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Effects of MK801 and neuroleptics on prepulse inhibition: re-examination in two strains of rats

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Abstract

Disruption of prepulse inhibition (PPI) induced by NMDA receptor antagonists, such as MK801, has been used as an animal model of positive and negative symptoms of schizophrenia. Previous studies suggested that atypical, but not typical, neuroleptics can selectively restore MK801-induced PPI disruption and that such selectivity may depend on strain differences. The present study re-examined PPI disruption by systemic MK801 in Wistar (WS) and Sprague–Dawley (SD) strains, and addressed the issue whether clozapine (atypical), compared to haloperidol (typical), effectively antagonizes MK801-induced PPI disruption. In addition, we tested the effects of bilateral microinfusion of MK801 into the ventral hippocampus in WS. Systemic MK801 disrupted PPI in both strains. Neither clozapine nor haloperidol antagonized MK801-induced PPI in either strain. Our clozapine data do not agree with previous reports of clozapine's ability to antagonize MK801-induced PPI disruption. Similar to previous results with SD, MK801 infusion into the ventral hippocampus failed to affect PPI in WS. In our view, the selective ability of atypical neuroleptics to restore PPI disruption by NMDA antagonists, and to serve as a tool for identifying possible atypical neuroleptics, requires further examination. PPI disruption with systemic MK801 may be due to the blockade of NMDA receptors in multiple brain sites. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Prepulse inhibition; NMDA; Dizocilpine; Hippocampus; Clozapine; Haloperidol; Schizophrenia

The phenomenon of prepulse inhibition (PPI) is the reduction of an acoustic startle response to an intense acoustic stimulus (pulse) that is immediately preceded by a stimulus of lower intensity (prepulse). The prepulse itself does not elicit a startle response, but is thought to activate inhibitory neuronal processes which attenuate or gate the startle response. Thus, PPI is a frequently used measure of sensorimotor gating in both humans and rats. Schizophrenic patients exhibit disruption of PPI and related measures, suggesting disruption of sensorimotor gating [5–7,10]. In rats, disruption of PPI can be induced by administration of either NMDA antagonists or dopamine agonists [13,18,19,26,28,34]. The similarity between pharmacologically induced PPI disruption in rats and PPI disruption exhibited by schizophrenic patients suggests that PPI disruption is an animal model of sensorimotor gating deficits with construct validity for schizophrenia.

Although NMDA antagonists and dopamine agonists produce a comparable disruption of PPI, the ability of neuroleptics to antagonize PPI disruption induced by these drugs may differ. Pretreatment with either a typical or an atypical neuroleptic, haloperidol and clozapine, respectively, antagonized PPI disruption produced by dopamine agonists, such as apomorphine [19,22,28,29,30,32]. In contrast, PPI disruption induced by NMDA antagonists, such as phencyclidine (PCP), MK801, and ketamine, was unaffected by pretreatment with a typical neuroleptic, haloperidol [8,11,12,14,26,34]. Pretreatment with atypical antipsychotics, however, has yielded conflicting results. For example, clozapine, olanzapine, seroquel, and remoxipiride antagonized PPI disruption by PCP or MK801 in Sprague–Dawley (SD) rats [1,4,12,25]. On the other hand, studies using SD and Wistar (WS) rats found that clozapine was as ineffective as haloperidol in antagonizing PPI disruption induced by MK801 and PCP [11,12,34,37]. It has been suggested that strain differences may contribute to such discrepancy in clozapine effects [34]. Thus, the question whether atypical neuroleptics possess the ability to antag-

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onize PPI disruption by NMDA receptor antagonists in different strains of rats requires further examination. Indeed, such selective effects of atypical neuroleptics on PPI can be used as a possible measure for identifying potential atypical neuroleptics.

Assessing PPI disruption induced by noncompetitive NMDA receptor antagonists is worthwhile for another reason: it may reveal whether different neural structures are involved in mediating PPI disruption by NMDA antagonists and by dopamine agonists (for review, see Ref. [15]). One theory emphasizes that disrupted PPI induced by dopamine agonists is due to an increased stimulation of dopamine (D2) receptors in the nucleus accumbens [17,28,29,39]. This notion is supported by findings that haloperidol, a potent dopamine D2 receptor antagonist, antagonized apomorphine-induced PPI disruption. Given that haloperidol failed to antagonize PPI disruption produced by noncompetitive NMDA receptor antagonists, the reversibility by haloperidol appears to be limited to PPI changes mediated by the dopaminergic system. Thus, it is conceivable that brain areas involved in NMDA-mediated expression of PPI may differ from the areas involved in dopamine-mediated effects. Although the precise target site for systemic MK801 is not clear, the hippocampus, which has a higher NMDA receptor expression and PCP binding sites than any other brain region [21,24], may be critically involved in modulation of PPI [17,29]. Moreover, stimulation of the NMDA receptors in the ventral hippocampus by NMDA infusion disrupts PPI [16,35,40]. Interestingly, however, bilateral infusion of NMDA receptor antagonist, MK801, into the dorsal, but not the ventral, hippocampus disrupted PPI in SD rats [2,3]. These findings suggest that within the hippocampus, there may exist a regional difference in NMDA receptor-mediated processes. Within the ventral hippocampus, overstimulation of NMDA receptors disrupts PPI, whereas a blockade of NMDA receptors in the same region may not affect PPI expression.

The present study examined MK801-induced PPI disruption. In the first part of the study, we re-examined the disruption of PPI by systemic MK801 in both WS and SD rats, addressing the issue whether clozapine, compared to haloperidol, is effective in antagonizing MK801-induced PPI disruption. Emphasis was placed on two issues: First, Bakshi et al.'s [4] findings suggested that a narrow range of effective doses of clozapine antagonized the PPI disruption by MK801 and PCP in SD rats. We approached this issue by testing various combinations of MK801 (0.05–0.1 mg/kg) and clozapine (5 and 10 mg/kg) on PPI in WS rats and by testing MK801 (0.1 mg/kg) and clozapine (5 and 10 mg/kg) in SD rats. Second, findings of Bakshi et al. [4] suggested that clozapine's effects may be limited to one half of the session and the higher prepulse intensities. We conducted additional statistical analyses to detect any subtle changes in the magnitude of MK801 effects that could be restricted to a narrow time window after drug administration and in response to different prepulse intensities. In the second part of the study, the effects on PPI of systemic administration of the lowest effective dose of MK801 (0.05 mg/kg) were compared with the effects of bilateral micro-infusion of MK801 (6.25 μg/0.5 μl/site) into the ventral hippocampus in WS rats. This experiment was conducted in order to assess whether the lack of effect of ventral hippocampal infusion of MK801 (6.25 μg/0.5 μl/site) on PPI reported in SD rats [2] is strain specific, or more general. Given the evidence that NMDA transmission within the ventral hippocampus is critical for PPI [16,35,40], we tested the hypothesis that the blockade of NMDA transmission in the ventral hippocampus would affect PPI in WS rats.

