

Neonatal Exposure to the Glutamate Receptor Antagonist MK-801: Effects on Locomotor Activity and Pre-pulse Inhibition Before and After Sexual Maturity in Rats

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Neonatal lesions of the ventral hippocampus in rats lead to post- but not pre-pubertal behavioural changes suggesting adolescent onset of dopaminergic hypersensitivity and providing an animal model of schizophrenia. Neonatal exposure to glutamate receptor antagonists produces accelerated apoptosis leading to neuronal loss in central nervous system structures including the hippocampus. This suggested that neonatal MK-801 might lead to behavioural changes like those reported following ventral hippocampal lesions. Thus, rats received MK-801 (0, 0.5, 1.0 mg/kg, i.p.) on post-natal day 3 (P3) and were tested pre- (P35) and post-pubertally (P56). MK-801 produced an increase in TUNEL staining in the hippocampus and other forebrain structures, confirming the induction of apoptosis. Results showed little difference in locomotor activity between neonatal saline- and MK-801-treated groups during habituation or following saline injection but increased activity was seen in the 0.5 mg/kg MK-801 group following amphetamine (1.5 mg/kg, i.p.) at P35 but not P56. In tests of pre-pulse inhibition (PPI), neonatal saline and MK-801 groups showed stable startle amplitudes, minimal responding to the pre-pulse stimuli alone, an increase in PPI with increases in pre-pulse intensity, and reduced PPI with apomorphine (0.1 mg/kg, s.c.). At P56, neonatal MK-801 groups tested following vehicle showed less sensitivity to changes in pre-pulse intensity. It was concluded that neonatal MK-801 increases apoptotic cell loss in the hippocampus but does not produce behavioural effects like those seen after neonatal ventral hippocampal lesions. However, neonatal MK-801 did lead to increases in locomotor activity in juveniles but not adults and reduced sensitivity to pre-pulse intensity in PPI tests in adulthood.

Keywords: Amphetamine; Apomorphine; Glutamate; Hippocampus; Locomotor activity; MK-801; Neurodevelopment; NMDA; Pre-pulse inhibition

In recent years it has been proposed that schizophrenia is a neurodevelopmental disorder characterized by a second trimester insult that results in abnormal development of the frontal cortex at the time of sexual maturation and subsequent dysregulation of the dopamine system in response to stress (Weinberger, 1995). Evidence for an early insult includes the observation of minor physical abnormalities and brain morphological abnormalities associated with schizophrenia (Csernansky and Bardgett, 1998; Raedler *et al.*, 1998), premorbid behavioural abnormalities in people subsequently developing schizophrenia (Jones and Done, 1997) and epidemiological reports of a weak but often significant association between maternal illness (e.g. influenza) during the second trimester and the development of schizophrenia in the offspring (review: Weinberger, 1995). The nature of the insult precipitating putative neurodevelopmental impairments remains unspecified.

Further support for the neurodevelopmental hypothesis of schizophrenia has come from the development of animal models using rats. It is noteworthy that the first post-natal week of neurodevelopment of the rat cortex corresponds to the second trimester of gestation in primates (Bayer, 1980). One model is based on the effects of neonatal [post-natal day 7 (P7)] ventral hippocampal lesions on behaviour and molecular changes in the prefrontal cortex after sexual maturity (Lipska and Weinberger, 2000). Observations include enhanced locomotor activity and responses to amphetamine (Lipska *et al.*, 1993)

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and impairments of sensory-motor gating assessed using the pre-pulse inhibition (PPI) test and its disruption by apomorphine (Lipska *et al.*, 1995) after sexual maturation but not before. Similar enhancements in responses to amphetamine (Segal and Janowsky, 1978) and PPI impairments are seen in schizophrenic patients (Braff *et al.*, 1978; Braff *et al.*, 1992; Bolino, 1994). A related animal model involves early post-natal isolation and leads to many similar deficits to those seen following P7 ventral hippocampal lesions (Powell and Geyer, 2002). In recent years, these models have become widely accepted as useful tools for studying possible mechanisms of schizophrenia (Hanlon and Sutherland, 2000; Kato *et al.*, 2000; Powell and Geyer, 2002).

A recent finding concerning glutamatergic function may be relevant to neurodevelopmental mechanism underlying schizophrenia. Olney and his co-workers (Ikonomidou *et al.*, 1999; Pohl *et al.*, 1999) found that perinatal transient blockade of glutamatergic N-methyl-D-aspartate (NMDA) receptors leads to accelerated apoptotic cell death. The specific brain regions affected maximally by this blockade depend on the precise time of administration of the NMDA receptor antagonist during perinatal development. Thus, NMDA antagonists given at birth (P0) resulted in increased apoptotic cell death in some diencephalic areas and in the dentate and CA1 regions of the hippocampus but not in other regions of the cortex; affected regions were showing the greatest rate of spontaneous apoptotic cell death at the time of injection. NMDA antagonist injection into rats at P7 resulted in maximally accelerated apoptotic cell death in cortical (cingulate, parietal, frontal) and dorsolateral thalamic regions; these regions showed the highest rate of spontaneous apoptotic cell death at P7 whereas this rate had declined sharply in other diencephalic regions. As NMDA receptors are involved in cell migration, neurite extension, branching and dendritic stabilization, their blockade may provide a mechanism for the putative neurodevelopmental insult that causes schizophrenia. Although Ikonomidou *et al.* (1999) did not specifically refer to schizophrenia, they suggested that the neonatal blockade of NMDA receptors may give rise to patterns of neuronal loss that contribute to a variety of neuropsychiatric illnesses. However, at present, it is not known whether acute exposure to an NMDA receptor antagonist around the time of birth indeed produces behavioural or neurochemical changes in adults like those produced by neonatal ventral hippocampal lesions.

The present investigation examined whether neonatal injections (P3) of the NMDA receptor antagonist MK-801 produce post-pubertal behaviours similar to those seen in schizophrenia. Behavioural tests included locomotor activity during habituation, following saline and following

amphetamine according to the protocol of Lipska *et al.* (1993) and PPI after injection of vehicle or apomorphine. It was hypothesized that neonatal MK-801 injections will lead to behavioural changes like those seen in neonatal ventral hippocampal lesion rats. Although neonatal exposure to MK-801 led to accelerated apoptosis in hippocampal and other telencephalic tissue and to behavioural changes at P35 or P56, the pattern of results was not like that seen in neonatal ventral hippocampal lesion rats.

METHODS

The Queen's University Animal Care Committee approved these experiments. All animals were treated in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University Policies.

