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Avoidance disruptive effect of clozapine and olanzapine is potentiated by increasing the test trials: Further test of the motivational salience hypothesis

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Abstract

Antipsychotic drugs suppress animals' ability to avoid an aversive stimulus in the conditioned avoidance response model (CAR). This behavioral effect is thought to reflect antipsychotic activity and is suggested to be mediated by a drug's action in attenuating the motivational salience of a conditioned stimulus (CS). In the present study, we tested whether atypical antipsychotic drugs clozapine and olanzapine act through this behavioral mechanism by manipulating the number of avoidance test trials. We reasoned that more CS trials in the present of clozapine or olanzapine would afford the drug more opportunities to decrease the motivational salience of the CS, thus avoidance decline would be greater with the increase of CS trials in each test session. In two separate experiments, adult male Sprague-Dawley rats were tested under clozapine (5.0 mg/kg, sc), olanzapine (0.5 mg/kg, sc) or vehicle (sterile water) for 6 consecutive days in three CS trial conditions (i.e. 3, 10, and 40 CS trials per session). Two days later, all rats were tested under the same 40-trial session after receiving clozapine (5.0 mg/kg, sc) or olanzapine (0.5 mg/kg, sc). Results show that repeated clozapine and olanzapine treatment persistently decreased avoidance response, and this effect was potentiated by the increase of number of CS trials in the test sessions, as the clozapine-treated or olanzapine-treated rats tested under the 40-trial or 10-trial condition had significantly lower avoidance and faster decline across-sessions than those tested under the 3-trial condition. This potentiated effect was not only seen in the total avoidance percentage, but also observed in the within-session decline pattern in the last three drug test sessions and in the final 40-trial test session. These findings suggest that the clinical efficacy of a drug can be enhanced by increasing the exposure of symptoms in the presence of the drug.

Keywords

Clozapine; Olanzapine; Conditioned avoidance response; Motivational salience; Within-session decline

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1. Introduction

Antipsychotic drugs (APDs), including typical (e.g. haloperidol, HAL) and atypical (e.g. clozapine, CLZ, and olanzapine, OLZ) classes, are effective in the treatment of psychotic symptoms (e.g. delusions, hallucinations). Research over the years has shown that actions at various receptor sites, notably dopamine D_2, serotonin 5-HT_{2A}, and/or 5-HT_{1A} receptors, are critically important for the therapeutic effect of antipsychotic drugs (Miyamoto et al., 2005). How they work at the behavioral level is not entirely clear. One idea is that antipsychotic treatment halts incentive learning and decreases aberrantly heightened motivational salience of stimuli (e.g. psychotic thoughts, abnormal perceptions) which typically grab a patient's attention and instigate a motivated response (Kapur et al., 2005). As a result, new aberrant beliefs are less likely to form and previously formed aberrant memories are more likely to be extinguished (Beninger, 2006, Kapur, 2003, Kapur, Mizrahi, 2005, Miller, 1987). Clinical evidence that patients under drug treatment are less likely to generate "erroneous" associations in the forms of delusions or hallucinations, and are less bothered by their symptoms is consistent with this idea (Kapur et al., 2006, Mizrahi et al., 2006). However, the precise treatment parameters and conditions (e.g. classes of drug, drug dose, dosing regimens, routes of administration, environmental settings, with or without psychotherapies, etc) that may modulate this putative action of antipsychotic drugs are not well understood due to the complexity and heterogeneity of clinical conditions (Kapur, 2003, Kapur, Mizrahi, 2005, Mizrahi, Kiang, 2006, Remington and Kapur, 2010).

In recent years, we have used the conditioned avoidance responding (CAR) model and examined the motivational salience attenuation action of APDs (Li et al., 2007). The CAR model is a commonly used preclinical test for antipsychotic drugs with the suppression of avoidance response as a behavioral index of antipsychotic activity (Li, Fletcher, 2007, Porsolt et al., 2010). It is also an instrumental incentive (negative) conditioning paradigm (Bolles and Grossen, 1970) which allows us to examine the role of motivational salience in psychotic-like behavior in animals (Li et al., 2008) and investigate the motivational salience attenuation action of APDs (Li et al., 2009, Zhang et al., 2011). In the CAR, a neutral stimulus (e.g. white noise) is presented in close temporal contiguity with a foot-shock (unconditioned stimulus, US), thus acquiring the motivational property of the US through an incentive salience attribution process and becoming a conditioned stimulus (CS). As a CS, it can instigate the motor response that is usually elicited by the US. One interesting finding on the motivational salience attenuation action of APDs comes from our recent study in which we tested three groups of rats under the same dose of HAL treatment (0.025 mg/kg, sc) but under three different CS trial conditions (3, 10, 40 CS trials) for 6 days (Li, Fletcher, 2007). We found that HAL-treated rats tested under the 40-trial test sessions showed a faster decline than those tested under the 10-trial and 3-trial sessions across the 6 days of testing. Two days later, when all groups were tested again in a 40-trial session after injected with HAL 0.025 mg/kg, the 40-trial HAL had significantly lower avoidance responding than the 3-trial group ($p=0.037$, one-tail). These findings suggest that the more exposures to the CS in the presence of HAL afford the drug more opportunities to decrease the motivational salience of the CS and lead to a faster decline in avoidance responding - a position compatible with the motivational salience attenuation hypothesis and also consistent with our other findings showing that rats previously treated with HAL and CLZ still show significantly lower avoidance responses even in the absence of drug (Li et al., 2004) and risperidone and OLZ have a stronger suppressive effect on a less salient CS2-elicited avoidance than a highly salient CS1-elicited avoidance (Li, He, 2009, Zhang, Fang, 2011).

The present study was designed to provide an additional test of the motivational salience attenuation action of APDs using the different CS trial testing paradigm (Li, Fletcher, 2007). If the avoidance disruptive effect of atypical drugs such as CLZ and OLZ is also modulated

by the number of CS trials, with more trials in each test session leading to more disruption, similar to what we have observed with HAL, it would provide strong support for the motivational salience attenuation hypothesis.

