


10-2012

# Drug–drug conditioning between citalopram and haloperidol or olanzapine in a conditioned avoidance response model: implications for polypharmacy in schizophrenia

Nathan L. Sparkman  
*University of Nebraska-Lincoln*

Ming Li  
*University of Nebraska-Lincoln, mli2@unl.edu*

Follow this and additional works at: <http://digitalcommons.unl.edu/psychfacpub>

 Part of the [Chemicals and Drugs Commons](#), and the [Experimental Analysis of Behavior Commons](#)

---

Sparkman, Nathan L. and Li, Ming, "Drug–drug conditioning between citalopram and haloperidol or olanzapine in a conditioned avoidance response model: implications for polypharmacy in schizophrenia" (2012). *Faculty Publications, Department of Psychology*. 706.  
<http://digitalcommons.unl.edu/psychfacpub/706>

This Article is brought to you for free and open access by the Psychology, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications, Department of Psychology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.



Published in final edited form as:

*Behav Pharmacol.* 2012 October ; 23(7): 658–668. doi:10.1097/FBP.0b013e328358590d.

## Drug–drug conditioning between citalopram and haloperidol or olanzapine in a conditioned avoidance response model: implications for polypharmacy in schizophrenia

Nathan L. Sparkman and Ming Li

Department of Psychology, University of Nebraska-Lincoln, Lincoln, Nebraska, USA

### Abstract

Patients with schizophrenia often have anxiety and depression, and thus are treated with multiple psychotherapeutic medications. This practice of polypharmacy increases the possibility for drug–drug interactions. However, the pharmacological and behavioral mechanisms underlying drug–drug interactions in schizophrenia remain poorly understood. In the present study, we adopted a preclinical approach and examined a less known behavioral mechanism, drug–drug conditioning (DDC) between haloperidol (a typical antipsychotic) or olanzapine (atypical antipsychotic) and citalopram (a selective serotonin reuptake inhibitor). A rat two-way conditioned avoidance response paradigm was used to measure antipsychotic activity and determine how DDC may alter the antipsychotic efficacy in this model. Following acquisition of the avoidance response, rats were then randomly assigned to receive vehicle, citalopram (10.0 mg/kg, intraperitoneally), haloperidol (0.05 mg/kg, subcutaneously), olanzapine (1.0 mg/kg, subcutaneously), combined haloperidol with citalopram, or combined olanzapine with citalopram treatment for seven avoidance test sessions. In comparison with antipsychotic treatment alone, combined treatment with citalopram potentiated the antiavoidance effect of olanzapine or haloperidol (to a lesser extent) during the seven drug-test sessions. In addition, repeated pairing of citalopram with haloperidol or olanzapine caused citalopram to show a newly acquired avoidance-disruptive effect. This effect was context specific because citalopram paired with haloperidol or olanzapine outside the avoidance testing context (i.e. home cages) did not show such an effect. These findings indicate that concurrent antidepressant and antipsychotic treatments may engender a DDC process that follows the general Pavlovian associative conditioning principles. They also indicate that adjunctive citalopram treatment may enhance the antipsychotic efficacy of haloperidol and olanzapine in the treatment of schizophrenia.

### Keywords

citalopram; depression; drug–drug conditioning; haloperidol; olanzapine; Pavlovian conditioning; polypharmacy; rat; schizophrenia

---

Correspondence to Ming Li, PhD, Department of Psychology, University of Nebraska-Lincoln, 238 Burnett Hall, Lincoln, NE 68588-0308, USA mli2@unl.edu.

### Conflicts of interest

There are no conflicts of interest.

## Introduction

Depression is a common comorbidity in schizophrenia, affecting an estimated 50% of patients (Buckley, 2008), and this symptom is often unabated by traditional antipsychotic regimens. Polypharmacy regimens have often sought to alleviate the depression-related symptoms by coprescribing an antidepressant along with an antipsychotic drug (Zink *et al.*, 2010). For example, citalopram (CIT), a selective serotonin reuptake inhibitors (SSRIs) used for the treatment of major depression (ZumBrunner and Jann, 1998; Sepehry *et al.*, 2007), is often used as an adjunctive therapy with traditional antipsychotics in the treatment of comorbid anxiety and depression symptoms in schizophrenia (Salokangas *et al.*, 1996; Friedman *et al.*, 2005). This practice of polypharmacy has raised some concerns on the efficacy, costs, and possible adverse effects of drug–drug interactions (Alfaro, 2001; Sandson *et al.*, 2005; Conley and Kelly, 2007; Rupnow *et al.*, 2007). Some reports suggest that antidepressants such as citalopram are effective in the treatment of depression in schizophrenia with concurrent antipsychotic drug treatment (Englisch *et al.*, 2009; Zisook *et al.*, 2009). However, other reports question the efficacy of add-on SSRI in the improvement of negative symptoms (Sepehry *et al.*, 2007). Overall, evidence on augmentation of antipsychotics with SSRIs remains inconclusive. Also, it is not clear whether the efficacy of antidepressants or antipsychotics is altered in the combined drug treatment regimens. Zink *et al.* (2010) report that there is an urgent need to conduct well-designed randomized-controlled trials on the use of antidepressants in schizophrenia. Without controlled trials and mechanistic investigations, it is difficult to assess the extent and nature of drug–drug interactions of antidepressants and antipsychotics in the treatment of schizophrenia (ZumBrunner and Jann, 1998).

In almost all polypharmacy studies, the focus has been on two major varieties of drug–drug interactions: pharmacodynamic interactions and pharmacokinetic interactions (Sandson *et al.*, 2005). Concurrent drug use can also result in a drug–drug conditioning (DDC) phenomenon that is less understood by psychiatrists. Similar to Pavlovian conditioning involving exteroceptive stimuli (e.g. light, sound, food, or shock), DDC is found to be mediated by the same learning principles (Revusky *et al.*, 1979; Taukulis and Brake, 1989). Taukulis and Brake (1989) reported that the anxiolytic effect of diazepam can be potentiated by repeatedly pairing it with chlorpromazine. They injected rats with diazepam (2.5 mg/kg), followed 30 min later by chlorpromazine (10.0 mg/kg). After 10–12 repeated drug pairings of this type, the anxiolytic responses of the animals to diazepam alone were tested in an elevated plus maze task. They found that rats previously conditioned with diazepam and chlorpromazine spent more time in the open arms than rats conditioned with either diazepam alone or chlorpromazine, followed 30 min later by diazepam (backward pairings). The results showed that the order of the drugs during the conditioning period was critical to developing the enhanced anxiolytic effect of diazepam. They later found the same effect with diazepam–haloperidol (HAL) pairings. Interestingly, other dopamine (DA) antagonists (thioridazine and pimozide) did not produce an enhanced conditioned response (Taukulis *et al.*, 1992). This enhanced anxiolytic effect is believed to be caused by the interdrug conditioning, an internal associative process, rather than pharmacological alteration because reversing the order of the drug treatment does not change the anxiolysis of diazepam.

Following a similar approach, we recently examined possible DDC between haloperidol (a typical antipsychotic) and chlordiazepoxide (a benzodiazepine anxiolytic) and the DDC between olanzapine (atypical antipsychotic) and chlordiazepoxide in a conditioned avoidance response (CAR) model (Li *et al.*, 2009). The CAR has been used for the detection of antipsychotic activity for more than 60 years and shows high predictive validity, as most antipsychotics at clinically relevant doses disrupt avoidance responding preferentially (Arnt, 1982; Franberg *et al.*, 2008; Porsolt *et al.*, 2010; Wadenberg, 2010). Our results show that the repeated concurrent chlordiazepoxide and olanzapine treatment attenuated the antiavoidance effect of olanzapine. However, chlordiazepoxide acquired a haloperidol-like property in disrupting avoidance responding after being paired repeatedly with haloperidol.

The present study used a similar DDC approach and examined how the antidepressant citalopram interacts with haloperidol or olanzapine (OLZ) in the CAR model. It is known that both haloperidol and olanzapine disrupt avoidance responding whereas citalopram has not been shown to disrupt avoidances effectively (Sun *et al.*, 2010). Therefore, it may be possible that following repeated pairings, citalopram may acquire the avoidance-disruptive effect of haloperidol or olanzapine. However, repeated combined treatment of citalopram with haloperidol or olanzapine may alter the effectiveness of haloperidol or olanzapine to disrupt avoidance. In the present study, citalopram served as a neutral cue [conditioning stimulus (CS)] that signals that the effects of haloperidol or olanzapine [unconditioned stimulus (US)] were imminent. Over time, through repeated pairings, citalopram acquired the avoidance-disruptive property of haloperidol and olanzapine, and also potentiated the avoidance-disruptive effect of these drugs. These findings indicate that adjunct citalopram treatment may enhance the antipsychotic efficacy of haloperidol and olanzapine.

## Methods

### Subjects

Male Sprague–Dawley rats (226–250 g upon arrival; Charles River Laboratories, Potage, Michigan, USA) were housed two per cage in 48.3 cm × 26.7 cm × 20.3 cm transparent polycarbonate cages under 12-h light/dark conditions (light on between 06:30 and 18:30 h). Room temperature was maintained at 21±1° with a relative humidity of 45–60%. Food and water were freely available. Animals were allowed at least 1 week of habituation to the animal facility before being used in the experiments. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska, Lincoln.

