

8-2014

Effect of Environmental Cues on Behavioral Efficacy of Haloperidol, Olanzapine and Clozapine in Rats

Tao Sun

Nanjing University School of Medicine

Xinfeng Liu

Nanjing University School of Medicine, xliu2@yahoo.com.cn

Ming Li

University of Nebraska-Lincoln, mli2@unl.edu

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



Part of the [Behavioral Neurobiology Commons](#), and the [Psychology Commons](#)

Sun, Tao; Liu, Xinfeng; and Li, Ming, "Effect of Environmental Cues on Behavioral Efficacy of Haloperidol, Olanzapine and Clozapine in Rats" (2014). *Faculty Publications, Department of Psychology*. 692.

<http://digitalcommons.unl.edu/psychfacpub/692>

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. 2014 August ; 25(4): 277–286. doi:10.1097/FBP.0000000000000047.

Effect of Environmental Cues on Behavioral Efficacy of Haloperidol, Olanzapine and Clozapine in Rats

Tao Sun^{1,2}, Xinfeng Liu^{1,*}, and Ming Li^{2,*}

¹Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China

²Department of Psychology, University of Nebraska-Lincoln, USA

Abstract

Previous studies have reported that context can powerfully modulate the inhibitory effect of an antipsychotic drug on phencyclidine (PCP)-induced hyperlocomotion (a behavioral test used to evaluate putative antipsychotic drugs). The present study investigated the experimental conditions under which environmental stimuli exert their influence through associative conditioning processes. Experiment 1 examined the extent to which prior antipsychotic treatment in the home cages affected a drug's ability to inhibit PCP-induced hyperlocomotion in a novel motor activity test apparatus. Five days of repeated haloperidol (0.05 mg/kg, sc) and olanzapine (2.0 mg/kg, sc) treatment in the home cages still potentiated their inhibition of PCP-induced hyperlocomotion (i.e. sensitization) assessed in a new environment, whereas the clozapine (10.0 mg/kg, sc) treatment enhanced the development of clozapine tolerance, indicating a lack of environmental modulation of antipsychotic efficacy. Experiment 2 assessed the impact of different numbers of antipsychotic administrations in either the home environment or test environment (e.g. 4, 2 or 0) on a drug's ability to inhibit PCP-induced hyperlocomotion. Repeated administration of clozapine (5.0 mg/kg, sc) or olanzapine (1.0 mg/kg, sc) for 4 consecutive days, regardless of where these treatments occurred, caused a similar level of inhibition on PCP-induced hyperlocomotion. However, 4-day haloperidol (0.03 mg/kg, sc) treatment in the test apparatus caused a significant higher inhibition than 4-day home cage treatment. Thus, more exposures to the test environment under the influence of haloperidol (but not clozapine or olanzapine) cause a stronger inhibition than fewer exposures, indicating a strong environmental modulation. Collectively, these findings suggest that prior antipsychotic treatment in one environment could alter later antipsychotic-like response assessed in a different environment under certain test conditions. Therefore, whether the circumstances surrounding antipsychotic drug administration exert a powerful control of the expression of antipsychotic-like efficacy is dependent on specific experimental and drug treatment factors.

*Corresponding address: Ming Li, PhD, Associate Professor, Department of Psychology, University of Nebraska-Lincoln, 238 Burnett Hall, Lincoln, NE 68588-0308, USA, Telephone: 402-472-3144, mli2@unl.edu, Xinfeng Liu, Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, 305 Zhongshan East Road, Nanjing 21002 (China), Tel. +86 25 8527 2631, Fax +86 25 8480 1861, xfliu2@yahoo.com.cn.

Conflicts of interest: None

Disclosures

All authors declare no conflict of interest.

Keywords

Haloperidol; Olanzapine; Clozapine; Phencyclidine; Locomotor activity; Sensitization; Tolerance; Contextual Control; rat

INTRODUCTION

Phencyclidine (PCP) is a psychotomimetic drug that induces various behavioral, emotional, and cognitive changes in animals by blocking neurotransmission at N-methyl-D-aspartate (NMDA)-type glutamate receptors. Many animal models of schizophrenia are developed based on this property of PCP (Javitt and Zukin, 1991; Chen *et al.*, 2011; Javitt *et al.*, 2012). Among those, PCP-induced hyperlocomotion is a widely used behavioral screening tool to identify potential antipsychotic-like compounds and study the behavioral and neurobiological mechanisms of antipsychotic action, as many commonly used antipsychotic drugs such as haloperidol, clozapine and olanzapine, but not anxiolytics or antidepressants, suppress PCP-induced hyperlocomotion upon acute drug administration (Redmond *et al.*, 1999; Porsolt *et al.*, 2010; Zhao *et al.*, 2012a). With repeated drug administration, antipsychotics (e.g. haloperidol, clozapine, or olanzapine) progressively potentiate inhibition of repeated PCP-induced hyperlocomotion and prolong this action over several test sessions (Sun *et al.*, 2009), whereas repeated administration of anxiolytics (e.g. chlordiazepoxide) or antidepressants (e.g. fluoxetine and citalopram) either does not affect PCP-induced hyperlocomotion or even enhances it (Redmond *et al.*, 1999). Thus, the repeated PCP-induced hyperlocomotion test is effective in distinguishing antipsychotic drugs from other psychotherapeutic drugs and in capturing the time course of an antipsychotic's clinical effects (Agid *et al.*, 2006).

Another interesting finding from the repeated PCP-induced hyperlocomotion studies is that when rats are later given a challenge dose of the drug, those that have been repeatedly treated with haloperidol or olanzapine exhibit a sensitization effect as they often have *lower* PCP-induced hyperlocomotion than vehicle-pretreated rats (Zhang and Li, 2012). In contrast, rats previously treated with clozapine exhibit *higher* PCP-induced hyperlocomotion than those that are treated with clozapine for the first time, indicating a tolerance-like effect (Feng *et al.*, 2013). These findings indicate that when an antipsychotic drug is given repeatedly and intermittently, there is often a long-term alteration (increase or decrease) in its behavioral efficacy, a phenomenon commonly associated with drugs of abuse (Pierce and Kalivas, 1997; Siegel *et al.*, 2000).

Recent evidence shows that the context surrounding drug administration can powerfully modulate behavioral efficacy in the PCP-induced hyperlocomotion model (Zhang and Li, 2012; Feng *et al.*, 2013). In those studies, we used a novel across-model transfer paradigm in which we first treated rats repeatedly with haloperidol, olanzapine or clozapine and tested them in a conditioned avoidance response model (another behavioral test with high predictive validity for drugs that have antipsychotic efficacy in humans) for 5 consecutive days. They were then tested for the expression of haloperidol and olanzapine sensitization and clozapine tolerance in the PCP-induced hyperlocomotion model. We found that prior

treatment of haloperidol, olanzapine or clozapine in the avoidance response model did not change their acute efficacy in the inhibition of PCP-induced hyperlocomotion. When tested in the PCP model, rats previously treated with these drugs did not show an immediate stronger (in the case of haloperidol and olanzapine) or weaker (in the case of clozapine) inhibition of PCP-induced hyperlocomotion than those treated with these drugs for the first time. However, when tested in the avoidance response model where the original antipsychotic treatment took place, rats previously treated with these drugs did show a stronger or weaker inhibition of avoidance response. These results suggest that behavioral effects of antipsychotic drugs are strongly modulated by the drug test environment and/or selected behavioral response, exhibiting a context-dependent feature.

