects of 0.5 mg/kg MK-801 (t.i.d.) when given to rat pups 7 days old. Also, pups were most vulnerable to MK-801 in the first week of life, with apoptosis falling off at 14 days and reaching undetectable levels by 21 days. These authors observed apoptosis after their treatment regimen in the hippocampus, dentate gyrus, subiculum, caudate, thalamus, hypothalamus, and in the cingulate, retrosplenial, and parietal cortices in addition to the frontal cortex (Ikonomidou et al., 1999). All of these areas were not examined in the present study, but we observed no evidence of apoptosis in the cingulate or retrosplenial cortices, caudate, nucleus accumbens, CA1, CA3 or dentate gyrus. One complicating factor in this comparison of studies is that the acute neurotoxicity caused by administration of PCP on PN 7 and 9 may have been missed in the present study because we looked for evidence of such only on PN 12, 24 h after the last injection on PN11. It has also been demonstrated that degenerating neurons were evident 8–24 h after the administration of MK-801, but not after 48 h (Ikonomidou et al., 1999). If this is true, then the appearance of TUNEL-positive cells observed here might have been the result of only the administration of PCP on PN 11 and thus an underestimate of the true extent of the neurotoxicity caused by this administration paradigm. This difference could also be related to the administration of three doses of PCP over a 5-day period compared to three doses of MK-801 given in 1 day.

PCP/MK-801 toxicity has been associated with signs of apoptosis as well as necrosis (Fix et al., 1993; Sharp et al., 1991; Zhang et al., 1996). We have previously reported an increase in GFAP-positive cells in the same region as TUNEL-positive cells following treatment of adult rats with chronic PCP (Johnson et al., 1998). In both studies, our interpretation of this observation is that if the magnitude of the apoptotic cell death is sufficiently great, neighboring 'non-professional' cells are inadequate to accomplish the required phagocytosis and removal of cellular debris and that this triggers a reactive astrocytosis. However, it is possible that PCP causes some necrotic cell death in addition to the apoptosis observed here using the TUNEL assay and in the Ikonomidou et al. (1999) study using the DeOlmos cupric silver stain technique.

There could be several mechanisms that underlie the neurotoxic effect of PCP. At the cellular level, one possibility is related to the observation that the survival of NMDA receptor-bearing neurons is regulated tightly by glutamatergic input. Thus, neurons that are deprived of this input for a sustained period may undergo degeneration (Ikonomidou et al., 1999). Another possibility could be related to the observation that acute ketamine administration has been reported to increase glutamate release, resulting in the stimulation of non-NMDA glutamatergic receptors (Moghaddam et al., 1997). If this mechanism were still operative after chronic PCP administration as suggested by others (Arvanov and Wang, 1999), this could well contribute to the observed neurotoxicity and associated behavioral effects. Discounting increased glutamate release, it is also possible that normal glutamatergic transmission during the period when PCP concentration is low is actually enhanced because of the up-regulation of the NR1 receptor subunit. The distribution and magnitude of the up-regulation of NR1 mRNA is essentially identical to what we have observed previously following chronic PCP administration of adult female rats (Johnson et al., 1998). Up-regulation of NR1 protein and NMDA receptor function following chronic PCP administration in male rats (Hanania et al., 1999) is similar in magnitude to the increase in NR1 protein observed here. However, NMDA receptor up-regulation alone is not sufficient to cause degeneration as we have observed in this and previous studies that this receptor subunit is up-regulated in regions such as the caudate that do not show signs of degeneration (Hanania et al., 1999; Johnson et al., 1998; Wang et al., 1999). Thus, in addition to increased function of NMDA receptors, other factors must also be involved. These factors also could involve the stage of synaptogenesis or the subunit composition of the NMDA receptor as suggested by others (Ikonomidou et al., 1999).