### Avoidance conditioning apparatus

Eight 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 W × 35.56 cm D × 63.5 cm H). 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 PVC partition with an arch-style doorway (15 cm H × 9 cm W at base). An aluminum hurdle (4 cm high) was placed between the two compartments; thus, 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 rat's location 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 (~ 74 dB) was provided by a ventilation fan affixed at the top corner of each isolation cubicle.

### **Experiment 1: Effects of repeated citalopram and haloperidol pairing on avoidance responding to citalopram and haloperidol**

The experiment comprised of three phases: avoidance training, DDC, and drug testing (see Fig. 1 for the procedural details).

**Avoidance training phase**—Forty rats were first handled and habituated to the avoidance conditioning apparatus for two 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 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, 32 rats that had fulfilled the training criterion (> 70% avoidance in each of the last two sessions) were used in the drug conditioning phase. They were matched and then assigned randomly to one of the four groups. The drug conditioning phase consisted of a 3-day cycle and was repeated seven times over a 21-day period. On day 1, each group was administered a double injection of one of the following combinations: CIT + VEH ( $n = 8$ ), VEH + HAL ( $n = 8$ ), CIT + HAL ( $n = 8$ ), and VEH + VEH ( $n = 8$ ). The first injection (CIT 10.0 mg/kg, or sterile water, 1.0 ml/kg, intraperitoneally) was administered 15 min before the second injection (HAL 0.05 mg/kg, or sterile water, 1.0 ml/kg, subcutaneously). One hour after the second injection, rats were placed in the avoidance conditioning boxes and tested. On day 2, rats in the CIT + VEH, VEH + HAL, and CIT + HAL groups received a single injection of HAL, CIT, and VEH, respectively, whereas the VEH + VEH group received a double injection of CIT and HAL separated by 15 min. Immediately after the injections, rats were returned to their home cages. No avoidance test was carried out on this day. The purpose 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 CIT, 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 DDC effect on avoidance behavior could be assessed. On day 3, all rats were

untreated and unhandled. Following the seven cycles of the conditioning procedure, all rats were retrained 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 CIT (10.0 mg/kg, intraperitoneally) and tested 75 min later. The next day, rats were retrained drug-free, and 1 day later, tested again under HAL (0.025 mg/kg, subcutaneously, – 60 min) to assess the HAL sensitization effect (Li *et al.*, 2010; Mead and Li, 2010; Zhang and Li, 2012). 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.

## **Experiment 2: Effects of repeated citalopram and olanzapine pairing on avoidance responding to citalopram and olanzapine**

This experiment was identical to experiment 1, except that HAL was replaced by OLZ. Forty rats were used, of which 32 rats that fulfilled the learning criterion were used in the drug testing. They were assigned to one of the following four groups: CIT + VEH ( $n = 8$ ), VEH + OLZ ( $n = 8$ ), CIT + OLZ ( $n = 8$ ), and VEH + VEH ( $n = 8$ ), and were subjected to the seven sessions of drug conditioning and two sessions of drug testing (the CIT test, followed by the OLZ 0.5 mg/kg test) to assess the CIT conditioning effect and the OLZ sensitization effect.

## **Drugs**

The injection solutions of HAL (5 mg/ml ampoules, Shanghai Xudong Haipu Pharmaceutical Co., Shanghai, China) and CIT (Toronto Research Chemicals Inc., Toronto, Ontario, Canada) were obtained by mixing drugs with sterile water. OLZ (a gift from the National Institute of Mental Health drug supply program) was dissolved in 1.5% glacial acetic acid in distilled water. HAL and OLZ were administered subcutaneously, whereas CIT was administered intraperitoneally. The doses of HAL (0.05 mg/kg) and OLZ (1.0 mg/kg) and their injection route were chosen on the basis of (a) previous work showing that at the chosen doses, HAL and OLZ injected subcutaneously produce a comparable progressive across-session decrease in avoidance responding (Li *et al.*, 2007) and (b) rat brain D<sub>2</sub> receptor occupancy data showing that both drugs induce clinically comparable levels of D<sub>2</sub> occupancy (65–80%) (Kapur *et al.*, 2003). The choice of the CIT dose (10 mg/kg) and its route of injection was made on the basis of the findings showing that (a) CIT (10 mg/kg) is ineffective in disrupting avoidance responding (Sun *et al.*, 2010) and (b) CIT at this dose is effective in several aversively conditioned paradigms, such as Pavlovian fear conditioning (Hashimoto *et al.*, 2009; Sun *et al.*, 2010).

This time interval between CIT and HAL or OLZ (15 min) was determined so that there was sufficient time for the drug effects of HAL or OLZ and CIT to overlap. The half-lives of CIT, HAL, and OLZ in rats are 3 h (Hyttel *et al.*, 1984), 1.5 h (Cheng and Paalzow, 1992), and 2.5 h (Aravagiri *et al.*, 1999), respectively. This arrangement ensures that the two drugs had sufficient concurrency of the effective drug states that would be suitable for DDC. A similar kind of DDC arrangement had been used by Taukulis and Brake (1989). To



determine possible behavioral sensitization induced by repeated HAL or OLZ treatment, HAL and OLZ were administered at half of their training doses during the last test session (0.025 and 0.5 mg/kg, respectively) (Li *et al.*, 2010; Zhang and Li, 2012).

## Statistics

The main dependent variable was the number of avoidance responses. All data are expressed as mean  $\pm$  SEM. Data from the DDC phase were first analyzed using a mixed-model repeated-measures analysis of variance (ANOVA) with CIT (CIT vs. VEH, two levels), HAL (HAL vs. VEH, two levels), or OLZ (OLZ vs. VEH, two levels) as the between-subjects factor and the test sessions (i.e. seven drug sessions) as the within-subjects factor, followed by post-hoc Fisher's protected least squared difference to identify the significant group differences. A similar repeated-measures analysis was used for the two consecutive drug-free retraining days. For the drug challenge test days (CIT, HAL, or OLZ challenge tests), because we had an a-priori hypothesis on the basis of our previous work (Li *et al.*, 2009), a series of planned comparisons instead of post-hoc tests were used to identify significant differences between groups. A conventional two-tailed level of significance at the 5% level was used.

## Results

### Experiment 1: Effects of repeated citalopram and haloperidol pairing on avoidance responding to citalopram and haloperidol

**CIT and HAL: conditioning**—HAL potently and progressively attenuated avoidance responding (Fig. 2a). There was a significant main effect of HAL [ $F(1,28) = 316.56$ ,  $P < 0.001$ ] as well as a significant HAL  $\times$  Session interaction [ $F(6,168) = 14.47$ ,  $P < 0.001$ ]. The main effect of CIT was marginally significant [ $F(1,28) = 4.17$ ,  $P = 0.05$ ], whereas the CIT  $\times$  Session interaction was not significant. Examination of the pattern of avoidance responding across sessions showed that rats treated with VEH + VEH maintained a high level of responding, whereas rats treated with CIT + VEH had somewhat attenuated responding. The VEH + HAL rats showed a rapid and progressive attenuation across test sessions and those treated with CIT + HAL tended to have the lowest levels of avoidance responding.

CIT did not alter the number of escape failures. However, there was a significant main effect of HAL [ $F(1,28) = 26.01$ ,  $P < 0.001$ ] and a significant HAL  $\times$  Session interaction [ $F(6,168) = 14.06$ ,  $P < 0.001$ ], wherein animals treated with HAL showed a progressive increase in their number of escape failures across test sessions (Fig. 2b).

**CIT and HAL: drug-free retraining**—During the subsequent two drug-free retraining sessions, rats that had received HAL treatment (i.e. VEH + HAL or CIT + HAL) recovered avoidance responding (Fig. 2a). There was a significant CIT  $\times$  HAL  $\times$  Session interaction [ $F(1,28) = 70.05$ ,  $P < 0.02$ ], a significant CIT  $\times$  Session interaction [ $F(1,28) = 7.05$ ,  $P < 0.02$ ], and a significant HAL  $\times$  Session interaction [ $F(1,28) = 31.54$ ,  $P < 0.001$ ], wherein rats treated with VEH + HAL performed the least number of avoidance responses on the first retraining day, whereas those that received CIT + HAL performed at intermediate levels and those that received VEH + VEH or CIT + VEH performed the highest number of

avoidance responses. On the first day of retraining, there were significant main effects of HAL [ $F(1,28) = 34.50, P < 0.001$ ] and CIT [ $F(1,28) = 6.30, P < 0.02$ ] and a significant CIT  $\times$  HAL interaction [ $F(1,28) = 6.88, P < 0.02$ ]. One-way ANOVA, followed by post-hoc analysis showed that rats that had been treated previously with CIT + HAL made significantly fewer avoidance responses than those treated previously with VEH + VEH or CIT + VEH ( $P < 0.05$ ). Furthermore, rats treated previously with VEH + HAL performed fewer avoidance responses than the VEH + VEH, CIT + VEH, or CIT + HAL rats. On the second retraining day, there was a significant main effect of HAL [ $F(1,28) = 9.75, P < 0.005$ ], wherein rats treated with HAL made significantly fewer avoidance responses than those not treated previously with HAL.