1. Method

1.1. Part 1. PPI disruption by systemic MK801 and pretreatment with neuroleptics

1.1.1. Subjects

Sixty-one male adult WS rats [Zur:Wist(HanIbm)] and 24 SD rats [Zur:SD(Crl:CD(SD)BR)], weighing 300–450 g, were used in this experiment. These two strains were bred in the same facility at the Research Unit Schwerzenbach, Switzerland. Animals were housed four to a cage under a reversed light–dark cycle (lights on: 19:00–07:00 hours) with free access to food and water. Before testing, rats were handled 5 min per day for at least 3 days. All experimental procedures occurred during the dark phase of the cycle. The experiments were carried out in accordance with Swiss federal regulations for animal experimentation.

1.1.2. Drug administration

MK801 (Merck, Sharp, and Dohme, UK) was dissolved in 0.9% saline and administered subcutaneously (0.05, 0.075, and 0.1 mg/kg, sc) 15 min prior to PPI testing. Haloperidol (5 mg/ml, Janssen-Cilag, Baar, Switzerland) was diluted with 0.9% saline. Clozapine (Novartis, Basel, Switzerland) was dissolved in 0.9% saline adjusted to pH 5.5 by addition of 0.1 M HCl and Na_2CO_3. Haloperidol (0.2 mg/kg, ip) and clozapine (5 and 10 mg/kg, ip) were administered intraperitoneally 45 and 30 min, respectively, prior to PPI testing. Saline (1 ml/kg) was used for control injections.

1.1.3. Experimental design

Each experiment included three groups of rats. The first group was pretreated with saline, followed by another saline injection (saline + saline). The second group received a saline pretreatment, followed by a single dose of MK801 (saline + MK801). The third group was pretreated with a single dose of either clozapine or haloperidol, followed by a single dose of MK801 (haloperidol + MK801 or clozapine + MK801). MK801 effect on PPI was measured by

comparing the saline + saline group with the saline + MK801 group. Pretreatment effects of neuroleptics were tested by comparing the neuroleptic-pretreated group (haloperidol + MK801; clozapine + MK801), and the saline-pretreated group (saline + MK801). All animals used in Part 1 were experimentally naive subjects. None of these animals was reused in other experiments.

1.1.4. PPI testing

The animals were tested in squads of four with startle chambers counterbalanced across the different experimental groups. The testing was conducted in four ventilated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), which contained a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a plexiglas frame. Acoustic pulses and prepulses were delivered via a speaker, which was mounted 24 cm above the tube. Movement inside the tube was detected by a piezoelectric accelerometer below the frame. The amplitude of the whole body startle to an acoustic pulse was defined as the average of 100 one-millisecond accelerometer readings collected from pulse onset. Delivery of the acoustic stimuli and recording of startle responses was controlled by a computer. Once the animals were placed inside the tube, the startle session started with a 5-min acclimatization period, with a background noise level of 68 dB(A), which was maintained throughout the session. Following the acclimatization period, four startle pulses [30 ms, 120 dB(A)] were presented. The four initial startle pulses served to achieve a relatively stable level of startle reactivity for the remainder of the test session because the most pronounced habituation of the startle response occurs during the first four pulse presentations [2,17]. The prepulses were broad band noise bursts of either 72, 76, 80, or 84 dB(A) and were 20 ms in duration. The interval between the prepulse and pulse was 80 ms. Each session consisted of six blocks of 11 trials. Each block included four different trial types: two pulse alone trials, four prepulses at different intensities followed by pulse, four prepulses alone at four intensities, and one no stimulus trial. The different trial types were presented pseudorandomly with a variable intertrial interval of 10–20 s. One session lasted about 23 min.

1.1.5. Data analysis

For each of the four ‘prepulse–pulse’ trial types, the percentage PPI (%PPI) was calculated: %PPI = 100 × [1 − (-(startle amplitude in prepulse trial/startle amplitude in pulse alone trial)]. The overall mean %PPI was calculated for the four prepulse intensities. The mean startle amplitude was calculated as the average response to the 12 ‘pulse-alone’ trials across the entire six blocks. The PPI values were calculated for the total duration of the test session as well as for the first and second half of the test session. Statistical analysis was conducted by ANOVA with the different combinations of injections for pretreatment and treatment as between-subjects factors and prepulse types and session halves as repeated measures. Pair-wise comparisons were made by Fisher’s PLSD tests.

1.2. Part 2. Effects of MK801 microinfusion into the ventral hippocampus on PPI: comparison with the effects of systemic MK801

1.2.1. Subjects

Twenty-six male adult WS rats (300–450 g) from the same breeding facility as in Part 1 were used in this experiment. All animals used in Part 2 were drug-naive but had been used in previous experiments involving saline infusion and PPI or open-field assessments. Of 26 rats, 10 rats were bilaterally implanted with cannulae in the ventral hippocampus and 16 unoperated rats were used as an additional comparison to assess the effects of systemic MK801 (0.05 mg/kg, sc). The animals were kept in single cages throughout the experiment. Other animal care and housing conditions were identical to those described in the Part 1 experiment.

1.2.2. Implantation of guide cannulae and intracerebral infusion

Rats were anesthetized with Nembutal (sodium pentobarbital, 50 mg/ml/kg, ip, Abbott, Chicago, IL) and placed in a stereotaxic frame. The skull was exposed, bregma and lambda were aligned in the same horizontal plane. Two small holes (1.5 mm in diameter) were drilled onto the skull and bilateral guide cannulae were implanted. The tips of the cannulae were aimed 1.5 mm above the ventral hippocampus: AP = − 5.2 mm, ML = ± 5 mm, DV = − 5 mm [20]. Guide cannulae were constructed from 26-gauge stainless-steel tubing (0.45 mm in outer diameter) held in a 5-mm perspex block. The guide cannulae were fixed onto the skull using dental cement. Three small stainless-steel skull screws served as anchors for the guide cannulae. Stainless-steel stylets, which extended 0.5 mm beyond the tips of the guide cannulae, were placed inside the guide cannulae to prevent occlusion. At least 7 days were allowed for a recovery period, during which the animals were handled and acclimatized to a simulation of the intracerebral infusion procedure.

For intracerebral microinfusions, the animals were gently held by the experimenter, stylets were removed, injector needles were inserted into the guide cannulae, and the tip of each injector needle was allowed to protrude 1.5 mm beyond the end of the guide cannula. The injector unit consisted of 34-gauge (0.22 mm) cannulae, held in a cuff of 26-gauge stainless-steel cannulae (0.45 mm outer diameter), which were connected via polyetheretherketone (PEEK) tubing to 10-μl Hamilton syringes. MK801 was dissolved in 0.9% sterile saline at a concentration of 6.25 μg/0.5 μl [2]. MK801 or saline was infused using an infusion pump (WPI, Sarasota, FL; model sp200i) at a speed of 0.5 μl/min. The volume of infusion was 0.5 μl per site. A 60-s diffusion period was allowed before removal of the injector needles

and replacement of the stylets. The animals were tested on PPI immediately after infusion.

1.2.3. Experimental design

This experiment included three groups. The first group of rats ($n=10$) had bilateral cannulae in the ventral hippocampus (vHippo), and received either vehicle (saline) or MK801 infusion. The dose of MK801 for infusion was the same dose used by Bakshi and Geyer [2]. The second group ($n=8$) of unoperated rats received systemic injection of MK801 (UNOP-MK801) to compare the systemic effects with intracerebral infusion effects on PPI. The dose of MK801 was the lowest effective dose used in the earlier experiment. The third group ($n=8$) of unoperated rats received systemic saline injection (UNOP-Sal) as a control. All systemic injections were given 15 min prior to PPI testing. MK801 (0.05 mg/ml) solution for systemic injection was prepared by diluting the solution (6.25 mg/0.5ml in concentration) that was used for intracerebral infusion.