Other possible mechanisms are suggested by the observation that olanzapine pretreatment prevented the neurotoxicity. That is, inasmuch as olanzapine is known to block dopamine D_1, D_2, D_3, D_4, serotonin 5-HT_{2A}, 5-HT_{2C}, norepinephrine α_1, histamine H_1, and all muscarinic receptor subtypes at relatively low concentrations ($K_i < 100$ nM) (Bymaster et al., 1996), it is possible that PCP-induced trans-synaptic activation of these receptors plays a role in the observed neurotoxicity. It is possible that PCP-induced activation of dopaminergic systems plays an important role in the neurotoxicity observed here. PCP is known to increase dopamine turnover in the olfactory and frontal cortices, (Moghaddam and Adams, 1998; Rao et al., 1990). Also, dopamine itself is neurotoxic (Luo et al., 1999). Perhaps the combination of high NMDA receptor density and increased extracellular dopamine concentration contributes to PCP-induced apoptosis. Additional studies using receptor-selective antagonists are needed to clarify the receptor and transmitter level mechanisms involved in the neurotoxicity observed in the present study.

Subcellular mechanisms of PCP-induced apoptosis

We have previously observed that NMDA-induced apoptosis of forebrain neurons *in vitro* was accompanied by an increase in the pro-apoptotic protein Bax and a decrease in the anti-apoptotic protein Bcl-X_L (Wang et al., 2000a). Since cultures pretreated with PCP were more sensitive to this effect of NMDA, we reasoned that a similar difference might be observed in the frontal cortex of PCP-treated rat pups. The confirmation of this hypothesis in this study suggests that this difference

could be the direct result of enhanced 'NMDAergic' transmission resulting from an increase in glutamate release (Arvanov and Wang, 1999) and/or an up-regulation of the NMDA receptor in PCP-treated rats (Hanania et al., 1999; Wang et al., 1999). The mechanism by which NMDA regulates Bax and Bcl-X_L is unknown, but the prevention of this effect *in vitro* by the addition of catalase and superoxide dismutase (Wang et al., 2000a) suggests the possible involvement of ROS. The link between the formation of ROS and regulation of either Bax or Bcl-X_L in this system is unknown. The tumor suppressor p53 and the transcription factor NF-κB are known to be sensitive to the redox state of the cell and both are induced by glutamate in primary cultures (Grilli et al., 1996; Uberti et al., 1998, 1999). As such, they are possible candidates for mediating this linkage. The pro-apoptotic tumor suppressor protein p53 is known to up-regulate Bax in several settings (Chao and Korsmeyer, 1998), which has been implicated in several p53-dependent models of apoptotic cell death (Cregan et al., 1999; Xiang et al., 1998). p53 also is known to suppress Bcl-2 (Miyashita et al., 1994), but an effect on Bcl-X_L has not been reported. Thus, there is no obvious link between NMDA-induced alteration in intracellular signals and the down-regulation of Bcl-X_L. In fact, increases in ROS appear to increase NF-κB, which in turn up-regulates Bcl-X_L in several settings (O'Neill and Kaltschmidt, 1997; Tamatani et al., 1999). This may suggest that Bcl-X_L is down-regulated by PCP treatment via a mechanism distinct from the ROS pathway. The impact of a reduction in Bcl-X_L is due to the ability of Bcl-X_L to form heterodimers with Bax. This would prevent Bax from promoting the release of cytochrome *c* from the mitochondria into the cytoplasm. Since the release of cytochrome *c* leads to the activation of caspases, a decrement in Bcl-X_L could then contribute to the ultimate demise of the cell.

If NF-κB proteins are involved in PCP-induced apoptosis, it is possible that PCP treatment would induce nuclear translocation of one or more NF-κB proteins. Electrophoretic mobility shift assays suggested that PCP increased the translocation of two or three NF-κB proteins. Whether the shifted bands represent p50/p50, p50/p65 or other NF-κB dimers is not known. Stimuli that increase ROS and the oxidative stress level are able to increase the translocation of NF-κB to the nucleus where it can act at specific DNA binding sites to affect the transcription of many target genes. However, it is difficult to interpret these data with certainty because of the fact that in many situations, NF-κB can play a protective role (Maggirwar et al., 1998; O'Neill and Kaltschmidt, 1997; Tamatani et al., 1999). One approach to sorting out the action of NF-κB relies on the effect of a peptide inhibitor of translocation, called SN50. In our hands, low concentrations of SN50 (0.3–2.5 μM) protected against NMDA-induced apoptosis *in vitro*, while inhibiting NMDA-induced NF-κB translocation (unpublished observations). It was recently demonstrated that neurotoxicity and NF-κB translocation caused by injection of dopamine into the brain was also prevented by preinjection of SN50 (Luo et al., 1999). This parallel may suggest a possible role for increased extracellular dopamine in PCP-induced neurotoxicity.

