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Original article

An Investigation of the Behavioral Mechanisms of Antipsychotic Action Using a Drug-Drug Conditioning Paradigm

Ming Li (mli2@unl.edu), Wei He and Alexa Mead

Antipsychotic drugs at noncataleptic doses selectively suppress conditioned avoidance response in rats. In our previous study, we had used a two-way active avoidance response paradigm to show that the antipsychotic-induced interoceptive state is one of the mechanisms underlying the avoidance-disruptive effect of antipsychotics. In this study, we sought to further examine this mechanism using a novel drug-drug conditioning procedure. We made use of the fact that both the typical neuroleptic haloperidol and the atypical neuroleptic olanzapine disrupt conditioned avoidance responding, whereas chlordiazepoxide (an anxiolytic) does not. We reasoned that if the antipsychotic interoceptive state is important in causing a disruption on avoidance responding (an index of antipsychotic efficacy), pairing chlordiazepoxide (a cueing drug conditional stimulus) with haloperidol or olanzapine (a cued drug unconditional stimulus) should engender chlordiazepoxide to exhibit this property and behave like an antipsychotic drug. Chlordiazepoxide exhibited an acquired antipsychotic-like property in disrupting avoidance responding after being repeatedly paired with haloperidol, but not with olanzapine. In contrast, it significantly attenuated the antiavoidance efficacy of olanzapine but not haloperidol after being repeatedly paired with these drugs. This study suggests that the haloperidol-induced interoceptive drug state is directly involved in its antiavoidance action, and chlordiazepoxide may attenuate the antiavoidance efficacy of antipsychotics (especially olanzapine). To the extent that the antiavoidance effect predicts clinical effects of antipsychotic treatment, this study suggests that the antipsychotic-induced interoceptive drug state may be an important behavioral mechanism mediating the clinical effects of antipsychotic treatments.

Keywords: antipsychotic drugs, chlordiazepoxide, conditioned avoidance response, drug-drug conditioning, polypharmacy, psychotherapeutic drug-drug interaction, rat, schizophrenia

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Introduction

Antipsychotic drugs (APDs) are effective in the treatment of positive symptoms of schizophrenia (Lieberman *et al.*, 2005). Research over the years has shown that actions at various receptor sites, notably dopamine D₂, serotonin 5-HT_{2A}, and 5-HT_{1A} receptors, are critically important for the therapeutic effect of both typical and atypical drugs (Seeman, 2000; Kapur and Mamo, 2003; Richtand *et al.*, 2007). It is still not well understood how this action at the neurobiological level translates into symptom improvement. This situation is peculiar given the fact that schizophrenia is a cluster of psychological symptoms, and the diagnosis and symptom improvement all manifests at the psychological level. The neurobiological level of explanations of antipsychotic action alone is clearly insufficient to account for the clinical effect of antipsychotic action (Miller, 1987; Kapur, 2003). To understand fully how antipsychotics work, a detailed understanding of the behavioral mechanisms of antipsychotic action is needed.

We recently took a preclinical approach and investigated this issue using a well-established preclinical animal model of antipsychotics-conditioned avoidance response (CAR) model (Li *et al.*, 2007, 2009; Mead and Li, in press). All currently available antipsychotics, at clinically relevant doses, selectively disrupt avoidance responding to a conditional stimulus (CS, *e.g.* white noise) without altering escape responding to an unconditional stimulus (US, *e.g.* footshock) (Arnt, 1982; Wadenberg *et al.*, 2001; Natesan *et al.*, 2006). Thus, an antiavoidance responding effect is frequently used as a validated behavioral index of 'antipsychotic' property. Using this model, we found that rats treated with haloperidol (HAL), risperidone (RIS) and olanzapine (OLZ) daily for 7 consecutive days showed a progressive across-session decline in avoidance responding, suggesting that antipsychotics may progressively attenuate the motivational salience of the CS (Wise, 2004; Li *et al.*, 2009). We also found that rats previously treated with HAL and OLZ, and retested under the same dose of drugs after their avoidances re-

covered to the predrug level, showed fewer avoidances than when they were first tested (Mead and Li, in press). This finding indicates that the interoceptive drug state induced by HAL may also play a role in causing a progressive decline in avoidance across sessions. On the basis of these findings, we proposed that antipsychotics may suppress avoidance responding through a dual action: (i) decreasing the motivational salience of stimuli; (ii) providing an interoceptive drug cue that allows the decreased salience of stimuli to be maintained over time.

In contrast to the well-documented salience attenuation effect of antipsychotics (Fouriez *et al.*, 1978; Berridge and Robinson, 1998; Dickinson *et al.*, 2000; Wise, 2004; Colpaert *et al.*, 2007), the notion that the drug-induced interoceptive state(s) may be involved in the antipsychotic effects is relatively new, although preclinical studies such as those based on drug discrimination and state-dependent learning have long recognized the distinct drug states induced by typical and atypical antipsychotics (Overton, 1979; Goudie *et al.*, 1998; Porter *et al.*, 2000b; Porter *et al.*, 2005). The primary goal of this study was thus to examine this mechanism further, using a novel drug-drug conditioning paradigm in the CAR model. In this study, we utilized the fact that both HAL (typical APD) and OLZ (atypical APD) disrupt conditioned avoidance responding, whereas chlordiazepoxide (CDP) does not (Sanger, 1985; Li *et al.*, 2004, 2007). If the antipsychotic-induced state is directly involved and critically important in causing a disruption on avoidance responding, pairing CDP with HAL or OLZ through a drug-drug conditioning procedure (Revusky *et al.*, 1989; Taukulis, 1996) should alter the intrinsic property of CDP and engender it to show a disruptive effect on avoidance responding. In Pavlovian terminology, the CDP was considered a CS drug, which signals to an organism that the effects of HAL or OLZ (US) are imminent. Any avoidance-disruptive effect exhibited by CDP after repeated pairing with HAL or OLZ would support the notion that antipsychotic-induced interoceptive state is 'directly' involved in the antipsychotic effect.

As psychotic fear and anxiety disturbances are seen at a relatively high frequency in patients with schizophrenia (Siris, 1994), anxiolytic drugs are frequently combined with antipsychotics in schizophrenic patients (ZumBrunnen and Jann, 1998). This practice of psychotropic polypharmacy has raised some concerns regarding the possible adverse effects of drug-drug interactions (Sandson *et al.*, 2005). This study also allowed us to examine how the repeated pairings of CDP with HAL and OLZ might alter the intrinsic drug efficacy of HAL and OLZ.