1.2.4. PPI testing

PPI testing spanned 4 test days, with 3 days between test days. On day 1, all rats were tested for baseline PPI without any infusions or injections. The unoperated rats were then matched by baseline PPI and divided into the UNOP-MK801 and the UNOP-Sal groups. On day 2, the vHippo group received vehicle infusion into the ventral hippocampus less than 2 min prior to a PPI session. The two UNOP groups were subjected to PPI testing without injection. On day 3, the vHippo group received MK801 infusion. A complete counterbalanced treatment sequence (vehicle-MK801, MK801-vehicle infusion) was not used. This was to avoid possible carryover effects of MK801. The UNOP-MK801 and UNOP-Sal groups received systemic injection of MK801 (0.05 mg/kg, ip) and saline, respectively, 15 min prior to the session. On day 4, all three groups received a PPI session to assess a possible residual drug effect from day 3. All other procedures for PPI testing were identical to those in the Part 1 experiment.

1.2.5. Histology

Upon completion of the experiment, rats were deeply anesthetized with an overdose of pentobarbital, and they were transcardially perfused with 0.9% NaCl solution, followed by 4% formalin solution. Each brain was extracted, post-fixed in 4% formalin solution, and cut in 40-μm coronal sections on a freezing microtome. Every fifth section through the ventral hippocampus was mounted on slides and stained with Cresyl violet. The sections were examined under a light microscope and the injection sites (i.e., the placement of injector tips) were verified after Paxinos and Watson [20].

1.2.6. Data analysis

The mean startle amplitude and %PPI were calculated as in Part 1. The resulting values were subjected to ANOVA with groups as a between-subjects factor and days, prepulse intensities, and session halves as repeated measures. Fisher's PLSD test was used for pair-wise comparisons.

2. Results

2.1. Part 1. PPI disruption by systemic MK801 and pretreatment with neuroleptics

In all experiments that involved systemic injections of MK801 and combinations of MK801 and neuroleptics, there was a significant main effect of pulse alone trials on startle amplitude, indicating habituation of the startle response over the 16 pulse presentations ($P<.001$). Overall %PPI increased with an increase in prepulse intensity, with the greatest %PPI at the highest prepulse intensity. This was reflected by a significant main effect of prepulse intensity on %PPI ($P<.001$).

2.2. Effects of systemic MK801 on PPI in WS rats

2.2.1. Startle response

ANOVA of the combined startle data of the saline + saline groups ($n=15$) and the saline + MK801 groups of three separate experiments using different MK801 doses (0.05, 0.075, and 0.1 mg/kg; $n=5–6$) did not yield a significant effect on the mean startle amplitude, $F(3, 27)=1.32$, $P>.05$.

2.2.2. Prepulse inhibition

Systemic injection of MK801 (0.05, 0.075, and 0.1 mg/kg) disrupted PPI (Fig. 1A). ANOVA yielded a significant main effect of MK801 treatment on %PPI, $F(3, 27)=14.96$, $P<.0001$. Post hoc pair-wise comparisons showed that all MK801 groups significantly differed from the saline + saline group. The data in Fig. 1 are combined data from the saline + saline groups ($n=15$) and the saline + MK801 groups of three separate experiments, using three different MK801 doses ($n=5–6$).

In further analysis, following the precedent in Bakshi et al. [4], we divided the PPI data into two half sessions. ANOVA revealed a significant effect of session half, $F(1, 27)=4.89$, $P<.05$ (see Fig. 1B). There was no significant interaction of treatment group and session half, $F(3, 27)=1.48$, $P>.05$.

2.3. Effect of clozapine and haloperidol pretreatment on MK801-induced PPI disruption in WS rats

2.3.1. Startle response

Experiments involving 0.05 or 0.075 mg/kg MK801 and pretreatment with clozapine failed to show a significant drug effect on the mean startle amplitude ($P>.05$). However, in one experiment involving 0.1 mg/kg MK801 and pretreatment with haloperidol (0.2

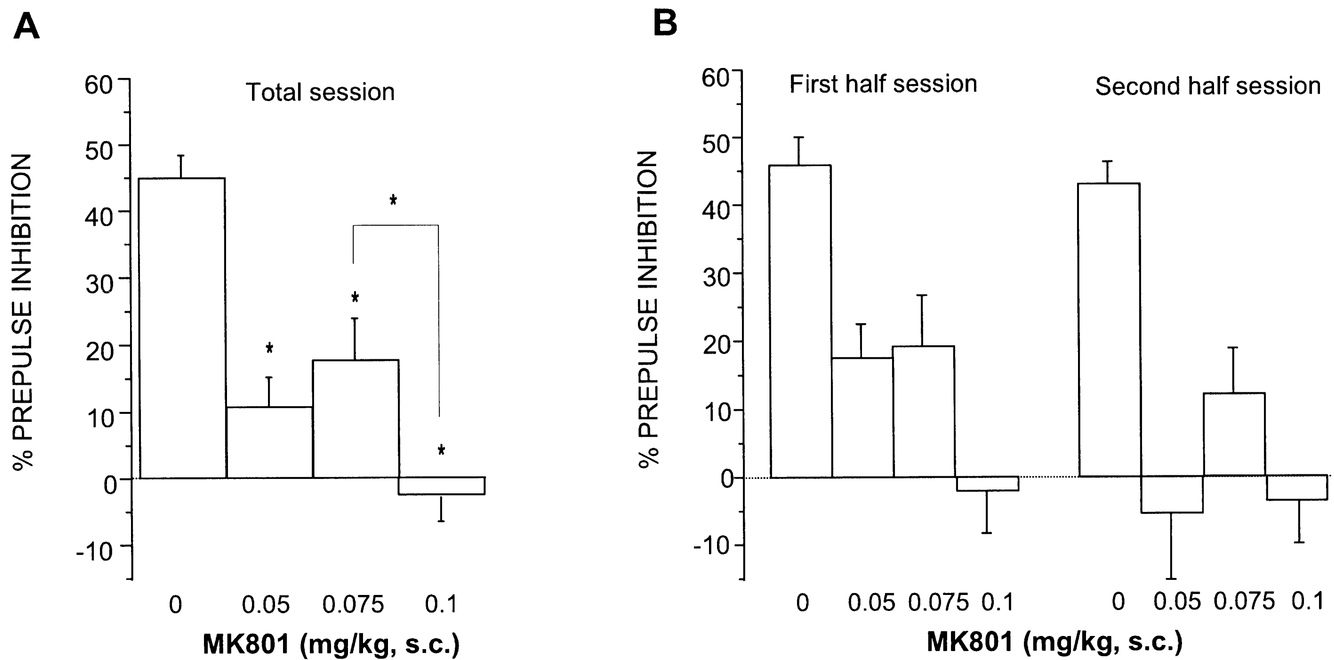

Fig. 1. Disruption of PPI by systemic MK801 (0.05, 0.075, and 0.1 mg/kg, sc) in WS rats. The numbers on the *Y* axis represent the mean %PPI. The numbers on the *X* axis represent different doses of MK801: 0.0 mg/kg ($n=15$); 0.05 mg/kg ($n=5$); 0.075 mg/kg ($n=5$); and 0.1 mg/kg ($n=6$). A dose of 0.0 mg/kg indicates saline injection. Asterisks denote significant differences compared to the saline controls ($P<.05$). Bars represent standard error of the mean. (A) Data are combined from all prepulse intensities for the entire session. Note a significant difference between the group that received 0.075 mg and the group that received 0.1 mg/kg MK801. (B) The session data are divided into two half sessions.

mg/kg) and clozapine (5 mg/kg), there was a significant treatment effect, $F(3,\ 17)=3.25$, $P<.05$, which was due to a higher mean startle amplitude of the saline + saline group, compared to other treatment groups ($P<.05$). There was no significant difference among the saline + MK801, haloperidol + MK801, or clozapine + MK801 groups ($P>.05$).