Animals

Timed pregnant Sprague-Dawley rats (Charles River, St. Constant, Quebec) arrived about 1 week prior to giving birth and were housed individually on cedar bedding in transparent plastic cages. The cages were kept in a temperature-controlled room ($20 \pm 1^\circ\text{C}$) with a 12-h light/dark cycle with lights on at 07:15. At the time of weaning (approximately P25), the pups were housed in same-sex pairs in similar caging on a 12-h reverse light/dark cycle with lights on at 19:00. From this time until the beginning of behavioural testing at P35 they were handled each day for approximately 5 min. Food and water were available *ad libitum* in the home cages.

Apparatus

Activity Monitors

Activity was measured as the number of breaks across 14 pairs of photocells positioned at a height of 5.0 (lower activity) and 15.0 cm (upper activity) above the metal rod floor in each of six experimental chambers ($50 \times 40 \times 40$ cm high) constructed from Plexiglas and housed in wooden, Styrofoam-insulated outer boxes. Each chamber was illuminated with a 2.5 W bulb and ventilated by a small fan that also provided background noise. Beam breaks were recorded on an experimenter-controlled circuit board connected to a Macintosh computer. For further details of the apparatus see Beninger *et al.* (1985).

Pre-pulse Inhibition

PPI was measured in a translucent plastic cage ($14 \times 16 \times 12$ cm high) housed in a wooden, Styrofoam-insulated outer box that was illuminated with

a 7.0 W bulb. The cage was placed on a horizontally oriented U-shaped thin aluminium platform outfitted with a pizoceramic disk at the base of the U that detected movement. The signal from the disk was sent to an amplifier circuit and then integrated, producing a voltage level output that was digitised at 1.0 ms intervals and averaged over 500 ms to provide a measure of startle amplitude, timed from the offset of the startle stimulus. The background sound pressure level was approximately 40 dB. The startle stimulus, broad-band noise in the range of 40–20,000 Hz for a duration of 20 ms, was set at 100 dB. Pre-pulse stimuli with the same physical characteristics were set at 50, 55, 60, and 65 dB, making them 10, 15, 20 and 25 dB over background. Timing, stimulus presentation and recording of startle amplitude was carried out automatically with the use of a microcontroller (Walter and Palya, 1988) connected to a 486-based computer.

Drugs

MK-801 (dizocilpine; [5R-10S]-[+]5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate salt), purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON), and dextroamphetamine sulphate (USP, Rockville, MD) were dissolved in saline. Apomorphine hydrochloride (Sigma–Aldrich Canada Ltd., Oakville, ON) was dissolved in distilled water with ascorbic acid (1.0 mg/ml).

Procedure

Neonatal Treatments

On P3, pups were injected with MK-801 (0.05 or 1.0 mg/kg, i.p.) or saline and then returned to their mothers. Separate litters were used for each dose and each drug dose group was compared to littermates that received saline. The weights of lesion and control rats were monitored throughout the testing period and did not differ significantly (data not shown).

Activity

All animals were tested twice, once around P35 and again around P56. For the 0.5 mg/kg MK-801 experiment, activity tests took place on P35–P37 and P56–P58. For the 1.0 mg/kg MK-801 experiment, activity tests took place on P35 and P62, before and after sexual maturity, respectively. For convenience, the test days will be referred to as P35 and P56 throughout the manuscript.

The procedure used for the assessment of activity followed that employed by Lipska *et al.* (1993). Lower and upper activity counts were recorded every 10 min. Activity was measured over 3.5 h using a protocol with three distinct phases. The habituation

phase had a duration of 1 h. Rats were then removed from the chamber, injected with saline (1.0 ml/kg, i.p.) and returned to the chamber for another 1-h session. Rats were again removed from the chamber, injected with amphetamine (1.5 mg/kg, i.p.) and tested for an additional 90 min.

Pre-pulse Inhibition

Rats in the 0.5 mg/kg experiment were tested once for PPI on P58–P61. Rats in the 1.0 mg/kg experiment were tested four times for PPI, twice on P29–P35 and twice on P54–P60, once each time immediately following injection of vehicle (1.0 ml/kg, s.c.) and once each time immediately following injection of apomorphine (0.1 mg/kg, s.c.). At each post-natal testing time, half of the rats were tested first with vehicle and then with apomorphine and vice versa for the other half. For convenience, the test days will be referred to as P35 and P56 throughout the manuscript.

During PPI testing the intertrial interval was a minimum of 30 s. Prior to the programmed onset of each trial, movement was monitored for 500 ms. If a startle amplitude of 10 or greater units was detected, the trial was aborted for 500 ms and then another pre-trial monitoring period began. Each PPI session began and ended with three presentations of the startle stimulus alone. Between these presentations, rats received 50 trials in 5 blocks of 10. Each 10-trial block consisted of the following: no stimulus, 10, 15, 20 and 25 dB over background pre-pulse alone, startle stimulus alone, and 10, 15, 20 and 25 dB over background pre-pulse plus startle stimulus. For these latter trials, the startle stimulus followed the pre-pulse stimulus after 90 ms (timed from the offset of the pre-pulse).

TUNEL Staining

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) staining (Gavrieli *et al.*, 1992) was carried out to detect degenerating cells in five rats at P4, 24 h following injection of saline or 0.5 mg/kg MK-801 on P3. When apoptotic cell death is occurring, DNA undergoes internucleosomal cleavage into fragments that can be detected by tissue staining. Rats were decapitated and their brains rapidly fixed in isopentane–liquid N₂ at –40°C. Brains were sectioned at 40 µm using a freezing micortome and stained using an ApoTag Peroxidase kit (InterGen Co., New York, NY). Sections were mounted, stained and examined by light microscopy.

Data Analyses

For each dose of neonatal MK-801, lower and upper activity were analysed separately. For each type of activity, habituation, saline and amphetamine scores

(counts/10 min) were analysed separately with 3-variable mixed design analyses of variance (ANOVA). Variables analysed were time, age at the time of testing and group. For PPI studies, 2-, 3- or 4-way mixed design ANOVA were used to analyse startle amplitude to the startle stimulus, startle amplitude to the pre-pulse stimuli and per cent PPI separately for each MK-801-dose group. Per cent PPI was calculated by expressing startle responses during each of the pre-pulse-plus-startle-stimulus conditions as a proportion of the mean startle response to the startle stimulus alone; this proportion was subtracted from 1.0 and multiplied by 100 to provide percent PPI. Where appropriate, ANOVA were followed by tests of simple main effects and pair-wise comparisons.

RESULTS

TUNEL Staining

A marked increase in TUNEL-positive cells was observed following neonatal MK-801 0.5 mg/kg treatment compared to neonatal saline-treated controls. While this increase was most noticeable in the striatum (Fig. 1A), there was also an increase in TUNEL-positive nuclei in the hippocampus (Fig. 1B). Results from rats treated neonatally with 1.0 mg/kg were similar (not shown).