It is conceivable that various experimental parameters such as schedule of injections, drug dose, and similarities between different environmental cues all play a role in the regulation of environmental control of antipsychotic efficacy. Because the situational impact on antipsychotic efficacy is a less studied phenomenon, in comparison to a long history and literature on research on the contextual control of behavioral effects of drugs of abuse (Siegel, 1975; 1977; Siegel *et al.*, 2000), it is necessary to delineate the exact conditions that facilitate or diminish the ability of antipsychotics to inhibit PCP-induced hyperlocomotion and compare this research to the large body of literature on drugs of abuse (Siegel, 1978; Poulos *et al.*, 1981; Vezina and Stewart, 1984; Anagnostaras and Robinson, 1996; Robinson *et al.*, 1998; Siegel *et al.*, 2000). One critical yet unaddressed question is whether it is possible to induce any change in behavioral efficacy of an antipsychotic drug even when prior treatment occurs in a different environment. In other words, is it possible to observe a context-independent change in antipsychotic efficacy due to repeated drug administration? If the answer to this question is yes, this would have important clinical implications. For example, if manipulation of environmental cues cannot completely prevent the occurrence of drug-induced change in behavioral efficacy, this would suggest that the repeated treatment effects may be mainly mediated by the neuroadaptive processes initiated by the interaction of a drug and its receptors (e.g. dopamine D₂ or serotonin 5-HT_{2A} receptors). Therefore, the treatment settings (e.g. home or hospital) may not be critical for the expression and maintenance of the therapeutic effects in patients.

In this study, we investigated possible conditions within which contextual control of the behavioral efficacy of haloperidol, olanzapine and clozapine operates in the PCP-induced hyperlocomotion model. Two approaches were explored. In Experiment 1, we assessed the extent to which prior antipsychotic treatment in one environment (Home cage) affected a drug's inhibition of PCP-induced hyperlocomotion in another environment (Test environment). In Experiment 2, we varied the number of pairings of antipsychotic treatment in either the home environment or test environment (e.g. 4, 2 or 0) and tested how a drug's inhibition of the PCP-induced hyperlocomotion was altered by different pairings.

METHODS

Subjects

All experimental treatments and procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln. Adult male Sprague-Dawley rats

(226–250 g upon arrival, Charles River, Portage, MI) 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 6:30 am and 6:30 pm). Room temperature was maintained at $22 \pm 1^\circ\text{C}$ with a relative humidity of 45–60%. Food and water was freely available. Animals were allowed at least 5 days of habituation to the animal facility before being used in experiments. All behavioral tests were conducted during the light phase.

Locomotor activity monitoring apparatus

This apparatus has been described before (Sun *et al.*, 2009; Zhao and Li, 2012; Feng *et al.*, 2013). Sixteen activity boxes were housed in a quiet room. The boxes were 48.3 cm × 26.7 cm × 20.3 cm transparent polycarbonate cages, which were similar to the home cages but were each equipped with a row of 6 photocell beams (7.8 cm between two adjacent photobeams) placed 3.2 cm above the floor of the cage. A computer with recording software (Aero Apparatus Sixbeam Locomotor System v1.4, Toronto, Canada) was used to detect the disruption of the photocell beams and recorded the number of beam breaks. All experiments were run during the light cycle.

Experiment 1: Effects of prior antipsychotic treatment in the home cages on the inhibitory effect of an antipsychotic drug on PCP-induced hyperlocomotion

This experiment examined the impact of the context on antipsychotic efficacy by testing how prior exposure to an antipsychotic drug in the home cage environment (Home environment) affects the drug's efficacy in the inhibition of PCP-induced hyperlocomotion in a motor activity test environment (Test environment). We compared rats that were repeatedly treated with haloperidol, clozapine or olanzapine in the home cages for 5 consecutive days with those that were treated with vehicle.

Forty-eight rats were randomly assigned to 1 of 7 groups. From Day 1 to 5, 4 groups of rats were injected with sterile water in their home cages for 5 consecutive days. They were denoted as the VEH+VEH ($n = 6$), VEH+HAL ($n = 7$), VEH+CLZ ($n = 7$) and VEH+OLZ ($n = 7$) groups. Three groups received haloperidol (0.05 mg/kg, $n = 7$), clozapine (10.0 mg/kg, $n = 7$), or olanzapine (2.0 mg/kg, $n = 7$) treatment in their home cages during this period. They were denoted as the HAL+HAL, CLZ+CLZ and OLZ+OLZ groups. From Day 6 to 10, all rats were tested for their PCP-induced hyperlocomotion for 5 consecutive days in the test apparatus. On each of the PCP test days, rats were first injected with either sterile water (for the VEH+VEH group), haloperidol (0.05 mg/kg, sc, for the VEH+HAL and HAL+HAL groups), clozapine (10 mg/kg, sc, for the VEH+CLZ and CLZ+CLZ groups), or olanzapine (2.0 mg/kg, sc, for the VEH+OLZ and OLZ+OLZ groups), and then immediately placed in the boxes for 30 min. Any drug effect during this period reflects the effect on spontaneous motor activity. At the end of the 30-min period, they were taken out and injected with PCP (3.20 mg/kg, sc) and placed back in the boxes for another 60 min. The drug effect during this period reflects the antipsychotic-like effect on PCP-induced increase of motor activity (an index of antipsychotic efficacy). Locomotor activity (number of photobeam breaks) was measured in 5 min blocks throughout the entire 90-min testing session.

Experiment 2: Effects of different numbers of drug exposure in the motor activity test apparatus on the inhibitory effect of an antipsychotic drug on PCP-induced hyperlocomotion

Experiment 1 did not control total drug exposure between each pair of drug groups. Therefore, the differential drug effects could be attributed either to this factor or to different drug-environment (home cage) pairings. In Experiment 2, we controlled the total number of drug exposure among different groups. In addition, we directly manipulated the number of drug-environment pairings. Ninety-six rats (run in two cohorts of 48) were first randomly assigned to 4 groups ($n = 24/\text{group}$) that were subjected to sterile water, haloperidol, clozapine or olanzapine treatment. Each treatment group was further divided randomly into 3 subgroups ($n = 8/\text{group}$) that differed in the number of drug exposures (i.e., 0, 2, and 4) in the motor activity test apparatus prior to the 3 PCP-induced hyperlocomotion probe tests.

For the 0-exposure subgroups (VEH-0, HAL-0, CLZ-0, OLZ-0), rats were injected with water, haloperidol (0.03 mg/kg, sc), clozapine (5.0 mg/kg, sc), or olanzapine (1.0 mg/kg, sc) followed by PCP (1.6 mg/kg, sc) 30 min later in their home cages for 4 consecutive days. The antipsychotic drugs and PCP were tested at a lower dose to explore the impact of drug dosage on the environmental modulation of antipsychotic efficacy in comparison to results from Experiment 1. In addition, a lower dose would better allow a detection of contextual control of a drug effect. One day after the last drug injection in the home cages, rats were tested in the 3 probe tests in the motor activity test apparatus. In each test, rats were injected with water, haloperidol (0.03 mg/kg, sc), clozapine (5.0 mg/kg, sc), or olanzapine (1.0 mg/kg, sc) followed by PCP (1.6 mg/kg, sc) 30 min later in the test apparatus. Their motor activities were recorded for the entire 90 min (30 min before and 60 min after the PCP injection), as done in our previous work (Zhang and Li, 2012). For these subgroups, they had 0-day experience with the test apparatus before the probe tests.