2. Materials and Methods

2.1 Animals

Adult male Sprague-Dawley rats (226–250 g upon arrival, Charles River, Portage, MI) were used. They were housed two per cage, in 48.3 cm × 26.7 cm × 20.3 cm transparent polycarbonate cages under 12-hr light/dark conditions (light on between 6:30 am and 6:30 pm). Room temperature was maintained at 22±1°C with a relative humidity of 45–60%. Food and water was available *ad libitum*. Animals were allowed at least one 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.

2.2 Drugs

CLZ and OLZ (gifts from the NIMH drug supply program) were dissolved in 1.0% glacial acetic acid in sterile distilled water (Kapur et al., 2003). All drugs were administrated subcutaneously (sc), 1 h before testing in a volume of 1.0 ml/kg body weight. We tested CLZ at 5.0 mg/kg in the first experiment and OLZ at 0.5 mg/kg in the second experiment. These doses were chosen based on the following considerations: (1) our pilot work and previous reports show that at these doses, CLZ and OLZ produce a reliable and comparable disruption on avoidance responding (Mead and Li, 2009, Zhang and Li, 2012); (2) both CLZ and OLZ at these doses also give rise to a clinically comparable range (40%–60%) striatal dopamine D_2 occupancy in rats (Kapur, VanderSpek, 2003, Wadenberg et al., 2001).

2.3 Two-way avoidance conditioning apparatus

Eight identical two-way shuttle boxes custom designed and manufactured by Med Associates (St. Albans, VT) 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 was divided into two equal-sized compartments by a partition with an arch style doorway (15 cm high × 9 cm wide at base). A barrier (4 cm high) was placed between the two compartments, so the rats had to jump from one compartment to the other. The grid floor consisted of 40 stainless-steel rods with a diameter of 0.48 cm, spaced 1.6 cm apart center to center, through which a scrambled footshock (US, 0.8 mA, maximum duration: 5 s) was delivered by a constant current shock generator (Model ENV-410B) and scrambler (Model ENV-412). The rat location and crossings between compartments were monitored by a set of 16 photobeams (ENV-256-8P) affixed at the bottom of the box (3.5 cm above the grid floor). Illumination was provided by two houselights mounted at the top of each compartment. The CS (i.e. 76 dB white noise) was produced by a speaker (ENV 224 AMX) mounted on the ceiling of the cubicle, centered above the shuttle box. Background noise (approximately 74 dB) was provided by a ventilation fan affixed at the top corner of each isolation cubicle. All training and testing procedures were controlled by Med Associates programs running on a computer.

2.4 Experiment

2.4.1 Experiment 1: Effects of CLZ treatment on avoidance responding under different CS trial conditions—This experiment examined the extent to which the avoidance disruptive effect of CLZ is modulated by the number of CS trials. Sixty rats (in 2 batches) were first handled and habituated to the CAR boxes for 2 days (20 min/day). Then

they were trained to acquire avoidance responding for 10 sessions (one/day) over a 2-week period. Each session consisted of 30 trials, and each trial started with a presentation of a white noise (CS) for 10 s, followed by a continuous scrambled footshock (0.8 mA, US, maximum duration = 5 s) on the grid floor. If a rat moved from one compartment to the other within the 10 s of CS presentation, it avoided the shock and this shuttling response was recorded as avoidance. If the rat remained in the same compartment for more than 10 s and made a crossing only after receiving the footshock, this response was recorded as escape. If the rat did not switch compartments during the entire 5 s presentation of the shock, the trial was terminated and escape failure was recorded. The total number of avoidance responses was recorded for each session. Inter-trial intervals varied randomly between 30s and 60s.

One day after the last training session, rats were first tested under the CS-only condition (no shock) to assess their baseline level of avoidance responding (40 trials of the CS presentations). The CS-only condition was used to ensure that all rats had a consistent and high level of CS-elicited avoidance response before the drug tests. Fifty rats that acquired a robust and persistent avoidance response (≥ 28 avoidances in 40 trials) were matched and randomly assigned to one of the following six groups based a 2 (CLZ vs. vehicle, VEH) × 3 (3, 10, 40 trials) between-subjects factorial design: CLZ 3-trial (n=8), VEH 3-trial (n=8), CLZ 10-trial (n=8), VEH 10-trial (n=9), CLZ 40-trial (n=8) and VEH 40-trial (n=9). During the next 6 consecutive days, the three CLZ groups were injected with the same dose of CLZ (5.0 mg/kg, sc, −60 min) but tested under three different CS-only trial conditions (3, 10 or 40 CS presentations per session, no shock). The three VEH groups received the same VEH (sterile distilled water, 1.0 ml/kg) treatment and tested under 3-trial, 10-trial or 40-trial condition. Two days after the last (6^{th}) drug test session, all rats were injected with the same dose of CLZ (5.0 mg/kg, sc), and their avoidance behavior was tested in a CS-only 40-trial session. This test was designed to further assess whether prior different trial test conditions altered the motivational salience of the CS in the long run.

2.4.2 Experiment 2: Effects of OLZ treatment on avoidance responding under different CS trial conditions—This experiment examined the extent to which the avoidance disruptive effect of OLZ is modulated by the number of CS exposure in the presence of the drug. The basic procedure was identical to that of Experiment 1 with the exception that CLZ was replaced by OLZ (0.5 mg/kg). Sixty rats (in 2 batches) were initially trained, of which 52 rats reached the training criterion and were used in the subsequent drug tests. They were randomly assigned to one of six groups: OLZ 3-trial (n=9), VEH 3-trial (n=8), OLZ 10-trial (n=8), VEH 10-trial (n=8), OLZ 40-trial (n=9), and VEH 40-trial (n=9) groups and tested under OLZ (0.5 mg/kg, sc, −60 min) or VEH for 6 consecutive days. Two days later, all rats were tested again in a CS-only 40-trial session 1 h after receiving 0.5 mg/kg OLZ treatment.

2.5 Statistical Analysis

All data were expressed as mean ± S.E.M. Avoidance performance was expressed as the percent avoidance (i.e. number of avoidance/total number of trials) or the absolute number of avoidance responses and were analyzed using a split-plot analysis of variance (ANOVA) with the between-subjects factors being treatment (CLZ or OLZ vs. VEH) and trial conditions (3, 10, 40), and the within-subjects factor being test day (6 days). Group differences on each specific test day were analyzed by one-way ANOVA followed by Fisher's LSD tests (>3 groups) or independent samples t tests (2 groups). Group differences on the final 40-trial CS-only test were analyzed by one-way ANOVA followed by Fisher's LSD tests (>3 groups) or independent samples t tests (2 groups). A conventional two-tailed level of significance at the 5% level was required. All data was analyzed using SPSS version 19.