Locomotor Activity: Neonatal MK-801 0.5 mg/kg Experiment

Lower Activity

Activity levels (counts/10 min) on the lower set of detectors during habituation, following saline and following amphetamine injection for rats treated neonatally with 0.5 mg/kg MK-801 ($n = 15$) or saline ($n = 10$) on P35 and P56 are presented in Fig. 2 (left panel). Both groups showed a gradual decrease in activity on both post-natal days during habituation, time effect $F(5, 105) = 78.01$, $p < 0.001$, and following saline injection, time effect $F(5, 105) = 17.80$, $p < 0.001$. Overall levels of activity were higher on P56 than on P35, post-natal day effect for habituation $F(1, 21) = 31.78$, $p < 0.001$; post-natal day effect for saline $F(1, 21) = 42.65$, $p < 0.001$; post-natal day effect for amphetamine $F(1, 21) = 34.50$, $p < 0.001$. Groups did not differ in habituation or after saline. Amphetamine stimulated activity in both groups but at P35 the stimulant effect was greater in the group treated neonatally with MK-801. In the ANOVA for the amphetamine data, these latter differences yielded a significant post-natal day \times group interaction, $F(1, 21) = 8.70$, $p < 0.01$. Subsequent separate analyses of groups at P35 and P56 showed that the neonatal MK-801 group was significantly more active following amphetamine at P35, $F(1, 23) = 4.15$, $p = 0.05$, but

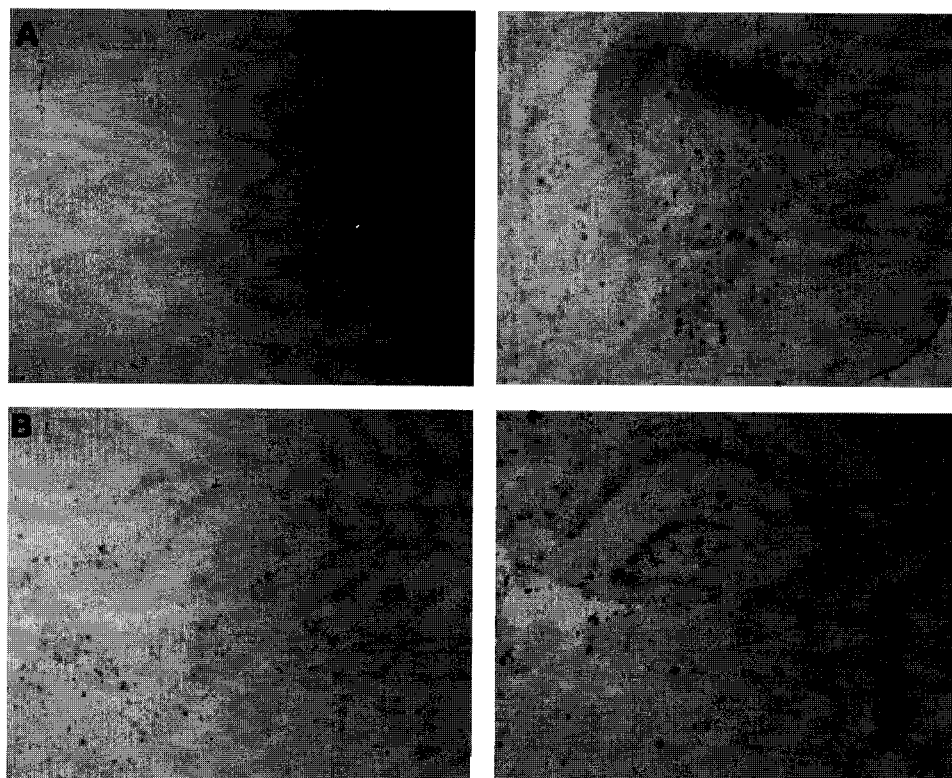


FIGURE 1 TUNEL staining in the striatum (A) and hippocampus (B) of rats treated neonatally with saline (left panels) or 0.5 mg/kg MK-801 (right panels). Drug-treated rats showed a large increase in staining in both structures.

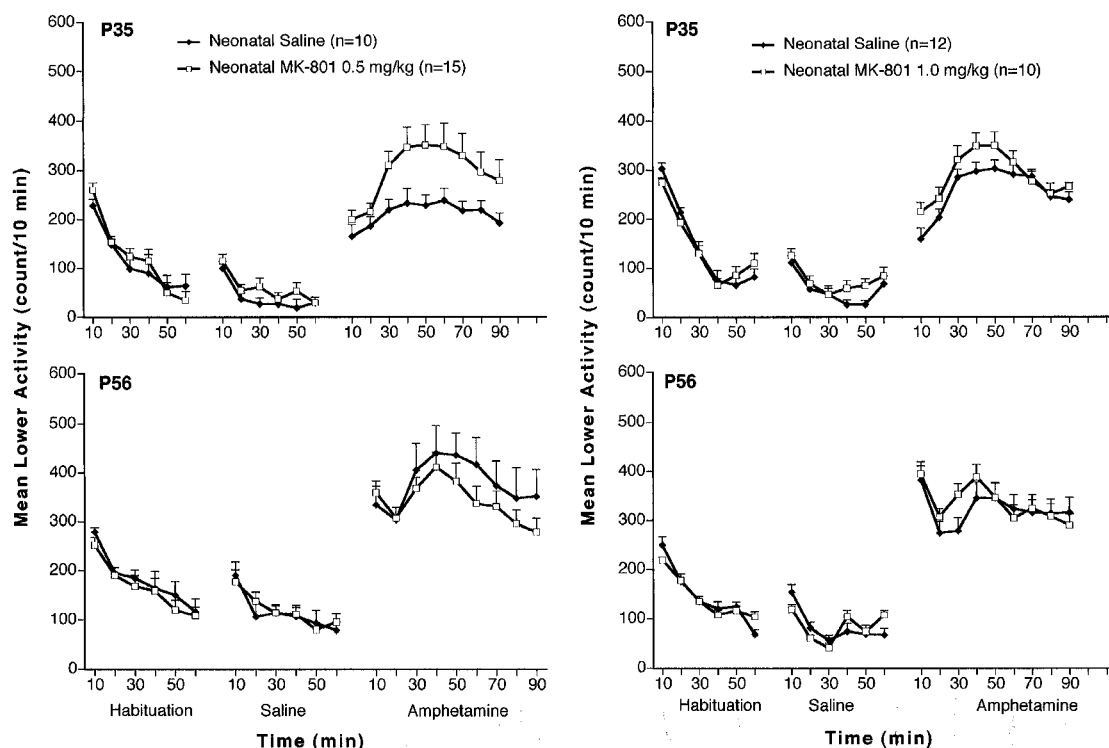


FIGURE 2 Mean (\pm SEM) lower activity (counts/10 min) during habituation and following saline and D-amphetamine (1.5 mg/kg, i.p.) injections in rats treated neonatally with MK-801 (0.5 mg/kg, left panel; 1.0 mg/kg, right panel) or saline on post-natal day 3 (P3) and tested on P35 and P56. Activity was significantly ($p < 0.05$) higher in the neonatal MK-801 0.5 mg/kg group at P35 following amphetamine.

not at P56, $F(1,23) < 1.0$, $p \geq 0.10$, revealing the source of the interaction.