For the 2-exposure subgroups (VEH-2, HAL-2, CLZ-2, OLZ-2), rats were injected with water, haloperidol (0.03 mg/kg, sc), clozapine (5.0 mg/kg, sc), or olanzapine (1.0 mg/kg, sc) and PCP (1.6 mg/kg, sc) at a 30-min interval in the home cages for the first two days, then in the test apparatus for the last two days; thus, they had a 2-day experience with the test apparatus prior to the 3 probe tests.

For the 4-exposure subgroups (VEH-4, HAL-4, CLZ-4, OLZ-4), rats had all of their 4-day drug treatments in the test apparatus prior to the 3 probe tests. As is apparent, the 3 subgroups in each treatment condition (e.g. HAL-0, HAL-2 and HAL-4) had an identical drug treatment history, and only differed in the number of exposures to the test environment prior to the probe tests. Thus, any possible differences in locomotion during the probe tests among the 3 treatment subgroups would reflect the impact of prior drug exposure in the test environment on the efficacy of a drug in the inhibition of spontaneous motor activity (i.e. 30 min before PCP injection) and PCP-induced hyperlocomotion (i.e. 60 min after PCP injection).

Drugs

The injection solution of haloperidol (5 mg/ml ampoules, 5 mg/ml ampoules, Sabex Inc. Boucherville, Quebec, Canada) was obtained by mixing drugs with sterile water. The injection solution of phencyclidine hydrochloride (gift from National Institute on Drug Abuse Chemical Synthesis and Drug Supply Program) was obtained by mixing drugs with 0.9% saline. Clozapine (gift from the NIMH drug supply program) and olanzapine (Toronto Research Chemical Inc., Canada) were dissolved in 1.5% glacial acetic acid distilled water. The doses of haloperidol (0.03 or 0.05 mg/kg), olanzapine (1.0 or 2.0 mg/kg), clozapine (5 and 10 mg/kg) and PCP (1.60 or 3.20 mg/kg) were chosen based on our previous work (Sun *et al.*, 2009; Sun *et al.*, 2010; Zhang and Li, 2012; Zhao *et al.*, 2012b; Feng *et al.*, 2013). All drugs were administered s.c. at 1.0 ml/kg.

Statistical Analysis

All data were expressed as mean + *SEM*. Data from the drug test sessions were analyzed using a mixed analysis of variance (ANOVA) with the between-subjects factors of drug group and/or drug exposures (e.g. 0, 2 or 4) and the within-subjects factor of test days, followed by post hoc Fisher's LSD tests. Differences between groups on the specific drug test days were analyzed using one-way ANOVAs, followed by post hoc LSD tests. For all analyses, $p < 0.05$ was considered statistically significant and all data were analyzed using SPSS version 21. Because the Fischer's LSD test does not correct for multiple comparisons, a few instances when p was close to 0.05 should be treated with caution.

RESULTS

Experiment 1: Effects of prior antipsychotic treatment in the home cages on the inhibitory effect of an antipsychotic drug on PCP-induced hyperlocomotion

Prior drug exposure in the home cage environment potentiated the acute inhibitory effect of haloperidol and olanzapine on spontaneous motor activity and PCP-induced hyperlocomotion, but not that of clozapine—Fig. 1 shows the mean motor activity of the 7 groups of rats during the 30-min pre- and 60-min post-PCP injection on the 1st drug test day. Acute haloperidol, olanzapine or clozapine treatment inhibited spontaneous motor activity (Fig. 1A) and PCP-induced hyperlocomotion (Fig. 1B). One-way ANOVA revealed a significant main effect of group in the first 30 min before PCP injection, $F(6, 41) = 27.20$, $p < 0.001$ and the 60 min after PCP injection, $F(6, 41) = 13.44$, $p < 0.001$. Post-hoc Fisher's LSD tests revealed that all the antipsychotic treated groups had significantly lower motor activity than the vehicle group (VEH+VEH) in the first 30 min, $p < 0.003$, and all but the VEH+HAL group also had significantly lower motor activity in the second 60 min, $p < 0.002$. The VEH+HAL had lower motor activity than the VEH+VEH group, but the difference was not significant.

Prior haloperidol or olanzapine, but not clozapine, treatment in the home cages also enhanced their inhibitory effect on spontaneous motor activity and PCP-induced hyperlocomotion on Day 1. Post-hoc Fisher's LSD tests on each pair of haloperidol, olanzapine and clozapine groups showed that the HAL+HAL group had significantly lower motor activity than the VEH+HAL in the 30 min before PCP, $p < 0.02$ and in the 60 min

after PCP injection, $p < 0.05$. Similarly, the OLZ+OLZ group also had significantly lower motor activity than the VEH+OLZ group in the first 30 min, $p < 0.02$ and in the 60 min after PCP injection, $p < 0.005$. In contrast, the two clozapine groups did not differ from each other during both periods. These findings suggest that prior haloperidol and olanzapine treatment, even in a different environment, still enhanced their acute inhibition of spontaneous motor activity and PCP-induced hyperlocomotion. The influence of prior clozapine treatment did not manifest an acute behavioral effect. These findings also indicate that the expression of haloperidol and olanzapine sensitization induced in the home cage environment, manifested as an enhanced inhibition of spontaneous motor activity and PCP-induced hyperlocomotion, was less dependent on the context in which the prior drug exposure occurred.

Prior clozapine treatment in the home cages enhanced the development of clozapine tolerance—

Fig. 2A shows the mean motor activity of the 7 groups of rats during the 30-min test period before PCP injection throughout the 5 days of drug testing. A mixed ANOVA revealed significant main effects of group, $F(6, 41) = 12.60$, $p < 0.001$, and test day, $F(4, 164) = 89.04$, $p < 0.001$, and a significant group \times day interaction, $F(24, 164) = 8.25$, $p < 0.001$. One-way ANOVAs on each test day indicated that on Day 1, all antipsychotic treated groups had significantly lower motor activity than the vehicle group, all $p < 0.005$, confirming their acute motor suppressive effect. But on Day 5, only the two olanzapine groups had significantly lower motor activity than the vehicle group, $p < 0.005$, whereas the CLZ+CLZ group had a significantly higher motor activity, $p < 0.001$, indicating that there was a gradual development of clozapine tolerance in the home cage clozapine treatment group. This observation was also supported by the finding that the CLZ+CLZ had significantly higher motor activity than the VEH+CLZ group on days 4 and 5, $p < 0.01$.

Fig. 2B shows the mean motor activity during the 60-min test period after PCP injection. Once again, a mixed ANOVA revealed significant main effects of group, $F(6, 41) = 27.08$, $p < 0.001$, and test day, $F(4, 164) = 10.58$, $p < 0.001$, and a significant group \times day interaction, $F(24, 164) = 4.359$, $p < 0.001$. One-way ANOVAs and post hoc tests on each test day indicated that all antipsychotic treated groups (except the VEH+HAL group on day 1) showed significantly lower motor activity than the vehicle group throughout the drug test period, all $p < 0.002$, confirming their motor suppressive effect on PCP-induced hyperlocomotion. Except on Day 1, each pair of haloperidol and olanzapine groups did not differ from each other throughout the rest of the test days. The CLZ+CLZ group had marginally higher motor activity than the VEH+CLZ groups on Day 5, but this difference failed to reach significance, $p = 0.078$.

