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## Repeated asenapine treatment produces a sensitization effect in two preclinical tests of antipsychotic activity

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### Abstract

Among several commonly used atypical antipsychotic drugs, olanzapine and risperidone cause a sensitization effect in the conditioned avoidance response (CAR) and phencyclidine (PCP)-induced hyperlocomotion paradigms – two well established animal tests of antipsychotic drugs, whereas clozapine causes a tolerance effect. Asenapine is a novel antipsychotic drug recently approved for the treatment of schizophrenia and manic disorders. It shares several receptor binding sites and behavioral features with other atypical antipsychotic drugs. However, it is not clear what type of repeated effect (sensitization or tolerance) asenapine would induce, and whether such an effect is transferrable to other atypicals. In this study, male adult Sprague-Dawley rats were first repeatedly tested with asenapine (0.05, 0.10 or 0.20 mg/kg, sc) for avoidance response or PCP (3.20 mg/kg, sc)-induced hyperlocomotion daily for 5 consecutive days. After 2–3 days of retraining/drug-free recovery, they were then challenged with asenapine (0.10 mg/kg, sc), followed by olanzapine (0.50 mg/kg, sc) and clozapine (2.50 mg/kg, sc). During the 5-day drug test period (the induction phase), repeated asenapine treatment progressively increased its inhibition of avoidance response and PCP-induced hyperlocomotion in a dose-dependent fashion. On the asenapine and olanzapine challenge tests (the expression phase), rats previously treated with asenapine still showed significantly lower avoidance response and lower PCP-induced hyperlocomotion than those previously treated with vehicle. An increased reactivity to clozapine challenge in prior asenapine-treated rats was also found in the PCP-induced hyperlocomotion test. These findings suggest that asenapine is capable of inducing a sensitization effect and a cross-sensitization to olanzapine and clozapine (to a lesser extent). Because the behavioral profile of asenapine in both tests is similar to that of olanzapine, but different from that of clozapine, we suggest that asenapine resembles olanzapine to a greater extent than clozapine in its therapeutic and side effect profiles.

### Keywords

Asenapine; Olanzapine; Clozapine; Conditioned avoidance response; Phencyclidine; Locomotor activity; Sensitization; Tolerance

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### Conflicts of interest

None.

### Disclosures

All authors declare no conflict of interest.

## 1. Introduction

Asenapine (US brand name: Saphris; EU brand name: Sycrest) is a new atypical antipsychotic medication recently approved for the acute and maintenance treatment of schizophrenia and treatment of acute manic or mixed episodes associated with bipolar I disorder (Tarazi and Neill, 2012; Tarazi and Shahid, 2009). It has a multiple receptor binding profile similar to those of other atypical antipsychotic drugs (e.g. clozapine, olanzapine), with higher antagonist action against serotonin receptors (5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>), adrenergic  $\alpha_1$  and  $\alpha_2$  receptors, histamine H<sub>1</sub> and H<sub>2</sub> receptors, and a relatively lower action on dopamine D<sub>2</sub> and D<sub>1</sub> receptors (Shahid et al., 2009). Clinical studies suggest that it is well tolerated and does not cause severe weight gains (Howland, 2011; Meltzer et al., 2009). Preclinical studies suggest that asenapine can dose-dependently enhance dopamine and acetylcholine release in the medial prefrontal cortex and hippocampus (Franberg et al., 2012; Huang et al., 2008), and may even possess a procognitive property (Elsworth et al., 2012; McLean et al., 2010; Snigdha et al., 2011).