Methods

Subjects

Male Sprague-Dawley rats (226-250 g upon arrival, Charles River, Portage, Michigan, USA) were housed two

per cage, in 48.3 × 6.7 × 20.3 cm transparent polycarbonate cages under 12-h light/dark conditions (light on between 6:30 am and 6:30 pm). Room temperature was maintained at 21 ± 11°C with a relative humidity of 55-60%. Food and water was freely available. Animals were allowed at least 1 week of habituation to the animal facility before being used in experiments. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

Avoidance conditioning apparatus

Six identical two-way shuttle boxes, custom designed and manufactured by Med Associates (St. Albans, Vermont, USA), were used. Each box was housed in a ventilated, sound-insulated isolation cubicle (96.52 cm wide × 35.56 cm deep × 63.5 cm high). Each box was 64 cm long, 30 cm high (from grid floor) and 24 cm wide, and divided into two equal-sized compartments by a white polyvinyl chloride partition with an arch-style doorway (15 cm high × 9 cm wide at base). An aluminum hurdle (4 cm high) was placed between the two compartments, so the rats had to jump from one compartment to enter the other. The grid floor consisted of 40 stainless-steel rods, spaced 1.6 cm apart center to center, through which a scrambled footshock (0.8 mA) was delivered by a constant current shock generator (Model ENV-410B) and scrambler (Model ENV-412). The location of the rat and motor activity were detected by a set of 16 photobeams (ENV-256-8P) affixed at the bottom of the box (3.5 cm above the grid floor). A speaker (ENV 224AMX) mounted on the ceiling of the cubicle, centered above the shuttle box, was used to provide a CS (76 dB white noise). All the training and testing procedures were controlled by Med Associates programs running on a computer. Background noise (approximately 74 dB) was provided by a ventilation fan affixed at the top corner of each isolation cubicle.

Drugs

The injection solutions of HAL (5 mg/ml ampoules, Saxe Inc., Boucheville, Quebec, Canada) and CDP (Sigma-Aldrich, St Louis, Missouri, USA) were obtained by mixing drugs with sterile water. OLZ (Toronto Research Chemical Inc., Canada) was dissolved in 1.5% glacial acetic acid in distilled water. HAL and OLZ were administered s.c., whereas CDP was administered intraperitoneally. The doses of HAL (0.05 mg/kg) and OLZ (1.0 mg/kg) and their injection route were chosen based on (i) our previous work showing that at the chosen doses, HAL and OLZ injected s.c. produce a comparable progressive across-session decline in avoidance responding (Li *et al.*, 2007); and (ii) rat brain D₂ receptor occupancy data showing that both drugs give rise to clinically comparable levels of D₂ occupancy (65-80%) (Kapur *et al.*, 2003). The choice of CDP dose (10 mg/kg) and its route of injection was based on the findings showing that (i) CDP (10 mg/kg) is ineffective in disrupting avoidance responding (Li *et al.*, 2004, 2007); (ii) CDP at this dose is effective in several aversively conditioned paradigms, such as Pavlov-

ian fear conditioning and passive avoidance responding (Klint, 1991; Joordens *et al.*, 1998); and (iii) at this dose, CDP produces a powerful internal drug cue (Colpaert, 1986).

Experiment 1: effects of repeated chlordiazepoxide and haloperidol pairing on avoidance responding to chlordiazepoxide and haloperidol

The experiment comprised of the following three phases: avoidance training, drug-drug conditioning, and drug testing.

Avoidance training phase

Forty-two rats were first handled and habituated to the avoidance conditioning apparatus for 2 days (30 min/day), and then trained for 10 consecutive days to acquire robust conditioned avoidance responding (> 70% avoidance trials). Each training session consisted of 30 discrete trials. Every trial was started by presenting white noise (CS, 76 dB) for 10 s, followed by a continuous footshock (US, 0.8 mA, maximum 5 s) on the grid floor. If a subject moved from one compartment into the other within the 10 s of CS presentation, the shock was prevented, and this shuttling response was recorded as 'avoidance' (a two-way avoidance). If the rat remained in the same compartment for more than 10 s and made a crossing upon receiving the footshock, this response was recorded as 'escape.' If the rat did not respond during the entire 5 s presentation of the shock, the trial was terminated and 'escape failure' was recorded. Intertrial intervals varied randomly between 30 and 60 s.

Drug conditioning phase

At the end of the training phase, rats ($n = 32$) that had reached the training criterion ($\geq 70\%$ avoidance in each of the last two sessions) were used in the drug conditioning phase. They were randomly assigned to one of four groups. On day 1, each group was given a double injection of one of the following combinations: CDP + vehicle (VEH) ($n = 8$), VEH + HAL ($n = 7$), CDP+HAL ($n = 7$), and VEH + VEH ($n = 10$). The first injection (CDP 10.0 mg/kg, or sterile water, 1.0 ml/kg, i.p.) was given 15 min before the second injection (HAL 0.05 mg/kg, or sterile water, 1.0 ml/kg, s.c.). One hour after the second injection, rats were placed in the avoidance conditioning boxes and tested. This time interval between CDP and HAL (15 min) was determined so that there was sufficient time for the drug effects of HAL and CDP to overlap and produce a robust drug-drug conditioning effect, given the half-life of CDP at 4-6 h (Koechlin *et al.*, 1965) and HAL at about 1.5 h (Cheng and Paalzow, 1992). A similar kind of drug-drug conditioning arrangement had been used by Taukulis and Brake (1989). On day 2, rats in the CDP + VEH, VEH + HAL, and CDP + HAL groups received a single injection of HAL, CDP, and VEH, respectively, whereas the Behavioral mechanisms of antipsychotic action Li *et al.* 3 VEH + VEH group received a double injection of CDP and HAL separated by 15 min. Immediately after the injections, rats were returned to their home cages. No avoidance test was done on this day. The pur-

pose of giving rats the drug treatments on day 2 and not testing them was to ensure that every rat received the same drug treatment (all rats had CDP, HAL, and VEH), although in different contexts (*e.g.* home cage vs. CAR boxes) and with different drug injection intervals (15 min vs. 24 h), so that the specific drug-drug conditioning effect on avoidance behavior could be assessed. On day 3, all rats were untreated and unhandled. This 3-day drug conditioning cycle repeated for seven times over a 21-day period, after which all rats were re-trained drug-free in two consecutive sessions to bring back a high level of avoidance responding.