3. Results

3.1 Experiment 1: Effects of CLZ treatment on avoidance responding under different CS trial conditions

3.1.1 Total avoidance percentage—Throughout the 6 days of testing, repeated CLZ treatment disrupted avoidance responding persistently, primarily under the 10-trial and 40-trial conditions. In addition, the 40-trial groups appeared to have lower avoidance than the 10-trial groups, which in turn, had lower avoidance than the 3-trial groups (Fig. 1A and B). A split-plot ANOVA on the mean percent avoidance revealed a main effect of treatment [$F(1, 44) = 110.319$, $p < 0.001$], a main effect of trial condition [$F(2, 44) = 29.977$, $p < 0.001$], and a significant interaction between the two [$F(2, 44) = 3.397$, $p = 0.042$]. There was also a main effect of test day [$F(5, 220) = 3.655$, $p = 0.003$] and a significant three-way interaction among test day, treatment and trial condition [$F(10, 220) = 2.063$, $p = 0.029$], but no significant test day × treatment interaction [$F(5, 220) = 1.402$, $p = 0.225$], nor test day × trial condition interaction [$F(10, 220) = 1.177$, $p = 0.307$].

To identify how the trial condition interacted with the CLZ treatment in decreasing avoidance response, we analyzed avoidance percentage data under the CLZ and VEH treatment separately. Under the CLZ treatment condition (Fig. 1A), a split-plot ANOVA revealed a main effect of trial condition [$F(2, 21) = 16.085$, $p < 0.001$], but no main effect of test day [$F(5, 105) = 1.671$, $p = 0.148$] or interaction between the two [$F(10, 105) = 1.057$, $p = 0.402$]. Post hoc LSD tests showed that the CLZ 40-trial and 10-trial groups had significantly lower avoidance than the CLZ 3-trial group, $ps \leq 0.001$. One-way ANOVAs followed by post hoc LSD tests on each test day revealed that the CLZ 40-trial group had significantly lower avoidance than the CLZ 3-trial group on all 6 days, all $ps < 0.016$. The CLZ 10-trial group had significantly lower avoidance than the CLZ 3-trial group on day 1, 2, 5 and 6, all $ps < 0.025$, suggesting that the avoidance disruptive effect of CLZ was enhanced by the increase of CS trials throughout the 6 days of testing.

Under the VEH treatment condition (Fig. 1B), there was a main effect of trial condition [$F(2, 23) = 16.482$, $p < 0.001$], a main effect of test day [$F(5, 115) = 6.203$, $p < 0.001$], and a significant interaction between the two [$F(10, 115) = 4.107$, $p < 0.001$]. Post hoc tests showed that the VEH 40-trial group had significantly lower avoidance than the VEH 10-trial and 3-trial groups, $ps \leq 0.001$. One-way ANOVAs followed by post hoc tests on each test day revealed that the VEH 40-trial group had significantly lower avoidance than the VEH 3-trial group on all 6 days except on day 2, and than the VEH 10-trial group on the last 4 days, $ps < 0.05$. The VEH 10-trial group had significantly lower avoidance than the VEH 3-trial group on day 4 and day 6, $ps < 0.05$. Overall, both CLZ treatment and more CS trials caused a decline in avoidance response throughout the 6 days of testing. The disruptive effect of CLZ was further potentiated by the increase of CS trials.

3.1.2 Within-session pattern of avoidance response—If CLZ indeed decreased the motivational salience of the CS, we would also expect to see a within-session decline in the drug test sessions. To illustrate this point, we selectively examined the avoidance data in each 10-trial block (a total of 4 blocks per session) on the last three drug test days from the two 40-trial groups (Fig. 2). The within-session decline pattern was clearly observed. On day 4, a split-plot ANOVA revealed a main effect of block [$F(3, 45) = 8.494$, $p < 0.001$], a main effect of treatment [$F(1, 15) = 40.341$, $p < 0.001$], but no significant block × treatment interaction [$F(3, 45) = 0.503$, $p = 0.682$]. In the CLZ 40-trial group, paired sample t tests revealed that the block 2, 3 or 4 was significantly different from the block 1, all $ps < 0.05$. In the VEH 40-trial group, the block 3 and 4 were also significantly different from the block 1, all $ps < 0.05$.

On day 5, the effects of block [$F(3, 45) = 8.524$, $p < 0.001$] and treatment [$F(1, 15) = 36.190$, $p < 0.001$] were significant, but their interaction was not [$F(3, 45) = 1.688$, $p = 0.183$]. Paired sample t tests revealed that the block 2 and 3 were significantly different from the block 1 in the CLZ 40-trial and VEH 40-trial group, all $ps < 0.05$.

Similar to day 4 and 5, there was a main effect of block [$F(3, 45) = 7.253$, $p < 0.001$], a main effect of treatment [$F(1, 15) = 134.434$, $p < 0.001$], but no significant block × treatment interaction [$F(3, 45) = 0.174$, $p = 0.913$] on day 6. Paired sample t tests revealed that the block 2, 3 or 4 was significantly different from the block 1 in the CLZ 40-trial group, all $ps < 0.05$. In the VEH 40-trial group, the block 2 and 3 were also significantly different from the block 1, all $ps < 0.05$.

Two days later, all groups were tested again in a 40-trial session after injected with CLZ 5.0 mg/kg. There was no significant group difference among the three CLZ groups on the total number of avoidances, $F(2, 21) = 1.190$, $p = 0.324$ (Fig. 3A), indicating that the 40- and 10-trial conditions at this low dose of CLZ did not induce a long-lasting alteration of motivational salience of the CS. Similarly, no group difference was noted among the three VEH groups, $F(2, 23) = 0.531$, $p = 0.595$. However, the single administration of CLZ still caused a within-session decline in avoidance responding across the four 10-trial test blocks in both the CLZ-treated groups, $F(3, 63) = 25.751$, $p < 0.001$; and the VEH groups, $F(3, 69) = 22.096$, $p < 0.001$ (Fig. 3B), a behavioral response pattern similar to what was shown during the repeated drug test phase.