In recent years, we have systematically investigated the repeated effects of antipsychotic treatment using preclinical tests of antipsychotic activity (Li et al., 2007, 2010; Sun et al., 2009). We have identified two behavioral patterns (*sensitization* and *tolerance*) commonly associated with chronic use of drugs of abuse in two independent paradigms of antipsychotic activity: the conditioned avoidance response (CAR) and phencyclidine (PCP)-induced hyperlocomotion (Feng et al., 2013; Li et al., 2012; Mead and Li, 2010; Qiao et al., 2013; Swalve and Li, 2012; Zhang and Li, 2012). Specifically, we show that repeated administration of haloperidol, olanzapine or risperidone daily for 5–7 days tends to cause a progressively increased inhibition of avoidance responding and PCP-induced hyperlocomotion (a sensitization effect). When rats are given a challenge dose of these drugs at a later point, they also exhibit a sensitization effect as they often make significantly *fewer* avoidance responses and exhibit *lower* PCP-induced hyperlocomotion than those that are treated with these drugs for the first time (Li et al., 2009a, b; Li et al., 2010; Mead and Li, 2010; Qiao et al., 2013; Sun et al., 2009; Zhao et al., 2012). In addition, we show that haloperidol-induced sensitization is transferable to olanzapine-induced sensitization and vice versa, an intriguing finding that may be useful in determining the similarities or differences of different antipsychotic drugs (Li et al., 2007; Mead and Li, 2010).

Clozapine is the only tested antipsychotic that displays a different behavioral pattern in these tests. During the daily drug test phase, repeated administration of clozapine causes no apparent sensitization or tolerance (Li et al., 2010; Qiao et al., 2013; Sun et al., 2009). But on the challenge test, a tolerance effect is often observed as rats previously treated with clozapine make significantly *more* avoidance responses and exhibit *higher* PCP-induced hyperlocomotion than those that are treated with clozapine for the first time (Feng et al., 2013; Li et al., 2010; Qiao et al., 2013). The exact nature of clozapine tolerance and its clinical relevance are not entire clear; it could be connected to its unique clinical profile (e.g. superior therapeutic efficacy and little extrapyramidal side effects) in some way (Meltzer, 2012).

Given the fact that an atypical antipsychotic drug exhibits either a sensitization or tolerance behavioral patterns in the CAR and PCP-induced hyperlocomotion tests, which may reveal their clinical properties and receptor mechanisms, to better understand the preclinical profile of asenapine, it is important to determine what type of repeated effect (sensitization or tolerance) asenapine would induce, and whether such an effect is transferable to other atypical antipsychotic drugs. Furthermore, only one study examined the acute effect of asenapine in the CAR (Franberg et al., 2008), and there is no reported study on its efficacy

in the PCP-induced hyperlocomotion test. The present study was also designed to address this deficiency.

## 2. Materials and methods

### 2.1. Animals

All experimental treatment 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 or 276–300 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 ± 1 °C with a relative humidity of 45–60%. Food and water was available *ad libitum*. Animals were allowed at least 5 days of habituation to the animal facility before being used in experiments. All behavioral tests took place between 9 am and 5 pm in the light cycle.

### 2.2. Drugs and choice of doses

Asenapine Maleate (a gift from the NIMH drug supply program) was dissolved in 0.9% saline. Doses of asenapine (0.05, 0.10 and 0.20 mg/kg) were determined based on previous studies showing that this dose range of asenapine causes a dose-dependent suppression of CAR but does not cause severe motor impairment (Franberg et al., 2008; Marston et al., 2009) and is effective to cause an increase of dopamine, acetylcholine and norepinephrine efflux in the medial prefrontal cortex and hippocampus (Huang et al., 2008). Both olanzapine (OLZ, 0.50 mg/kg) and clozapine (CLZ, 2.50 mg/kg) (gifts from the NIMH drug supply program) were dissolved in distilled sterile water with 1.0–1.5% glacial acetic acid. Phencyclidine hydrochloride (PCP, gift from the NIDA Chemical Synthesis and Drug Supply Program) was dissolved in 0.9% saline. The dose of PCP (3.20 mg/kg) was chosen based on our previous work (Sun et al., 2009, 2010; Zhang and Li, 2012; Zhao et al., 2012). This dose of PCP is shown to induce a robust hyperlocomotion effect without causing severe stereotypy (Gleason and Shannon, 1997; Kalinichev et al., 2008). All drugs were administered subcutaneously (sc) at 1.0 ml/kg.

### 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. Locomotor activity monitoring apparatus

This apparatus has been described before (Feng et al., 2013; Sun et al., 2009; Zhao and Li, 2012). 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 record the number of beam breaks. All experiments were run during the light cycle.