Drug-testing phase

The drug-testing phase started 24 h after the last retraining session. Rats were first injected with CDP (10.0 mg/kg, i.p.) and tested 75 min later. The next day, rats were retrained drug-free, and 1 day later, tested again under HAL [0.05 mg/kg, subcutaneously (s.c.), - 60 min]. For both drug tests, the same conditioned avoidance procedure was used except that only the CS was presented in the 30 trials. No shock US was ever presented. The following figure illustrates the general experimental procedure (Figure 1).

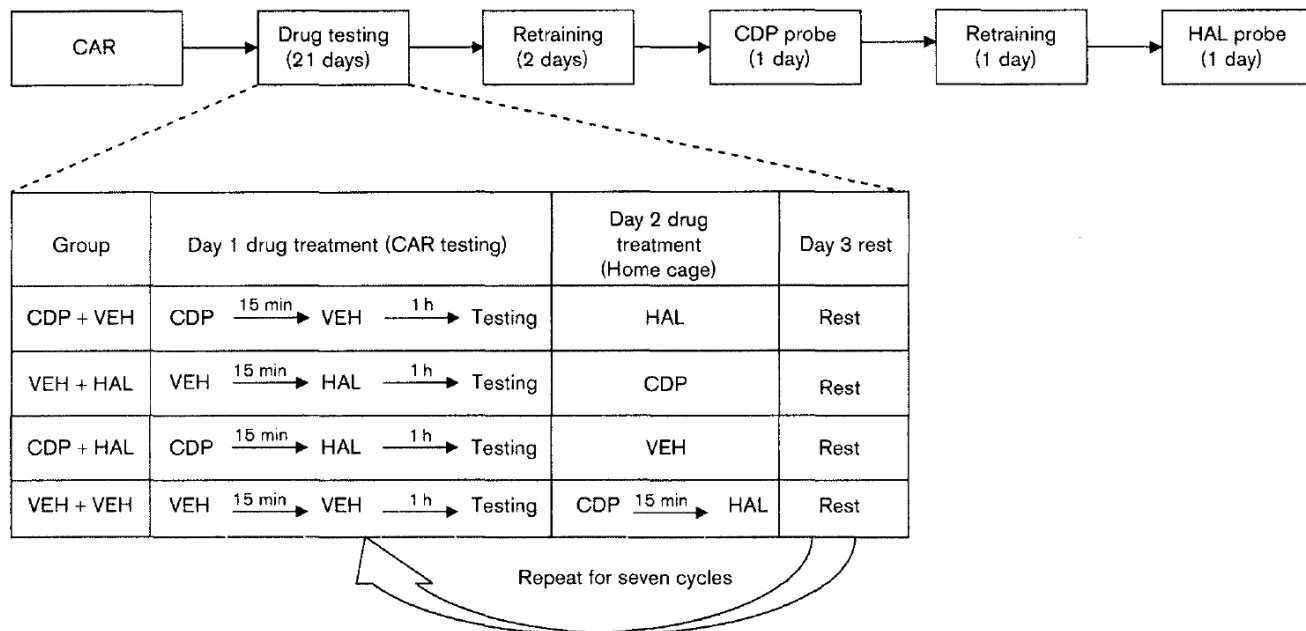
Experiment 2: effects of repeated chlordiazepoxide and olanzapine pairing on avoidance responding to chlordiazepoxide and olanzapine

This experiment was identical to experiment 1 except that HAL was replaced by OLZ. Forty-two rats were used, of which 30 reached learning criterion. They were then randomly assigned to one of the following four groups: CDP + VEH ($n = 6$), VEH + OLZ ($n = 8$), CDP+OLZ ($n = 9$), and VEH + VEH ($n = 7$), and were subjected to the seven sessions of drug conditioning and two sessions of drug testing (the CDP test followed by the OLZ test).

Experiment 3: reexamining the effects of repeated chlordiazepoxide and haloperidol pairing on avoidance responding to chlordiazepoxide and haloperidol

As the CDP + HAL (experiment 1) and CDP + OLZ pairing (experiment 2) produced different results, we re-examined the effects of repeated CDP and HAL pairing on avoidance responding to CDP and HAL. Twenty rats that had experienced the same white noise and footshock in a Pavlovian fear-conditioning paradigm were used. It should be noted that none of the rats were ever exposed to any drug before this experiment and that there was at least a 2-week window between the previous experiment and this one (unpublished experiment). The basic procedure was the same as the one used in the previous two experiments. First, all rats were trained in 10 avoidance conditioning sessions. At the end of the training Figure 2 phase, 15 rats reached the learning criterion and were randomly assigned to two groups: CDP + HAL ($n = 8$) and VEH+HAL ($n = 7$). They were then subjected to the seven sessions of drug conditioning and two sessions of drug testing (the CDP test followed by the HAL test).

Fig. 1



A schematic depiction of the experimental procedure in experiment 1. CAR, conditioned avoidance response; CDP, chlordiazepoxide; HAL, haloperidol; VEH, vehicle.

Statistics

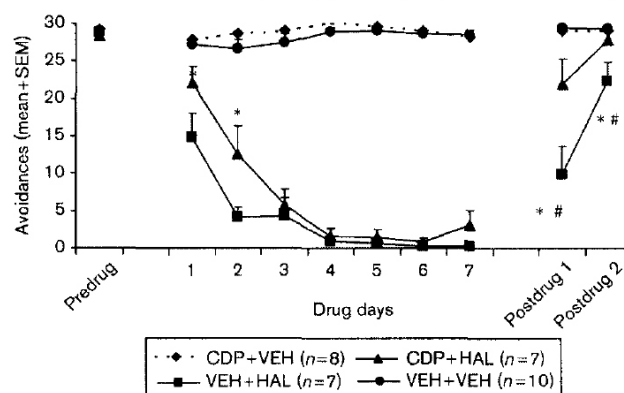
The main dependent variable was the number of avoidance responses (expressed as mean ± SEM). Data from the drug-drug conditioning phase were first analyzed using a repeated-measures analysis of variance (ANOVA) with treatments (i.e. four groups) as a between-subjects factor and the test sessions (i.e. seven drug sessions) as a within-subjects factor, followed by post-hoc Tukey's honestly significant difference test to detect the group differences. If a significant group difference was detected, one-way ANOVAs were then used to specify the difference for each drug session. Data from the drug-testing phase were analyzed separately using one-way ANOVAs, followed by Tukey's test. A conventional two-tailed level of significance at the 5% level was required.