3.2 Experiment 2: Effects of OLZ treatment on avoidance responding under the different CS trial conditions

3.2.1 Total avoidance percentage—Throughout the 6 days of drug testing (Fig. 4), repeated OLZ treatment progressively increased its disruption of avoidance response, especially in the 10-trial and 40-trial condition. A split-plot ANOVA revealed a main effect of treatment [$F(1, 46) = 21.400$, $p < 0.001$], a main effect of trial condition [$F(2, 46) = 8.319$, $p = 0.001$], a main effect of test day [$F(5, 230) = 16.955$, $p < 0.001$]. In addition, there was a significant test day × treatment interaction [$F(5, 230) = 7.006$, $p < 0.001$] and a significant test day × trial condition interaction [$F(10, 230) = 3.016$, $p = 0.001$], but no significant treatment × trial condition interaction [$F(2, 46) = 1.331$, $p = 0.274$] nor test day × treatment × trial condition three-way interaction [$F(10, 230) = 1.049$, $p = 0.403$]. Post hoc tests indicated that the 40-trial condition had significantly lower avoidance responses than the other two conditions, $ps < 0.035$. These analyses support the observation that OLZ caused an across-session decline in avoidance response and avoidance performance declined across sessions at different rates under the different trial conditions.

Next, we compared avoidance performance across the three different trial conditions under either OLZ or VEH. First, under the OLZ treatment condition (Fig. 4A), a split-plot ANOVA revealed a main effect of trial condition [$F(2, 23) = 6.050$, $p = 0.008$], a main effect of test day [$F(5, 115) = 15.949$, $p < 0.001$], and a significant interaction between the two [$F(10, 115) = 2.172$, $p = 0.024$]. Post hoc LSD tests showed that the OLZ 40-trial group had significantly lower avoidance than the OLZ 3-trial group, $p < 0.05$. One-way ANOVAs followed by post hoc LSD tests on each test day revealed that the OLZ 40-trial group had significantly lower avoidances than the OLZ 3-trial group on all 6 days except on day 1 and day 3, and than the OLZ 10-trial group on the last test day, $ps < 0.05$. The OLZ 10-trial group had significantly lower avoidance than the OLZ 3-trial group on day 2 and day 6, $ps < 0.05$. This analysis suggests that the avoidance disruptive effect of OLZ was enhanced by the increase of CS trials across the 6 days of testing.

In contrast, under the VEH condition (Fig. 4B), there was no main effect of the trial condition [$F(2, 23) = 2.366$, $p = 0.116$], no main effect of test day [$F(5, 115) = 2.184$, $p = 0.061$], and no significant interaction between the two [$F(10, 115) = 1.688$, $p = 0.092$], suggesting that different number of CS trials did not significantly affect avoidance response over time under the vehicle condition. Collectively, OLZ treatment caused a progressive decline in avoidance response throughout the 6 days of testing, and this disruptive effect was further potentiated by the increase of the CS test trials. Contrary to the results from Experiment 1, the increase of CS trial numbers did not cause an increase in avoidance decline under the vehicle treatment.

3.2.2 Within-session pattern of avoidance response—Analysis of the within-session response pattern on the last three drug days revealed an apparent across-block decline in the OLZ group (Fig. 5). On day 4, a split-plot ANOVA revealed a main effect of block [$F(3, 48) = 7.529$, $p < 0.001$], a main effect of treatment [$F(1, 16) = 18.382$, $p = 0.001$], but no significant block × treatment interaction [$F(3, 48) = 0.969$, $p = 0.415$]. Paired sample t tests revealed that the block 3 and 4 were significantly different from the block 1 in the OLZ 40-trial group, all $ps < 0.05$. No block difference was noted in the VEH 40-trial group.

Similarly on day 5, there was a main effect of block [$F(3, 48) = 4.880$, $p = 0.005$], a main effect of treatment [$F(1, 16) = 17.891$, $p = 0.001$], but no significant block × treatment interaction [$F(3, 48) = 0.148$, $p = 0.931$]. Paired sample t tests showed that the block 2 was significantly different from the block 1, $p < 0.05$ in the OLZ 40-trial group. No block difference was noted in the VEH 40-trial group, $ps > 0.112$.

On day 6, there was also a main effect of block [$F(3, 48) = 11.209$, $p < 0.001$], a main effect of treatment [$F(1, 16) = 11.866$, $p = 0.003$], but no significant block × treatment interaction [$F(3, 48) = 2.090$, $p = 0.114$]. Paired sample t tests showed that the block 2, 3 and 4 were significantly different from the block 1 in the OLZ 40-trial group, $ps < 0.05$, while in the VEH 40-trial group, only the block 2 was significantly different from the block 1, $ps < 0.05$.

On the final 40-trial CS-only OLZ challenge test when all rats were injected with OLZ 0.5 mg/kg (Fig. 6), the three OLZ groups did not differ from each other on the total number of avoidances, $F(2, 23) = 0.539$, $p = 0.590$ (Fig. 6A), again, indicating no long-lasting alteration of motivational salience of the CS. Similarly, no group difference was noted among the three VEH groups, $F(2, 23) = 0.473$, $p = 0.629$. Like CLZ, acute injection of OLZ still caused a within-session decline across the four 10-trial test blocks in both the OLZ-treated groups, $F(3, 69) = 45.890$, $p < 0.001$; and the VEH groups, $F(3, 69) = 39.339$, $p < 0.001$ (Fig. 6B).

4. Discussion

The present study examined the effect of repeated CLZ and OLZ treatment on avoidance responding under different CS trial conditions in an attempt to delineate the behavioral mechanisms of action of CLZ and OLZ in the CAR model (e.g. motivational salience attenuation). The rationale was that if CLZ and OLZ decrease avoidance response by attenuating the motivational salience of the CS, increasing the number of CS trials in each test session would enhance their disruptive effects given the fact that more CS trials afford more opportunities for CLZ and OLZ to attenuate its salience. Our results are consistent with this hypothesized action of antipsychotic drugs, although the findings from both experiments may be subjected to alternative explanations (e.g. motor fatigue or state-dependent learning, etc).

