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# Clozapine's Effects on Phencyclidine-Induced Disruption of Prepulse Inhibition of the Acoustic Startle Response

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WILEY, J. L. *Clozapine's effects on phencyclidine-induced disruption of prepulse inhibition of the acoustic startle response.* PHARMACOL BIOCHEM BEHAV **49**(4) 1025–1028, 1994. – The reduction in magnitude of the startle reflex in response to a loud noise produced by prior presentation of a stimulus of lower intensity is known as prepulse inhibition (PPI). PPI may be disrupted by a variety of drugs, most notably by dopaminergic agonists such as apomorphine and by phencyclidine (PCP), and related noncompetitive *N*-methyl-D-aspartate (NMDA) antagonists. Apomorphine-induced disruption of PPI is antagonized by both typical and atypical neuroleptics. The present study examined the effects of the atypical neuroleptic, clozapine, alone and in combination with PCP, on PPI in rats. The results of previous studies suggest that disruption of PPI by PCP and similar drugs is not sensitive to antagonism by typical neuroleptics such as haloperidol. The results of the present study show that clozapine's effect on PCP-induced disruption of PPI is also limited. The failure of clinically effective antipsychotics of diverse chemical classes to block the effects of PCP on PPI of acoustic startle suggest that the effects of PCP in this procedure may represent a model of attentional deficits observed in treatment-resistant schizophrenia.

Clozapine Startle Phencyclidine

PREPULSE inhibition (PPI) is the phenomenon whereby exposure to a weak acoustic stimulus presented a few milliseconds before a sudden loud noise will decrease the magnitude of the resulting startle response (8). In humans, the degree of PPI is believed to reflect sensorimotor integration, including the ability to modify a motor response in response to preceding sensory stimuli. Indeed, subjects with acute schizophrenia exhibit less PPI than do subjects without psychiatric disorder, suggesting that schizophrenia may involve deficient capacity to gate or inhibit attention to incoming sensory information (3). In animals, a similar deficit can be produced by administration of dopamine agonists such as amphetamine and apomorphine or noncompetitive phencyclidine-like *N*-methyl-D-aspartate (NMDA) antagonists such as phencyclidine (PCP) or dizocilpine [for a review, see (6)]. On the basis of such similaries, at least one article has suggested that disruption of PPI of the acoustic startle response in rats may represent an animal model of attentional deficits observed in acute schizophrenia (4). Interestingly, PCP or high doses of amphetamine can produce subjective psychotomimetic effects in humans who abuse these drugs (1,5).

Previous studies have shown that neuroleptics used to treat schizophrenia can reverse the disruption of PPI produced by dopamine agonists with a relative potency corresponding to their clinical potency (14,16). This effect is believed to result from the dopamine-blocking properties of neuroleptics and has been observed with both typical neuroleptics (those that produce extrapyramidal motor side effects) and atypical neuroleptics (those that do not) (13,15,16). Less is known about the interaction of neuroleptics with the effects of PCP-like drugs on PPI. If PCP-induced disruption of PPI represents an animal model of schizophrenia [e.g., (4)], then one would expect that treatments that alleviate the clinical condition (e.g., neuroleptics) would be effective in the animal model. The results of such tests, thus far, have not been promising. The typical neuroleptic, haloperidol, has been tested as an antagonist of PCP-induced disruption of PPI, with negative results (9). Similarly, others (7) have shown that both haloperidol and the atypical neuroleptic, clozapine, fail to reverse the disruption of PPI produced by dizocilpine (MK-801). The present study examines the effects of clozapine against PCP-induced disruption of PPI. The purpose of the study is to

determine the generality of clozapine's interaction with PCP in this model under different laboratory conditions and experimental parameters.

## METHOD

### *Subjects*

Twelve adult male Sprague-Dawley rats (240–265 g), obtained from Charles River (Wilmington, MA), were weighed and handled daily for 2 weeks after delivery before testing began. The rats were individually housed in a temperature-controlled (20–22°C) environment with 12 L : 12 D cycle (lights on at 0700 h) and were transported to the laboratory for startle sessions. Water and standard rodent chow were freely available in the home cages.