Upper Activity

Activity levels (counts/10 min) on the upper set of detectors during habituation, following saline and following amphetamine injection for rats treated neonatally with 0.5 mg/kg MK-801 ($n = 15$) or saline ($n = 10$) on P35 and P56 are shown in Fig. 3 (left panel). As was the case for lower activity, both groups showed a gradual decrease in activity on both post-natal days during habituation, time effect $F(5,105) = 30.87$, $p < 0.001$, and following saline injection, time effect $F(5,105) = 2.80$, $p < 0.05$ and upper activity overall was higher at P56 than at P35, post-natal day effect for habituation $F(1,21) = 76.86$, $p < 0.001$; post-natal day effect for saline $F(1,21) = 24.49$, $p < 0.001$; post-natal day effect for amphetamine $F(1,21) = 10.74$, $p < 0.01$. Amphetamine stimulated activity in both groups and the stimulant effect of amphetamine was greater in the group neonatally treated with MK-801 when tested at P35 but not when tested at P56. In the ANOVA of the amphetamine data, the 3-way interaction of time, post-natal day and group attained a p -value of 0.055: $F(8,168) = 1.95$, $p = 0.055$. This result suggested that group differences depended on both time and post-natal day (Fig. 3). Subsequent analyses of groups and

time at each post-natal day revealed that group differences neared significance at P35, $F(1,23) = 3.87$, $p = 0.06$, but not at P56, $F(1,23) < 1.0$, $p \geq 0.10$. Results suggest a greater response to amphetamine in the group treated neonatally with MK-801 when tested at P35 but not when tested at P56.

The ANOVA for the habituation sessions also yielded a significant post-natal day \times group interaction, $F(5,105) = 2.59$, $p < 0.05$. Subsequent two-way ANOVA analysing time and group effects at each of P35 and P56 yielded a significant group effect at P56 only, $F(1,23) = 4.25$, $p = 0.05$. Thus, the rats treated neonatally with MK-801 were more active than the neonatal saline controls during habituation at P56 but not at P35.

Summary

In groups treated neonatally with 0.5 mg/kg MK-801 or saline, lower and upper activity showed a gradual decline during habituation and following saline treatment and an increase following amphetamine. Activity levels were higher at P56 than at P35. Upper activity levels during habituation were higher in the group treated neonatally with MK-801 than in the neonatal saline group at P56 but not at P35 and not in the saline session. Amphetamine produced a significantly greater increase in lower activity and a near-significantly greater increase in upper activity

TABLE III Mean startle amplitude (\pm SEM) at P35 and P56 for the neonatal MK-801 1.0 mg/kg experiment following presentation of the startle stimulus alone on 3 occasions prior to PPI testing (Prior), on 5 occasions during PPI testing (During), and on 3 occasions following PPI testing (After) for sessions preceded by injections of vehicle or apomorphine (Apo: 0.10 mg/kg)

Group	<i>n</i>	Injection	Prior	During	After
P35					
Neonatal saline	17	Vehicle	25.4 \pm 6.0	16.8 \pm 4.0	14.1 \pm 2.7
		Apo	14.9 \pm 2.6	16.3 \pm 3.3	17.6 \pm 4.7
Neonatal MK-801	18	Vehicle	18.1 \pm 3.0	23.1 \pm 4.9	17.9 \pm 4.0
		Apo	21.2 \pm 3.1	22.1 \pm 3.6	15.9 \pm 3.6
P56					
Neonatal saline	17	Vehicle	24.5 \pm 2.9	33.4 \pm 5.5	32.7 \pm 9.9
		Apo	33.1 \pm 8.3	35.4 \pm 6.8	36.9 \pm 9.7
Neonatal MK-801	18	Vehicle	29.8 \pm 5.4	39.9 \pm 5.2	28.1 \pm 6.0
		Apo	29.7 \pm 4.7	23.1 \pm 3.5	24.8 \pm 4.5

ANOVA confirmed a significant main effect of pre-pulse intensity, $F(3, 72) = 5.63$, $p < 0.01$, with non-significant group and interaction effects. Planned individual ANOVA for each group revealed a significant pre-pulse effect for the saline group, $F(3, 36) = 6.21$, $p < 0.01$, but not for the MK-801 group, $F(3, 36) = 2.00$, $p > 0.10$. Thus, neonatal treatment with MK-801 0.5 mg/kg reduced the normal systematic effect of different pre-pulse intensities on PPI when testing took place at P56.

PPI: Neonatal MK-801 1.0 mg/kg Experiment

Startle Amplitude

PPI studies were done on 1.0 mg/kg MK-801 and control rats at P35 and P56 and at both ages all rats were tested following injection of vehicle and apomorphine. Mean startle amplitude was measured three times prior to PPI testing, five times during the PPI protocol, and three times following PPI testing. To evaluate the stability of the startle amplitude during PPI testing, ANOVA compared the means for these three assessments (Table III) following injection of vehicle or apomorphine for each group at each post-natal testing time. These ANOVA failed to yield any significant effects showing that the magnitude of the startle response did not change significantly

during testing or between the vehicle and apomorphine test. For analysis of the PPI effect, the startle responses to the 11 startle stimuli alone were averaged and this mean was used to calculate percent PPI (see below). Thus, the amplitude of the startle response was stable within testing sessions.

No Stimulus and Pre-pulse Alone

Presentation of the pre-pulse stimuli alone during the PPI protocol produced no greater response than that measured in the no-stimulus condition for either the neonatal saline or MK-801 groups at P35 or P56 (Table IV). Generally, the startle amplitude was higher during tests following apomorphine than during those following vehicle but the amplitude of the response was still only about a fifth of that seen following the startle stimulus (cf. Table III). Four 2-way ANOVA, one for each group at each post-natal time, with injection (vehicle or apomorphine) and stimulus condition as the variables analysed, revealed no significant main effects of stimulus condition. Injection prior to testing was significant for the neonatal saline group at P35, $F(1, 16) = 5.13$, $p < 0.05$, and at P56, $F(1, 16) = 5.44$, $p < 0.05$, revealing the reliability of the increase in startle amplitude produced by apomorphine. The only significant interaction was for the neonatal MK-801

TABLE IV Mean startle amplitude (\pm SEM) at P35 and P56 for the neonatal MK-801 1.0 mg/kg experiment following 5 presentations of no stimulus or the pre-pulse stimuli alone during PPI testing (10, 15, 20 or 25 dB over background noise) for sessions preceded by injections of vehicle or apomorphine (Apo: 0.10 mg/kg)