In Experiment 1, we showed that repeated CLZ treatment persistently decreased avoidance response, consistent with our previous finding (Li et al., 2010). More importantly, this CLZ effect was potentiated by the increase of number of CS trials in the test sessions, as the CLZ-treated rats tested under the 40-trial and 10-trial conditions had significantly lower avoidance than those tested under the 3-trial condition throughout the six days of testing. This potentiated effect was not only seen in the total avoidance percentage, but also reflected in the within-session decline pattern, which is typically regarded as the consequence of extinction of motivational salience (Wise, 2004, Wise et al., 1978). These findings suggest that CLZ may have attenuated the ability of the CS to instigate avoidance response (i.e. its motivation salience). Interestingly, the CS trial condition itself also had an extinction-like effect on avoidance response, with more CS trials causing a faster across-session decline. Thus the potentiated effect between CLZ and CS trials can also be viewed as CLZ facilitating the extinction of avoidance response, a finding consistent with previous reports with chlorpromazine (Miller et al., 1957) as well as HAL and CLZ (Li, Parkes, 2004).

In Experiment 2, we observed that repeated OLZ treatment progressively enhanced its disruption of avoidance response, a finding consistent with our previous work (Li, Fletcher, 2007, Li, He, 2009, Li et al., 2012, Li, Sun, 2010, Mead and Li, 2010, Zhang, Fang, 2011, Zhang and Li, 2012). Similar to that of CLZ, this effect was also enhanced by the increase of number of CS trials, as OLZ-treated rats tested under the 40-trial condition had a faster decline than those tested under the 10- or 3-trial condition (Fig. 4A). This potentiation by the CS trials was also seen in the with-session decline pattern on the last three drug days as well as in the final challenge test in both OLZ- and vehicle-treated rats. As a within-session decline has often been used as evidence supporting the reinforcement attenuation effect of drugs (Dickinson et al., 2000, Fouriezos et al., 1978), the fact of both CLZ and OLZ caused this pattern of response change indicates that the CS was gradually losing its motivational ability under the influence of CLZ and OLZ.

One alternative explanation on the within-session decline is the motor fatigue. It was possible that rats tested under the 40-trial condition became fatigued as the trials progressed. This explanation is not supported by the following two observations. First, the VEH 40-trial rats in both experiments made no fewer avoidance responses (often more) in the 4^{th} block than in the 2^{nd} and 3^{rd} blocks (Figures 3 and 6). Second, the VEH 40-trial rats in Experiment 2 did not make significantly lower avoidance than the VEH 10-trial or VEH 3-trial rats. If motor fatigue was the cause of the within-session decline, we would expect to see lower avoidance in the 4^{th} block than in other blocks and lower avoidance in the 40-trial group than in other groups, opposite to the ones presented above. Another alternative relates to the state-dependent learning (Overton, 1979). That is, rats may have adapted to a learning state consisting of an association between a drug's effects and avoidance behavior in the CAR. Thus, an increase in the trial numbers may lead to an increase of the number of occasions to strengthen this association, resulting in an increase in the inhibition of avoidance responding under a more trials condition. This alternative explanation needs to be carefully examined in the future study.

One unexpected discrepancy between Experiment 1 and 2 was the avoidance performance in the VEH groups. In Experiment 1, the VEH 40-trial group had significantly lower avoidance than the VEH 10-trial and 3-trial groups, whereas no such group difference was found in Experiment 2. We do not have a solid explanation for this difference. One possibility is the batch difference and time of experiment as the two experiments consisting of four batches of rats were conducted at different times of the year. As a piece of supporting evidence, we did notice that rats in Experiment 1 had a significantly higher baseline level of avoidance response than those in Experiment 2: the mean avoidance percentage at the baseline in

Experiment 1 was 93.76%, while it was 97.13% in Experiment 2 (Independent sample t test, t (101) = 2.488, $p = 0.014$, 2-tailed).

The overall finding from the present study is consistent with our previous work on the motivational salience attenuation action of APDs (Li, Fletcher, 2007, Li, He, 2009, Zhang, Fang, 2011) as well as others (Beninger et al., 1980a, 1980b, Davis et al., 1961). Davis et al. (1961) first trained rats to escape footshock in the shuttle box, then confined them in one side of the box and exposed them to 15 trials of the buzzer-footshock pairing under either chlorpromazine or saline. When tested without drug, the previously chlorpromazine-treated rats made significantly fewer avoidance responses than the saline rats, demonstrating that it was the action of chlorpromazine on the motivational salience of the buzzer that contributed to the lower avoidance in the chlorpromazine-treated rats. Beninger et al. (1980a, b) showed that avoidance responding under pimozide showed an extinction-like gradual decline with repeated drug testing. We have shown that HAL treatment also caused a within-session decline in avoidance responding (Li, Fletcher, 2007); and CLZ, OLZ and risperidone disrupt avoidance response elicited by a less salient conditioned stimulus (CS2) to a greater extent than avoidance elicited by a more salient stimulus (CS1) (Li, He, 2009, Li, Sun, 2012, Zhang, Fang, 2011). Clearly, these observations, together with the present ones, suggest that the motivational salience attenuation action of APDs is perhaps the *critical* action of APDs on avoidance.

Although the main finding that the avoidance disruptive effect of CLZ and OLZ was potentiated by the increase of number of CS test trials is consistent with the motivational salience attenuation hypothesis, it could also be viewed as a phenomenon of the "rate-dependent drug effects" (Barrett and Bergman, 2008, Leander, 1975, Leander and McMillan, 1974). It is well known that the baseline response rate is an important determinant of the behavioral effects of drugs. A drug's behavioral effect and the direction of its effect can be quite different under different test conditions (Barrett, 2002, Barrett and Bergman, 2008, Dews, 1976, McMillan and Katz, 2002, Spealman et al., 1983). Therefore, the different drug trial groups (e.g. 3, 10 or 40 trials) may have shown different avoidance decline rates because of their different baseline avoidance numbers, in addition to the salience attenuation action of APDs. Based on our recent study showing that the baseline rate of avoidance responses does not influence the basic avoidance-disruptive effect of OLZ and risperidone (another antipsychotic drug) (Zhang, Fang, 2011), we speculate that the motivational salience attenuation action is a more plausible cause. Other reports in the literature also find that the effects of some antipsychotic drugs, such as thioridazine, chlorpromazine and haloperidol can be largely independent of the type of schedule or the type of consequent event that maintains response (Spealman, Kelleher, 1983, Wenger, 1979).