Results

Experiment 1: effects of repeated chlordiazepoxide and haloperidol pairing on avoidance responding to chlordiazepoxide and haloperidol

Figure 2 shows the number of avoidance responses made by the rats in the four groups during the seven

Fig. 2



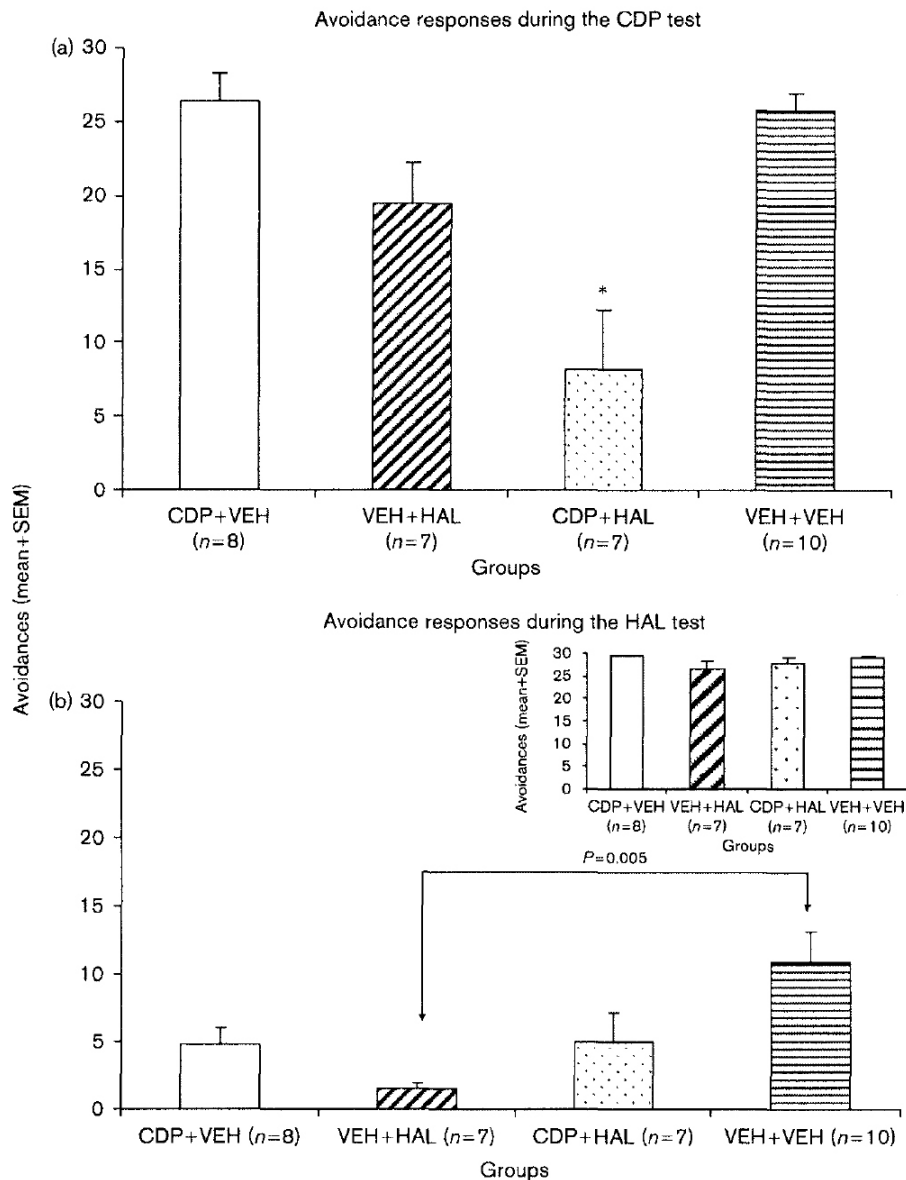
Number of avoidance responses made by the rats in the four groups during the seven drug conditioning sessions and two drug-free retraining sessions from experiment 1. Repeated haloperidol (HAL) treatment significantly disrupted avoidance responding across the seven daily test sessions. This effect was attenuated by chlordiazepoxide (CDP) in the first two sessions. Rats that received the combination of CDP and HAL treatment during the conditioning phase recovered faster than those that only received HAL when the treatment was stopped. Repeated CDP treatment by itself had no effect on avoidance responding. * $P < 0.05$ for comparisons between the two HAL groups. # $P < 0.05$ for comparisons between the vehicle (VEH) + HAL and the VEH + VEH group.

drug conditioning sessions and two drug-free retraining sessions. The two HAL groups (e.g. the CDP + HAL and VEH + HAL) showed a progressive across-session decrease in avoidance responding under drug and a quick

recovery when the treatment was stopped. The other two groups (e.g. the CDP + VEH and VEH + VEH) maintained a high level of avoidance responding throughout the entire drug conditioning phase. For the seven drug conditioning sessions, a two-way ANOVA ('treatments' \times 'sessions') showed a significant effect of 'treatments' [$F_{(3,28)} = 322.85, P < 0.001$], 'sessions' [$F_{(6,168)} = 28.04, P < 0.001$], and a significant 'treatments' \times 'sessions' interaction [$F_{(18,168)} = 15.173, P < 0.001$]. Post-hoc Tukey tests revealed that the CDP + HAL and VEH + HAL groups were significantly

different from the CDP + VEH and VEH + VEH groups (all P values < 0.001). Interestingly, the CDP + HAL group also differed significantly from the VEH + HAL group ($P = 0.045$). Individual one-way ANOVA revealed that on the first two drug conditioning days, the CDP + HAL rats displayed higher numbers of avoidance responses than the rats in the VEH + HAL group (day 1: $P < 0.05$; day 2: $P < 0.05$), indicating that CDP may have attenuated the HAL effect, at least at the early stage of paired drug treatment. This attenuation was also reflected in the two

Fig. 3



Number of avoidance responses during the chlordiazepoxide (CDP) test (a) and haloperidol (HAL) test (b) from experiment 1. (a) CDP significantly inhibited avoidance responding in the CDP + HAL group during the CDP test ($*P < 0.05$ for post-hoc group comparisons with other three groups). (b) HAL significantly inhibited avoidance responding in all four groups, but to a lesser extent in the vehicle (VEH) + VEH group. The graph in (b) depicts the avoidance responses during the retraining session between the two drug tests. No significant group difference was detected during this session.

subsequent retraining sessions, as the CDP + HAL group reinstated avoidance responding much faster than the VEH + HAL group (CDP + HAL vs. VEH + HAL: day 1: $P < 0.01$; day 2: $P < 0.05$). Furthermore, the CDP + HAL group showed no significant difference when compared with the CDP + VEH and VEH + VEH groups (all P values > 0.09), whereas the VEH + HAL did (all P values < 0.01).