Group	<i>n</i>	Injection	No stimulus	10 (dB)	15 (dB)	20 (dB)	25 (dB)
P35							
Neonatal saline	17	Vehicle	2.6 \pm 0.6	2.4 \pm 0.6	2.5 \pm 0.7	1.4 \pm 0.3	1.9 \pm 0.4
		Apo*	3.2 \pm 0.6	2.9 \pm 0.5	4.3 \pm 1.0	2.9 \pm 0.6	4.3 \pm 1.0
Neonatal MK-801	18	Vehicle	2.6 \pm 0.5	3.0 \pm 0.5	2.7 \pm 0.4	2.8 \pm 0.5	3.0 \pm 0.5
		Apo	3.3 \pm 0.6	3.2 \pm 0.5	3.7 \pm 0.5	3.6 \pm 0.5	3.7 \pm 0.4
P56							
Neonatal saline	17	Vehicle	4.2 \pm 0.7	3.4 \pm 0.6	4.2 \pm 0.8	4.4 \pm 0.7	4.8 \pm 0.8
		Apo*	5.9 \pm 1.0	6.0 \pm 0.9	5.5 \pm 0.7	4.8 \pm 0.6	6.3 \pm 0.9
Neonatal MK-801	18	Vehicle	3.9 \pm 0.7	2.7 \pm 0.4	4.9 \pm 0.8	3.9 \pm 0.6	4.4 \pm 0.8
		Apo**	5.0 \pm 0.7	5.0 \pm 0.7	4.1 \pm 0.4	5.5 \pm 0.8	3.9 \pm 0.3

*Significantly higher startle amplitude vs. vehicle by ANOVA ($p < 0.05$). **Significant interaction of injection (vehicle or Apo) \times stimulus condition by ANOVA ($p < 0.05$).

TABLE I Mean startle amplitude (\pm SEM) at P56 for the neonatal MK-801 0.5 mg/kg experiment following presentation of the startle stimulus alone on 3 occasions prior to PPI testing (Prior), on 5 occasions during PPI testing (During), and on 3 occasions following PPI testing (After)

Group	n	Prior	During	After
Neonatal saline	13	32.3 \pm 6.3	30.0 \pm 5.2	35.1 \pm 7.0
Neonatal MK-801	13	29.0 \pm 4.1	21.5 \pm 3.3	35.2 \pm 8.3

interaction at P56 approached significance, $F(8,160) = 1.91$, $p = 0.06$; this suggests that the amphetamine session at P56 contributed most strongly to the time \times group interaction seen when the analyses combined the data from the two post-natal test times.

Summary

In groups treated neonatally with 1.0 mg/kg MK-801 or saline, lower and upper activity showed a gradual decline during habituation and following saline treatment and an increase following amphetamine. Activity levels generally were higher at P56 than at P35. In both groups combined, amphetamine had a bigger effect in the early part of the session at P56 than it did at P35. No group differences were seen in either activity measure during habituation or following saline at P35 or P56. Following amphetamine, upper activity was significantly greater in the early part of the sessions at P35 and P56 combined in the group that had received MK-801 on P3. Thus, neonatal MK-801 appeared to lead to an increase in the initial upper activity response to amphetamine but no significant effect of sexual maturation was seen on this increase. However, additional analyses suggested that group differences in upper activity response to amphetamine in the early part of the session at P56 contributed most strongly to the observed effect.

PPI: Neonatal MK-801 0.5 mg/kg Experiment

Startle Amplitude

PPI studies were done on 0.5 mg/kg MK-801 and control rats at P56 only. Mean startle amplitude was measured three times prior to PPI testing, five times during the PPI protocol, and three times following PPI testing. To evaluate the stability of the startle

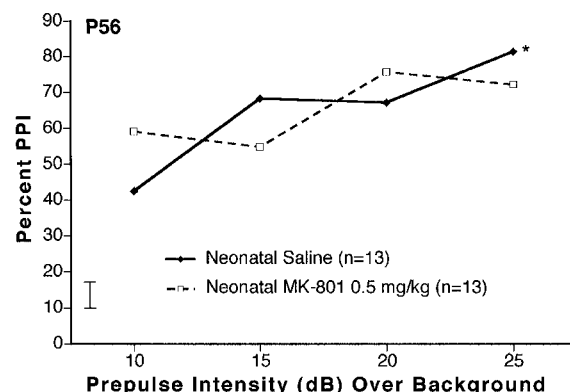


FIGURE 4 Percent pre-pulse inhibition (PPI) in neonatal MK-801 (0.5 mg/kg) and saline-treated rats produced by pre-pulse intensities of 10, 15, 20 and 25 dB above background at P56. The bar on the left indicates the average SEM. *Significant ($p < 0.01$) increase in PPI with increasing pre-pulse intensity.

amplitude over testing, ANOVA compared the means for these three assessments (Table I); no significant differences were found for the neonatal saline, $F(2,24) < 1.0$, $p > 0.10$, or MK-801 groups, $F(2,24) = 1.27$, $p > 0.10$. Additional analyses of these data using the means for every stimulus presentation (not shown) similarly yielded no significant effects. For analysis of the PPI effect, the startle responses to the 11 startle stimuli alone were averaged and this mean was used to calculate percent PPI (see below). Thus, the amplitude of the startle response was stable throughout testing.

No Stimulus and Pre-pulse Alone

Presentation of the pre-pulse stimuli alone during the PPI protocol produced no greater response than that measured in the no-stimulus condition for either the neonatal saline or MK-801 groups (Table II). A 2-variable mixed design ANOVA with stimulus condition and groups as the variables analysed yielded no significant main effects or interaction. Thus, presentation of the pre-pulse stimuli alone had no effect on the startle response.

PPI

Percent PPI is shown in Fig. 4. Both groups showed greater PPI with increasing intensity of the pre-pulse but groups did not appear to differ from one another.