Neurotoxicity and olanzapine protection

There was a strong rationale for the use of olanzapine in this study. First, as mentioned previously, olanzapine is active against the positive, negative and cognitive symptoms of schizophrenia (Beasley et al., 1996; Purdon et al., 2000). Second, olanzapine has been shown to block several behavioral effects of NMDA antagonists, including hyperactivity (Gleason and Shannon, 1997), acute PCP-induced neurotoxicity (Farber et al., 1996) and inhibition of PPI by acute PCP (Bakshi et al., 1994). Olanzapine was used at a dose that produces about 85% and 100% occupation of the 5-HT_2 and D_2 receptors *in vivo*, respectively (Zhang and Bymaster, 1999), thereby making this dose reasonable and relevant for study in the rat. Finally, olanzapine is known to have no direct effect on the NMDA receptor (Bymaster et al., 1999), thereby ruling out a trivial explanation for the effects of olanzapine that were observed.

Since olanzapine also blocks the increase in NR1 mRNA, it is possible that the olanzapine-sensitive receptors are involved in the up-regulation mechanism. Currently, little is known about the regulation of NR1 transcription. The NR1 gene is known to be expressed in a neuron-specific manner and to include in its promoter region one GSG motif known to be recognized by a group of immediate-early genes called NGFI-A, B and C (or ERG 1, 2 and 3), one AP-1 site and two SP-1 motifs (Bai and Kusiak, 1993, 1995). Thus, activation of olanzapine-sensitive receptors by PCP administration could play a role in NR1 regulation, but further conjecture is premature at this point. However, it is worth noting that treatment of forebrain cortical cultures with PCP for 48 h resulted in a 47% increase in NR1 message (Wang et al., 2000a). A similar finding has been reported following treatment of cortical cultures with ethanol, another non-competitive NMDA antagonist (Hu et al., 1996). This is of interest because the number of synapses, and therefore the potential role of non-NMDA, olanzapine-sensitive receptors, is much more limited than found *in vivo*. This may suggest that blockade of the NMDA receptor and its downstream elements such as increased intracellular Ca^{2+} alone may be sufficient to trigger increased transcription of the NR1 gene.

The neuroprotective effect of olanzapine could also involve a newly discovered ability of clozapine and olanzapine to block the inhibitory effect of PCP on the NMDA receptor ion channel complex (Arvanov and Wang, 1999; Arvanov et al., 1999; Wang et al., 2000b). This mechanism is incompletely understood, but is thought to involve antagonism of 5-HT_{2A} receptors and a subsequent increase in NMDA receptor phosphorylation by protein kinases (Arvanov and Wang, 1998; Wang et al., 2000b). While this mechanism could play a role in the prevention of PCP's effects, it would seem unlikely to explain the ability of olanzapine post-treatment to reverse the effect of PCP on PPI. However, it has also been shown that therapeutic concentrations of

olanzapine and clozapine can potentiate the function of the NMDA receptor by a mechanism that also involves activation of presynaptic NMDA receptors by phosphorylation and subsequent release of glutamate, activation of postsynaptic α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate and/or kainate receptors, and finally relief of Mg^{2+} blockade of postsynaptic NMDA receptor (Wang et al., 2000b). In this fashion, olanzapine may be able to compensate for the overall hypoglutamatergic state caused by either chronic PCP in this model or that found in schizophrenia. This compensatory increase in 'NMDAergic' function may be sufficient to reverse the deficit caused by PCP-induced cortical neurotoxicity.

Delayed acquisition of spatial alternation task

Acquisition of the delayed spatial alternation task was slower in rats treated with PCP than in those treated with saline. Several issues must be considered in interpretation of these preliminary results. First, although we did not measure running speed in the maze, our unsystematic observation was that the PCP-treated rats were slower at traversing the maze than were saline-treated rats. The effect of this slower running speed was to prolong the length of delay between reaching the choice point in the maze on successive trials. Since length of delay affects accuracy in this task (Carter et al., 1995), motor impairment and its consequences cannot be completely eliminated as explanations for the slowed acquisition of PCP-treated rats. Second, dysfunction of prefrontal cortical regions could also be contributing to the results. The results of other experiments included here demonstrate apoptosis in frontal areas at PN 12 in similarly treated female rats. Previous research has shown that neonatal lesions of the medial prefrontal cortex affect later performance in delayed spatial alternation, although the effect may be on the rate of spontaneous alternation rather than on learning (Freeman and Stanton, 1992). Third, terminal accuracy of the two groups of rats is not significantly different, suggesting that acquisition, although slower, does occur in the PCP-treated rats.