On escape failures, across the two-day retraining period (Fig. 2b), there were significant effects of CIT [ $F(1,28) = 5.10, P < 0.05$ ], HAL [ $F(1,28) = 9.52, P < 0.005$ ], and the CIT  $\times$  HAL interaction [ $F(1,28) = 10.05, P < 0.005$ ]. In addition, there was a significant HAL  $\times$  Session interaction [ $F(1,28) = 9.75, P < 0.005$ ]. Post-hoc analysis showed that during the first retraining session, rats that had been treated in the shuttle box with VEH + HAL had a greater number of escape failures than those treated with CIT + HAL, CIT + VEH, or VEH + VEH.

**CIT and HAL: CIT challenge test**—During the CIT test, all rats were treated with CIT (10.0 mg/kg, intraperitoneally) to determine the DDC effects of CIT to HAL (Fig. 3). Planned comparisons of the four conditioning groups showed that rats treated with VEH + VEH or CIT + VEH did not differ from each other and maintained a high level of avoidance responding. Rats that had been treated previously with VEH + HAL also did not differ significantly from these two groups. However, rats had been conditioned to CIT + HAL showed the fewest avoidance responses and were significantly different from the VEH + VEH or the CIT + VEH rats ( $P$  values  $< 0.05$ ). This indicates that CIT + HAL conditioning resulted in CIT acquiring the avoidance-disrupting effects of HAL. In addition, this effect was specific to the conditioned effects of the drugs in the testing context because rats that received CIT + HAL pairings in their home cage did not show this effect.

**CIT and HAL: HAL challenge test**—Following a subsequent retraining day (no group differences present; Fig. 4, inset), all rats were administered a 0.025 mg/kg HAL injection and their avoidances were tested 1 h later to assess HAL sensitization (Fig. 4) (Li *et al.*, 2010). Planned comparisons showed no difference between rats treated previously with CIT + VEH or VEH + VEH. However, rats treated previously with VEH + HAL or CIT + HAL performed significantly fewer avoidance responses than the CIT + VEH or VEH + VEH groups ( $P$  values  $< 0.05$ ). There were also no differences between the two HAL groups. These data indicate that repeated administration of HAL induced a long-lasting sensitization effect in avoidance disruption, consistent with our previous finding (Li *et al.*, 2007; Zhang and Li, 2012). In addition, CIT did not alter the efficacy of HAL even after repeated pairings.



## Experiment 2: Effects of repeated citalopram and olanzapine pairing on avoidance responding to citalopram and olanzapine

**CIT and OLZ: conditioning**—Repeated administration of OLZ potently and progressively attenuated avoidance responding across the seven drug test sessions (Fig. 5a). In contrast, rats treated with VEH + VEH or CIT + VEH maintained a high level of responding, and they did not differ from one another. There was a significant main effect of OLZ [ $F(1,28) = 316.56, P < 0.001$ ] and a significant OLZ  $\times$  Session interaction [ $F(6,168) = 13.76, P < 0.001$ ]. In addition, there was a significant main effect of CIT [ $F(1,28) = 316.56, P < 0.001$ ] and a significant CIT  $\times$  Session interaction [ $F(6,168) = 30.05, P < 0.01$ ], wherein CIT reduced the number of avoidance responses. This was especially evident during the first four drug-test sessions.

Analysis of escape failures showed that repeated administration of OLZ progressively increased the number of escape failures across sessions (Fig. 5b). Rats treated with VEH + VEH or CIT + VEH maintained very low levels of escape failures and they did not differ from one another. There was a significant main effect of OLZ [ $F(1,28) = 34.37, P < 0.001$ ] and a significant OLZ  $\times$  Session interaction [ $F(6,168) = 13.87, P < 0.001$ ].

**CIT and OLZ: drug-free retraining**—During the subsequent two drug-free retraining sessions, rats that had received OLZ treatment recovered at a slower rate compared with VEH-treated rats or rats that received CIT alone. A repeated-measures ANOVA showed a significant main effect of OLZ [ $F(1,28) = 16.66, P < 0.001$ ; Fig. 5a]. Rats that had been treated previously with VEH + OLZ or CIT + OLZ made significantly fewer avoidance responses on both days. The VEH + OLZ and CIT + OLZ groups were significantly different from the VEH + VEH or CIT + VEH groups ( $P$ 's  $< 0.05$ ) on the first retraining day. The CIT + OLZ group was still significantly different from the VEH + VEH or the CIT + VEH group on the second retraining day ( $P < 0.05$ ).

In terms of escape failures, there was a significant main effect of OLZ [ $F(1,28) = 4.412, P < 0.05$ ; Fig. 5b]; however, the difference between group means was less than one trial and was probably not psychologically significant.

**CIT and OLZ: CIT challenge test**—Following two retraining days, all rats were treated with CIT to determine the DDC effect of CIT to OLZ (Fig. 6). Rats treated with VEH + VEH or CIT + VEH during the DDC sessions maintained a high level of avoidance responding and they did not differ from one another. Also, rats treated with VEH + OLZ were not different from any group. However, planned comparisons showed that rats that had been treated previously with CIT + OLZ made significantly fewer avoidance responses than CIT + VEH ( $P < 0.005$ ) or VEH + VEH ( $P < 0.005$ ). This indicates that CIT + OLZ conditioning resulted in CIT acquiring the avoidance-disrupting effects of OLZ. In addition, this effect was specific to the conditioned effects of the drugs in the testing context because animals that experienced CIT + OLZ pairings in their home cage did not show this effect.

**CIT and OLZ: OLZ challenge**—On the subsequent retraining day, although they achieved an average of 25.5 avoidances, rats conditioned with CIT + OLZ still made significantly fewer avoidance responses than those conditioned with CIT + VEH or VEH +

VEH ( $P < 0.05$ ; Fig. 7, inset). To examine the long-term sensitization effect of OLZ (Li *et al.*, 2010; Mead and Li, 2010; Zhang and Li, 2012), all rats were administered a 0.5 mg/kg OLZ injection and their avoidances were tested (Fig. 7). One-way ANOVA showed a significant effect of group [ $F(3,28) = 13.73$ ,  $P < 0.001$ ] and subsequent planned comparisons showed that rats treated previously with VEH + OLZ or CIT + OLZ during the DDC phase made significantly fewer avoidance responses than those treated previously with VEH + VEH ( $P$  values  $< 0.05$ ). Interestingly, rats previously conditioned to CIT + VEH also made fewer avoidance responses than the VEH + VEH-treated rats ( $P < 0.001$ ), but more than the rats conditioned to CIT + OLZ ( $P < 0.04$ ) or VEH + OLZ ( $P < 0.02$ ). These results indicate that CIT + VEH treatment also enhanced rats' sensitivity to the avoidance-disruptive effect of OLZ. However, this effect was weak in comparison with the effects of OLZ treatment and was dependent on the context of the drug experience, as the VEH + VEH rats receiving CIT + OLZ pairings in their home cage did not show this enhanced sensitivity.

## Discussion

In two separate studies, we examined how the SSRI CIT interacted behaviorally with the typical antipsychotic HAL or the atypical antipsychotic OLZ as a means to examine the impact of polypharmacy treatment in schizophrenia. Our results clearly show that when two psychotropic drugs are used together, their behavioral effects could be altered by a DDC mechanism. For example, CIT by itself does not have an intrinsic disruptive effect on the CAR (Figs 2a and 5a) (Sun *et al.*, 2010): however, after repeated pairings with HAL or OLZ, it acquired an antiavoidance property (Figs 3 and 6). This 'acquired' avoidance-disruptive effect of CIT was attributed specifically to DDC, wherein CIT functioned as the drug CS and HAL or OLZ as the drug US (Taukulis and Brake, 1989), and could not be attributed to the simple pharmacological effects of the drugs, as no such effect was found in the control groups (e.g. the CIT + VEH, VEH + HAL, or VEH + OLZ), even though they received the same numbers of CIT and HAL or OLZ injections separated by 24 h. We also found that, to induce the antiavoidance effect in the CIT group, CIT + HAL or CIT + OLZ pairing had to occur within the context of avoidance testing. Rats that received this pairing in their home cages (i.e. VEH + VEH rats) did not show altered drug efficacy in the CAR procedure. These findings indicate that the drug conditioning is context specific. They also indicate that the drug conditioning effects follow the same general associative conditioning principles found in a typical Pavlovian conditioning paradigm in which a deliberate CS-US pairing is required (Domjan, 2005).