Figure 3a shows the number of avoidance responses during the CDP test. Both the CDP + VEH and VEH + VEH groups exhibited a high level of avoidance responding (the average was 26.4 and 25.7 avoidances, respectively), as did the VEH + HAL group (mean number: 19.4), even though there was no shock (only white noise) present during this test. This finding was consistent with the data from the drug conditioning phase, showing that CDP itself has no effect on avoidance responding. In contrast, the CDP + HAL group exhibited the lowest avoidance responses (8.1). One-way ANOVA revealed that the CDP + HAL group was significantly different from the other three groups (all P values < 0.025), strongly suggesting that CDP produced a significant inhibition of avoidance responding. In other words, CDP 'acquires' a HAL-like property (e.g. disrupting avoidance responding) after being repeatedly paired with HAL. Prior CDP + HAL pairing in the absence of avoidance testing (e.g. the VEH + VEH group) did not change its property.

After a retraining session [pre-HAL session: no group difference was detected, $F_{(3,28)} = 2.16$, $P = 0.12$, Figure 3b], all rats were tested for their avoidance responses under HAL in a CS-only session (Figure 3b). In comparison with the pre-HAL session, avoidance responding was apparently lower in all groups. A 4×2 repeated-measures ANOVA ('treatment' \times 'sessions') showed a significant effect of 'treatment' [$F_{(3,28)} = 5.34$, $P < 0.005$], 'sessions' [$F_{(1,28)} = 640.48$, $P < 0.001$], and a significant 'treatment' \times 'sessions' interaction [$F_{(3,28)} = 3.45$, $P < 0.05$]. One-way ANOVA focusing on the HAL test session revealed no significant group difference except between the VEH + HAL and VEH + VEH groups ($P < 0.005$), indicating a strong HAL experience effect consistent with our previous finding (Li *et al.*, 2007). More importantly, no significant group difference was detected between the VEH + HAL and CDP + HAL groups, indicating that the efficacy of HAL had not been altered even after being repeatedly paired with CDP.

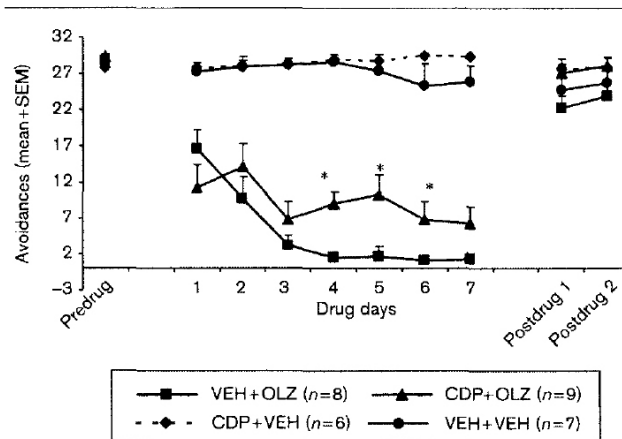
Experiment 2: effects of repeated chlordiazepoxide and olanzapine pairing on avoidance responding to chlordiazepoxide and olanzapine

Figure 4 shows the number of avoidance responses made by the rats in the four groups during the seven drug conditioning sessions and two drug-free retraining sessions. Similar to what was seen in experiment 1, the two OLZ-treated groups (e.g. the CDP + OLZ and VEH + OLZ) showed a progressive across-session decrease in

avoidance responding under drug and a quick recovery when OLZ treatment was stopped. The other two groups (e.g. the CDP + VEH and VEH + VEH) maintained a high level of avoidance responding throughout the entire drug conditioning phase. For the seven drug conditioning sessions, a two-way ANOVA ('treatments' \times 'sessions') showed a significant effect of 'treatments' [$F_{(3,26)} = 69.05$, $P < 0.001$], 'sessions' [$F_{(6,156)} = 6.91$, $P < 0.001$], and a significant 'treatments' \times 'sessions' interaction [$F_{(18,156)} = 4.29$, $P < 0.001$]. Post-hoc two-group comparisons revealed that both OLZ-treated groups were significantly different from the other two groups (all P values < 0.001), indicating a potentiated inhibitory effect of repeated OLZ treatment on avoidance responding. This potentiated effect appears to be attenuated to an extent by CDP, as the CDP + OLZ group showed less of a decrease than the VEH + OLZ group. Independent samples t -tests on each drug conditioning day revealed that there were significant differences between the two groups on day 4 ($P < 0.001$), day 5 ($P < 0.02$), and day 6 ($P < 0.05$).

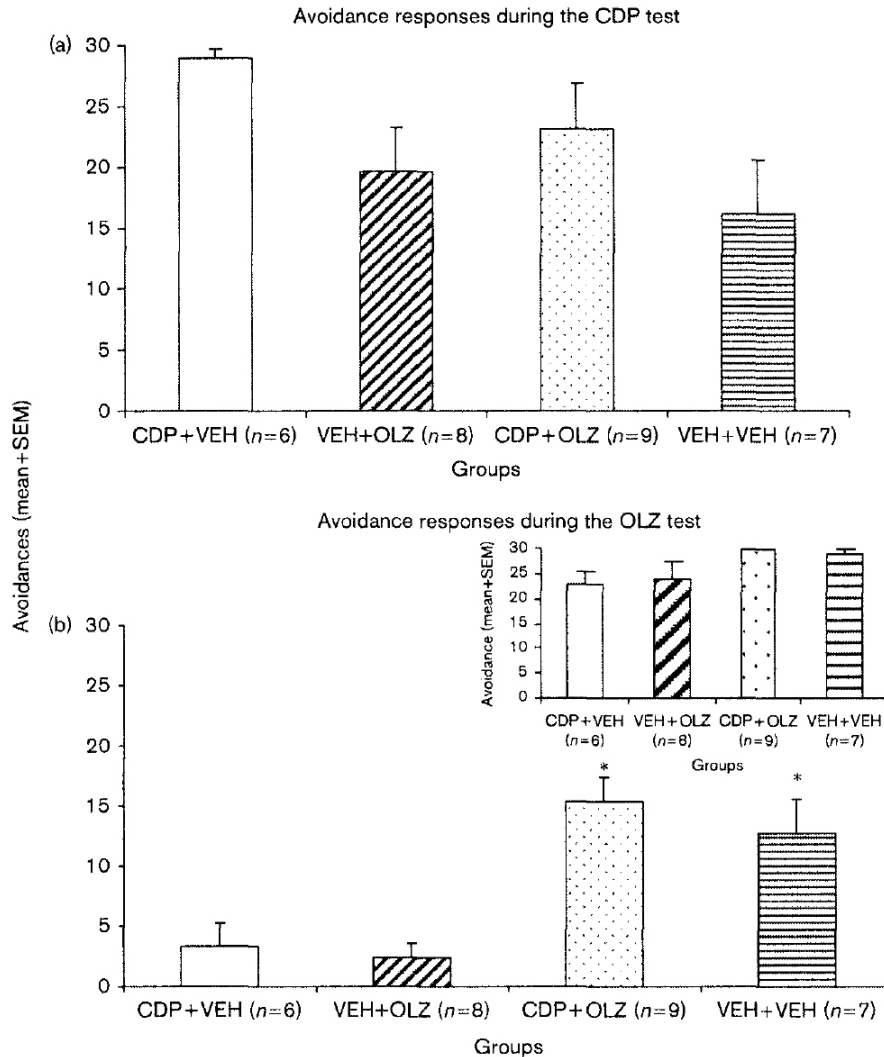
Figure 5a shows the number of avoidance responses during the CDP test. All four groups showed a comparably high level of avoidance responding. One-way ANOVA did not show any significant group difference [$F_{(3,26)} = 1.97$, NS], suggesting that CDP given alone or in combination with OLZ did not change its action on avoidance behavior. After a retraining session [pre-OLZ session: no group difference was detected, $F_{(3,26)} = 2.77$, $P = 0.061$, Figure 5b],