To better reveal the different impacts of home cage haloperidol and olanzapine exposure from that of clozapine exposure, we illustrated the time course of the motor activity in response to haloperidol, olanzapine or clozapine treatment for each drug pair groups (e.g. VEH+HAL and HAL+HAL) together with the vehicle group in 5-min blocks on Day 1 and Day 5 (Fig. 3). The potentiated inhibition of prior haloperidol and olanzapine treatment on the first day was indexed by the finding that the HAL+HAL and OLZ+OLZ groups had consistently higher motor activity throughout the 90-min test period than the respective VEH+HAL and VEH+OLZ groups on Day 1. Similarly, the diminished impact of prior

clozapine on the last day was shown by the consistently reduced motor activity in the CLZ +CLZ group compared to the VEH+CLZ group on Day 5.

Experiment 2: Effect of different numbers of drug exposure in the motor activity test apparatus on the inhibitory effect of an antipsychotic drug on PCP-induced hyperlocomotion

Prior haloperidol exposure in the test apparatus enhanced its efficacy in the inhibition of PCP-induced hyperlocomotion, while prior clozapine and olanzapine did not—Figure 4 shows the mean motor activity in the 30 min before and 60 min after PCP injection of the 12 subgroups on their 3 consecutive probe tests. All groups of rats had identical past drug treatment history by this time but different numbers of exposures to the test environment. For example, the 4-exposure subgroups had 4 days of antipsychotic (sterile water) + PCP treatment in the test apparatus (pairing) before the probes; the 2-exposure subgroups had 2 days of pairing, and the 0-exposure subgroups had not been exposed to the test apparatus. To examine how different numbers of antipsychotic and test environment pairings (e.g. 4, 2 or 0) affected a drug's ability to suppress spontaneous motor activity and PCP-induced hyperlocomotion, we first conducted a mixed ANOVA (4 drug conditions \times 3 exposure conditions \times 3 test days). In the first 30 min of motor activity testing, there were significant main effects of treatment, $F(3, 84) = 11.15, p < 0.001$, and number of test apparatus exposures, $F(2, 84) = 25.20, p < 0.001$, and a significant treatment \times exposure interaction, $F(6, 84) = 4.23, p < 0.001$. The main effect of test days was also significant, $F(2, 168) = 33.06, p < 0.001$, as was the test \times exposure interaction, $F(4, 168) = 22.63, p < 0.001$. In the 60 min after PCP injection, a mixed ANOVA showed significant main effects of treatment, $F(3, 84) = 37.25, p < 0.001$, and number of test apparatus exposures, $F(2, 84) = 3.18, p < 0.05$, but no significant treatment \times exposure interaction, $F(6, 84) = 1.48, NS$. The main effect of test days was significant, $F(2, 168) = 6.11, p = 0.005$, as were the test \times treatment interaction, $F(6, 168) = 3.38, p = 0.005$, and the test \times exposure interaction, $F(4, 168) = 7.72, p < 0.001$.

Because of the significant treatment \times exposure interaction and test \times exposure interaction, in order to specify how different numbers of test environment exposures affected each antipsychotic drug efficacy differently, we conducted mixed ANOVAs comparing the 3 subgroups within each treatment condition over the 3 probe test days (e.g. HAL-0, HAL-2 and HAL-4). For the 3 vehicle subgroups, a mixed ANOVA revealed significant main effects of group, $F(2, 21) = 18.86, p < 0.001$, and test, $F(2, 42) = 9.15, p < 0.001$, and a significant group \times test interaction, $F(4, 42) = 8.11, p < 0.001$ in the first 30 min. One-way ANOVAs followed by post hoc tests found that the VEH-0 subgroup had significantly higher motor activity than the VEH-2 and VEH-4 subgroups on the first two days of testing, $p < 0.01$. Because the VEH-0 group had no prior experience of the test apparatus before the probe tests, the higher motor activity in this subgroup relative to the other 2 vehicle subgroups could be attributed to the novelty-induced increase in motor activity. In the second 60 min, neither the group effect, $F(2, 21) = 0.59, NS$, nor the group \times test interaction, $F(4, 42) = 2.176, p = 0.088$, were significant. For the 3 haloperidol subgroups, a mixed ANOVA revealed significant main effects of group, $F(2, 21) = 8.99, p < 0.002$, and test, $F(2, 42) = 13.08, p < 0.001$, and a significant group \times test interaction, $F(4, 42) = 7.73, p <$

0.001 in the first 30 min. The HAL-4 subgroup had significantly lower motor activity than the HAL-0 group on the first day, $p < 0.005$, and lower motor activity than the HAL-0 group on the second day, $p < 0.01$. In the second 60 min, the group effect was significant, $F(2, 21) = 6.44$, $p < 0.01$, as were the test effect was significant, $F(2, 42) = 11.63$, $p < 0.001$, and the group \times test interaction, $F(4, 42) = 4.18$, $p < 0.01$. The HAL-4 subgroup had significantly lower motor activity than the HAL-0 group on the first day, $p < 0.005$ and the last day, $p < 0.05$. These results suggest that repeated haloperidol exposure in the test apparatus enhanced the efficacy of HAL in the inhibition of spontaneous motor activity and PCP-induced hyperlocomotion in the same environment.

For the 3 clozapine subgroups, a mixed ANOVA showed no significant main effect of group, $F(2, 21) = 0.86$, *NS*, but a significant group \times test interaction, $F(4, 42) = 3.44$, $p < 0.02$ in the first 30 min. One-way ANOVAs on each test day did not find a significant group effect, all $p > 0.06$. In the following 60 min, the group effect, the test effect and their interaction were all nonsignificant, $p > 0.075$. These results indicate that repeated clozapine exposure in the test apparatus did not alter its efficacy in the inhibition of spontaneous motor activity and PCP-induced hyperlocomotion in the same test environment.

For the 3 olanzapine subgroups, in the first 30 min, a mixed ANOVA revealed significant main effects of group, $F(2, 21) = 8.86$, $p < 0.002$, and test, $F(2, 42) = 14.51$, $p < 0.001$, and a significant group \times test interaction, $F(4, 42) = 6.65$, $p < 0.001$. The OLZ-0 subgroup had significantly higher motor activity than the other two OLZ subgroups on the first day, $p < 0.001$, and higher motor activity than the OLZ-2 subgroup on the third day, $p < 0.05$. In the following 60 min, the group effect, the test effect and their interaction were all nonsignificant, $p > 0.24$. These results suggest that repeated olanzapine exposure in the test apparatus primarily enhanced its efficacy in the inhibition of spontaneous motor activity.

Taken together, results from this experiment suggest that repeated pairing of haloperidol and olanzapine treatment with the test environment enhanced their efficacy of inhibition of spontaneous motor activity. Repeated pairing of haloperidol treatment with the test environment also enhanced its inhibition of PCP-induced hyperlocomotion, while repeated clozapine or olanzapine and environment pairing had little effect on this measure.

DISCUSSION

The present study provides evidence that specific experimental parameters and drug treatment play a role in determining whether the contextual cues associated with antipsychotic drug administration would exert control over the expression of antipsychotic efficacy. In Experiment 1, we found that repeated administration of haloperidol (0.05 mg/kg, sc) and olanzapine (2.0 mg/kg, sc), even in the home cages, potentiated their inhibition of PCP (3.20 mg/kg, sc)-induced hyperlocomotion in the motor activity test boxes (a novel environment). Furthermore, relative to the VEH+CLZ group, pretreatment with clozapine in the home cage decreased its effectiveness on spontaneous activity over the 5-day test period in the motor test environment. These findings suggest that under certain test conditions, antipsychotic sensitization or tolerance could be less impacted by environmental cues. This conclusion was consistent with our finding in Experiment 2 that repeated administration of

clozapine (5.0 mg/kg, sc) or olanzapine (1.0 mg/kg, sc) for 4 consecutive days, regardless of where these treatments occurred (e.g. 2 days in the home cage and 2 days in the test apparatus; or 4 days in the home cage and 0 day in the test apparatus), had a similar level of inhibition on PCP (1.6 mg/kg, sc)-induced hyperlocomotion over the 3-day probe test period. In contrast, the observation that rats that received 4-day haloperidol (0.03 mg/kg, sc) treatment in the test apparatus had significantly lower motor activity than those that received 0-day treatment, in spite of their identical drug treatment history, does support the notion that the environment associated with repeated drug administration could powerfully modulate the expression of antipsychotic efficacy.