In addition, the combined treatment of CIT with HAL or OLZ potentiated the avoidance-disruptive effect of HAL and OLZ during the DDC phase. This effect may be mediated by the pharmacological mechanisms associated with the pharmacokinetic or pharmacodynamic interactions of the drugs. Pharmacokinetically, most antipsychotics and SSRIs are metabolized through the CYP isozyme system (mainly CYP1A2, CYP2D6, and CYP3A4) and, as a result, SSRIs can inhibit the metabolism of antipsychotic drugs (ZumBrunnen and Jann, 1998). In the case of CIT (CYP2C19, CYP2D6, and CYP3A4) (Spina *et al.*, 2008), extant evidence suggests that the impact of pharmacokinetic interaction is minimal. Combined treatment with CIT and HAL (Syvalahti *et al.*, 1997) or CIT and OLZ (Botts *et al.*, 2008) did not cause significant changes in the plasma concentrations of HAL or OLZ in

humans and possibly in rats. This leaves the pharmacodynamic interaction as the most likely factor contributing toward the enhanced antiavoidance effect. Indeed, several studies have reported that the central (brain) effects of HAL and OLZ are enhanced by CIT or other SSRIs. For example, Waldmeier and Delini-Stula (1979) reported that CIT potentiated the increase in striatal deaminated DA metabolites (homovanillic acid and 3,4-dihydroxyphenylacetic acid) induced by HAL. Behaviorally, CIT also potentiated HAL-induced catalepsy and its antagonism of apomorphine-induced stereotypies. Huang *et al.* (2006) found that CIT increased extracellular DA and norepinephrine efflux in rat medial prefrontal cortex induced by risperidone. Others have also shown that fluoxetine increases the release of DA and norepinephrine in the medial prefrontal cortex induced by OLZ (Koch *et al.*, 2004). These augmented neurochemical effects by CIT on HAL or OLZ may explain the potentiated antiavoidance effect in the present study and the potentiated therapeutic effects on affective symptoms in the clinic (Zink *et al.*, 2010). Because the antipsychotic action, as well as the antiavoidance effect of HAL and OLZ, is shown to be mediated by the antagonism of D<sub>2</sub> receptors (Wadenberg *et al.*, 2001; Li *et al.*, 2010), CIT may increase the antagonistic action of HAL and OLZ on D<sub>2</sub> receptors through its selective inhibition of reuptake of 5-HT and increase of 5-HT release in the medial prefrontal cortex (Huang *et al.*, 2006). Another possibility is that the increased level of 5-HT by CIT treatment may stimulate 5-HT<sub>2C</sub> receptors to exert a disruptive effect on avoidance responding. This hypothesis is supported by the evidence showing that 5-HT<sub>2C</sub> receptor agonists such as 2,5-dimethoxy-4-iodo-amphetamine (DOI), 1-(3-chlorophenyl) piperazine (mCPP), and the 5-HT<sub>2A/2C</sub> receptor agonist D-LSD disrupt the avoidance response (Wadenberg and Hicks, 1999; Li *et al.*, 2010). It is also consistent with the findings that the activation of 5-HT<sub>2C</sub> receptors decreases DA release in the nucleus accumbens and cell firing in the ventral tegmental area (Di Giovanni *et al.*, 2000; Di Matteo *et al.*, 2002), the mesolimbic DA system that is implicated in the CAR (Wadenberg and Hicks, 1999).

In recent years, we have shown that the repeated administration of HAL and OLZ induces a behavioral sensitization in the CAR model (Li *et al.*, 2010; Mead and Li, 2010; Zhang and Li, 2012). This sensitization effect can be found in a challenge test (i.e. re-exposure to the drug) in which antipsychotic-treated animals show a stronger response (i.e. lower avoidance) to the drug than drug-naïve animals (Mead and Li, 2009; Zhang *et al.*, 2011; Zhang and Li, 2012). It is also long-lasting, producing an effect that can be observed up to 3 weeks later (Mead and Li, 2009) and is subject to contextual and behavioral controls (Zhang and Li, 2012). The results from the present study are consistent with these previous observations (Figs 4 and 7). In the current study, rats treated with HAL or OLZ (i.e. VEH + HAL or CIT + HAL; VEH + OLZ or CIT + OLZ) in the CAR testing apparatus showed enhanced responses to a challenge dose of HAL or OLZ compared with those treated with the same drugs outside of the CAR apparatus. More interestingly, CIT treatment did not alter this long-term treatment effect of HAL or OLZ, as there was no significant difference between the two HAL (VEH + HAL or CIT + HAL) or two OLZ (VEH + OLZ or CIT + OLZ) groups on the challenge tests. Our recent work indicates that the activation of 5-HT<sub>2A/2C</sub> receptors by DOI (a 5-HT<sub>2A/2C</sub> receptor agonist) can attenuate HAL-induced and (to a lesser extent) OLZ-induced sensitization of avoidance responding (Li *et al.*, 2010). The failure of CIT, but not DOI, to modulate HAL and OLZ sensitization might reflect differences

between changes in the synaptic levels of 5-HT produced by reuptake inhibition and the direct stimulation of 5-HT<sub>2A/2C</sub> receptors.

As mentioned in the Introduction section, most schizophrenic patients are treated with multiple psychotherapeutic drugs (ZumBrunnen and Jann, 1998; Zink *et al.*, 2010). Combined SSRIs and antipsychotic therapies are also used widely in the treatment of major depressive disorders and especially in hard-to-treat and treatment-refractory patients (DeBattista and Hawkins, 2009). There are also drugs (e.g. Symbyax) with this drug combination built in (DeBattista and DeBattista, 2010). The current studies are important in understanding the psychological interactions associated with the polypharmacy treatment of schizophrenia-related spectrum disorders and comorbidities. The methodology of these studies effectively models the acute antipsychotic actions of drugs and their progressive effectiveness over time. The dynamics of drug efficacy may be of considerable importance in predicting both acute and long-term behavioral outcomes. As the current studies indicate, these drugs can have behavioral interactions that are well beyond the traditionally considered pharmacological interactions. These studies show that SSRIs, such as CIT, may augment the behavioral effects of both typical and atypical antipsychotics, although to date, this interaction has not been observed in a clinical setting. It is important to consider that at clinically relevant doses, drugs may interact at a behavioral level in ways that may either be efficacious or harmful in real-world use. Furthermore, these studies reinforce the idea that the experiential context of drug action may be an important part of drug efficacy and may play a role in drug maintenance and symptom relapse.

Besides its contribution in providing a preclinical approach to the study of polypharmacy in the treatment of schizophrenia, the present study is also important because it extends psychopharmacology research on antipsychotic drugs utilizing a Pavlovian DDC paradigm (Li *et al.*, 2009). First, it shows that an instrumental conditioned active motor behavior can also be used as a valid index to evaluate the drug conditioning effect. This is different from many previous drug conditioning studies that typically use simple physiological measures or reactive responses, 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). Second, as discussed in our previous publication (Li *et al.*, 2009), it introduces a new approach to examine the effects of DDC. In many drug conditioning studies, the drug conditioning effect is often indexed by some change in one or more of the intrinsic properties of the CS drug (Taukulis, 1996). For example, in a series of studies on the diazepam–haloperidol or diazepam–chlorpromazine conditioning (Taukulis and Brake, 1989; Taukulis *et al.*, 1992), the drug conditioning was evidenced by the altered drug properties of diazepam, such as enhanced hypothermia, reduced muscle relaxation, and enhanced anxiolytic effect. In the present study, the conditioning effect was observed in the newly ‘acquired’ avoidance-disruptive effect of CIT, which is not an intrinsic drug property of CIT. This approach provides an unequivocal demonstration of the DDC effect. Finally, it indicates that SSRIs can also be used as a CS drug to study the behavioral and neurobiological mechanisms underlying drug–drug interactions. Together with our previous work with chlordiazepoxide

(Li *et al.*, 2009), it appears that DDC may be a general process applicable to multiple psychotropic drugs.

## Conclusion

Our results show that the concurrent use of CIT with HAL or OLZ caused a potentiation of the avoidance-disruptive effect of both antipsychotic drugs. Conversely, the behavioral effect of CIT was altered by HAL or OLZ through a DDC process, so that CIT acquired an additional avoidance-disruptive effect (an antipsychotic-like effect) after being combined repeatedly with HAL or OLZ. Our work provides a preclinical approach to examine the extent and mechanisms of drug–drug interactions among antipsychotics and antidepressants in the treatment of schizophrenia.

## Acknowledgments

This study was funded by a NIMH grant (R01MH085635) to Professor Ming Li. We thank Paul Nabity and Natasha Swalve for their editorial work.