Fig. 4



Number of avoidance responses made by the rats in the four groups during the seven drug conditioning sessions and two drug-free retraining sessions from experiment 2. Repeated olanzapine (OLZ) treatment significantly disrupted avoidance responding across the seven daily test sessions. Repeated chlordiazepoxide (CDP) treatment had no effect on avoidance responding. VEH, vehicle.

Fig. 5



Number of avoidance responses during the chlordiazepoxide (CDP) test (a) and olanzapine (OLZ) test (b) from experiment 2. (a) CDP did not significantly affect avoidance responding during the CDP test. There was no significant group difference. (b) OLZ significantly inhibited avoidance responding, but only in the CDP + vehicle (VEH) and VEH + OLZ group. The two groups treated with the combination of CDP and OLZ during the drug conditioning phase showed less of a decrease in avoidance responding. The graph in (b) depicts the avoidance responses during the retraining session between the two drug tests. No significant group difference was detected during this session.

all rats were tested for their avoidance responses under OLZ in a CS-only session. In comparison with the pre-OLZ session, avoidance responses were significantly decreased by OLZ, especially in the CDP + VEH and VEH + OLZ groups. A 4×2 two-way ANOVA ('treatment' \times 'sessions') showed a significant effect of 'treatment' [$F_{(3,26)} = 8.11, P < 0.001$], 'sessions' [$F_{(1,26)} = 149.25, P < 0.001$], and a significant 'treatment' \times 'sessions' interaction [$F_{(3,26)} = 5.04, P < 0.01$]. Post-hoc two-group comparisons revealed that the CDP + OLZ and VEH + VEH groups were significantly different from the CDP + VEH and VEH + OLZ groups (all P values < 0.05). The CDP + VEH group did not differ from the VEH + OLZ group. As the CDP + OLZ and VEH + VEH groups all received a double drug

treatment (e.g. CDP and OLZ pairing), this result suggests that OLZ might lose its antipsychotic action after being repeatedly paired with CDP.

Experiment 3: reexamining the effects of repeated chlordiazepoxide and haloperidol pairing on avoidance responding to chlordiazepoxide and haloperidol

Figure 6 shows the number of avoidance responses during the seven drug conditioning sessions and two drug-free retraining sessions. Both groups showed a progressive across-session decrease in avoidance responding under drug, and a recovery when HAL was stopped. For the seven drug conditioning sessions, a two-way ANOVA ('treatments' \times 'sessions') showed a

significant effect of 'sessions' [$F_{(6,78)} = 6.36, P < 0.001$], but not 'treatments' [$F_{(1,13)} = 0.048, NS$], or 'treatments' \times 'sessions' interaction [$F_{(6,78)} = 0.806, NS$]. During the two subsequent retraining sessions, it appeared that the CDP + HAL group reinstated avoidance behavior much faster than the VEH + HAL group and had higher mean numbers of avoidances on both days. This group difference was significant on the first day ($P < 0.05$), but failed to reach a significant level on the second day.

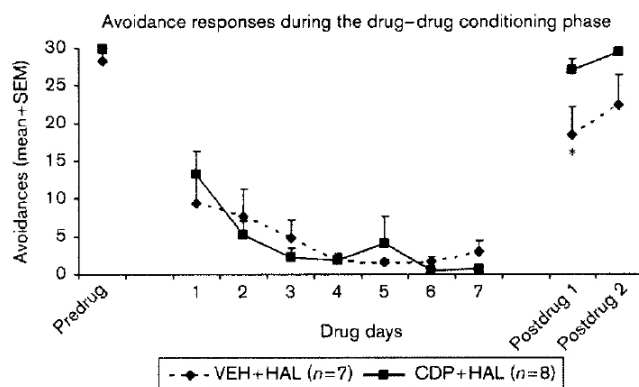
The pattern was reversed on the CDP test (Figure 7a). The CDP + HAL group showed many fewer avoidances than the VEH + HAL group, and this difference was statistically significant [$t(13) = 3.00, P < 0.01$]. This result confirmed the finding from experiment 1 and supported the notion that CDP might 'acquire' an antipsychotic property after being repeatedly paired with HAL.

After a retraining session [pre-HAL session: no group difference was detected, $t(13) = -1.82, NS$, Figure 7b], all rats were tested for their avoidance responses under HAL in a CS-only session (Figure 7b). As can be seen in Figure 7b, in comparison with the pre-HAL session, avoidance responding was significantly decreased by HAL [$F_{(1,13)} = 173.26, P < 0.001$]. There was no significant difference between groups [$F_{(1,13)} = 2.49, NS$], indicating that the efficacy of HAL was not altered even if it had been repeatedly paired with CDP.