The contextual control of the behavioral efficacy of an antipsychotic drug is a relatively less studied topic. Evidence supporting both context independence and context dependence has been reported in the literature. For example, Sanger (1985) reported that repeated haloperidol or clozapine treatment, whether inside the test environment or outside, has little impact on the development of haloperidol sensitization or clozapine tolerance in a conditioned avoidance response model. However, in a similar conditioned avoidance response test, we showed that home-cage haloperidol (0.05 mg/kg) and olanzapine (1.0 mg/kg) treatment did not increase their behavioral efficacy of inhibition of avoidance response, indicating a context-dependent feature (Li *et al.*, 2009; Sparkman and Li, 2012). Similarly, Schmidt's group also reported that intermittent haloperidol treatment and repeated catalepsy testing caused an intensification of catalepsy over time and that this increase was completely context specific, as context changes abolished catalepsy sensitization (Amtage and Schmidt, 2003; Klein and Schmidt, 2003). Therefore, the environmental impact of a drug effect is dependent on the specific test conditions, including the selected behavioral responses, drug types, drug doses, number of drug treatments, etc. It is not a question of whether environmental cues play a role in the regulation of a drug effect, but under what exact conditions.

This point was also supported in our recent study of the environmental and behavioral controls of the expression of clozapine tolerance (Feng *et al.*, 2013). Using an across-model transfer paradigm, we first treated rats repeatedly with clozapine (2.5–10.0 mg/kg, sc) in either the conditioned avoidance response task or the PCP (1.6 mg/kg, sc)-induced hyperlocomotion test for 5 consecutive days. We then switched them to a different test and tested them under clozapine for another 5 days. When switching from the avoidance task to the PCP test, rats previously treated with clozapine in the avoidance task did not show an immediately weakened inhibition of PCP-induced hyperlocomotion compared to those treated with clozapine for the first time, but showed a significantly weaker inhibition over time. This finding matches perfectly with our finding from Experiment 1, suggesting that change of environment only affects the acute effect of clozapine, but has no effect on its tolerance effect.

In our previous study (Sun *et al.*, 2009), we showed that repeated administration of clozapine at 10.0 mg/kg maintained its inhibition of spontaneous motor activity and PCP-induced hyperlocomotion over the 5 test days, a finding replicated in the present study (Figure 2B). Because the VEH+PCP group showed a progressive increase in motor activity over days, the persistent inhibition in the VEH+CLZ group indicates a sensitization-like

effect, especially during the repeated drug test phase. This sensitization-like effect seems weaker in the CLZ+CLZ group relative to the VEH+CLZ group, as indicated by the significantly higher motor activity in the CLZ+CLZ group compared to the VEH+CLZ group in the first 30 min of testing on Day 4 and 5, and marginally significant higher motor activity in the second 60 min of testing on Day 5. Although we interpreted this finding as reflecting the home cage clozapine-induced tolerance development, it could also be interpreted as the consequence of home cage clozapine treatment that prevented the appearance of a sensitization-like effect. The exact nature of such a prior clozapine effect needs to be further investigated.

Studies on contextual control of drug effects typically compare a “paired” group (a group that receives drug injection in the test environment) with an “unpaired” group (a group that receives vehicle injection in the test environment, and drug in the home cage) (Poulos and Hinson, 1982; Amtage and Schmidt, 2003). The influence of environment is assessed on a test day, when all animals receive a challenge injection of the drug in the test environment. If a stronger drug effect was detected in the “paired” group, this would suggest that environmental stimuli have an influence on the drug effect (Robinson *et al.*, 1998). Experiment 2 used this approach and found that only repeated pairings of haloperidol treatment in the test apparatus enhanced the efficacy of haloperidol in the inhibition of spontaneous motor activity and PCP-induced hyperlocomotion, as the HAL-4 subgroup had significantly lower motor activity than the HAL-0 group in the 30 min and second 60 min test periods. This enhanced HAL effect occurred because HAL, administered at this low dose (0.03 mg/kg) in the test environment for the first time, was unable to inhibit novelty-induced increase in motor activity as shown in the HAL-0 group. In contrast, repeated clozapine/olanzapine pairings in the test environment produced little alteration of their effects. These results further suggest that the specific conditions govern the impact of environmental cues on a drug effect. Under the current condition, the circumstances surrounding drug administration had a large impact on the behavioral efficacy of haloperidol, but not that of clozapine and olanzapine. The reason for this differential impact on different antipsychotic drugs is not entirely clear. One possibility is that haloperidol has a stronger antagonist action on D₂ receptor and tighter D₂ receptor binding than olanzapine and clozapine (Kapur and Seeman, 2000), and multiple behavioral effects mediated by dopamine systems are typically modulated by environmental cues (Anagnostaras and Robinson, 1996; Robinson *et al.*, 1998; Anagnostaras *et al.*, 2002).

One limitation of the present study is that only one dose of each antipsychotic drug was tested in each experiment. Therefore, the results from this study should be considered together with, and also in the context of, evidence from our previous studies (Li *et al.*, 2009; Sparkman and Li, 2012; Zhang and Li, 2012; Feng *et al.*, 2013). Overall the evidence seems to support the notion that environmental cues and behavioral responses could have a powerful impact on the inhibitory effect of an antipsychotic drug on PCP-induced hyperlocomotion and on conditioned avoidance responding (two well-validated behavioral measures of antipsychotic-like activity). But such an impact could be limited by certain test conditions, such as the degree of similarity between different test environments, drug doses, and number of drug treatments.

Acknowledgments

We would like to thank Ms. Shinn-Yi Chou and Ms. Natasha Swalve for their thoughtful comments on an earlier version of this manuscript. Research reported in this paper was supported by the National Institute of Mental Health of the National Institutes of Health under award number **R01MH085635** to Professor Ming Li. We thank the two anonymous reviewers for their insightful comments.