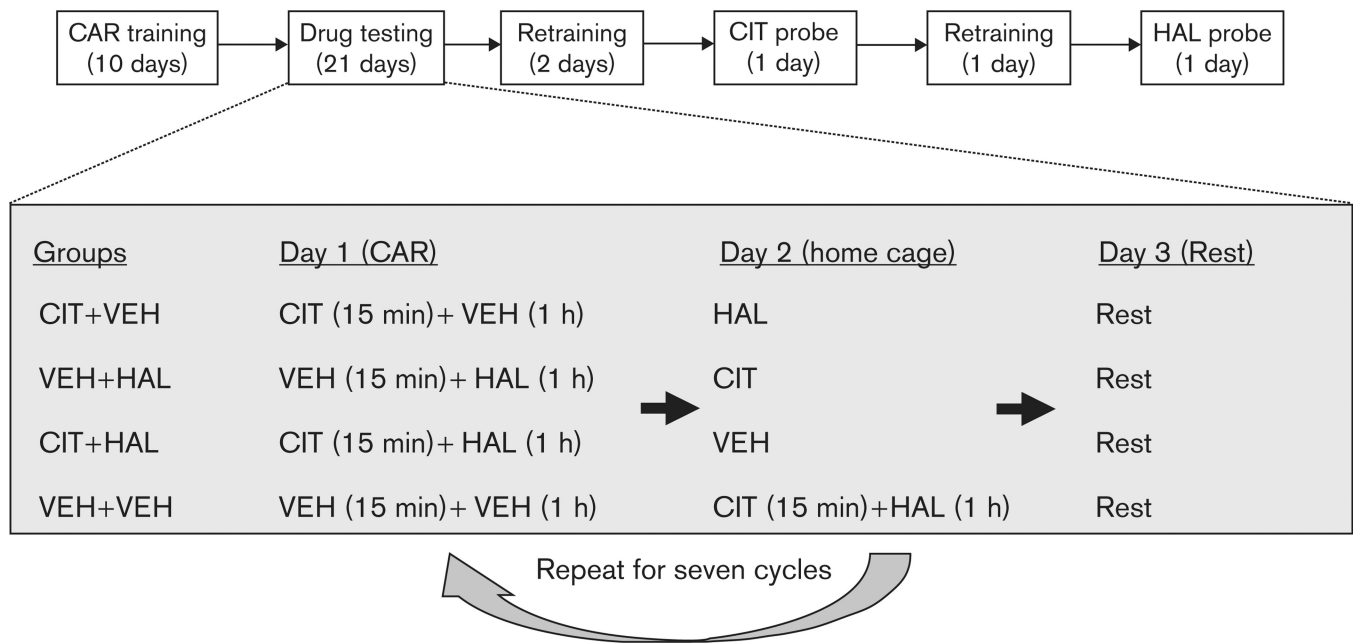
## References

- Alfaro CL. Emerging role of drug interaction studies in drug development: the good, the bad, and the unknown. *Psychopharmacol Bull.* 2001; 35:80–93. [PubMed: 12397858]
- Aravagiri M, Teper Y, Marder SR. Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharm Drug Dispos.* 1999; 20:369–377. [PubMed: 10870093]
- Arnt J. Pharmacological specificity of conditioned avoidance response inhibition in rats: inhibition by neuroleptics and correlation to dopamine receptor blockade. *Acta Pharmacol Toxicol (Copenh).* 1982; 51:321–329. [PubMed: 6129770]
- Biederman GB, Davey VA. Antisickness conditioning using a nausea-producing nondrug cue. *Behav Neurosci.* 1993; 107:215–217. [PubMed: 8383501]
- Botts S, Diaz FJ, Santoro V, Spina E, Muscatello MR, Cogollo M, et al. Estimating the effects of co-mediations on plasma olanzapine concentrations by using a mixed model. *Prog Neuropsychopharmacol Biol Psychiatry.* 2008; 32:1453–1458. [PubMed: 18555573]
- Buckley PF. Factors that influence treatment success in schizophrenia. *J Clin Psychiatry.* 2008; 69(Suppl 3):4–10.
- Cheng YF, Paalzow LK. Linear pharmacokinetics of haloperidol in the rat. *Biopharm Drug Dispos.* 1992; 13:69–76. [PubMed: 1554878]
- Conley RR, Kelly DL. Drug-drug interactions associated with second-generation antipsychotics: considerations for clinicians and patients. *Psychopharmacol Bull.* 2007; 40:77–97. [PubMed: 17285099]
- Davey VA, Biederman GB. Methodological issues in drug–drug conditioning in rats: nonassociative factors in heart rate and avfail. *Behav Neurosci.* 1991; 105:850–859. [PubMed: 1663759]
- DeBattista C, DeBattista K. Safety considerations of the use of second generation antipsychotics in the treatment of major depression: extrapyramidal and metabolic side effects. *Curr Drug Saf.* 2010; 5:263–266. [PubMed: 20394571]
- DeBattista C, Hawkins J. Utility of atypical antipsychotics in the treatment of resistant unipolar depression. *CNS Drugs.* 2009; 23:369–377. [PubMed: 19453199]
- Di Giovanni G, Di Matteo V, Di Mascio M, Esposito E. Preferential modulation of mesolimbic vs. nigrostriatal dopaminergic function by serotonin(2C/2B) receptor agonists: a combined in vivo electrophysiological and microdialysis study. *Synapse.* 2000; 35:53–61. [PubMed: 10579808]
- Di Matteo V, Cacchio M, Di Giulio C, Esposito E. Role of serotonin(2C) receptors in the control of brain dopaminergic function. *Pharmacol Biochem Behav.* 2002; 71:727–734. [PubMed: 11888564]

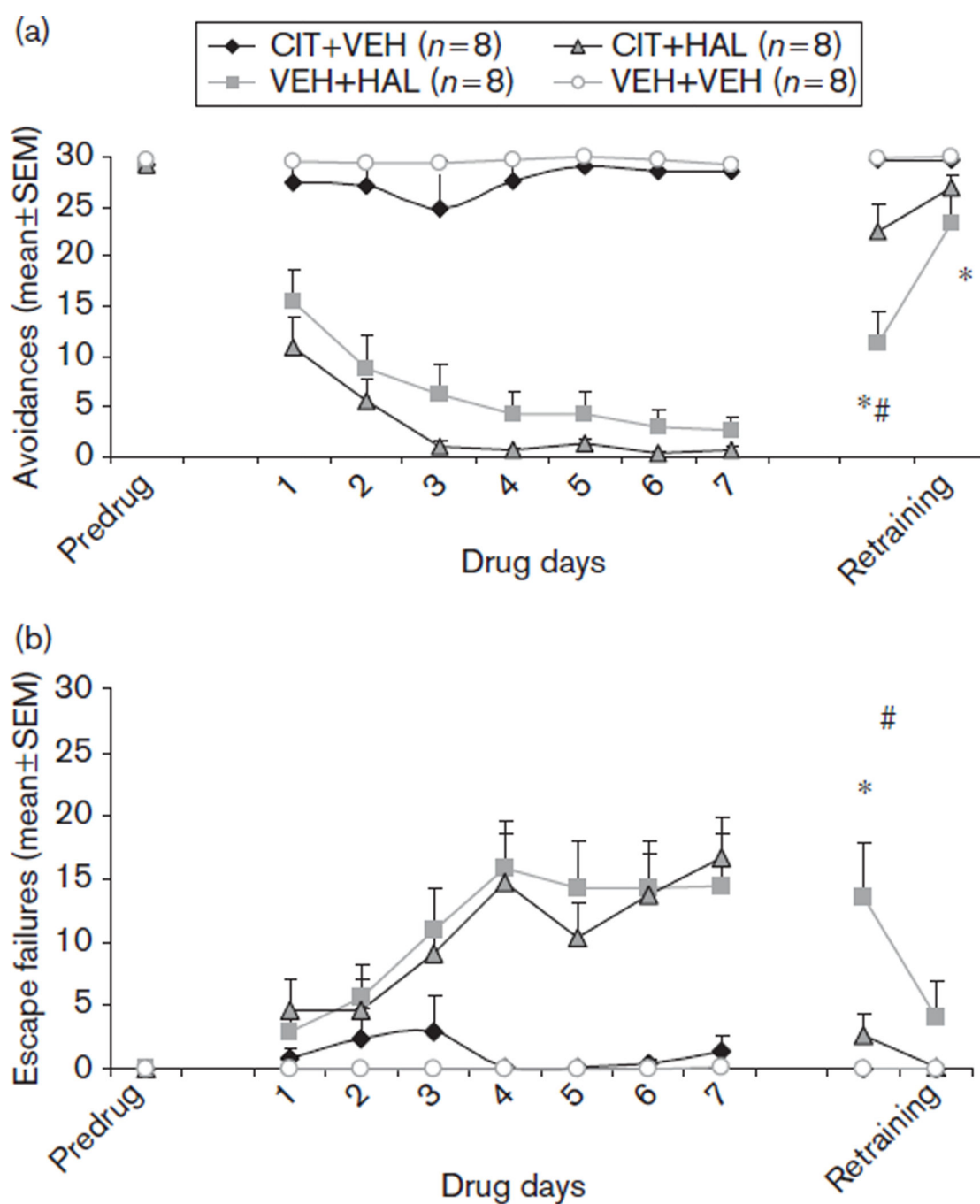
- Domjan M. Pavlovian conditioning: a functional perspective. *Annu Rev Psychol.* 2005; 56:179–206. [PubMed: 15709933]
- Englisch S, Knopf U, Scharnholz B, Kuwilsky A, Deuschle M, Zink M. Duloxetine for major depressive episodes in the course of psychotic disorders: an observational clinical trial. *J Psychopharmacol.* 2009; 23:875–882. [PubMed: 18583440]
- Franberg O, Wiker C, Marcus MM, Konradsson A, Jardemark K, Schilström B, et al. Asenapine, a novel psychopharmacologic agent: preclinical evidence for clinical effects in schizophrenia. *Psychopharmacology (Berl).* 2008; 196:417–429. [PubMed: 17940749]
- Friedman JI, Ocampo R, Elbaz Z, Parrella M, White L, Bowler S, et al. The effect of citalopram adjunctive treatment added to atypical antipsychotic medications for cognitive performance in patients with schizophrenia. *J Clin Psychopharmacol.* 2005; 25:237–242. [PubMed: 15876902]
- Hashimoto S, Inoue T, Muraki I, Koyama T. Effects of acute citalopram on the expression of conditioned freezing in naive versus chronic citalopram-treated rats. *Prog Neuropsychopharmacol Biol Psychiatry.* 2009; 33:113–117. [PubMed: 19010374]
- Huang M, Ichiwaka J, Li Z, Dai J, Meltzer HY. Augmentation by citalopram of risperidone-induced monoamine release in rat prefrontal cortex. *Psychopharmacology (Berl).* 2006; 185:274–281. [PubMed: 16521036]
- Hyttel J, Overo KF, Arnt J. Biochemical effects and drug levels in rats after long-term treatment with the specific 5-HT-uptake inhibitor, citalopram. *Psychopharmacology (Berl).* 1984; 83:20–27. [PubMed: 6429698]
- Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. *J Pharmacol Exp Ther.* 2003; 305:625–631. [PubMed: 12606608]
- Koch S, Perry KW, Bymaster FP. Brain region and dose effects of an olanzapine/fluoxetine combination on extracellular monoamine concentrations in the rat. *Neuropharmacology.* 2004; 46:232–242. [PubMed: 14680761]
- Li M, Fletcher PJ, Kapur S. Time course of the antipsychotic effect and the underlying behavioral mechanisms. *Neuropsychopharmacology.* 2007; 32:263–272. [PubMed: 16738541]
- Li M, He W, Mead A. An investigation of the behavioral mechanisms of antipsychotic action using a drug–drug conditioning paradigm. *Behav Pharmacol.* 2009; 20:184–194. [PubMed: 19322074]
- Li M, Sun T, Zhang C, Hu G. Distinct neural mechanisms underlying acute and repeated administration of antipsychotic drugs in rat avoidance conditioning. *Psychopharmacology (Berl).* 2010; 212:45–57. [PubMed: 20623111]
- Mead A, Li M. Avoidance-suppressing effect of antipsychotic drugs is progressively potentiated after repeated administration: an interoceptive drug state mechanism. *J Psychopharmacol.* 2009; 24(7): 1045–1053. [PubMed: 19329544]
- Mead A, Li M. Avoidance-suppressing effect of antipsychotic drugs is progressively potentiated after repeated administration: an interoceptive drug state mechanism. *J Psychopharmacol.* 2010; 24:1045–1053. [PubMed: 19329544]
- Porsolt RD, Moser PC, Castagne V. Behavioral indices in antipsychotic drug discovery. *J Pharmacol Exp Ther.* 2010; 333(3):632–638. [PubMed: 20200119]
- Reilly S, Revusky S. Drug–drug heart rate conditioning in rats: effective USs when pentobarbital is the CS. *Pharmacol Biochem Behav.* 1992; 42:633–643. [PubMed: 1325056]
- Revusky S, Taukulis HK, Peddle C. Learned associations between drug states: attempted analysis in Pavlovian terms. *Physiol Psychol.* 1979; 7:352–363.
- Revusky S, Davey V, Zagorski M. Heart rate conditioning with pentobarbital as a conditioned stimulus and amphetamine as an unconditioned stimulus. *Behav Neurosci.* 1989; 103:296–307. [PubMed: 2706076]
- Rupnow MF, Greenspan A, Gharabawi GM, Kosik-Gonzalez C, Zhu Y, Stahl SM. Incidence and costs of polypharmacy: data from a randomized, double-blind, placebo-controlled study of risperidone and quetiapine in patients with schizophrenia or schizoaffective disorder. *Curr Med Res Opin.* 2007; 23(11):2815–2822. [PubMed: 17910802]