### *Procedure*

The apparatus and general procedure have been described previously (10). Briefly, rats were transported to the laboratory and were injected with clozapine (0, 3, or 10 mg/kg, IP) 100 min before the startle session and with PCP (0 or 5 mg/kg, SC) 80 min presession. Rats were returned to their home cages after each injection. Five minutes before the start of the startle session, each rat was placed in a clear Plexiglas cylinder (8.2 cm diameter) which rested on a Plexiglas panel (10 × 20 cm) in the startle chamber (San Diego Instruments, San Diego, CA). Each of the three chambers was illuminated by a 15-watt houselight mounted in the ceiling above the cylinder. Acoustic stimuli were produced by a super tweeter, mounted 24 cm above the cylinder. A computer with SR-Lab software and interface (San Diego Instruments) was used to present stimuli and to record data.

After being placed in the chambers, rats were allowed a 5-min adaptation period, during which they were exposed to 69 dB background noise. This background noise continued throughout the session. Each startle session consisted of 31 trials (average intertrial interval = 45 s). During the first trial, rats were exposed to a 122 dB acoustic stimulus. Subsequent trials were divided into six types: 122 dB pulse alone (PA), 80 dB prepulse alone (PP), 69 dB background noise (NOSTIM), 80 dB prepulse with a duration of 3 ms (3M-PP), 10 ms (10M-PP), or 30 ms (30M-PP) followed by 122 dB pulse. A startle session was comprised of a first trial and five of each of the six other types of trials presented in mixed order. Startle pulse duration was held constant at 40 ms. A 160 ms delay was imposed between prepulse and pulse stimuli. During the course of the study, rats received each combination of the three pretreatments (VEH, clozapine 3 mg/kg or 10 mg/kg) and two treatments (SAL, PCP 5 mg/kg) for a total of six startle sessions. Order of presentation was determined by a randomized Latin square. Startle sessions were conducted twice weekly with at least 72 h between tests. The dose range for clozapine (0–10 mg/kg) was chosen based on previous research that showed that acute administration of doses at the lower end of the range did not decrease responding in operant procedures, whereas doses at the higher end produced suppression of operant responding (18,19).

### *Drugs*

PCP HCl (National Institute on Drug Abuse, Rockville, MD) was dissolved in 0.9% saline. Dosage of PCP refers to the salt. Clozapine (Sandoz, East Hanover, NJ) was prepared in a vehicle solution of lactic acid (5–10 drops) and distilled water. Clozapine doses refer to the free base. Both drugs were given at a volume of 1 ml/kg of body weight. PCP was administered subcutaneously; clozapine was injected intraperitoneally.

### *Statistical Analysis*

Startle score was defined as the average of 100 1-ms voltage readings. A 3 (pretreatment) × 2 (treatment) repeated measures analysis of variance (ANOVA) was performed on mean startle scores for pulse alone trials. Mean startle scores for NOSTIM and prepulse alone trials were less than 10 in every case and are not presented. Prepulse inhibition (PPI) was calculated for prepulse plus pulse trials as a percentage of pulse alone scores [(startle score for pulse alone trial − startle score for pulse following prepulse)/startle score for pulse alone trial] × 100. A 3 (pretreatment) × 2 (treatment) × 3 (duration of prepulse) repeated measures ANOVA was performed on mean %PPI. Tukey post hoc tests ($\alpha = 0.05$) were used to analyze differences revealed by ANOVAs. All data were analyzed with general linear model procedures (SAS Institute, Cary, NC).