CLZ and OLZ possess a much more potent antagonism on the 5-$HT_{2A/2C}$ receptors in addition to relatively weak antagonism on D_2 receptors (Meltzer et al., 2003). It is thus possible that their disruptive effect on CAR could be attributed to their action on D_2 receptors alone (Kapur and Seeman, 2001, Wadenberg, Soliman, 2001) or its dual action on both 5-$HT_{2A/2C}$ and D_2 receptors (Meltzer et al., 1989) or even effects on other receptors (e.g. D_1, D_4, 5-HT_{1A}). Our recent work indicates that CLZ disrupts avoidance response primarily by blocking 5-$HT_{2A/2C}$ receptors, whereas OLZ appears to exert its disruptive effect primarily by blocking dopamine D_2 receptors (Li, Sun, 2012, Li, Sun, 2010). We found that pretreatment of 1–2, 5-dimethoxy-4-iodo-amphetamine (DOI, a selective 5-$HT_{2A/2C}$ agonist, 2.5 mg/kg, sc), but not quinpirole (a selective dopamine $D_{2/3}$ agonist, 1.0 mg/kg, sc), attenuated CLZ-induced disruption of avoidance responding, whereas pretreatment of quinpirole, but not DOI, attenuated that effect of OLZ. In light of the present findings, we suggest that CLZ may attenuate motivational salience of the CS by blocking 5-

HT_{2A} receptors, which in turn decreases dopamine release (Ichikawa et al., 2001, Millan et al., 1998). OLZ may decrease motivational salience of the CS by directly blocking dopamine D_2 receptors as D_2 blockade is known to decrease motivational salience of stimuli in the control of behavior (Berridge, 2007).

In conclusion, the present study provides support for the motivational salience attenuation action of OLZ and CLZ in the CAR model by showing that their avoidance disruptive effect can be potentiated by increasing the number of CS trials. It is clinically relevant as the experimental approach described in this study could be potentially used to examine the interactive effects of psychotherapy (e.g. exposure therapy) and pharmacotherapy (e.g. antipsychotic treatment) and associated mechanisms at the behavioral and brain levels. If our findings were any indication of such an interaction in the treatment of psychosis (avoidance as a proxy), it appears that combined treatment would yield an optimal result.

Acknowledgments

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Highlights

- Repeated clozapine and olanzapine treatment persistently decreased avoidance response in rats.
- Their avoidance disruptive effect was potentiated by the increase of number of test trials.
- The potentiation was observed also in the within-session decline of avoidance responding pattern.

Fig. 1A

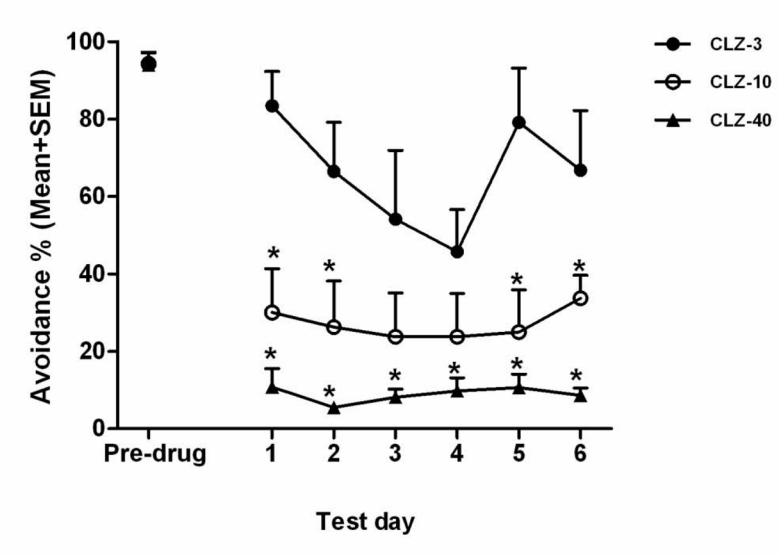

Fig. 1B

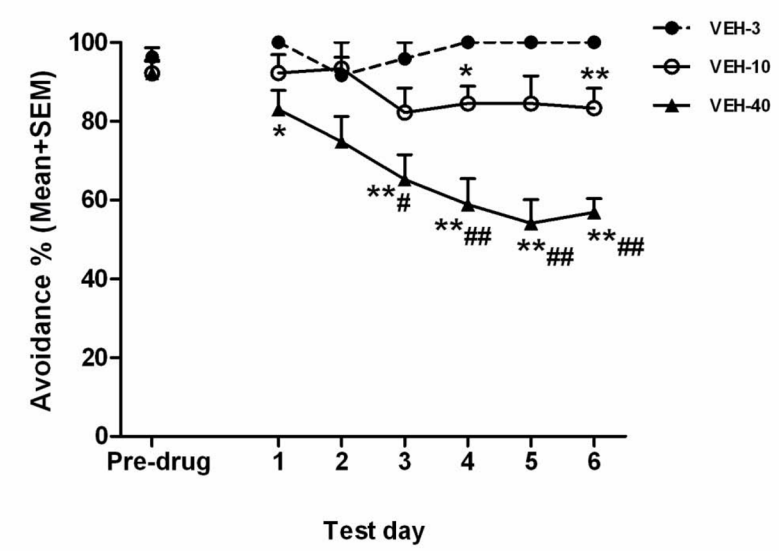

Figure 1.

Percentage of avoidance response (Mean + S.E.M) made by rats treated with CLZ 5.0 mg/kg (**A**) or vehicle (VEH) (**B**) and tested under the 3 CS trial conditions (3, 10, or 40 trials/day) on the pre-drug day and throughout the 6 test days. $^{*}p < 0.05$, $^{**}p < 0.001$ in comparison to the respective 3-trial group, $^{\#}p < 0.05$, $^{\#\#}p < 0.001$ in comparison to the respective 10-trial group.