TABLE II Mean startle amplitude (\pm SEM) at P56 for the neonatal MK-801 0.5 mg/kg experiment following 5 presentations of no stimulus or the pre-pulse stimuli alone during PPI testing (10, 15, 20 or 25 dB over background noise)

Group	n	No stimulus	10 (dB)	15 (dB)	20 (dB)	25 (dB)
Neonatal saline	13	3.2 \pm 0.6	3.2 \pm 0.6	2.8 \pm 0.6	3.7 \pm 0.7	3.4 \pm 0.7
Neonatal MK-801	13	2.3 \pm 0.5	2.8 \pm 0.7	3.5 \pm 0.8	3.5 \pm 0.8	2.7 \pm 0.7

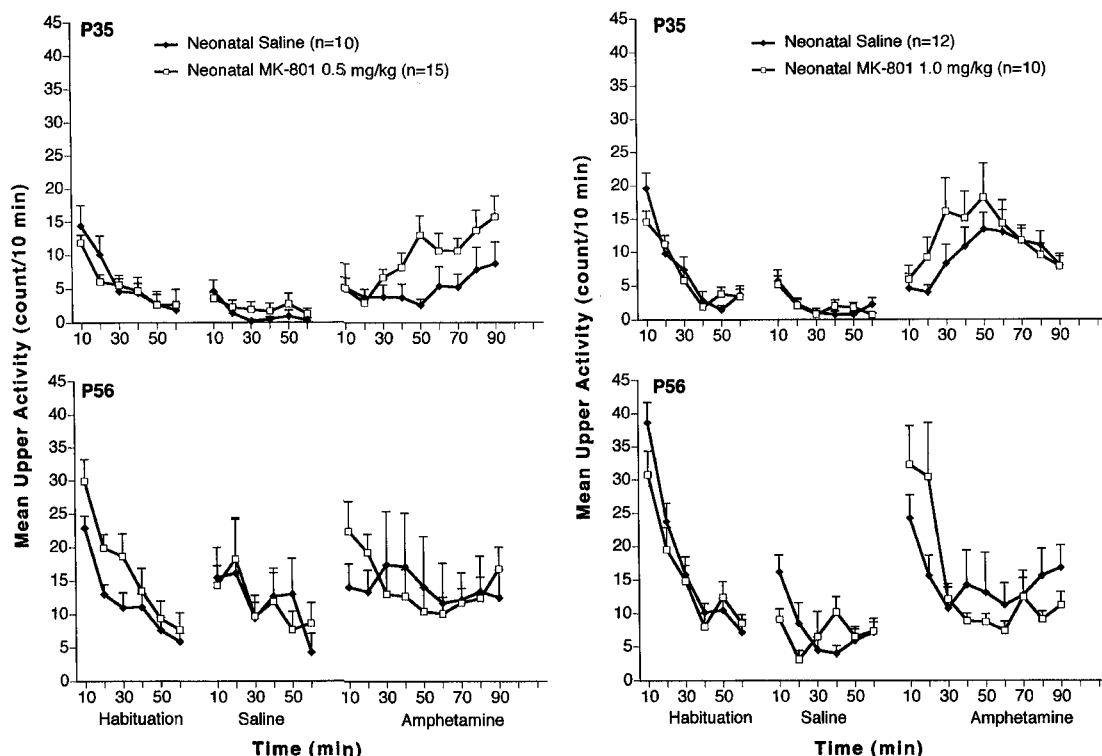


FIGURE 3 Mean (\pm SEM) upper activity (counts/10 min) during habituation and following saline and D-amphetamine (1.5 mg/kg, i.p.) injections in rats treated neonatally with MK-801 (0.5 mg/kg, left panel; 1.0 mg/kg, right panel) or saline on post-natal day 3 (P3) and tested on P35 and P56. Activity was near significantly ($p = 0.06$) higher in the neonatal MK-801 0.5 mg/kg group at P35 following amphetamine. Activity was significantly ($p < 0.05$) higher in the neonatal MK-801 1.0 mg/kg group in the first 20 min of the amphetamine session when post-natal days were combined.

at P35, but no significant group differences were seen at P56.

Locomotor Activity: Neonatal MK-801 1.0 mg/kg Experiment

Lower Activity

Activity levels (counts/10 min) on the lower set of detectors during habituation, following saline and following amphetamine injection for rats treated neonatally with 1.0 mg/kg MK-801 ($n = 10$) or saline ($n = 12$) on P35 and P56 are shown in Fig. 2 (right panel). Both groups showed a gradual decrease in activity on both post-natal days during habituation, time effect $F(5, 100) = 114.47$, $p < 0.001$, and following saline injection, time effect $F(5, 100) = 13.13$, $p < 0.001$. Amphetamine stimulated activity and the stimulant effect of amphetamine was greater in the early part of the session on P56 than it was in that part of the session on P35. This description is supported by a significant interaction of time \times post-natal day, $F(8, 160) = 6.93$, $p < 0.001$. There was no significant difference between the groups.

Upper Activity

Activity levels (counts/10 min) on the upper set of detectors during habituation, following saline and

following amphetamine injection for rats treated neonatally with 1.0 mg/kg MK-801 ($n = 10$) or saline ($n = 12$) on P35 and P56 are shown in Fig. 3 (right panel). As was the case for lower activity, both groups showed a gradual decrease in activity on both post-natal days during habituation, time effect $F(5, 100) = 53.52$, $p < 0.001$, and following saline injection, time effect $F(5, 100) = 4.46$, $p = 0.001$.

Amphetamine stimulated activity in both groups and the stimulant effect of amphetamine in the neonatal MK-801 group vs. the neonatal saline group was greater in the early part of the session on P56 than it was in that part of the session on P35. Analysis of upper activity after amphetamine yielded two significant interactions: time \times day, $F(8, 160) = 8.87$, $p < 0.001$, and time \times group, $F(8, 160) = 1.99$, $p = 0.05$. The time \times day interaction occurs when groups are combined and reflects the higher levels of activity in the first 20 min at P56 vs. P35 following amphetamine injection, as was seen in lower activity (above). The time \times group interaction occurs when post-natal days are combined and reflects the higher level of activity in the first 20 min of the group treated neonatally with MK-801 vs. the neonatal saline group (Fig. 3, right panel). Separate 2-way ANOVA done for each post-natal amphetamine test session revealed a significant time effect for each group and the time \times group

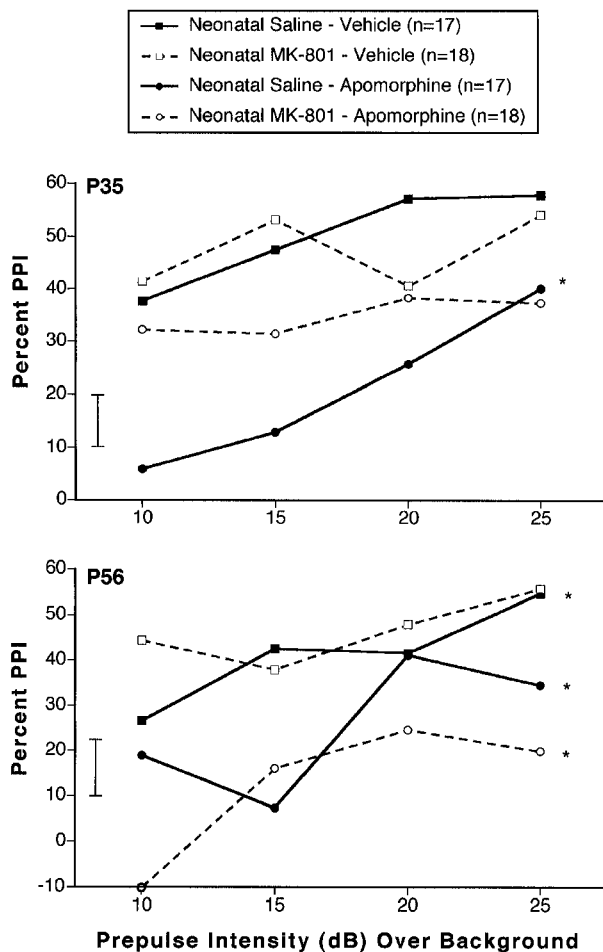


FIGURE 5 Percent pre-pulse inhibition (PPI) in neonatal MK-801 (1.0mg/kg) and saline-treated rats produced by pre-pulse intensities of 10, 15, 20 and 25 dB above background at P35 and P56 following treatment with vehicle and apomorphine (0.1 mg/kg, s.c.). The bars on the left in each panel indicate the average SEM. *Significant ($p < 0.01$) increase in PPI with increasing pre-pulse intensity.