Reduced prepulse inhibition with perinatal PCP

For perinatal treatment with PCP to have some face validity as a model of schizophrenia, it is necessary for the resulting behaviors to have some relevance to the disease. Schizophrenic patients are known to have difficulty in filtering information from their surroundings and have deficits in sensorimotor gating as measured by PPI (Braff et al., 1992). Rats and healthy humans also show deficits in PPI following treatment with NMDA antagonists (Bakshi et al., 1994; Karper et al., 1994; Mansbach and Geyer, 1989). Clozapine and olanzapine have been shown to prevent PCP-induced PPI deficits (Bakshi and Geyer, 1995; Bakshi et al., 1994), but haloperidol does not (Keith et al., 1991). This profile suggests that PPI deficits may be a reasonable model of the negative symptoms of schizophrenia. With this in mind, we postulated that perinatal PCP treatment should produce a deficit in baseline PPI. To our knowledge, the deficit in basal PPI observed in this study is the first reported following chronic drug treatment. The ability of olanzapine pretreatment to prevent the development of this deficit in PPI further supports the hypothesis that perinatal PCP may also model the negative symptoms of schizophrenia. This notion was further supported by another experiment showing that olanzapine not only could prevent the deficit in PPI when administered just prior to PCP, but was also able to prevent the effects of chronic PCP when administered for 12 days beginning 1 day after PCP administration.

While olanzapine could prevent the effects of PCP by either blocking relevant receptors downstream of the primary effect of PCP or simply by preventing the blocking effect of PCP as mentioned above, its ability to reverse the effects of PCP suggests a different mechanism. It is conceivable that chronic olanzapine is able to compensate for the effects of PCP by enhancing pathways 'parallel' to those disrupted by PCP treatment, e.g. by up- or down-regulating various dopamine receptors (See et al., 1996; Tarazi et al., 2001). An interesting possibility is that antipsychotics such as haloperidol, clozapine and olanzapine have been reported to facilitate NMDA function (Arvanov and Wang, 1998; Leveque et al., 2000; Wang et al., 2000b) by phosphorylation mechanisms involving protein kinase A (Leveque et al., 2000), protein kinase C and/or calmodulin-dependent kinase II (Wang et al., 2000b) with the latter most likely involving the 5-HT_{2A} antagonist properties of clozapine and olanzapine (Arvanov and Wang, 1998). Thus, if subchronic PCP produces a hypoglutamatergic state either by simply blocking NMDA receptors or by killing NMDA receptor-bearing glutamatergic neurons as proposed here, then this phosphorylation mechanism could compensate for the loss of 'NMDAergic' tone.

Enhanced locomotor activity

Acute PCP administration to rats leads to increased locomotor activity, ataxia, rearing, stereotypy, and head weaving (Castellani and Adams, 1981). Psychomotor stimulants such as cocaine and amphetamine are well known to increase locomotor activity and stereotypic behavior in rodents. At high doses, these drugs mimic certain positive signs of schizophrenia in humans. Therefore, although there are false positives such as nicotine, locomotor activation in rodents is postulated to predict the capacity of a drug to induce psychoses in man (Wolf, 1998) and PCP-induced increases in locomotor activity are believed to be related to the positive clinical manifestations of schizophrenia (Adams and Moghaddam, 1998; Steinpreis et al., 1994). Repeated administration of PCP in rats causes enhanced locomotor activation upon PCP challenge (Johnson et al., 1998; Kitaichi et al., 1995; Xu and Domino, 1994). The resulting behavioral sensitization to chronic administration of PCP was blunted by sulpiride, haloperidol, olanzapine or risperidone pretreatment (Johnson et al., 1998; Kitaichi et al., 1995; Phillips et al., 2001). Antagonism of sensitization by these antipsychotic agents lends validity to PCP sen-

sitization as an animal model of schizophrenia. The current data demonstrating that olanzapine pretreatment prevents the sensitizing effect of perinatal PCP suggest that this paradigm may also be valid. Although there are no specific pharmacological antagonism data available in this model, the well-known role of dopamine D_2-like receptors in locomotor sensitization suggests that this may be a better model of positive symptoms of schizophrenia than of negative symptoms.