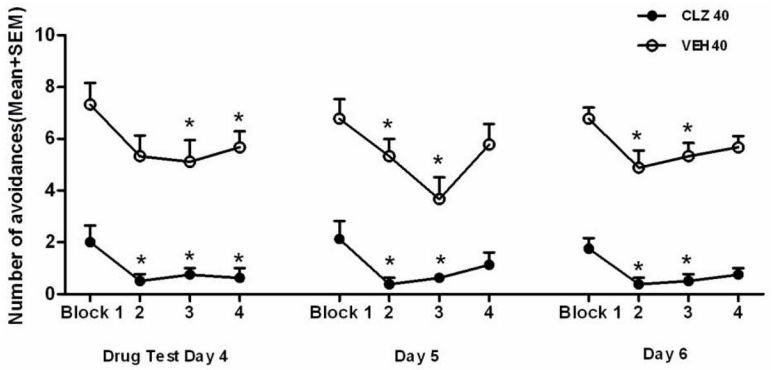

Figure 2.

Number of avoidance responses across the four 10-trial blocks made by rats treated with CLZ 5.0 mg/kg (**A**) or vehicle (VEH) (**B**) and tested under the 40 trial condition on the last 3 test days. $^{*}p < 0.05$ in comparison to the respective block 1.

Fig. 3A

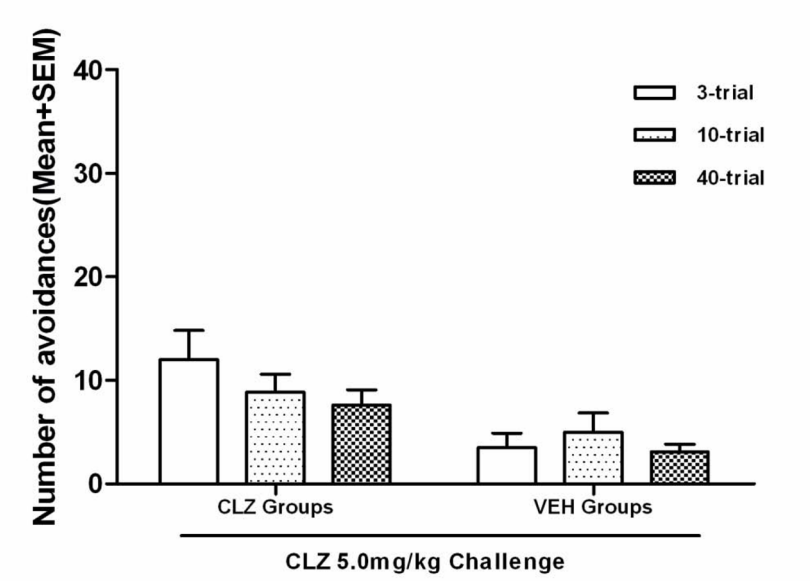

Fig. 3B

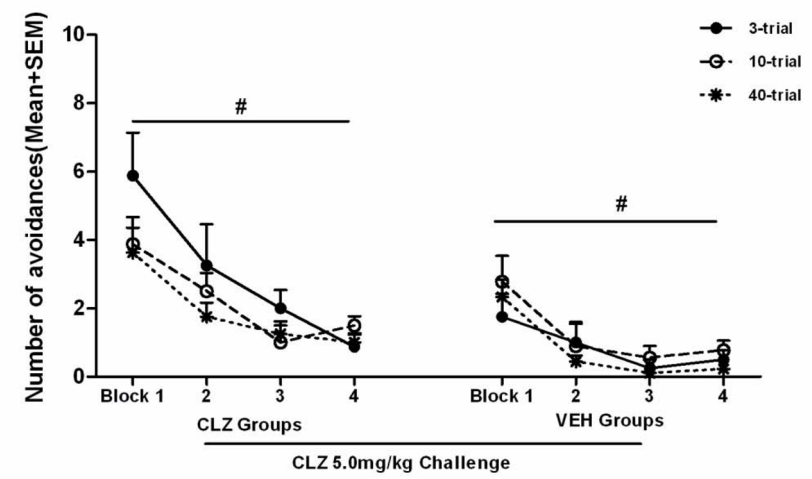

Figure 3.

(A) Total number of avoidance responses and (**B**) number of avoidances across the four 10-trial blocks made by rats on the final 40 trial CS-only test in which all rats were treated with CLZ 5.0 mg/kg. $^{\#}p < 0.050$ indicates a significant effect of block.