2.3.2. Prepulse inhibition

Neither clozapine (5 mg/kg) nor haloperidol (0.2 mg/kg) pretreatment reversed MK801 (0.1 mg/kg)-induced PPI disruption. Both the clozapine + MK801 and the haloperidol + MK801 groups showed a similar degree of PPI disruption as the saline + MK801 group, which showed a marked PPI disruption, compared to the saline + saline group (Fig. 2A). ANOVA indicated a significant main effect of group on %PPI, $F(3,\ 17)=6.42$, $P<.005$. Pair-wise comparisons indicated no significant differences between clozapine- and haloperidol-treated groups. When the PPI data were divided into two half sessions, MK801 effects on PPI during the first and the second half were comparable (Fig. 2B). There was no significant half-session effect nor an interaction between session half and prepulse intensity.

Neuroleptic-pretreatment effects were further examined with different combinations of clozapine and MK801. Combinations were used to detect any narrow dose range within which clozapine might antagonize MK801-induced PPI disruption. As shown in Fig. 3, both 0.05 mg/kg and 0.075 mg/kg of MK801 markedly disrupted PPI, whereas pretreatment with either 5 mg or 10 mg/kg clozapine failed to restore PPI disruption (Fig. 3A and C). ANOVA yielded a significant main effect of group [for Fig. 3A, $F(3,\ 16)=8.89$, $P<.005$; and for Fig. 3C, $F(3,\ 16)=6.47$, $P<.005$]. This was due to a difference between the saline + saline and the saline + MK801 (0.05 mg or 0.075 mg) groups ($P<.05$, all groups $n=5$).

To further examine the time course of clozapine effects, which may contribute a subtle change to the magnitude of PPI over time, the data were divided into two half sessions. For the experiment involving 0.05 mg/kg MK801, there was no significant effect of session half. ANOVA yielded a significant interaction of session half and group, $F(3,\ 16)=3.69$, $P<.05$, reflecting the fact that PPI disruption within the group pretreated with 5 mg/kg clozapine was more severe during the first half of the session as compared to the second half (Fig. 3B). For the experiment involving 0.075 mg/kg MK801, ANOVA yielded a significant effect of session half, $F(1,\ 16)=18.51$, $P<.001$, and a significant interaction of session half and group, $F(3,\ 16)=5.82$, $P<.01$, which may be due to a more severe PPI disruption during the second half in the MK801 group pretreated with 5 mg/kg clozapine (Fig. 3D).

The half session data were further divided by different prepulse intensities. For the experiment involving 0.05 and

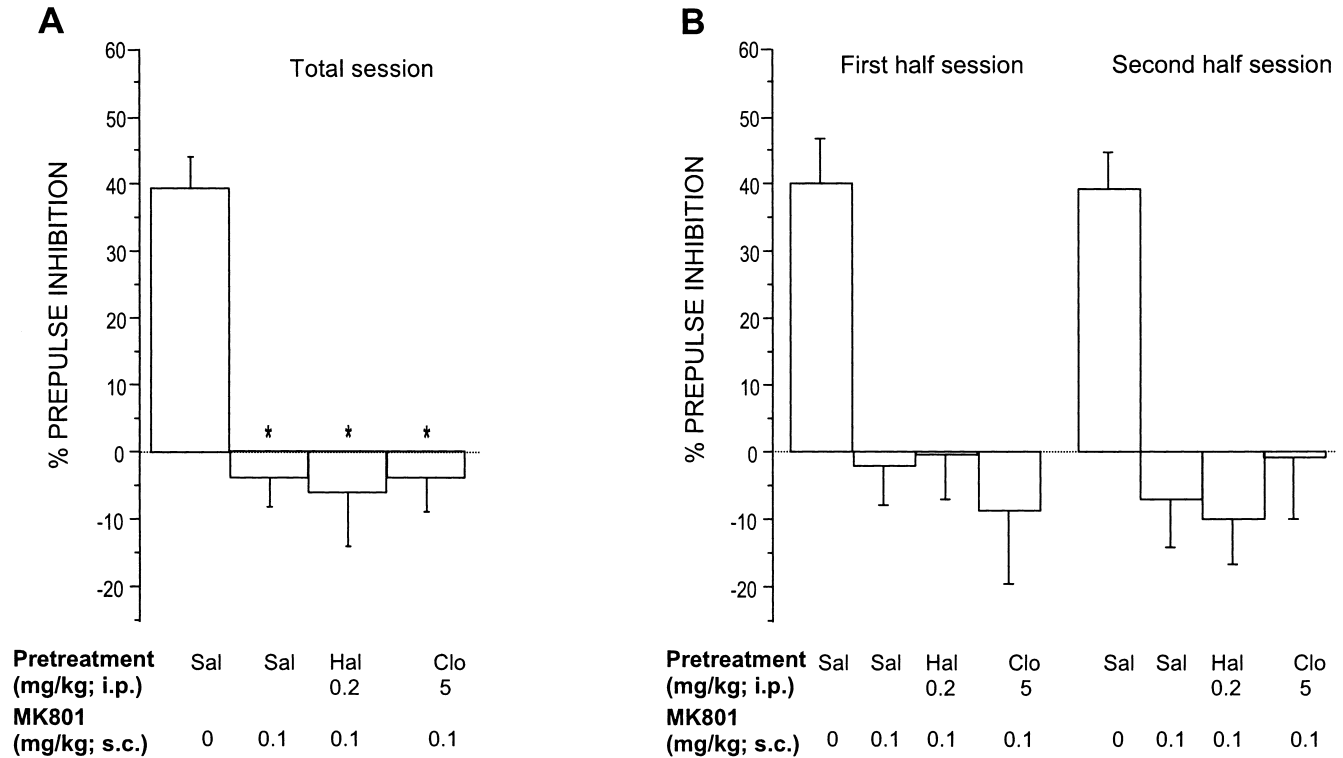

Fig. 2. Effects of pretreatment with either haloperidol (0.2 mg/kg, ip) or clozapine (5 mg/kg, ip) on PPI disruption by MK801 in WS rats. The numbers on the Y axis represent the mean %PPI. The numbers on the X axis represent different pretreatment-doses of neuroleptics and MK801 (0.1 mg/kg, sc, $n=5$ for each group). A dose of 0.0 mg/kg ($n=6$) indicates the saline injection. Sal: saline; Hal: haloperidol; Clo: clozapine. Asterisks denote significant differences compared to the saline controls (saline + saline). Bars represent standard error of the mean. (A) Data collapsed over all prepulse intensities for the entire session. (B) The session data are divided into two half sessions.