## 2.5. Experiment 1: effects of repeated asenapine treatment on avoidance response

This experiment examined whether repeated asenapine treatment induces a sensitization or tolerance effect in the CAR model and whether prior asenapine treatment would alter the avoidance-disruptive effect of olanzapine and clozapine (i.e. cross-sensitization or tolerance). The experiment was comprised of the following three phases: *Phase 1: Avoidance training and 5 days of repeated asenapine testing*; *Phase 2: Asenapine challenge test*; and *Phase 3: Olanzapine and clozapine challenge tests*.

### 2.5.1. Phase 1: avoidance training in CAR and 5 days of repeated asenapine testing

Forty rats were first habituated to the CAR boxes for 2 days (30 min/day). They were then trained for conditioned avoidance responding for 10 days/sessions. Each session consisted of 30 trials, and each trial started by presenting 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. An avoidance response was recorded if a subject moved from one compartment into the other within the 10 s of CS presentation. An escape response was recorded if the rat remained in the same compartment for more than 10 s and made a crossing upon receiving the footshock. If the rat did not respond during the entire 5 s presentation of the shock, the trial was terminated and the intertrial intervals started. The total number of avoidance responses was recorded for each session. Inter-trial intervals varied randomly between 30 and 60 s.

At the end of the training session, 35 rats reached the training criterion (70% avoidance in each of the last 2 sessions). They were first matched on avoidance performance on the last training day (i.e. pre-drug) to create blocks of rats ( $n = 4$  rats/block) that were approximately equal in performance. Within each block, they were then randomly assigned to 1 of 4 groups: asenapine 0.05 mg/kg (ASE 0.05,  $n = 9$ ), asenapine 0.10 mg/kg (ASE 0.10,  $n = 8$ ), asenapine 0.20 mg/kg (ASE 0.20,  $n = 9$ ) and vehicle (VEH,  $n = 9$ ), and tested daily under the CS-only (no shock, 30 trials/session) condition for 5 consecutive days, following the same procedure as employed before (Feng et al., 2012, 2013; Swalve and Li, 2012; Zhang and Li, 2012). On each test day, rats were first injected with asenapine or saline; 30 min later, they were placed in the CAR boxes and tested.

**2.5.2. Phase 2: asenapine challenge test**—One day after the last (5th) asenapine test day, all rats were retrained drug-free for 1 session under the CS-only (no shock) condition, followed by another under the CS-US condition to bring their avoidance responses back to the pre-drug level. These 2 retraining sessions also ensured that all groups had a comparable level of avoidance responding before the asenapine challenge test, which occurred 1 day after the 2nd retraining session. On the challenge day, all rats were injected with asenapine at 0.10 mg/kg and tested for avoidance performance, in the CS-only condition (30 trials) 30 min later.

**2.5.3. Phase 3: olanzapine and clozapine challenge tests**—Two days after the asenapine challenge test, all rats were once again retrained for 2 sessions (1 under the CS-only and 1 under the CS-US condition), followed by the olanzapine challenge test 1 day later. Similar to the asenapine challenge test, all rats were injected with olanzapine 0.50 mg/kg and tested for avoidance performance, in the CS-only condition (30 trials) 1 h later. This challenge procedure has been successfully used in our previous studies (Li et al., 2012, 2010; Sparkman and Li, 2012; Swalve and Li, 2012; Zhang and Li, 2012). Two days later, all rats were retrained and challenged under clozapine (2.50 mg/kg, sc). The basic procedure was identical to that of olanzapine. Fig. 1 summarizes the entire experimental procedure and groups at different phases of the experiment.

## 2.6. Experiment 2: effects of repeated asenapine treatment on the PCP-induced hyperlocomotion

This experiment examined how repeated asenapine (0.05, 0.10, 0.20 mg/kg, sc) treatment affects the PCP-induced hyperlocomotion. It was designed to examine the generality of asenapine sensitization across different tests of antipsychotic activity. The entire experiment was comprised of the following three phases: *Phase 1: Five days of repeated asenapine testing*; *Phase 2: Asenapine challenge test*; and *Phase 3: Olanzapine and clozapine challenge tests*.