References

- Agid O, Seeman P, Kapur S. The "delayed onset" of antipsychotic action--an idea whose time has come and gone. *J Psychiatry Neurosci*. 2006; 31:93–100. [PubMed: 16575424]
- Amtage J, Schmidt WJ. Context-dependent catalepsy intensification is due to classical conditioning and sensitization. *Behav Pharmacol*. 2003; 14:563–567. [PubMed: 14557724]
- Anagnostaras SG, Robinson TE. Sensitization to the psychomotor stimulant effects of amphetamine: modulation by associative learning. *Behav Neurosci*. 1996; 110:1397–1414. [PubMed: 8986341]
- Anagnostaras SG, Schallert T, Robinson TE. Memory processes governing amphetamine-induced psychomotor sensitization. *Neuropsychopharmacology*. 2002; 26:703–715. [PubMed: 12007741]
- Chen J, Wang Z, Li M. Multiple 'hits' during postnatal and early adulthood periods disrupt the normal development of sensorimotor gating ability in rats. *J Psychopharmacol*. 2011; 25:379–392. [PubMed: 20093319]
- Feng M, Sui N, Li M. Environmental and behavioral controls of the expression of clozapine tolerance: Evidence from a novel across-model transfer paradigm. *Behav Brain Res*. 2013; 238:178–187. [PubMed: 23092709]
- Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry*. 1991; 148:1301–1308. [PubMed: 1654746]
- Javitt DC, Zukin SR, Heresco-Levy U, Umbricht D. Has an angel shown the way? Etiological and therapeutic implications of the PCP/NMDA model of schizophrenia. *Schizophr Bull*. 2012; 38:958–966. [PubMed: 22987851]
- Kapur S, Seeman P. Antipsychotic agents differ in how fast they come off the dopamine D2 receptors. Implications for atypical antipsychotic action. *J Psychiatry Neurosci*. 2000; 25:161–166. [PubMed: 10740989]
- Klein A, Schmidt WJ. Catalepsy intensifies context-dependently irrespective of whether it is induced by intermittent or chronic dopamine deficiency. *Behav Pharmacol*. 2003; 14:49–53. [PubMed: 12576881]
- 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]
- Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res Brain Res Rev*. 1997; 25:192–216. [PubMed: 9403138]
- Porsolt RD, Moser PC, Castagne V. Behavioral indices in antipsychotic drug discovery. *J Pharmacol Exp Ther*. 2010
- Poulos CX, Hinson R. Pavlovian conditional tolerance to haloperidol catalepsy: evidence of dynamic adaptation in the dopaminergic system. *Science*. 1982; 218:491–492. [PubMed: 6889765]
- Poulos CX, Hinson RE, Siegel S. The role of Pavlovian processes in drug tolerance and dependence: implications for treatment. *Addict Behav*. 1981; 6:205–211. [PubMed: 7293843]
- Redmond AM, Harkin A, Kelly JP, Leonard BE. Effects of acute and chronic antidepressant administration on phencyclidine (PCP) induced locomotor hyperactivity. *Eur Neuropsychopharmacol*. 1999; 9:165–170. [PubMed: 10082243]
- Robinson TE, Browman KE, Crombag HS, Badiani A. Modulation of the induction or expression of psychostimulant sensitization by the circumstances surrounding drug administration. *Neurosci Biobehav Rev*. 1998; 22:347–354. [PubMed: 9579324]
- Siegel S. Evidence from rats that morphine tolerance is a learned response. *J Comp Physiol Psychol*. 1975; 89:498–506. [PubMed: 425]

- Siegel S. Morphine tolerance acquisition as an associative process. *J Exp Psychol Anim Behav Process.* 1977; 3:1–13. [PubMed: 845541]
- Siegel S. Pavlovian conditioning analysis of morphine tolerance. *NIDA Res Monogr.* 1978:27–53. [PubMed: 418352]
- Siegel S, Baptista MA, Kim JA, McDonald RV, Weise-Kelly L. Pavlovian psychopharmacology: the associative basis of tolerance. *Exp Clin Psychopharmacol.* 2000; 8:276–293. [PubMed: 10975617]
- Sparkman NL, Li M. Drug-drug conditioning between citalopram and haloperidol or olanzapine in a conditioned avoidance response model: implications for polypharmacy in schizophrenia. *Behav Pharmacol.* 2012
- Sun T, Hu G, Li M. Repeated antipsychotic treatment progressively potentiates inhibition on phencyclidine-induced hyperlocomotion, but attenuates inhibition on amphetamine-induced hyperlocomotion: relevance to animal models of antipsychotic drugs. *Eur J Pharmacol.* 2009; 602:334–342. [PubMed: 19059234]
- Sun T, Zhao C, Hu G, Li M. Iptakalim: a potential antipsychotic drug with novel mechanisms? *Eur J Pharmacol.* 2010; 634:68–76. [PubMed: 20184878]
- Vezina P, Stewart J. Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. *Pharmacol Biochem Behav.* 1984; 20:925–934. [PubMed: 6463076]
- Zhang C, Li M. Contextual and behavioral control of antipsychotic sensitization induced by haloperidol and olanzapine. *Behav Pharmacol.* 2012; 23:66–79. [PubMed: 22157143]
- Zhao C, Li M. Neuroanatomical substrates of the disruptive effect of olanzapine on rat maternal behavior as revealed by c-Fos immunoreactivity. *Pharmacol Biochem Behav.* 2012; 103:174–180. [PubMed: 22960130]
- Zhao C, Sun T, Li M. Neural basis of the potentiated inhibition of repeated haloperidol and clozapine treatment on the phencyclidine-induced hyperlocomotion. *Prog Neuropsychopharmacol Biol Psychiatry.* 2012a
- Zhao C, Sun T, Li M. Neural basis of the potentiated inhibition of repeated haloperidol and clozapine treatment on the phencyclidine-induced hyperlocomotion. *Prog Neuropsychopharmacol Biol Psychiatry.* 2012b; 38:175–182. [PubMed: 22476004]