group at P56, $F(4, 68) = 2.58$, $p < 0.05$, reflecting the higher amplitude response following apomorphine in the no-stimulus, 10 and 20 dB condition but lower response in the 15 and 25 dB condition. Thus, the pre-pulse stimuli alone produced no greater amplitude response than the no-stimulus condition and the amplitude of these small responses was greater following apomorphine.

PPI

At both P35 and P56, the amount of PPI generally increased with pre-pulse intensity and treatment with apomorphine decreased the amount of PPI (Fig. 5). Following vehicle injection, the neonatal saline and MK-801 groups showed a similar amount of PPI over pre-pulse intensities at both P35 and P56 but the MK-801 group showed less change over pre-pulse intensities than the control group. The same groups when treated with apomorphine were more

variable; at P35, the neonatal MK-801 group was less sensitive to apomorphine at the three lower intensity pre-pulses and at P56 it was more sensitive at three of the four pre-pulse intensities.

A 4-variable mixed-design ANOVA with independent groups and repeated measures on post-natal day, injection (vehicle or apomorphine) and pre-pulse intensity yielded main effects of injection, $F(1, 33) = 19.39$, $p < 0.001$, and pre-pulse intensity, $F(3, 99) = 14.47$, $p < 0.001$, confirming that apomorphine reduced PPI and that PPI increased with pre-pulse intensity. There was also a significant 4-way interaction, $F(3, 99) = 2.69$, $p = 0.05$. This interaction suggests that the effect of pre-pulse intensity depended on group, injection and post-natal test day. Two 3-way ANOVA, one at P35 and the other at P56, were carried out to isolate the source of the interaction. Results revealed the expected main effects of injection and pre-pulse intensity in each; the only other significant effect was the 3-way interaction of group \times injection \times pre-pulse intensity at P56, $F(3, 99) = 2.76$, $p < 0.05$. Thus, the 4-way interaction occurred because the 3-way interaction was significant at P56 but not at P35.

A set of four planned one-way ANOVA of the pre-pulse intensity effect for each of the groups following vehicle and apomorphine at P56 revealed a significant effect in three of them: neonatal saline with vehicle, $F(3, 48) = 2.78$, $p = 0.05$; neonatal saline with apomorphine, $F(3, 48) = 3.00$, $p < 0.05$; neonatal MK-801 with apomorphine, $F(3, 51) = 3.30$, $p < 0.05$. These analyses suggest that one source of the interaction was the differential effects of pre-pulse intensity in the neonatal MK-801 group treated with vehicle before test vs. the neonatal saline group treated with vehicle and both groups in the apomorphine condition at P56 (Fig. 5). A similar set of four planned one-way ANOVA of the pre-pulse intensity effect for each of the groups following vehicle or apomorphine at P35 revealed a significant effect only in the neonatal saline group treated with apomorphine, $F(3, 48) = 3.63$, $p < 0.05$. Results suggest that both groups were not sensitive to pre-pulse intensity following vehicle at P35 and that the neonatal MK-801 group continued to show this insensitivity when treated with apomorphine whereas the neonatal saline group showed pre-pulse intensity sensitivity after apomorphine at P35.

PPI: Summary

For the neonatal MK-801 0.5 mg/kg experiment, PPI was only tested at P56 without apomorphine. It was found that increasing pre-pulse intensity led to increased PPI for the neonatal saline group but not for the neonatal MK-801 group. For the neonatal MK-801 1.0 mg/kg experiment, there was an overall strong effect of pre-pulse intensity on PPI,

and apomorphine reduced PPI. The effects of pre-pulse intensity interacted with group and injection at P56 but not at P35 and analyses of the pre-pulse intensity effect for each group with and without apomorphine at P56 revealed that only the neonatal MK-801 group treated with vehicle before testing failed to show a significant pre-pulse intensity effect. This was the same group at the same post-natal testing time (and tested without apomorphine) that failed to show a significant pre-pulse intensity function in the neonatal MK-801 0.5 mg/kg experiment. Results suggest that when tested for PPI at P56 following vehicle, rats treated neonatally with MK-801 at either dose are less sensitive to changes in pre-pulse intensity. Further results from the P35 tests of neonatal MK-801 1.0 mg/kg rats suggest that they were less sensitive than saline controls to changes in pre-pulse intensity especially following apomorphine. Overall, results suggest that neonatal treatment with MK-801 reduces sensitivity to changes in pre-pulse intensity in PPI tests.

DISCUSSION

Results can be summarized as follows. Neonatal treatments on P3 with MK-801 resulted in accelerated apoptotic cell loss in a number of brain regions including the hippocampus. In tests of activity, neonatal MK-801 and control groups showed habituation over time and increased activity following amphetamine. These groups had higher activity counts on P56 than on P35. No group differences were seen during habituation or following saline at either test time except that the neonatal MK-801 0.5 mg/kg group had greater upper activity during habituation tested on P56. Following amphetamine, the neonatal MK-801 0.5 mg/kg group showed greater lower and upper activity at P35. The neonatal MK-801 1.0 mg/kg group showed an elevated response to amphetamine in the first 20 min of the P35 and P56 tests combined but additional analyses suggested that the effect was largely influenced by activity levels at P56. In PPI tests, startle amplitude was stable across testing and pre-pulse stimuli alone, like the no-stimulus condition, produced minimal startle. Treatment with apomorphine enhanced startle in the no-stimulus and pre-pulse alone conditions but the magnitude of startle was still much smaller than that seen with the startle stimulus. In all conditions combined, PPI was observed and increased systematically with increasing intensity of the pre-pulse. Apomorphine reduced the magnitude of the PPI effect. Both groups treated neonatally with MK-801 (0.5 or 1.0 mg/kg) failed to show a significant change in percent PPI with increasing pre-pulse intensity when tested with vehicle at P56.