**2.6.1. Phase 1: five days of repeated asenapine testing**—Forty-eight rats were randomly assigned to 1 of 5 groups: VEH + VEH (saline + saline,  $n = 16$ ), VEH + PCP (saline + PCP 3.20 mg/kg,  $n = 8$ ); ASE 0.05 + PCP (ASE 0.05 mg/kg + PCP 3.20 mg/kg,  $n = 8$ ), ASE 0.1 + PCP (ASE 0.10 mg/kg + PCP 3.20 mg/kg,  $n = 8$ ) and ASE 0.2 + PCP (ASE 0.20 mg/kg + PCP 3.20 mg/kg,  $n = 8$ ). All rats were first handled and habituated to the locomotor activity apparatus for 2 days (30 min/day). On each of the next 5 consecutive days, they were first injected with vehicle (saline), ASE 0.05, 0.10, or 0.20 mg/kg and then immediately placed in the boxes for 30 min. At the end of the 30-min period, they were taken out and injected with vehicle (saline) or PCP (3.20 mg/kg, sc) and placed back in the boxes for another 60 min. Locomotor activity (number of photobeam breaks) was measured in 5 min intervals throughout the entire 90-min testing session.

**2.6.2. Phase 2: asenapine challenge test**—Two days after the last (5th) ASE test, all rats were returned to the locomotor activity boxes for 1 re-habituation session (30 min), followed by the asenapine challenge test 1 day later. On the challenge day, the VEH + VEH group was split into two subgroups ( $n = 8$ /subgroup): the VEH + VEH-1 group and VEH + VEH-2 group. All rats were first injected with asenapine 0.10 mg/kg and then immediately placed in the locomotor activity boxes for 30 min. At the end of the 30-min period, they were taken out and injected with either saline (VEH + VEH-1,  $n = 8$ ) or PCP (3.20 mg/kg) (VEH + VEH-2 and other rats) and placed back in the boxes for another 60 min.

**2.6.3. Phase 3: olanzapine and clozapine challenge tests**—Three days after the last asenapine challenge test, all rats were returned to the locomotor activity boxes for 1 re-habituation session (30 min), followed by the olanzapine challenge test 1 day later. On the challenge test day, all rats were first injected with olanzapine 0.50 mg/kg and then immediately placed in the motor activity boxes for 30 min. At the end of the 30-min period, rats were taken out and injected with saline (VEH + VEH-1) or PCP (3.20 mg/kg), and placed back in the boxes for another 60 min.

The basic procedure of clozapine challenge test was identical to that of olanzapine. The dose of clozapine administration was 2.50 mg/kg Fig. 2 summarizes the experimental procedure and groups at different phases of the experiment.



## 2.7. Statistical analysis

All data were expressed as mean + SEM. Data from the 5 drug test sessions (e.g. avoidance response and PCP-induced hyperlocomotion) were analyzed using a factorial repeated measures analysis of variance (ANOVA) with the between-subjects factor being drug group and the within-subjects factor being test session, followed by post hoc LSD tests. Differences between groups on the specific drug test days and on the challenge tests 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 19.

## 3. Results

### 3.1. Experiment 1: effects of repeated asenapine treatment on avoidance response

**3.1.1. Phase 1: five days of repeated asenapine testing**—Fig. 3 shows the mean number of avoidance responses made by rats in the four groups on the last training (pre-drug) day and 5 drug test days. There was no group difference on the last training day (pre-drug). Throughout the 5 drug test days, asenapine increased its suppression of avoidance response progressively in a dose-dependent way. Two-way repeated measures ANOVA revealed a main effect of group,  $F(3, 31) = 42.445$ ,  $p < 0.001$ ; day,  $F(4, 124) = 42.061$ ,  $p < 0.001$  and a significant group  $\times$  day interaction,  $F(12, 124) = 5.603$ ,  $p < 0.001$ . Post hoc LSD tests show that all three asenapine groups made significantly fewer avoidance responses than the VEH group, all  $ps < 0.001$ ; and the ASE 0.10 and ASE 0.20 groups also had significantly lower avoidance than that of the ASE 0.05 group, all  $ps < 0.001$ . The difference between the ASE 0.10 and ASE 0.20 group was marginally significant,  $p = 0.05$ . One-way ANOVAs on each test day revealed that the ASE 0.10 and ASE 0.20 groups had significantly lower avoidance than the VEH group and the ASE 0.05 group on all 5 days,  $ps < 0.005$ , while the ASE 0.05 group showed significantly lower avoidance than the VEH group on last three drug test days,  $ps < 0.001$ .