Discussion

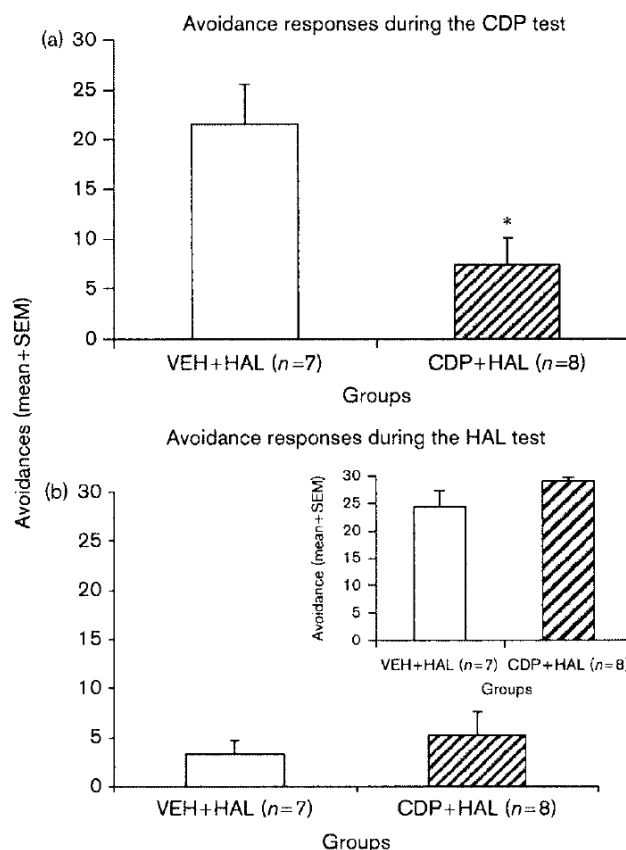
In the three experiments, we used a novel drug-drug conditioning paradigm and examined the role of anti-

Fig. 6



Number of avoidance responses made by the rats in the two groups during the seven drug conditioning sessions and two drug-free retraining sessions from experiment 3. Repeated haloperidol (HAL) treatment significantly disrupted avoidance responding across the seven daily test sessions. Rats that received the combination of chlordiazepoxide (CDP) and HAL treatment during the conditioning phase recovered faster than those that only received HAL when the treatment was stopped. $*P < 0.05$ for comparisons between the two groups. VEH, vehicle.

Fig. 7



Number of avoidance responses during the chlordiazepoxide (CDP) test (a) and haloperidol (HAL) test (b) from experiment 3. (a) CDP significantly inhibited avoidance responding in the CDP + HAL group during the CDP test, $*P < 0.05$. (b) HAL significantly inhibited avoidance responding both groups. The graph in (b) depicts the avoidance responses during the retraining session between the two drug tests. No significant group difference was detected during this session. VEH, vehicle.

psychotic-induced interoceptive state in the therapeutic effects of antipsychotic treatment. Results from experiments 1 and 3 suggest that the HAL-induced interoceptive state may be involved in its disruptive effect on avoidance responding, as evidenced by the finding showing that CDP + HAL pairing produced an anti-avoidance (e.g. antipsychotic-like) drug activity in CDP. This 'acquired' HAL activity of CDP is attributed to specific drug-drug conditioning with CDP functioning as the drug CS, and HAL as the drug US (Taukulis and Brake, 1989). It is not simply because of pharmacological effects of the drugs, as no such effect was found in two control groups (the CDP + VEH and VEH + HAL), even though they received the same numbers of CDP and HAL injections separated by 24 h. We also showed that, to induce the anti-avoidance effect in CDP, CDP + HAL pairing had to occur within the context of avoidance testing. The same pairing in the home cage (the VEH + VEH group) did not change the drug activity of CDP, indicating an important

interaction between drug treatment and targeted behavior as well as the direct involvement of HAL-induced drug state in the disruption of avoidance responding.

Unexpectedly, we did not find the same effect with CDP + OLZ pairing from experiment 2. CDP + OLZ pairing did not change the drug activity of CDP on avoidance responding. However, it did change the efficacy of OLZ, making it less effective in disrupting avoidance behavior when it was tested alone. This effect is because of drug-drug conditioning, as the control groups without conditioning (the CDP + VEH and VEH + OLZ) did not show such an effect. In addition, the contexts within which the CDP + OLZ conditioning occurred were not critical because even the drug pairing in the home cage achieved a similar effect (Figure 5b). Overall, the present findings reveal an interesting 'double dissociation' between the effect of CDP pairing with typical antipsychotic HAL and atypical OLZ. CDP + HAL pairing changed the drug activity of CDP, but did not change that of HAL. CDP exhibited an acquired antiavoidance effect after being repeatedly paired with HAL. In contrast, CDP + OLZ pairing changed the drug activity of OLZ, but not that of CDP. OLZ became less effective in disrupting avoidance behavior after repeated pairing.

This study provides additional evidence showing that the HAL-induced interoceptive state is related to its antiavoidance effect, a finding consistent with our previous work (Li *et al.*, 2007; Mead and Li, in press). The differential effect of CDP + HAL and CDP + OLZ pairing is surprising, given the fact that both HAL and OLZ are equally efficacious in the treatment of psychosis (Lieberman *et al.*, 2003) and share a similar molecular mechanism in blocking dopamine D₂ receptors (Kapur and Seeman, 2000). At the chosen doses, they also produced a comparable level of avoidance disrupting effect over the seven drug conditioning days (Figs 2 and 4). One possible explanation for the differential effect of CDP + HAL and CDP + OLZ pairing is that HAL and OLZ may induce different interoceptive states that are differently influenced by CDP. Pharmacologically, HAL is primarily a D₂ receptor antagonist (Kapur *et al.*, 1996), and it binds 'tightly' to the D₂ receptor and dissociates slowly (Kapur and Seeman, 2001), whereas OLZ has a moderate antagonist effect on the D₂ receptor but a high antagonist effect on the 5-HT_{2A} serotonergic, α₁ adrenergic, m₁ muscarinic, and H₁ histaminic receptors (Bymaster *et al.*, 1999; Miyamoto *et al.*, 2005) (Table 1). It is possible that the OLZ state is a compound cue that is mediated by its antagonism against multiple receptors (*e.g.* D₂, 5-HT_{2A}, α₁, m₁ and H₁, etc.), whereas the HAL state is a single cue that is primarily mediated by antagonism against D₂ receptors. Drug discrimination studies seem to support this notion (Goudie and Taylor, 1998; Porter *et al.*, 2000a; Cole *et al.*, 2007; Goudie *et al.*, 2007). Using a two-lever drug discrimination paradigm, Porter *et al.* (2000a) found that the dopamine D₂ antagonists chlorpromazine and