- Salokangas RK, Saarijarvi S, Taiminen T, Kallioniemi H, Lehto H, Niemi H, et al. Citalopram as an adjuvant in chronic schizophrenia: a double-blind placebo-controlled study. *Acta Psychiatr Scand*. 1996; 94:175–180. [PubMed: 8891083]
- Sandson NB, Armstrong SC, Cozza KL. An overview of psychotropic drug-drug interactions. *Psychosomatics*. 2005; 46:464–494. [PubMed: 16145193]
- Sepehry AA, Potvin S, Elie R, Stip E. Selective serotonin reuptake inhibitor (SSRI) add-on therapy for the negative symptoms of schizophrenia: a meta-analysis. *J Clin Psychiatry*. 2007; 68:604–610. [PubMed: 17474817]
- Spina E, Santoro V, D'Arrigo C. Clinically relevant pharmacokinetic drug interactions with second-generation antidepressants: an update. *Clin Ther*. 2008; 30:1206–1227. [PubMed: 18691982]
- Sun T, He W, Hu G, Li M. Anxiolytic-like property of risperidone and olanzapine as examined in multiple measures of fear in rats. *Pharmacol Biochem Behav*. 2010; 95:298–307. [PubMed: 20167232]
- Syvalahti EK, Taiminen T, Saarijarvi S, Lehto H, Niemi H, Ahola V, et al. Citalopram causes no significant alterations in plasma neuroleptic levels in schizophrenic patients. *J Int Med Res*. 1997; 25:24–32. [PubMed: 9027670]
- Taukulis HK. Pavlovian conditioning to a diazepam cue with yohimbine as the unconditional stimulus. *Neurobiol Learn Mem*. 1996; 65:223–232. [PubMed: 8616586]
- Taukulis HK, Brake LD. Therapeutic and hypothermic properties of diazepam altered by a diazepam-chlorpromazine association. *Pharmacol Biochem Behav*. 1989; 34:1–6. [PubMed: 2626441]
- Taukulis HK, Fillmore MT, Ruggles JL. Neuroleptic-induced changes in the anxiolytic and myorelaxant properties of diazepam in the rat. *Pharmacol Biochem Behav*. 1992; 41:13–21. [PubMed: 1347171]
- Wadenberg ML. Conditioned avoidance response in the development of new antipsychotics. *Curr Pharm Des*. 2010; 16:358–370. [PubMed: 20109144]
- Wadenberg ML, Hicks PB. The conditioned avoidance response test reevaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? *Neurosci Biobehav Rev*. 1999; 23:851–862. [PubMed: 10541060]
- Wadenberg ML, Soliman A, VanderSpek SC, Kapur S. Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. *Neuropsychopharmacology*. 2001; 25:633–641. [PubMed: 11682246]
- Waldmeier PC, Delini-Stula AA. Serotonin–dopamine interactions in the nigrostriatal system. *Eur J Pharmacol*. 1979; 55:363–373. [PubMed: 572774]
- Wilkin LD, Cunningham CL, Fitzgerald RD. Pavlovian conditioning with ethanol and lithium: effects on heart rate and taste aversion in rats. *J Comp Physiol Psychol*. 1982; 96:781–790. [PubMed: 6292269]
- Zhang C, Li M. Contextual and behavioral control of antipsychotic sensitization induced by haloperidol and olanzapine. *Behav Pharmacol*. 2012; 23:66–79. [PubMed: 22157143]
- Zhang C, Fang Y, Li M. Olanzapine and risperidone disrupt conditioned avoidance responding by selectively weakening motivational salience of conditioned stimulus: further evidence. *Pharmacol Biochem Behav*. 2011; 98:155–160. [PubMed: 21194545]
- Zink M, Englisch S, Meyer-Lindenberg A. Polypharmacy in schizophrenia. *Curr Opin Psychiatry*. 2010; 23:103–111. [PubMed: 20051861]
- Zisook S, Kascow JW, Golshan S, Fellows I, Solorzano E, Lehman D, et al. Citalopram augmentation for subsyndromal symptoms of depression in middle-aged and older outpatients with schizophrenia and schizoaffective disorder: a randomized controlled trial. *J Clin Psychiatry*. 2009; 70:562–571. [PubMed: 19192468]
- ZumBrunnen TL, Jann MW. Drug interactions with antipsychotic agents: Incidence and therapeutic implications. *CNS Drugs*. 1998; 9:381–401.