Fig. 4A

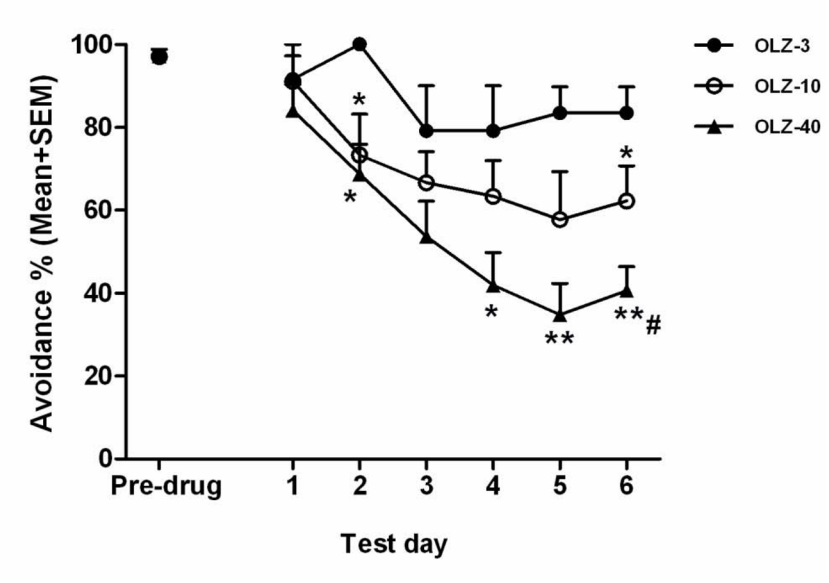

Fig. 4B

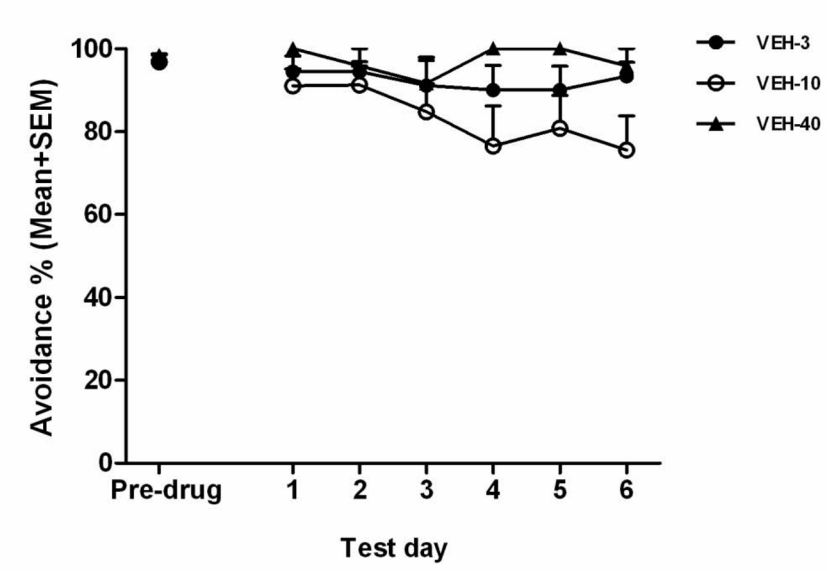

Figure 4.

Percentage of avoidance response (Mean + S.E.M) made by rats treated with olanzapine (OLZ) 0.5 mg/kg (**A**) or vehicle (VEH) (**B**) and tested under the 3 CS trial conditions (3, 10, or 40 trials/day) on the pre-drug day and throughout the 6 test days. $^{*}P < 0.05$, $^{**}P < 0.001$ in comparison to the respective 3-trial group, $^{\#}P < 0.05$ in comparison to the respective 10-trial group.

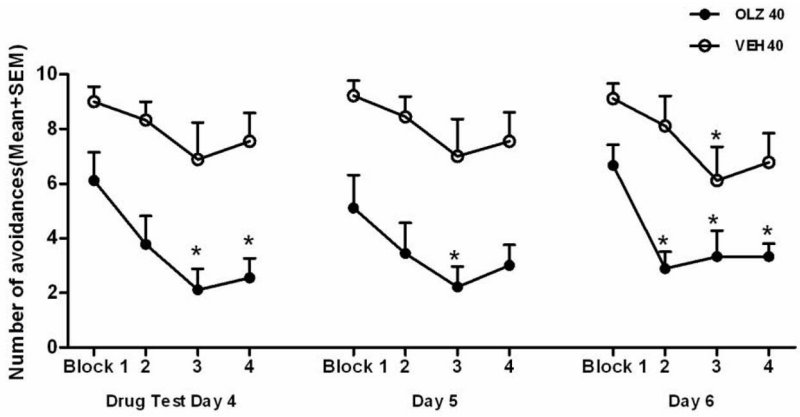

Figure 5.

Number of avoidance responses across the 4 10-trial blocks made by rats treated with clozapine (CLZ) 5.0 mg/kg (A) or vehicle (VEH) (B) and tested under the 40 trial condition on the last 3 test days. $^{*}P < 0.05$ in comparison to the respective block 1.

Fig. 6A

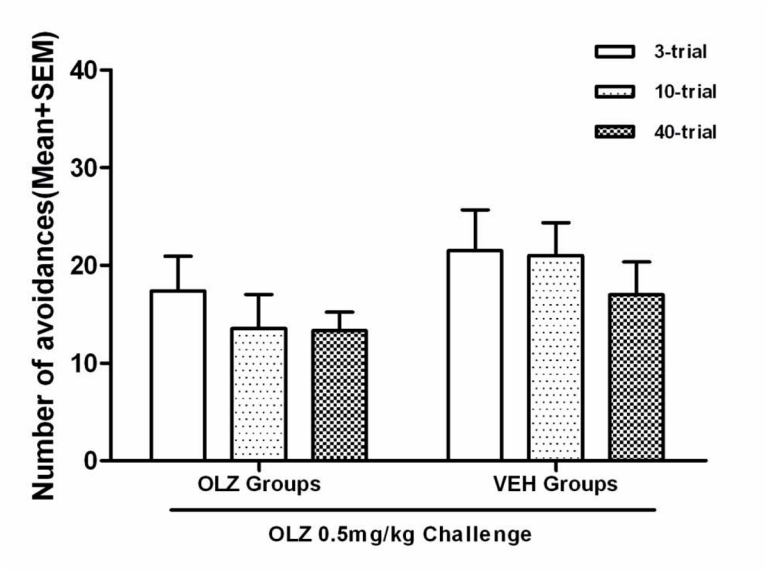

Fig. 6B

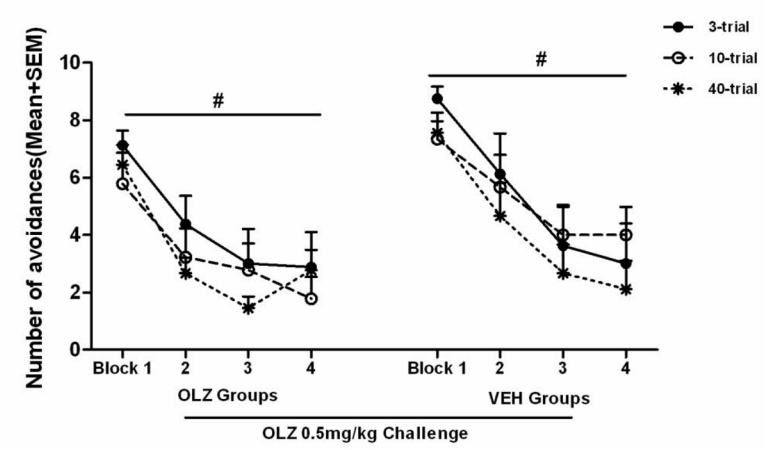

Figure 6.

(A) Total number of avoidance responses and (**B**) number of avoidances across the four 10-trial blocks made by rats on the final 40 trial CS-only test in which all rats were treated with OLZ 0.5 mg/kg. $^{\#}p < 0.050$ indicates a significant effect of block.