0.075 mg/kg MK801, ANOVA yielded no significant interaction of session half and prepulse intensity.

2.4. Effect of MK801 and clozapine pretreatment on PPI in SD rats

MK801 effects on PPI and clozapine-pretreatment effects on MK801-induced PPI disruption were further tested in SD rats, with 0.1 mg/kg MK801 and 5 and 10 mg/kg clozapine. These were the same doses tested in WS rats in our study, as well as in the study of Bakshi et al. [4], which demonstrated clozapine's ability to antagonize MK801 (0.1 mg/kg)-induced PPI disruption in SD rats.

2.4.1. Startle response

Consistent with our recent report [36], SD rats showed a substantially lower mean startle amplitude (±S.E.M.) (165±9, $n=6$), compared to WS rats (850±38, $n=15$) across 12 pulse-alone trials, $t(19)=4.43$, $P<.001$. Consistent with a previous report of Bakshi et al. [4], we found that MK801 (0.1 mg/kg) treatment produced an increase in mean startle amplitude, which was blocked by clozapine pretreatment, $F(3, 20)=9.49$, $P<.001$. The mean startle amplitudes for different groups were: 165±9 for saline + saline; 404±60 for saline + MK801; 193±22 for clozapine (5 mg/kg) + MK801; and 183 + 30 for clozapine (10 mg/kg) + MK801.

2.4.2. Prepulse inhibition

Like WS rats, SD rats showed disrupted PPI after systemic MK801, compared to the saline + saline group. Pretreatment with either 5 mg or 10 mg/kg of clozapine failed to reverse MK801-induced PPI disruption (Fig. 4A). ANOVA yielded a significant main effect of group on %PPI, $F(3, 20)=5.62$, $P<.001$. Pair-wise comparisons showed no significant differences between clozapine-treated groups and the saline + MK801 group. Again, drug-treatment effects on PPI during the first and the second half of the session were comparable (Fig. 4B). There was no significant interaction of session half and group. We also analyzed the half session data for different prepulse intensities and found no significant interaction of session half and prepulse intensity.

2.5. Part 2. Effects of MK801 microinfusion into the ventral hippocampus on PPI in WS rats: comparison with the effects of systemic MK801

2.5.1. Intracerebral infusion sites

Histological analysis showed that the tips of the infusion cannulae were located within or around the

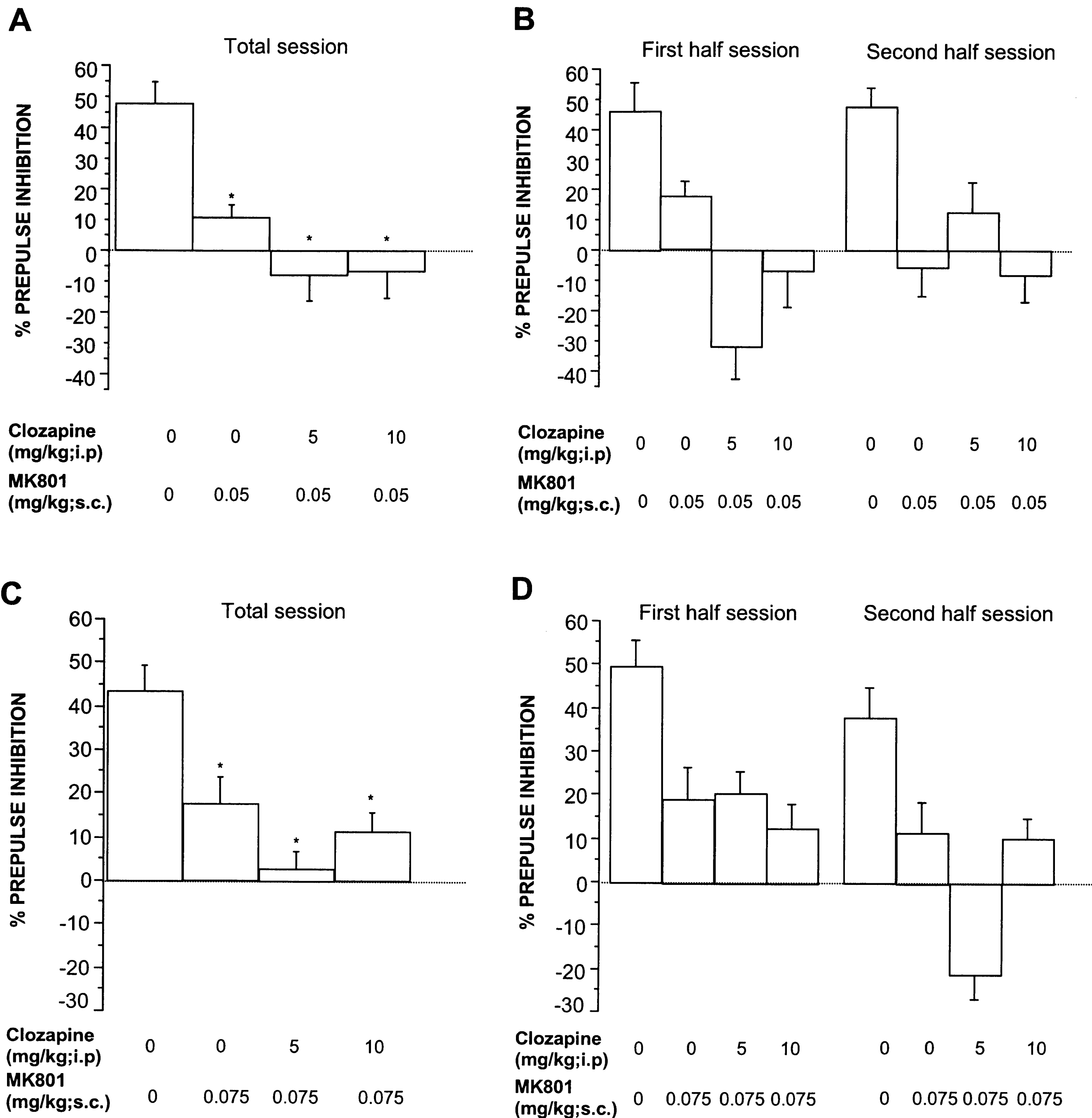

Fig. 3. Effects of pretreatment with clozapine (5 mg or 10 mg/kg, ip) on PPI disruption by MK801 (0.05 mg and 0.075 mg/kg, sc) in WS rats. The numbers on the Y axis represent the mean %PPI. The numbers on the X axis represent doses of clozapine and MK801. A dose of 0.0 mg/kg indicates the saline injection. Asterisks denote significant differences compared to the saline controls (saline + saline). Bars represent standard error of the mean. All groups: $n = 5$. Data from the group that received 0.05 mg/kg MK801 are shown in A and B: combined data from all prepulse intensities for the entire session (A); two half session data (B). Data from the group that received 0.075 mg/kg MK801 are shown in C and D: combined data from all prepulse intensities for the entire session (C); two half session data (D).

border of the ventral hippocampus in 10 cannulated rats (Fig. 5). In all cases, damage resulting from cannulae implantation was restricted to the area closely surrounding the guide cannulae.