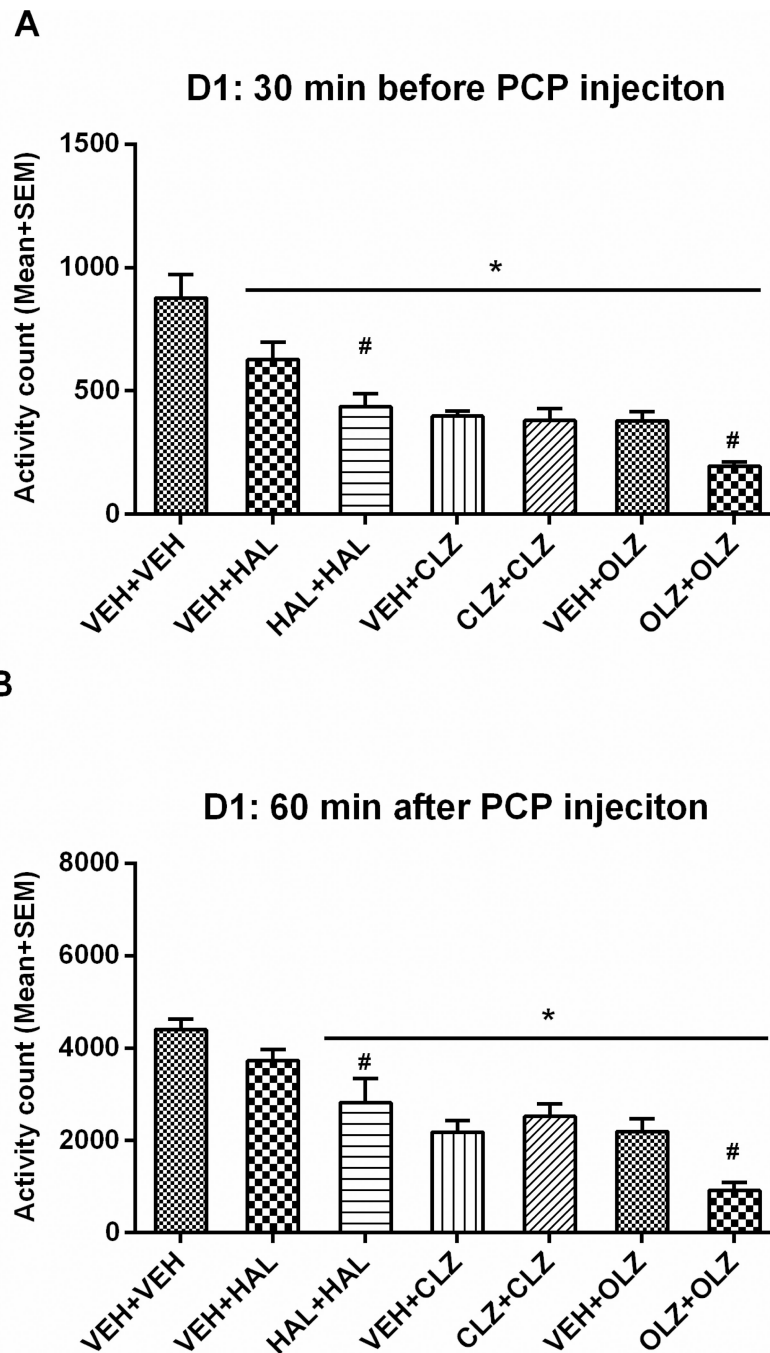


Figure 1. Effect of home cage antipsychotic treatment on the acute inhibitory effect of antipsychotic drugs on PCP-induced hyperlocomotion tested in a new motor activity apparatus Locomotor activity was measured for 30 min after sterile water (VEH), haloperidol (0.05 mg/kg), clozapine (10.0 mg/kg), or olanzapine (2.0 mg/kg) injection (**A**) and 60 min after PCP (3.2 mg/kg) injection (**B**) and expressed as mean + SEM for each group. * $p < 0.05$ relative to the VEH+VEH group; # $p < 0.05$ relative to the corresponding control groups that were treated with vehicle in the home cages (i.e. VEH+HAL or VEH+OLZ).

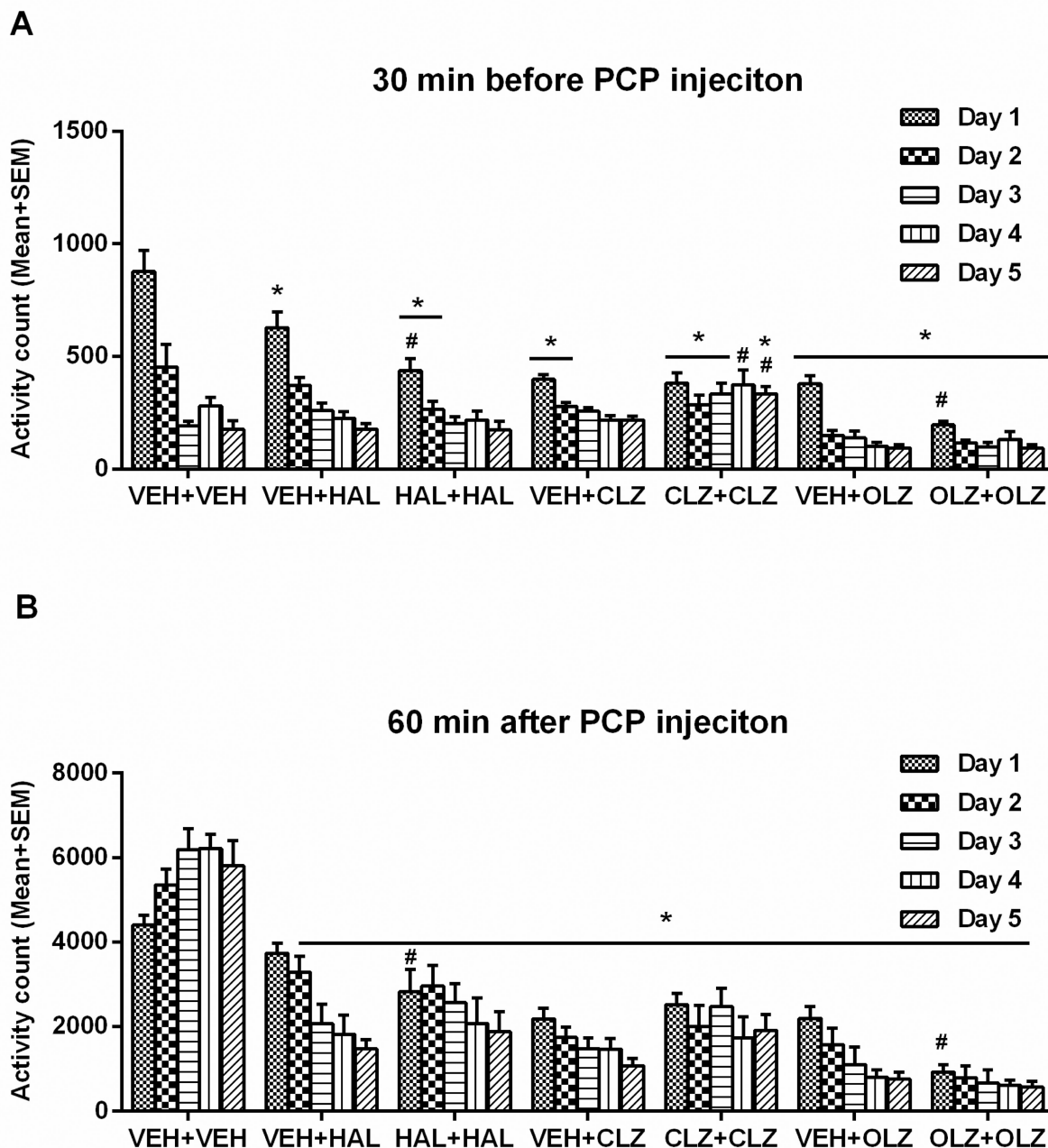


Figure 2. Effect of home cage antipsychotic treatment on the inhibitory effect of antipsychotic drugs on PCP-induced hyperlocomotion throughout the 5 days of drug testing

(A) Locomotor activity was measured for 30 min after sterile water (VEH), haloperidol (0.05 mg/kg), clozapine (10.0 mg/kg), or olanzapine (2.0 mg/kg) injection and expressed as mean + SEM for each group. (B) Locomotor activity was measured for 60 min after PCP injection and expressed as mean + SEM. * $p < 0.05$ relative to the VEH+VEH group; # $p < 0.05$ relative to the corresponding control groups that were treated with vehicle in the home cages (i.e. VEH+HAL or VEH+OLZ).