## RESULTS

Figure 1 shows mean startle scores (± SEM) for pulse alone trials as a function of clozapine and PCP doses. A significant interaction between clozapine and PCP was not observed, $F(2, 22) = 0.52$, $p = 0.60$; hence, comparisons among the individual cell means (i.e., single bars on Fig. 1) were inappropriate. On the other hand, post hoc analyses of the main effects for clozapine, $F(2, 22) = 9.72$, $p = 0.0009$, and PCP, $F(1, 11) = 13.36$, $p = 0.004$, revealed that 10 mg/kg clozapine and 5 mg/kg PCP independently decreased startle scores during pulse alone trials compared to their respective vehicle conditions. The 3 mg/kg dose of clozapine did not change mean pulse alone startle scores.

Figure 2 shows mean %PPI (± SEM) as a function of clozapine dose, PCP dose, and prepulse duration. ANOVA revealed main effects for clozapine, $F(2, 22) = 15.44$, $p = 0.0001$, and prepulse duration, $F(2, 22) = 19.61$, $p = 0.0001$, and a significant clozapine × PCP interaction, $F(2, 22) =$

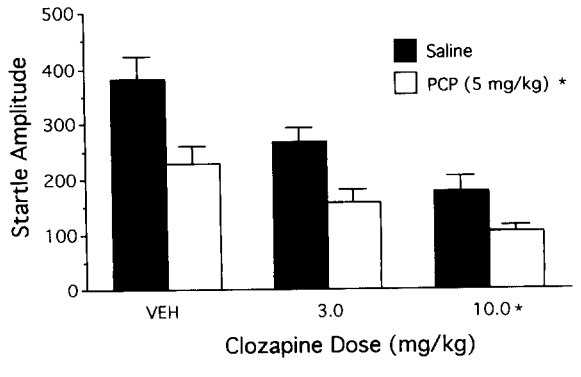


FIG. 1. Mean startle amplitude scores (± SEM) are shown for pulse alone trials following injection with clozapine (0, 3, or 10 mg/kg) and PCP (0 or 5 mg/kg). Because the clozapine × PCP interaction was not statistically significant, comparisons among the individual cell means (i.e., single bars) were inappropriate; hence, an asterisk indicates a significant ($p < 0.05$) main effect of a drug dose (collapsed across doses of the other drug) compared to the respective vehicle.

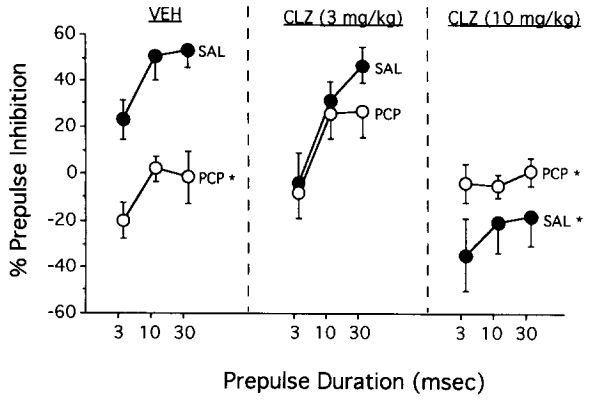


FIG. 2. Mean percentage of prepulse inhibition (± SEM) are shown for pulse + prepulse trials as a function of pretreatment (clozapine dose), treatment (PCP or saline), and prepulse duration. Positive numbers indicate a higher degree of prepulse inhibition than do negative numbers. Significant differences revealed by analysis of the clozapine × PCP interaction are indicated by asterisks. An asterisk indicates that mean %PPI (collapsed across prepulse duration) is significantly different from the mean %PPI following administration of VEH + SAL ($p < 0.05$).

7.21, $p = 0.004$. None of the other main effects or interactions were significant, although the main effect for PCP approached significance, $F(1, 11) = 4.55$, $p = 0.056$. Prepulse durations of 3 ms significantly decreased %PPI compared to longer durations of 10 or 30 ms. Because the clozapine × PCP interaction was significant, the results of post hoc analysis of the clozapine main effect are not reported. Post hoc analysis of the clozapine × PCP interaction showed decreases in %PPI (compared to VEH + SAL) for rats that received VEH + PCP or clozapine (10 mg/kg) with either dose of PCP (0 or 5 mg/kg). The magnitude of decreases produced by PCP and clozapine (10 mg/kg) was not significantly different. The decreases in %PPI produced by clozapine were only statistically significant when compared to the VEH + SAL condition; %PPI following injection with 10 mg/kg clozapine and 5 mg/kg PCP did not differ from responding after administration of the vehicle and PCP or the clozapine and saline combinations.