2.5.2. Startle response

The mean startle amplitudes did not differ across the experimental groups, $F(2, 23) = 1.61$, $P > .05$, or across experimental days, $F(3, 69) = 0.49$, $P > .05$. There was a

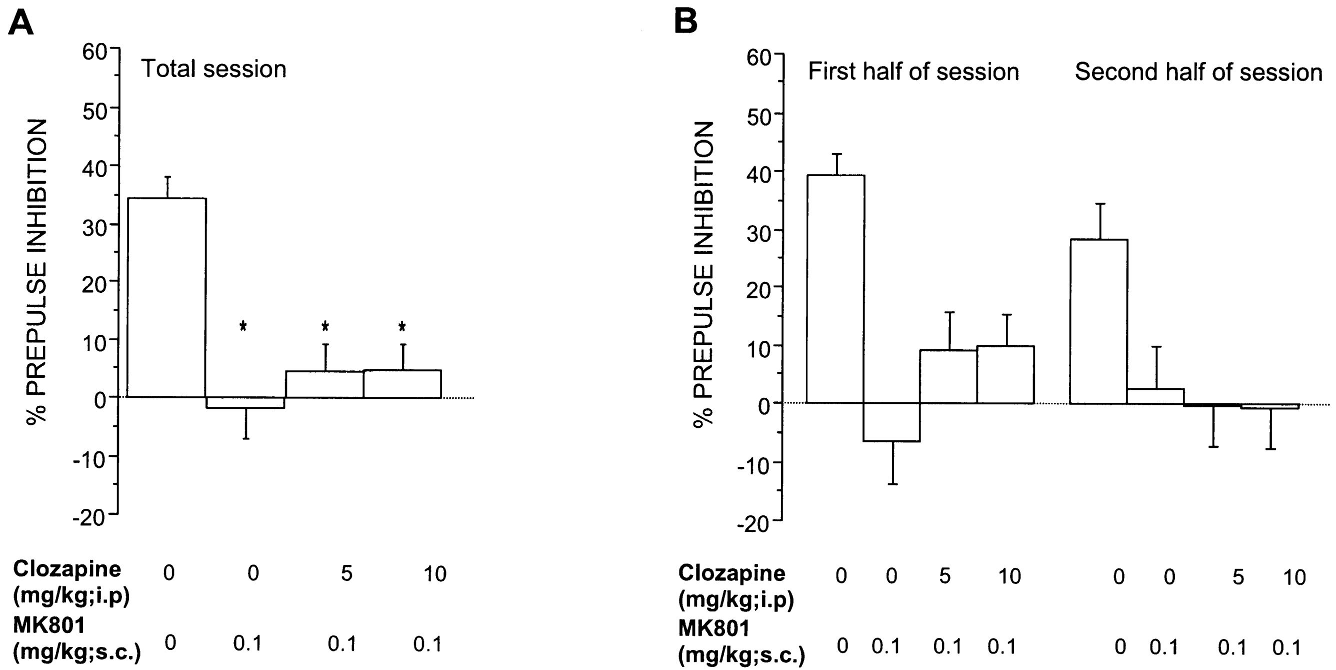

Fig. 4. Effects of pretreatment with clozapine (5 mg or 10 mg/kg, ip) on startle response and PPI disruption by MK801 (0.1 mg/kg, sc) in SD rats. The numbers on the *X* axis represent doses of clozapine and MK801. A dose of 0.0 mg/kg indicates the saline injection. Asterisks denote significant differences compared to the saline controls (saline + saline). Bars represent standard error of the mean. All groups: $n = 6$. (A) Combined PPI data from all prepulse intensities for the entire session. (B) Two half session data.

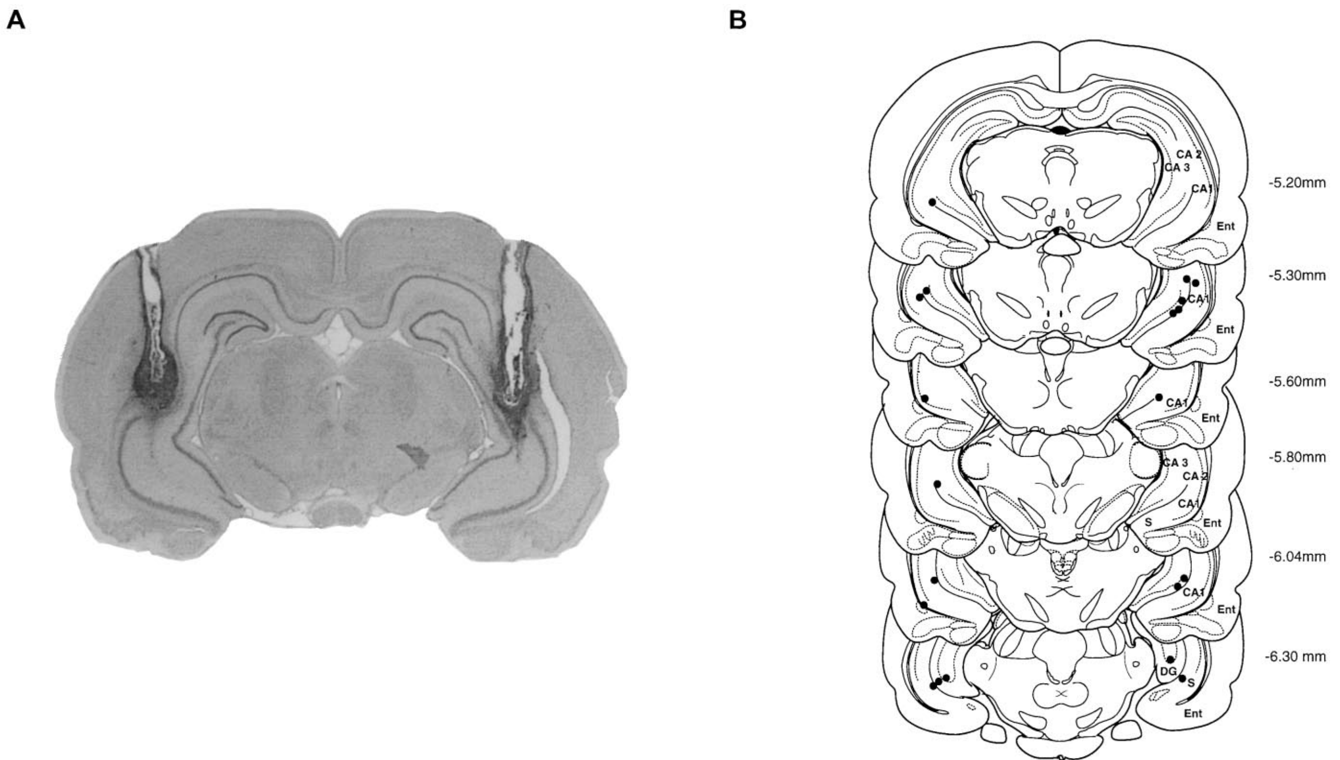

Fig. 5. Histological location of infusion sites in the ventral hippocampus of WS rats. (A) Photomicrographic image of a coronal brain section with the tracks of bilateral guide cannulae. (B) Estimated location of the injector tips. Numbers on each histological section represent the distance from bregma. CA1, CA2, and CA3, CA1, CA2, and CA3 field of the hippocampus; DG, dentate gyrus; Ent, entorhinal cortex; S, subiculum. Adapted from Paxinos and Watson [20].

significant main effect of pulse alone trials on startle amplitude, indicating habituation of the startle response over the 16 pulse presentations, $F(15, 345) = 27.0$, $P < .0001$.