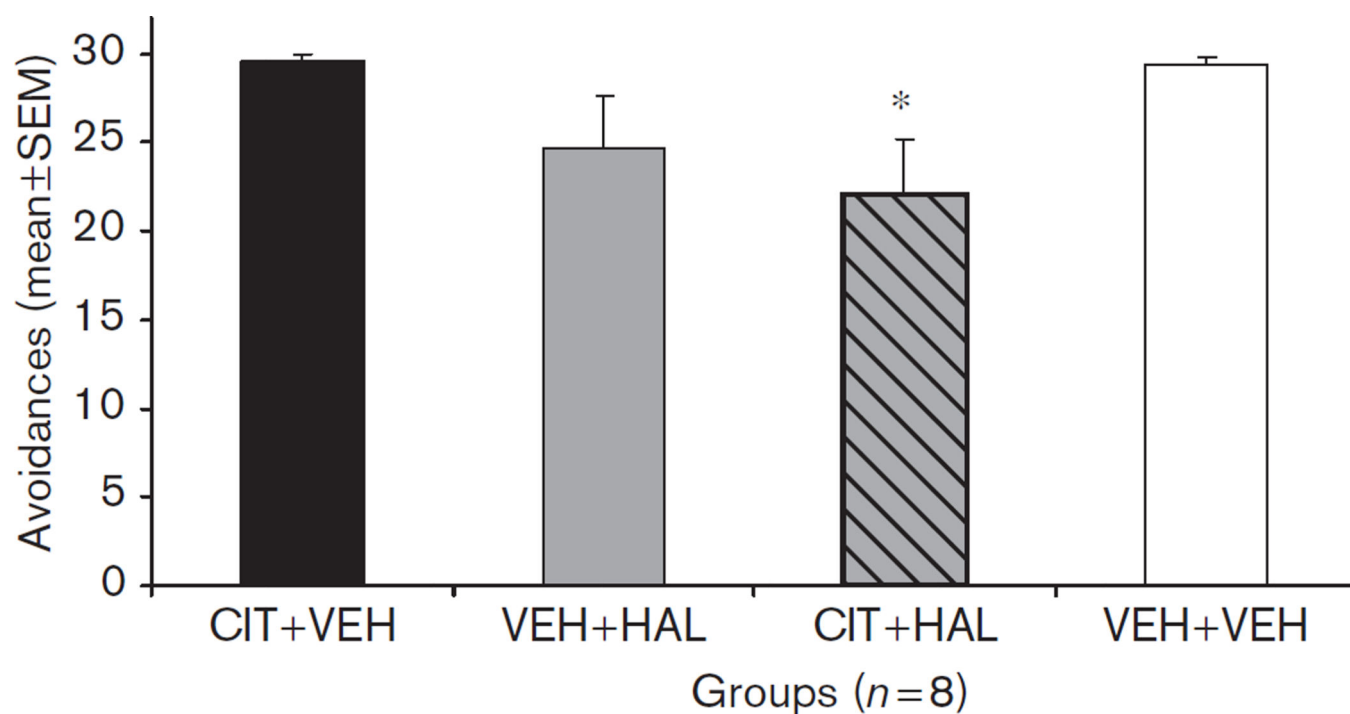
**Fig. 1.**

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



**Fig. 2.**

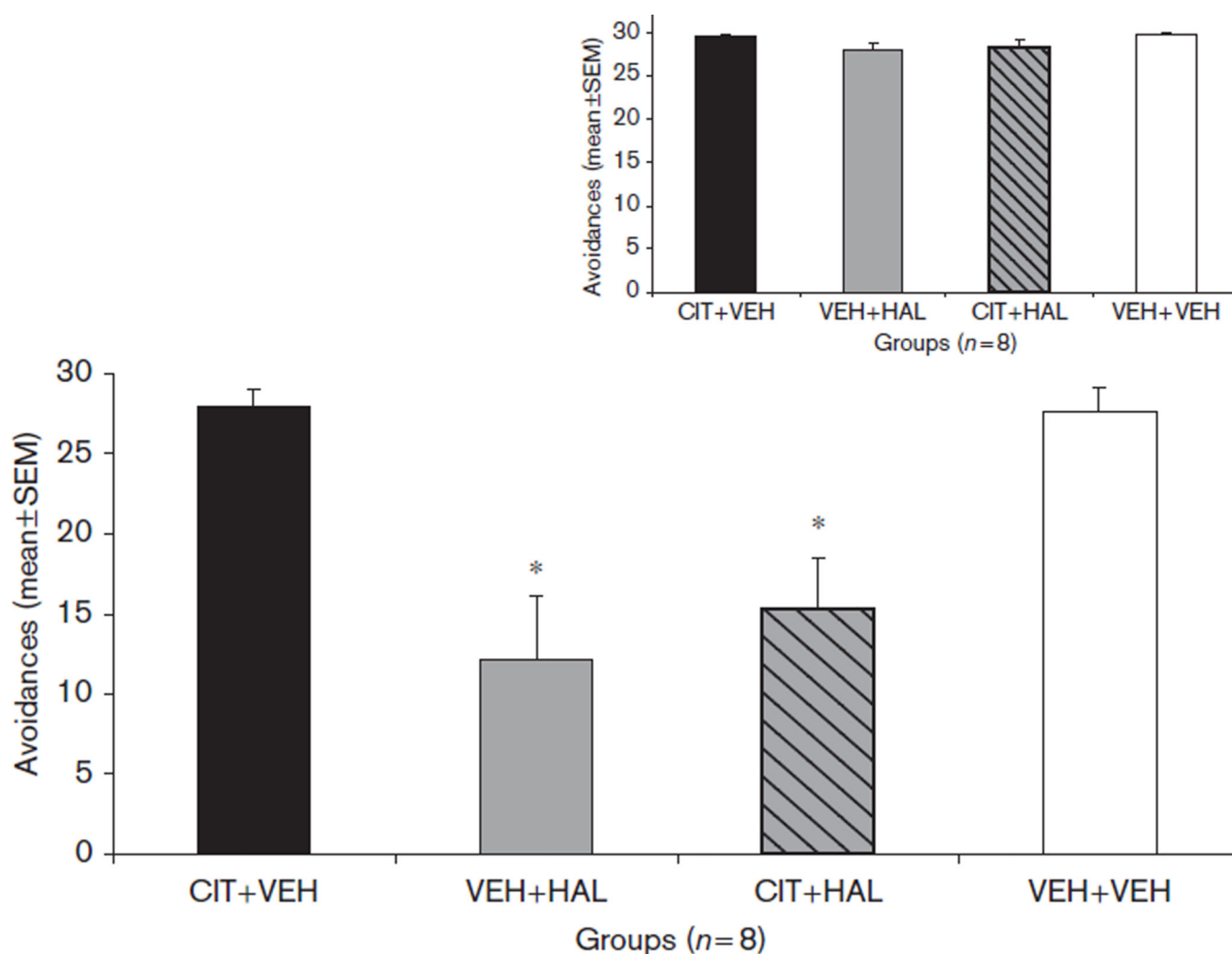
Experiment 1: Number of avoidances (a) and escape failures (b) made by the rats in the four groups during the last pre-drug session, seven drug conditioning sessions, and two drug-free retraining sessions. Points represent mean ± SEM. \*Differs significantly ( $P < 0.05$ ) from CIT + VEH and VEH + VEH; #differs significantly ( $P < 0.05$ ) from CIT + HAL. CIT, citalopram; HAL, haloperidol; VEH, vehicle.



**Fig. 3.**

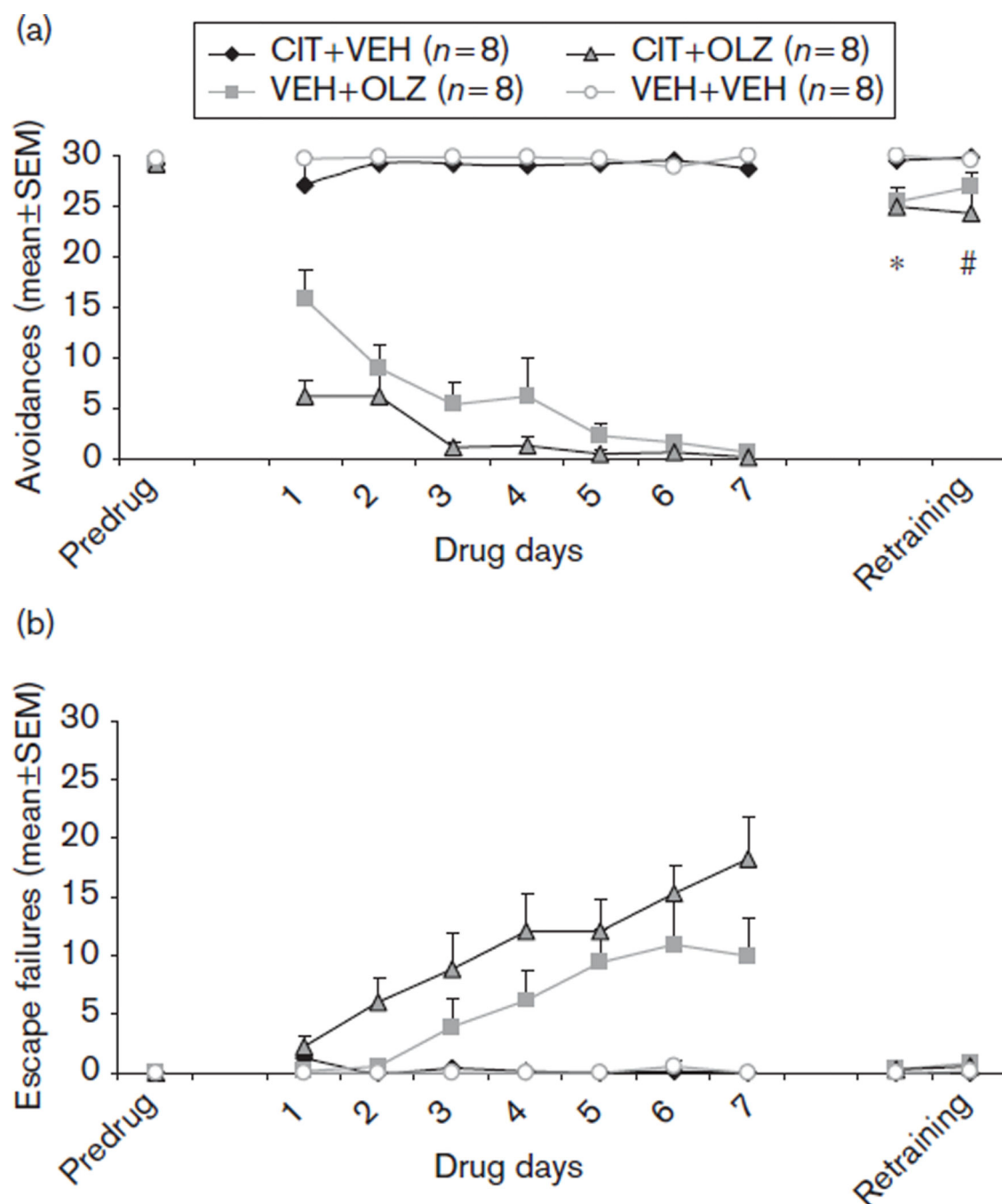
Experiment 1: Number of avoidances during the CIT challenge test. All rats were injected with CIT (10 mg/kg, intraperitoneally) and tested 75 min later. Points represent mean±SEM.