**3.1.2. Phase 2: asenapine challenge test**—Fig. 4A shows the number of avoidance responses on the pre-drug day and the asenapine challenge day. No significant group difference was detected on the pre-drug day. On the challenge day when all rats were injected with asenapine 0.10 mg/kg, the three ASE groups made fewer avoidance responses than the VEH group. One-way ANOVA confirmed a main effect of group,  $F(3, 31) = 6.047$ ,  $p = 0.002$ . Post hoc LSD tests showed that the ASE 0.10 and ASE 0.20 groups were significantly different from the VEH group,  $p = 0.003$  and  $0.001$ , respectively, and the ASE 0.20 group was also significantly different from the ASE 0.05 group,  $p = 0.027$ .

**3.1.3. Phase 3: olanzapine and clozapine challenge tests**—Fig. 4B shows the number of avoidance responses on the pre-drug day and the olanzapine challenge test day. Before the OLZ challenge, there was no significant group difference. On the challenge day when all rats were injected with OLZ 0.50 mg/kg, the three ASE groups once again made fewer avoidance responses than the VEH group, indicating a cross sensitization to olanzapine. One-way ANOVA showed a significant effect of group,  $F(3, 31) = 4.645$ ,  $p = 0.009$ . Post hoc LSD tests showed that the ASE 0.05, ASE 0.10 and ASE 0.20 groups were significantly different from the VEH group,  $p = 0.043$ ,  $0.001$  and  $0.034$ , respectively. For the clozapine challenge (Fig. 4C), although no significant group difference on the pre-drug day and on the CLZ (2.50 mg/kg) challenge day was found,  $ps > 0.522$ , the two ASE groups (0.10 and 0.20 mg/kg) did exhibit non-significantly lower avoidance than the VEH group.

Collectively, results from this experiment suggest that repeated asenapine treatment induced a sensitization and a cross-sensitization effect to olanzapine in conditioned avoidance response model. But there is no cross-sensitization to clozapine.

### 3.2. Experiment 2: effects of repeated asenapine treatment on the PCP-induced hyperlocomotion

**3.2.1. Phase 1: five days of repeated asenapine testing**—Fig. 5A shows the mean locomotor activity of the rats of five groups during the 60-min test period after vehicle or PCP injection throughout the 5 days of drug testing. Two-way repeated measures ANOVA revealed a significant main effect of group,  $F(4, 43) = 70.443, p < 0.001$ ; day,  $F(4, 172) = 6.013, p < 0.001$  and a significant group  $\times$  day interaction,  $F(16, 172) = 2.389, p = 0.003$ . Post hoc LSD tests revealed that the VEH + PCP group was significantly different from the VEH + VEH group,  $p < 0.001$ , indicating a strong psychomotor activation effect of this dose of PCP. The low ASE (0.05 mg/kg) group had significantly *higher* locomotor activity, whereas the high ASE (0.20 mg/kg) group had significantly *lower* motor activity than the VEH + PCP group, all  $ps < 0.001$ . In addition, the three ASE groups differed significantly from each other, all  $ps < 0.001$ . These findings imply a dose-dependent inhibition of the PCP-induced hyperlocomotion. This inhibition was not complete, as the three ASE groups still differed significantly from the VEH + VEH group, all  $ps < 0.002$ .