Deficiencies in perinatal PCP treatment as a model of schizophrenia

The relevance of PCP-induced apoptosis as a model of schizophrenia at this time is not completely clear. As recently reviewed by Lewis (1997), there are several reports indicating that schizophrenia is associated with a decrease in cortical volume, an increase in cell packing density and a decrease in the neuropil and the number of synapses in certain regions including the dorsolateral frontal cortex. There is also evidence of a decrease in the number of a small subpopulation of prefrontal cortical neurons (Benes et al., 1991), but in general, there is no evidence that there is a major loss of cortical neurons in schizophrenia (Lewis, 1997). Currently, there is no evidence that apoptosis plays a role in schizophrenia. However, it should be pointed out that apoptosis occurs normally during development, but because of phagocytosis of cell remnants by neighboring cells, there is no trace of evidence that this has occurred in the adult brain. Thus, the possibility that apoptosis plays a role in schizophrenia, perhaps as a mechanism in aberrant development, cannot be ruled out.

Further, it could be argued that since perinatal PCP treatment does not produce deficits in the anterior cingulate and hippocampus, this paradigm does not adequately model schizophrenia. Although no deficits in these regions were observed 24 h after treatment cessation, it is possible that examination of the brain at either earlier or later times might have revealed such changes. As mentioned above, this is possible because of the very efficient manner in which phagocytosis by neighboring cells remove evidence of apoptosis, a program which can be executed in a period of only 12 h. When adult brains were examined 72 h following chronic PCP treatment, TUNEL-positive cells were found only in the olfactory tubercles and piriform cortex (Johnson et al., 1998). However, when examined after 24 h, TUNEL-positive cells were also found in the anterior cingulate and dorsolateral frontal cortex (unpublished observations). Therefore, in order to determine the full extent of apoptosis in this model it will be necessary to examine brains approximately every 12 h during the course of treatment and for several days afterwards.

To date there are no animal models that completely mimic all aspects of schizophrenia. Acute PCP as a schizophrenia model has shortcomings because the neuroanatomical and imaging evidence supporting the developmental or neurodegenerative nature of schizophrenia and the chronic nature of the disease are not represented. It also requires the presence of the drug. The use of subchronic PCP administration as a model also can be challenged on many bases. However, it is our impression that there is much that can be learned from this model. For example, although the mechanisms underlying cortical dysfunction in schizophrenia and the PCP-induced behavioral deficits observed with this model are of different origin, the similarities in the overall functional deficits may be of value in designing novel pharmacological approaches that are targeted at increasing cortical function. In addition, this model could be of value in the study of cortical and subcortical plasticity following the partial loss of neurons in the dorsolateral and olfactory cortices at a critical period of development.

Summary

We have presented evidence showing that perinatal PCP treatment results in long-lasting alterations in acquisition of a delayed spatial learning task, sensorimotor gating and locomotor activity that may be related to behavioral changes observed in schizophrenia. Evidence is also presented suggesting that these behavioral disturbances could be related to the loss of neurons in the cortex. The loss of cortical glutamatergic neurons that regulate subcortical dopaminergic input to the cortex could account for the ability of this model to mimic the hypoglutamatergic state thought to be critical in schizophrenia. Finally, results suggest that neurotoxicity in this model could be the consequence of up-regulation of the NR1 subunit of the NMDA receptor, which then could lead to the altered regulation of Bax and Bcl-X_L, two proteins known to play a critical role in the regulation of apoptotic cell death. In all, it appears that perinatal treatment of rats with PCP results in a model that may be relevant to schizophrenia. Further investigation is essential to determine the molecular, cellular and systems mechanisms involved in PCP-induced behavioral alterations. This knowledge could result in the development of novel approaches for the treatment and perhaps prevention of schizophrenia in high-risk individuals.

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