2.5.3. Prepulse inhibition

ANOVA with the data of all 4 days yielded a highly significant effect of prepulse intensity on %PPI, $F(3, 23) = 122.29$, $P < .0001$, reflecting a gradual increase of PPI with higher prepulse intensities. There was no interaction between prepulse intensities and days ($P > .05$) or prepulse intensities and treatment groups ($P > .05$).

PPI was disrupted by systemic injection of MK801 but was not affected by bilateral MK801 infusion into the ventral hippocampus on day 3 (Fig. 6). While all three groups exhibited equal levels of PPI on the days without MK801 administration (days 1, 2, and 4), PPI at the three higher prepulse intensities was significantly disrupted on day 3 in the rats that received systemic application of MK801 (UNOP-MK801). The rats that received MK801 infusion into the ventral hippocampus (vHippo), however, exhibited the same intact PPI as the unoperated rats that received a saline injection (UNOP-Sal). The ANOVA with the data from all 4 days yielded a significant effect of day, $F(3, 23) = 7.345$, $P < .001$. Analysis of PPI at the three higher prepulse intensities on day 3 yielded a significant effect of group, $F(2, 23) = 4.11$, $P < .05$. Post hoc pair-wise comparisons revealed that PPI at the three higher prepulse intensities was significantly disrupted in the UNOP-MK801 group as compared to the UNOP-Sal and vHippo groups ($P < .05$ and $P < .05$, respectively), while there was no difference between the latter two groups ($P > .05$).

3. Discussion

3.1. Part 1. PPI disruption by systemic MK801 and effects of pretreatment with clozapine and haloperidol

3.1.1. Startle response

In the present study and in our previous report [36], the mean startle amplitude of WS rats was higher than that of SD rats. Such a strain difference in startle amplitude was also reported by others [33]. In the present study, the ranges of weights of the two strains were comparable. Thus, a higher startle response in WS rats compared with SD rats may reflect strain-related difference, rather than

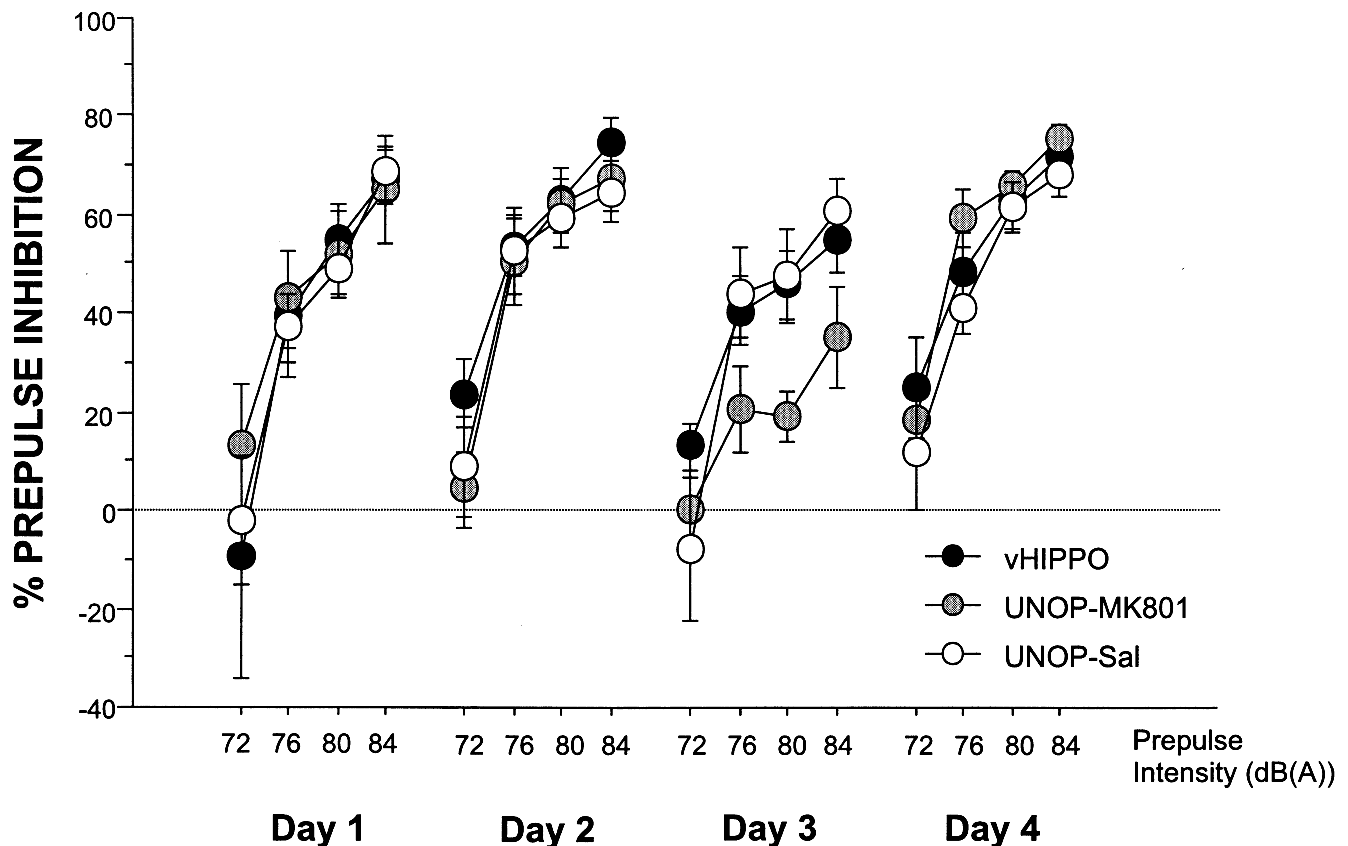

Fig. 6. Effects of bilateral MK801 infusion into the ventral hippocampus on PPI, compared with effects of systemic MK801 injection. Day 1 and Day 4: all groups received a PPI session without any treatment. Day 2: vHippo group ($n = 10$) received a bilateral vehicle infusion into the ventral hippocampus; both UNOP groups ($n = 8$, each group) received no treatment. Day 3: the vHippo group received a bilateral MK801 infusion (6.25 μg/0.5 μl/site); the UNOP-MK801 and UNOP-Sal groups received a systemic injection of MK801 (0.05 mg/kg) and saline, respectively.

variables such as weight. We also found strain-dependent drug effects on startle amplitude. Our results, consistent with findings of Bakshi et al. [4], showed that in SD rats MK801 (0.1 mg/kg) increased startle amplitude, and clozapine (5 mg/kg) reversed this increase. In WS rats, however, we found that MK801 had no consistent effect on mean startle. It is noteworthy that apomorphine (a dopamine agonist) increased the startle amplitude in SD rats but not in WS rats [15,33]. Thus, strain-dependent drug effects on the startle response appear to exist between these strains.