TUNEL staining results were consistent with the reports of Ikonomidou *et al.* (1999) and Pohl *et al.* (1999) showing that perinatal transient blockade of glutamatergic NMDA receptors leads to accelerated apoptotic cell death. Ikonomidou *et al.* (1999) showed that treatment on P3 with MK-801 0.5 mg/kg enhanced apoptotic cell death in a number of regions of the hippocampus including CA1, dentate gyrus and subiculum as well as other forebrain regions such as the striatum. Our results similarly showed increased TUNEL staining, indicative of increased apoptosis, in the hippocampus and striatum. It is noteworthy that neonatal (P3) injections of MK-801 resulted in cell loss in the ventral hippocampal region where neonatal excitotoxic lesions have been shown to lead to post-pubertal behavioural changes similar to some of the behavioural changes seen in schizophrenia (Lipska *et al.*, 1993; 1995). This suggested the hypothesis of the present studies, that rats given neonatal injections of MK-801 might show the same types of behavioural deficits. However, results did not support this hypothesis.

Neonatal lesion and control groups showed patterns of locomotor activity like those reported previously including a gradual decrease during habituation and following saline (Beninger *et al.*, 1985) and increased activity following amphetamine (Segal and Kuczenski, 1994). The observation of elevated activity counts at P56 compared to P35 is in good agreement with the similar observations of Lipska *et al.* (1993) in neonatal ventral hippocampus lesion and control animals. Results might indicate increased levels of activity with increasing age; alternatively, the larger size of the rats at P56 may have contributed to the observation of higher lower and upper activity counts.

The effects of neonatal MK-801 on activity appeared to depend on dose and age at the time of testing. The most striking observation was in rats treated neonatally with 0.5 mg/kg; they showed an elevated lower and upper activity response to amphetamine at P35 but not at P56. The 1.0 mg/kg group showed an elevated upper (but not lower) activity response to amphetamine in the first 20 min of testing when data from P35 and P56 were combined but the effect seemed to be most prominent at P56. Results from the 0.5 mg/kg MK-801 group are unlike those of Lipska *et al.* (1993) in two important ways: (1) they show changed sensitivity to amphetamine before sexual maturity, not after and (2) lower and upper activity changes were seen only in the amphetamine phase and not in the habituation and saline phases. Lipska *et al.* (1993) observed increased activity in their neonatal ventral hippocampal lesion animals in all three test phases following sexual maturity suggesting that it was not just the response to amphetamine that was elevated

in their animals but activity in general. The present findings with 0.5 mg/kg MK-801 given neonatally, showing an elevation in response to amphetamine at P35 without an increase in activity in the other two phases suggest that sensitivity to amphetamine was increased by the neonatal treatment. However, the pre-pubertal emergence of this behavioural effect and its disappearance post-pubertally is unlike the behavioural pattern seen in schizophrenia. The observed pattern suggests that seen in disorders involving a rectification of symptoms around the time of sexual maturity.

The observations of an elevated upper activity response to amphetamine in the first 20 min of testing at P56 in the neonatal 1.0 mg/kg group and of elevated upper activity during habituation tested on P56 in the neonatal 0.5 mg/kg group suggest that neonatal MK-801 treatments may have led to a tendency to greater activity post-pubertally. However, these effects were only seen in upper activity in one of the three activity test phases bringing their reliability into question.

In PPI experiments, the pre-pulse stimuli alone did not produce a startle response. As has been reported previously, PPI increased systematically with increasing intensity of the pre-pulse (Li *et al.*, 1998) and apomorphine reduced the magnitude of the PPI effect (Davis *et al.*, 1990). Lipska *et al.* (1995) found that neonatal ventral hippocampal lesion rats showed increased sensitivity to a 0.1 mg/kg dose of apomorphine at one pre-pulse intensity at P56 but not at P35. In the present experiment, we found no significant change in sensitivity to apomorphine at P35 or P56 in rats that received neonatal MK-801 1.0 mg/kg. As was the case in activity experiments, this finding shows that neonatal treatment with a glutamate receptor antagonist does not produce the schizophrenia-like behavioural profile seen in neonatal ventral hippocampal lesion animals.

The main finding from the present PPI experiments was that at P56 both neonatal MK-801 dose groups, when treated with vehicle, failed to show a significant change in percent PPI with increasing pre-pulse intensity. The 1.0 mg/kg group also failed to show a significant effect of pre-pulse intensity in the vehicle condition at P35 but neither did the control group (P35 PPI was not tested for the 0.5 mg/kg MK-801 group). Results suggest that neonatal exposure to MK-801 altered sensitivity to the pre-pulse stimuli in the vehicle condition. The nature of this alteration is unclear but observation of Figs. 4 and 5 suggest that at the lowest pre-pulse intensity (10 dB over background), the PPI effect was greater in the neonatal MK-801 groups. This might suggest that the neonatal treatments led to increased sensitivity to pre-pulse stimuli.

From the results of the activity and PPI studies, it is clear that neonatal treatments with MK-801 do not

produce the same behavioural effects as neonatal ventral hippocampal lesions. Although neonatal injections of MK-801 and neonatal ventral hippocampal lesions have in common a damaging effect on hippocampal cells, these treatments also lead to a number of different effects. Thus, neonatal ventral hippocampal lesions produce a local effect restricted to the region of the injection whereas neonatal injections of MK-801 have a more diffuse effect. In the present study, TUNEL staining showed increased apoptotic cell death in the hippocampus and striatum and in previous studies similar treatments with MK-801 and other NMDA receptor antagonists have been found to lead to apoptotic cell death in regions of the cortex and the dorsal thalamus (Ikonomidou *et al.*, 1999; Pohl *et al.*, 1999). Additionally, neonatal ventral hippocampal lesions produce excitotoxic cell death whereas neonatal injections of MK-801 enhance an ongoing natural process of programmed cell death by blocking the usual tropic influence of glutamate receptors (Pearce *et al.*, 1987). These differential effects of the two types of neonatal treatments may have influenced their differential behavioural outcomes.

CONCLUSIONS

Neonatal exposure to MK-801 leads to apoptotic cell loss in forebrain structures and developmental behavioural changes. Thus, amphetamine-stimulated locomotor activity is enhanced prior to, but not following sexual maturity in rats receiving 0.5 mg/kg MK-801 but not in rats receiving 1.0 mg/kg on P3. Neonatal exposure to 1.0 mg/kg MK-801 did not significantly alter the disruptive effect of apomorphine on PPI tested before or after sexual maturity. However, the usual systematic increase in PPI with increases in pre-pulse intensity was not seen after sexual maturity in animals treated neonatally with either dose of MK-801 and tested following injection of vehicle. Overall, results suggest that neonatal blockade of NMDA receptors may alter sensitivity to amphetamine and to mild (pre-pulse) stimuli. However, it is unclear at present how these changes might contribute to neuropsychiatric illnesses and further studies in this respect are needed.

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