Table 1 Relative neurotransmitter receptor affinities for haloperidol and olanzapine at therapeutic doses adapted from (Miyamoto *et al.*, 2005)

Receptors	Haloperidol	Olanzapine
D ₁	+	++
D ₂	++++	++
D ₃	+++	+
D ₄	+++	++
5-HT _{2A}	+	+++
5-HT _{2C}	-	++
5-HT ₆	-	++
5-HT ₇	-	-
α ₁	+++	++
α ₂	-	+
H ₁	-	+++
m ₁	-	+++
DA transporter		++
NA transporter		++

-, minimal to none; +, low; ++, moderate; +++, high; +++++, very high.

thioridazine substituted for OLZ in producing OLZ-appropriate responding in rats, as did the muscarinic cholinergic antagonist scopolamine and the 5-HT_{2A/2C} serotonergic antagonist ritanserin. This finding suggests that the antagonism of either dopamine D₂ receptors, muscarinic receptors, or 5-HT_{2A/2C} receptors is sufficient to mimic the OLZ-induced interoceptive state. Colpaert *et al.* (2007) used a two-lever, food-rewarded drug discrimination paradigm and found that HAL was more efficacious than OLZ in inducing 'win-shift' response pattern, possibly because of its strong D₂ receptor antagonism. As the 'antipsychotic' action, as well as the antiavoidance effect is thought to be mediated by antagonism against D₂ receptors (Wadenberg *et al.*, 2001; Seeman, 2006), the compound cue mediated by multiple receptor actions of OLZ is apparently less effective in bestowing an antipsychotic property to CDP than the single cue mediated by D₂ blockade by HAL. This is because other discriminative cues within this compound cue may obscure the 'antipsychotic' cue. Within the same line of reasoning, it is also possible that the differential effects of CDP + HAL and CDP + OLZ pairing is because of the differences between the dopamine D₂ receptor bindings of HAL and OLZ. The binding affinity of HAL at D₂ receptors is six to seven times greater than that of OLZ (Richelson and Souder, 2000), so although they may share a similar molecular mechanism in blocking D₂ receptors, they are not equally efficacious at this site. This distinct molecular binding profile of OLZ opens up the possibility that the discrepant findings represent a pharmacological effect between CDP and OLZ that was not present between CDP and HAL.

Another unexpected finding is that repeated concurrent CDP and OLZ treatment attenuated the antiavoidance effect of OLZ (Figure 5b), but not HAL (Figure 3b)

and Figure 7b) in the drug-alone testing, implying that the antipsychotic efficacy of OLZ, but not HAL, could be potentially attenuated by CDP in the clinic. One clinical report (Wolkowitz *et al.*, 1989) and one preclinical report (Keller *et al.*, 1976) seem to suggest that benzodiazepines may augment the drug effects of typical antipsychotics during the combined drug treatment. Our results did not show such an effect. Owing to limited research on this issue, it is premature to draw a definite conclusion. Future work with more vigorous controls and a wide selection of different types of benzodiazepines and antipsychotics is needed.

Besides its contribution to understanding the behavioral mechanisms of antipsychotics, this study is important because it also provides an approach to study drug-drug interactions in the treatment of schizophrenia. Most schizophrenic patients are treated with multiple psychotherapeutic drugs, such as antipsychotics, selective serotonin reuptake inhibitors, and benzodiazepines to control their diverse symptoms and comorbid anxiety and depression (ZumBrunnen and Jann, 1998). One recent report found that concurrent prescriptions for anxiolytic medications with antipsychotics grew by more than two-thirds from 1995 to 1999 in the state of New Hampshire (Clark *et al.*, 2002). This practice of psychotropic polypharmacy has raised some concerns regarding the efficacy, costs, and possible adverse effects of drug-drug interactions (Alfaro, 2001; Sandson *et al.*, 2005; Rupnow *et al.*, 2007). However, because current clinical data come mostly from case reports and limited uncontrolled studies, it is difficult to assess the extent and nature of drug-drug interactions in schizophrenia (ZumBrunnen and Jann, 1998) and determine their advantages or disadvantages. Our findings that the antiavoidance efficacy of HAL is actually attenuated by CDP during the early treatment phase (experiment 1, although not confirmed in experiment 3) and the long-term antiavoidance efficacy of OLZ is also attenuated by CDP seem to suggest that cautions need to be taken in monitoring the clinical responses of patients during the early stage of combined drug treatment and when benzodiazepines are discontinued.

This present study is also important because it extends research utilizing a Pavlovian Drug-drug conditioning paradigm in the following two directions. First, it introduces a new behavioral model to assess associative conditioning involving two drug cues. In many drug conditioning studies, conditional responses are usually some basic physiological or simple reactive behaviors, such as drug-induced thermic effects, heart rate, stomach emptying, muscle relaxation, or taste aversions (Wilkin *et al.*, 1982; Revusky *et al.*, 1989; Davey and Biederman, 1991; Reilly and Revusky, 1992; Biederman and Davey, 1993). This study shows that even instrumental conditioned active motor behavior can be used as a valid index to evaluate the conditioned drug effect. Second, it introduces a new approach to examine the effects of drug-drug condi-

tioning. In many drug conditioning studies, the drug conditioning effect is often indexed by some change in one or more of the properties of the CS drug (Taukulis, 1996). For example, in a series of studies on the diazepam-HAL or diazepam-chlorpromazine conditioning (Taukulis and Brake, 1989; Taukulis *et al.*, 1992), the drug conditioning was evidenced by the changed drug properties of diazepam, such as enhanced hypothermia, diminished muscle relaxation, and enhanced anxiolytic effect. As rightly pointed out by Taukulis (1996), this approach occasionally poses a challenge in the explanation for the drug conditioning phenomenon, because the specific unconditional effects of the 'signaled' drug (drug US) have not always been specified in advance. In this study, the conditioning effect was seen in the newly 'acquired' antipsychotic property in CDP, which is not an intrinsic drug property of CDP, and can only be attributed to the unconditioned effect of HAL. This approach provides an unequivocal demonstration of the drug-drug conditioning effect.

In summary, our results show that the HAL-induced interoceptive state is an important behavioral mechanism responsible for the maintenance of its antiavoidance effect and possibly antipsychotic effect over time. Concurrent use of CPD with antipsychotics, especially with OLZ, may cause a long-term attenuation of the antiavoidance effect of OLZ through a drug-drug interaction mechanism. The model introduced in this study may be useful in delineating behavioral mechanisms of antipsychotic action and assessing polypharmacy involving drug-drug conditioning in the treatment of schizophrenia.

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