\*Differs significantly ( $P < 0.05$ ) from CIT + VEH and VEH + VEH. CIT, citalopram; HAL, haloperidol; VEH, vehicle.



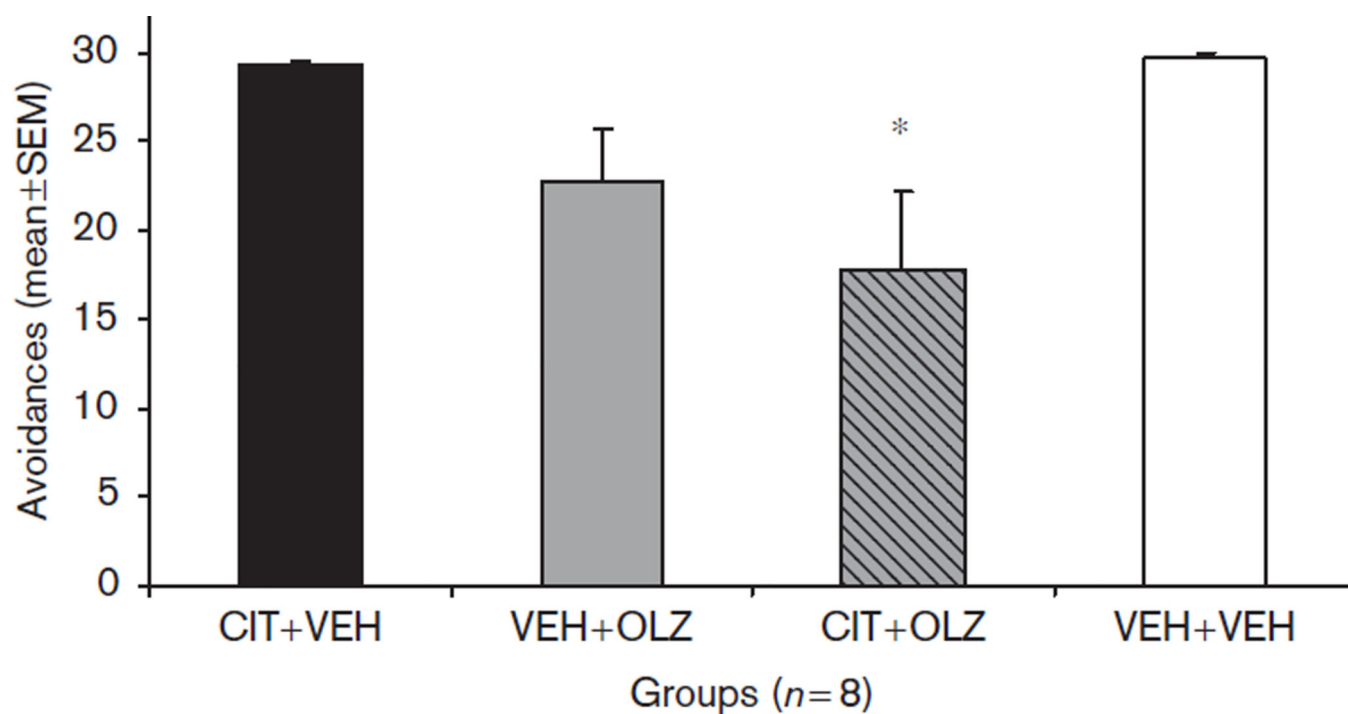
**Fig. 4.**

Experiment 1: Number of avoidances during the HAL challenge test. All rats were injected with HAL (0.025 mg/kg) and tested 60 min later. Points represent mean  $\pm$  SEM. \*Differs significantly ( $P < 0.05$ ) from CIT + VEH and VEH + VEH. Inset shows the number of avoidances from the drug-free retraining session conducted 1 day before. CIT, citalopram; HAL, haloperidol; VEH, vehicle.

**Fig. 5.**

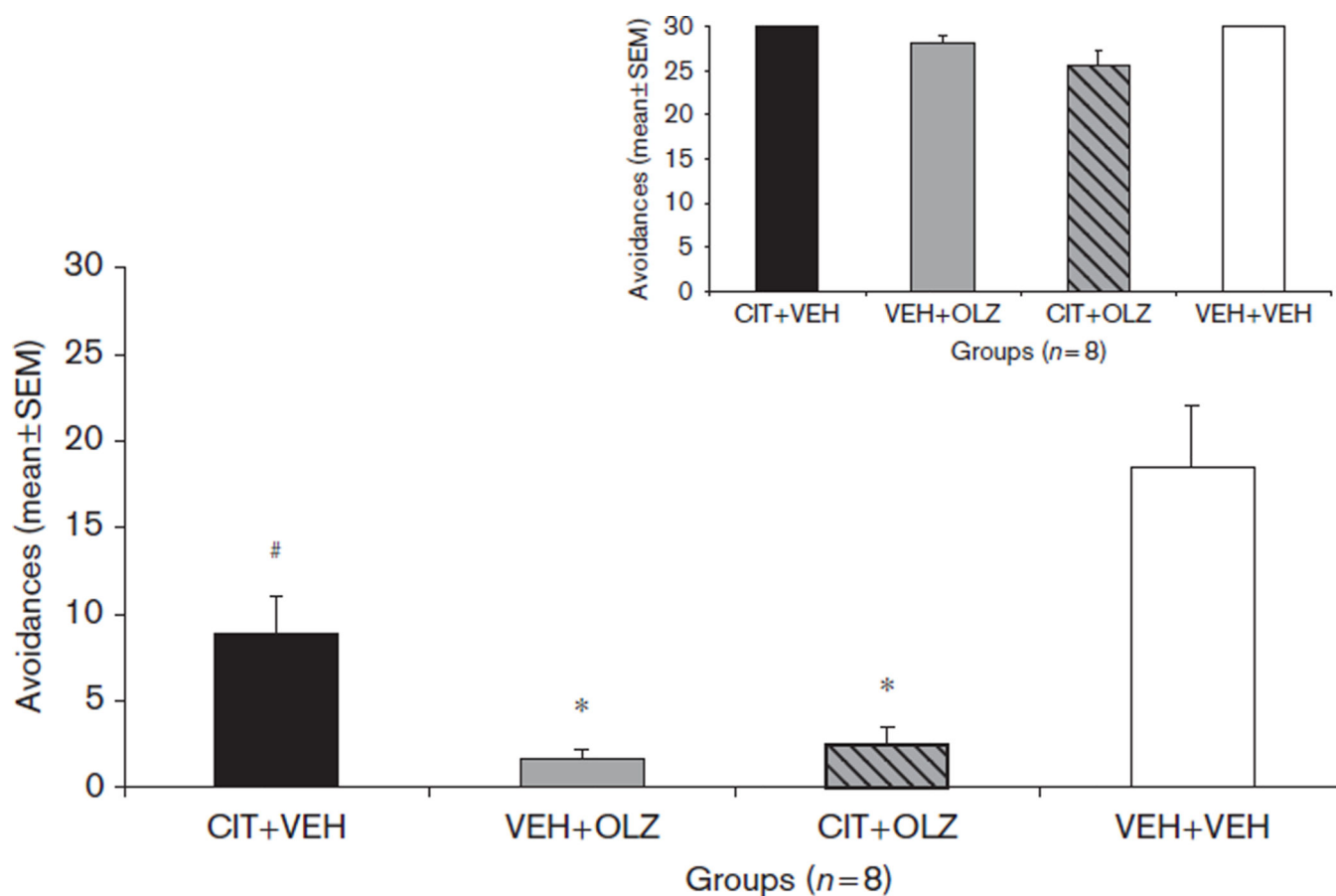
Experiment 2: Number of avoidances (a) and escape failures (b) made by the rats in the four groups during the last predrug session, seven drug conditioning sessions, and two drug-free retraining sessions. Points represent mean±SEM. \*VEH + OLZ and CIT + OLZ differ significantly ( $P < 0.05$ ) from the VEH + VEH or CIT + VEH; #CIT + OLZ differ significantly from the VEH + VEH and CIT + VEH. CIT, citalopram; HAL, haloperidol; OLZ, olanzapine; VEH, vehicle.





**Fig. 6.**

Experiment 2: During the CIT challenge test. All rats were injected with CIT (10 mg/kg, intraperitoneally) and tested 75 min later. Points represent mean ± SEM. \*Differs significantly ( $P < 0.05$ ) from CIT + VEH and VEH + VEH. CIT, citalopram; OLZ, olanzapine; VEH, vehicle.



**Fig. 7.**

Experiment 2: Number of avoidances during the OLZ challenge test. All rats were injected with OLZ (0.5 mg/kg) and tested 60 min later. Points represent mean ± SEM. \*Differs significantly ( $P < 0.05$ ) from CIT + VEH and VEH + VEH. #Differs significantly ( $P < 0.05$ ) from VEH + OLZ, CIT + OLZ and VEH + VEH. Inset shows the number of avoidances from the drug-free retraining session conducted 1 day before. CIT, citalopram; OLZ, olanzapine; VEH, vehicle.