## DISCUSSION

As expected, PCP disrupted PPI. This effect is consistent with previous studies that have found that noncompetitive PCP-like NMDA antagonists, but not competitive NMDA antagonists, disrupt PPI of the acoustic startle response (10,11). Visual inspection of the present data (left panel, Fig. 2) shows that PCP's effect on %PPI was observed at all three prepulse durations.

The effects of clozapine alone on %PPI were dose dependent. Although the 3 mg/kg dose of clozapine did not change the degree of PPI (compared to %PPI following vehicle administration), the 10 mg/kg dose of clozapine reduced %PPI. This dose of clozapine also decreased the startle amplitude during pulse alone trials. Although the method of calculating %PPI in the present study was designed to reduce problems with differences in magnitude of startle reactivity during pulse alone trials [see (15)], it does not entirely eliminate these problems. It is possible that the present results reflect a floor effect in which responding during pulse alone trials was so low that only a small absolute increase in startle amplitude during pulse + prepulse trials was necessary to produce a large decrease in %PPI. Swerdlow et al. (16) observed a similar independent decrease in PPI following injection with high doses of clozapine. In contrast, other researchers have reported that clozapine independently enhanced PPI (7,15). Because enhancement of PPI by clozapine is opposite that produced by schizophrenia, the disorder it is used to treat this effect has been suggested to represent a screening procedure for testing novel antipsychotic drugs that does not depend upon their antagonism of the actions of dopaminergic agonists (7,15). The results of the present study suggest that facilitation of PPI is not a reliable effect of clozapine and may depend upon the parameters of the acoustic startle procedure [see also (12)]. The present study differed from the previously mentioned studies in the variable (vs. standard) duration of prepulse stimuli and the absolute differences between pulse and prepulse stimulus intensities that enhanced (7,15) vs. inhibited (present study) %PPI. In addition, preinjection intervals for clozapine and PCP were considerably longer in the present study.

Although clozapine did not significantly change the effect of PCP on %PPI, the increase in %PPI produced by 3 mg/kg clozapine in combination with 5 mg/kg PCP represents an attenuation of the effects of 5 mg/kg PCP alone on %PPI. This apparent increase is created by the combination of a slight increase in %PPI following injection with 3 mg/kg clozapine and PCP and a slight decrease in %PPI after administration of 3 mg/kg clozapine and saline. Thus, although the results of the present study suggest that this dose of clozapine may have a tendency to reverse PCP's disruption of PPI, the effect is not robust and is accompanied by a tendency toward decreases in startle amplitude during pulse alone trials.

A preliminary report of another study has shown that clozapine reversed PCP-induced disruption of PPI (2). This effect was not dose dependent and was observed at a single dose of clozapine (5 mg/kg). Further tests with dopamine and serotonin antagonists suggested that clozapine's effect was not mediated via $D_1$, $D_2$, or 5-$HT_2$ receptors, suggesting that clozapine's reversal of PCP's disruption of PPI in this study may involve complex actions at several receptor sites (2).

The results of the present study suggest that clozapine's reversal of PCP-induced disruption of PPI is not robust effect. In clinical practice, the antipsychotic effect of clozapine typically is observed only after repeated administration; hence, it is possible that chronic dosing with clozapine would enhance its effect on PCP-induced disruption of PPI. Alternatively, the effects of PCP on PPI may represent a model of treatment-resistant schizophrenia, in that the majority of the research with this model [(7,9,15), results of present study] suggest that it is not sensitive to the effects of clinically effective antipsychotic drugs. The schizophrenic-like state produced by PCP in humans may represent a subtype of psychosis with distinct etiology and pharmacology.

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