To identify the behavioral patterns of the effects of repeated drug treatment (e.g. ASE and PCP) on the motor activity throughout the 5 drug test days, we compared activity data in 5-min blocks on day 1 with those on day 5 for each drug group (Fig. 5B–F). Two-way repeated measures ANOVA on the 5-min block data revealed a main effect of block,  $F(11, 154) = 11.318, p < 0.001$ , and block  $\times$  day interaction,  $F(11, 154) = 6.845, p < 0.001$ , but no main effect of day,  $F(1, 14) = 0.813, p = 0.382$  in the VEH + PCP group. The main effect of block and block  $\times$  day interaction were also significant in the ASE 0.05 + PCP group, all  $ps < 0.001$ . For the ASE 0.20 + PCP group, the main effects of block, day, and block  $\times$  day interaction were all significant, all  $ps < 0.001$ , suggesting that repeated asenapine treatment progressively strengthened its inhibition of PCP-induced hyperlocomotion.

**3.2.2. Three re-habituation sessions**—On the 1st, 2nd and 3rd re-habituation days before the asenapine, olanzapine and clozapine challenge tests, all rats were placed in the locomotor activity boxes for 30 min with no drug treatment, and one-way ANOVA did not find any significant group difference, all  $ps > 0.066$  (data not shown).

#### 3.2.3. Phase 2 and 3: asenapine, olanzapine and clozapine challenge tests—

Fig. 6A shows the mean locomotor activity during the 60-min test period after the vehicle or PCP injection on the asenapine challenge test. First, an independent samples  $t$  test found that the VEH + VEH-2 group (treated with PCP) had significantly higher locomotor activity than the VEH + VEH-1 group (treated with saline),  $t(14) = -5.108, p = 0.001$ . A one-way ANOVA on the PCP-treated groups (5 groups excluding the VEH + VEH-1 group) found a main effect of group,  $F(4, 35) = 3.358, p = 0.02$ . Post hoc tests showed that the ASE 0.20 + PCP group were significantly different from the VEH + VEH-2, VEH + PCP and ASE 0.05 + PCP groups, all  $ps < 0.038$ , suggesting that prior asenapine treatment at 0.20 mg/kg enhanced its inhibition of the PCP-induced hyperlocomotion.

Fig. 6B shows the mean locomotor activity during the 60-min test period after the vehicle or PCP injection on the olanzapine challenge test day. An independent samples  $t$  test found that the VEH + VEH-2 group had significantly higher motor activity than the VEH + VEH-1 group,  $t(14) = -11.149, p < 0.001$ . A one-way ANOVA on the PCP-treated groups found a main effect of group,  $F(4, 35) = 6.525, p < 0.001$ . Post hoc LSD tests showed that the ASE



0.10 + PCP group and ASE 0.20 + PCP group had a significantly lower locomotor activity than the VEH + VEH-2 group, all  $ps < 0.001$ , and the VEH + PCP group,  $p = 0.048$  and  $p = 0.004$ , respectively. The ASE 0.20 + PCP group also had significantly lower locomotor activity than the ASE 0.05 + PCP group,  $p = 0.012$ . These results suggest that repeated asenapine treatment caused a cross-sensitization to olanzapine, a finding similar to what was observed in the CAR model.

Fig. 6C shows the mean locomotor activity during the 60-min test period after the vehicle or PCP injection on the clozapine challenge test day. Once again, the VEH + VEH-2 group had significantly higher locomotor activity than the VEH + VEH-1 group,  $t(14) = -11.849$ ,  $p < 0.001$ . One way ANOVA on the PCP-treated groups revealed a main effect of group,  $F(4, 35) = 10.968$ ,  $p < 0.001$ . Post hoc LSD tests showed that the ASE 0.10 + PCP group and ASE 0.20 + PCP group had significantly lower locomotor activity than the VEH + VEH-2 group, all  $ps < 0.001$  and the VEH + PCP group, all  $ps < 0.016$ , indicating that prior asenapine treatment increased behavioral sensitivity to clozapine. The ASE 0.20 + PCP group also had significantly lower locomotor activity than the ASE 0.05 + PCP group,  $p = 0.001$ .

Collectively, results from this experiment suggest that repeated asenapine treatment induced a sensitization effect and a cross-sensitization effect to both olanzapine and clozapine in the PCP-induced hyperlocomotion model.