3.1.2. Prepulse inhibition

We found a robust disruption of PPI by MK801 in both WS and SD strains. This is consistent with previous studies in SD rats [1,4,8,11,13,14,18] and in WS rats [34]. In addition, we found a marked disruption of PPI following low doses of 0.05 and 0.075 mg/kg MK801 in WS rats. Our findings are in agreement with findings of Johansson et al. [13] who showed that 0.05 mg/kg MK801 (the lowest dose we used) disrupted PPI in SD rats. Our data are not in agreement with one report, showing that only a higher dose (0.1 mg/kg), but not a lower dose (0.075 mg/kg), of MK801 produced a significant PPI disruption in WS rats [34].

Neither clozapine nor haloperidol pretreatment restored MK801-induced PPI disruption in the present study. In WS rats, two doses of clozapine (5 and 10 mg/kg) consistently failed to antagonize PPI disruption induced by different doses of MK801 (0.05, 0.075, and 0.1 mg/kg). Similarly, in SD rats, clozapine (5 and 10 mg/kg) failed to reverse MK801 (0.1 mg/kg)-induced PPI disruption. Our results are in agreement with previous reports that 5 mg/kg of clozapine showed no effects on PPI disruption following 0.1 mg/kg MK801 in both SD [11] and WS rats [34]. Our results, however, are inconsistent with the findings of Bakshi et al. [4] that 5 mg/kg clozapine antagonized PPI disruption by 0.1 mg/kg MK801 in SD rats. Inconsistent results between different laboratories may be attributed to strain differences or to methodological differences between the different studies [11,15,27,31,34]. In our experiments, different strains were tested under identical conditions.

When our PPI data from WS rats were divided into two half sessions and analyzed for different intensities, we failed to observe the intensity-dependent or time-dependent clozapine effects in our results, involving either strain. In fact, we observed an opposite tendency, that is, a deterioration of PPI during the second half session in the clozapine-pretreated WS rats that received 0.075 mg/kg MK801. Considering that there was a minimal difference in methodology between Bakshi et al. [4] and the present study, we cannot rule out the possibility that the discrepancies in results may reflect subject variation due to differences between rat breeders or suppliers [31]. Nevertheless, given that the clozapine effect of MK801-induced PPI disruption reported by Bakshi et al. [4] was relatively mild, a parsimonious interpretation of our results is that the ability of clozapine to restore MK801-induced PPI disruption may not critically depend on strain differences.

Our finding that haloperidol (0.2 mg/kg) pretreatment failed to antagonize PPI disruption produced by 0.1 mg/kg MK801 in WS rats confirms and extends previous reports that 0.3 mg/kg haloperidol did not antagonize PPI disruption by 0.1 mg/kg MK801 in WS rats [34]. Similarly, pretreatment with either 0.1 mg or 0.5 mg/kg haloperidol was ineffective in antagonizing PPI disruption produced by 0.5 mg or 0.1 mg/kg MK801 in SD rats [11,14]. Taken together, these findings indicate that, unlike dopamine agonist-induced PPI disruption, which may be due to overstimulation of D2 receptors in the nucleus accumbens [17,29,39], MK801-induced PPI disruption may be mediated via a different mechanism. Nevertheless, the selective ability of atypical neuroleptics to restore PPI disruption by NMDA receptor antagonists and to serve as a tool in screening for possible atypical neuroleptic compounds [1,4,12,25,26] needs to be further examined.

3.2. Part 2. Effects of MK801 microinfusion into the ventral hippocampus on PPI in WS rats: comparison with the effects of systemic MK801

In contrast to a marked disruption of PPI with systemic MK801, bilateral infusion of a high dose of MK801 (6.25 μg/0.5 μl/site) into the ventral hippocampus did not affect PPI. Our finding in WS rats is in agreement with previous findings of Bakshi and Geyer [2] that infusion of the same dose of MK801 into the ventral hippocampus failed to affect PPI in SD rats. Though stimulation of NMDA receptor-mediated transmission in the ventral hippocampus disrupts PPI [16,35,40], blockade of NMDA transmission within the ventral hippocampus may not affect the expression of PPI.

Our results, however, do not rule out the possibility that the ventral hippocampus is involved in PPI disruption induced by systemic MK801. MK801 blocks NMDA receptors non-competitively in different brain regions, including the medial prefrontal cortex, nucleus accumbens, amygdala, and both the ventral and dorsal hippocampus [21,24], all of which are implicated in the regulation of PPI (for review, see Refs. [17,29]). Thus, it is conceivable that NMDA receptor blockade in multiple brain regions with systemic injection could affect several brain regions, and thereby act synergistically to yield a severe PPI disruption. By the same token, MK801 infusion into a specific brain region may not yield the marked changes in PPI shown after systemic injection. In fact, a recent report showed that bilateral MK801 infusions (6.25 μg/0.5 μl/site) into the amygdala [2] (see also Ref. [9]) or dorsal hippocampus produced a relatively weak, but significant PPI disruption, whereas infusions into the nucleus accumbens, ventral hippocampus, medial prefrontal cortex, or

dorsomedial thalamus had no significant effects on PPI [2]. The authors argued that a lack of infusion effect in these brain regions may have been due to the involvement of multiple limbic regions in PPI. Interestingly, results from our laboratory [38] indicate that bilateral infusions of MK801 into the dorsal hippocampus was not sufficient to disrupt PPI in WS rats. Thus, the recent view that blockade of NMDA receptors in multiple brain regions is involved in MK801-induced PPI disruption deserves further testing.

4. Conclusions

Systemic administration of MK801 yielded reliable results, with disruption of PPI in both WS and SD strains. In WS rats, the MK801-induced PPI disruption was not antagonized by pretreatment with either haloperidol or clozapine, a typical neuroleptic and an atypical neuroleptic, respectively. Similarly, in SD rats, clozapine failed to reverse MK801-induced PPI disruption. Our data do not indicate that strain difference contributes to a selective ability of clozapine to reverse MK801 effects on PPI. Although, at the present time, our data suggest that MK801-induced PPI disruption may not be a reliable tool with which to differentiate between typical and atypical neuroleptic compounds, our findings provide additional evidence that underlying mechanisms for NMDA receptor antagonist-induced PPI disruption could be essentially dopamine independent. Thus, given the construct validity of disrupted PPI for an animal model of schizophrenia [28], understanding of such a dopamine-independent mechanism may lead to new insights into treatment for the cases of schizophrenia which do not respond to any neuroleptic treatment and which constitute up to 40% of schizophrenic illnesses [23]. MK801 infusion into the ventral hippocampus did not affect PPI in the present study. The lack of effects on PPI following MK801 infusion in the ventral hippocampus, which expresses a considerably higher NMDA receptor density than other brain areas, suggests that severe PPI disruption with systemic MK801 may be due to additive effects resulting from blockade of NMDA receptors in multiple brain sites. Our view is in line with the notion that MK801-induced PPI disruption resembles schizophrenic pathology, which reflects abnormalities in several different limbic regions.

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