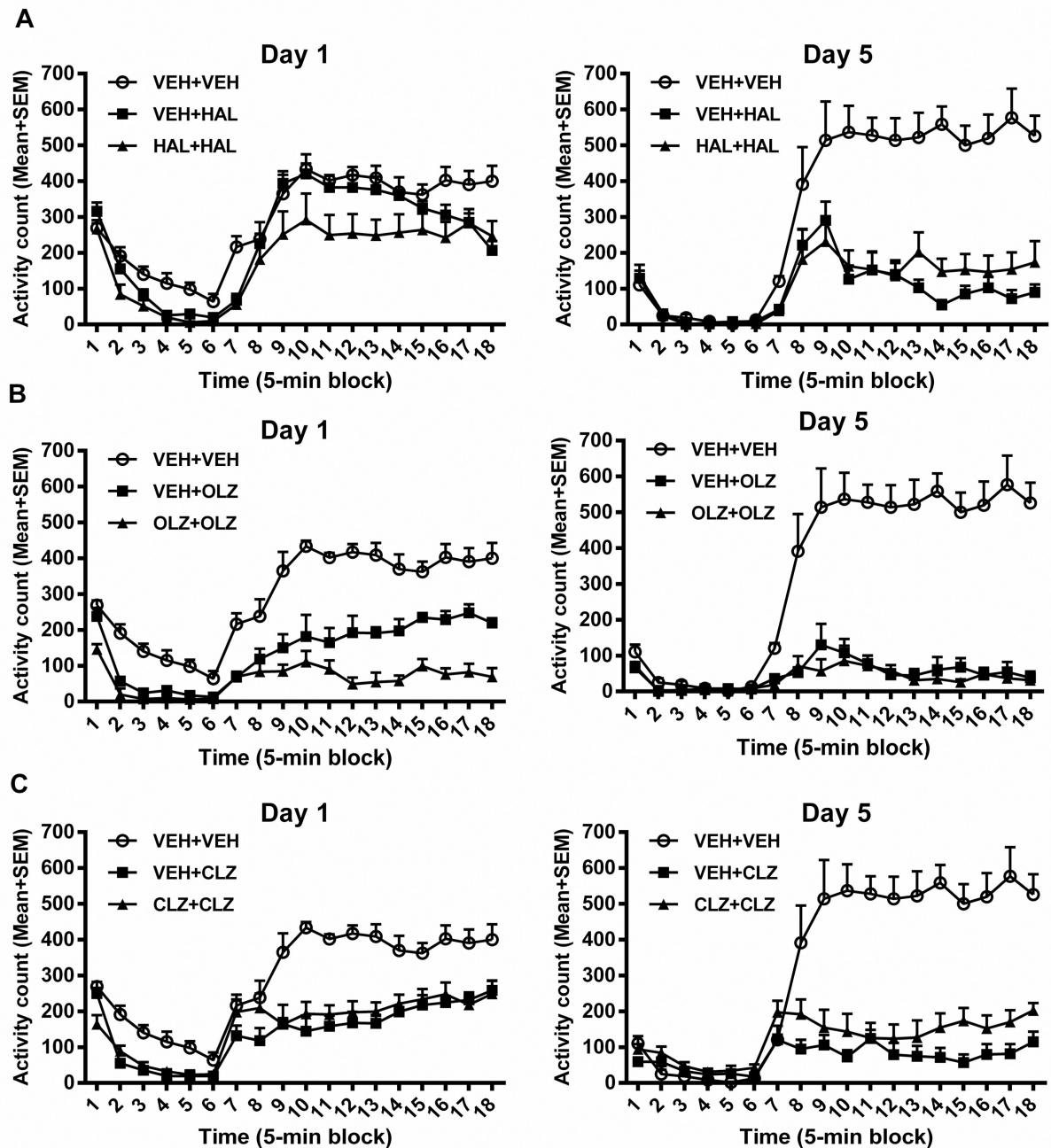


Figure 3. Effect of home cage antipsychotic treatment on the inhibitory effect of antipsychotic drugs on PCP-induced hyperlocomotion on Day 1 and Day 5 of drug testing

Locomotor activity was measured in 18 5-min blocks for the two haloperidol groups (VEH+HAL and HAL+HAL) (A), two olanzapine groups (VEH+OLZ and OLZ+OLZ) (B), and two clozapine groups (VEH+CLZ and CLZ+CLZ) (C), together with the vehicle groups.

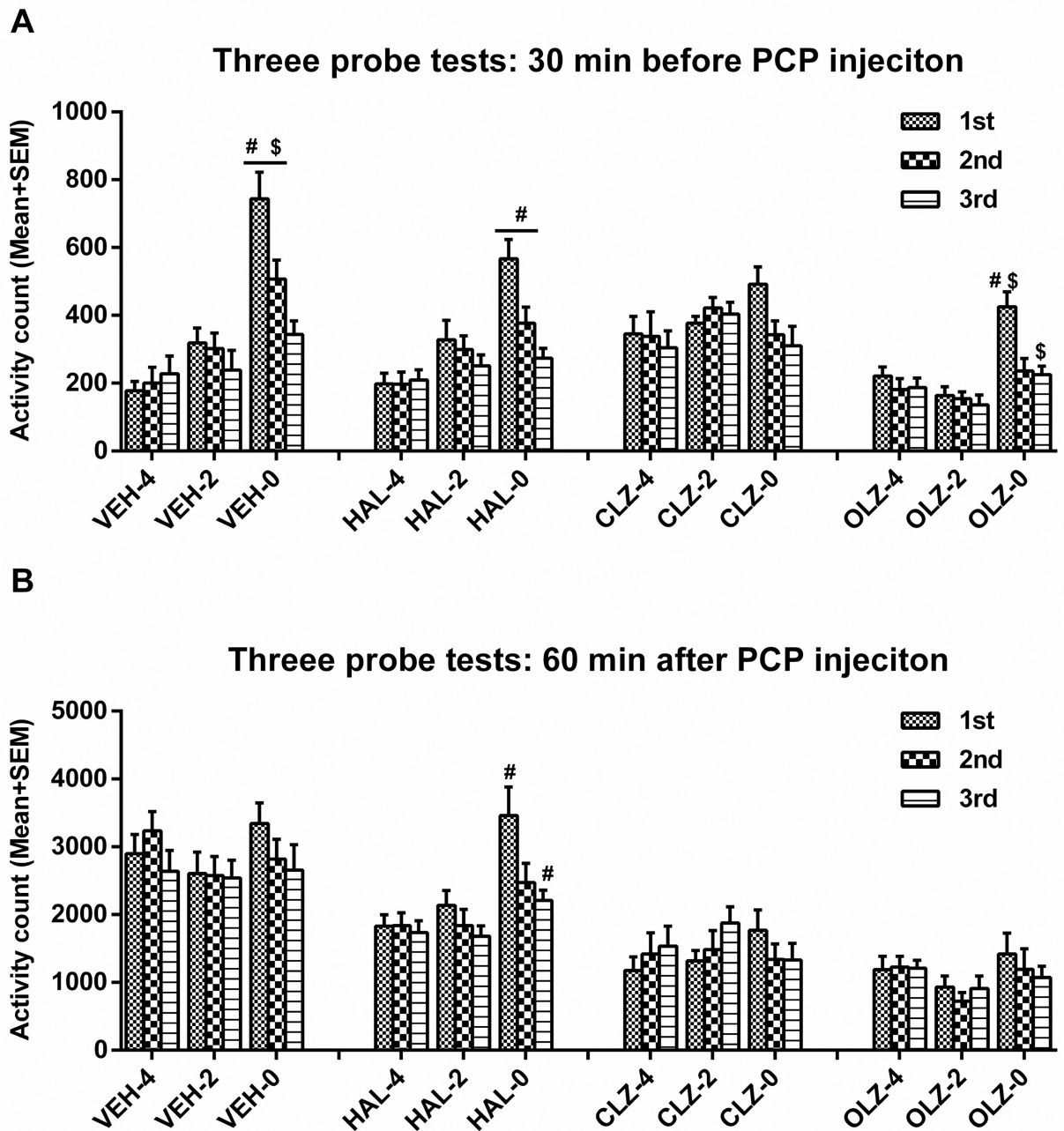


Figure 4. Prior haloperidol exposure in the test apparatus enhanced its efficacy of inhibition of PCP-induced hyperlocomotion, while prior clozapine and olanzapine did not

Locomotor activity in the 30 min before (A) and 60 min after PCP injection (B) of the 12 subgroups across the three days of PCP-induced hyperlocomotion testing are expressed as mean + SEM. # $p < 0.05$ relative to the corresponding 4-exposure subgroup; \$ $p < 0.05$ relative to the corresponding 2-exposure subgroup.