#### 4. Discussion

Unlike the majority of behavioral studies of asenapine, which typically focus on its acute efficacy, the present study examined the impact of repeated asenapine treatment on the behavioral responsiveness to asenapine, olanzapine and clozapine. We addressed two questions: (1) what kind of repeated effect (sensitization or tolerance) would asenapine induce? (2) is asenapine-induced sensitization effect transferrable to other atypicals such as olanzapine and clozapine? Using the two independent behavioral tests of antipsychotic activity, we demonstrated that repeated asenapine treatment induced a sensitization effect in its disruption of avoidance response and inhibition of PCP-induced hyperlocomotion. This effect was observed during the repeated drug test period (the induction phase) and in the challenge test (the expression phase). Furthermore, prior asenapine treatment also increased sensitivity to olanzapine treatment in both tests and to clozapine in the PCP-induced hyperlocomotion test. These findings reveal that similar to haloperidol, olanzapine and risperidone, but unlike clozapine, asenapine is an antipsychotic drug capable of inducing a long-lasting increased sensitivity to antipsychotic treatment in the CAR and PCP tests. Because of its sensitization profile and its ability to generate a cross-sensitization to olanzapine, asenapine may share a similar therapeutic and side effect profile with olanzapine, rather with clozapine, despite the fact that it shares many receptor binding profiles with clozapine (Meltzer et al., 2009; Shahid et al., 2009).

Previous work on the avoidance-disruptive effect of asenapine reports that acute asenapine at 0.10 and 0.20 mg/kg, but not at 0.05 mg/kg, produces a significant suppression of CAR at both 20 and 90 min after administration (Franberg et al., 2008). Our results are consistent with this finding, as we showed that on the 1st day of drug testing, only the 0.1 and 0.2 mg/kg asenapine, but not 0.05 mg/kg significantly disrupted avoidance response at 30–60 min (the test lasted about 30 min) after administration. However, with repeated drug administration and avoidance testing, 0.05 mg/kg asenapine started to significantly suppress avoidance response on the 3rd day and maintained its suppression throughout the remaining days. This finding clearly suggests that repeated drug exposure could alter drug sensitivity. This point is even more conspicuous when we inspect the avoidance response data on the

asenapine challenge test. On the pre-drug test, all 4 groups of rats (vehicle and 3 asenapine treatment groups) had a comparably high level of avoidance responding. However, on the challenge test, only those who had been exposed to asenapine at 0.10 and 0.20 mg/kg showed significantly lower avoidance than the other groups. The reason that the cross-sensitization to olanzapine did not show a dose-dependence is not clear (see Fig. 4B). Prior asenapine challenge test and/or specific experimental parameters (e.g. olanzapine challenge dose, number of test trials, etc.) may have made this effect less detectable. Of note, the asenapine-induced sensitization in the avoidance test is similar to the sensitization induced by many other antipsychotic drugs tested so far (Li et al., 2012, 2010; Mead and Li, 2010; Sparkman and Li, 2012; Swalve and Li, 2012; Zhang and Li, 2012), but different from that of clozapine, which tends to induce a tolerance effect (i.e. prior clozapine exposure decreases its ability to suppress avoidance response) (Feng et al., 2013; Li et al., 2012, 2010; Qiao et al., 2013).

In the present study, we also examined the acute and repeated effects of asenapine treatment on the PCP-induced hyperlocomotion, which, to our knowledge, has not been explored before. Upon acute administration, most antipsychotic drugs inhibit *acute* PCP-induced hyperlocomotion (Arnt, 1995; Gleason and Shannon, 1997), a feature often used to determine the potential antipsychotic activity. Our previous work suggests that the PCP-induced hyperlocomotion test using a repeated drug administration regimen is better in detecting antipsychotic action, capturing the time course of the antipsychotic effect and differentiating antipsychotics from anxiolytics (Sun et al., 2009; Zhao et al., 2012). Recent work suggests that it is also useful in detecting antipsychotic-induced sensitization and tolerance, as prior treatment of haloperidol and olanzapine often causes an increased responsiveness to these drugs so that their inhibition of PCP-induced hyperlocomotion becomes stronger, whereas prior treatment of clozapine causes a decreased responsiveness to clozapine re-exposure so that its inhibition becomes weaker (Feng et al., 2013; Qiao et al., 2013; Zhang and Li, 2012). Our finding that asenapine caused a sensitization effect in this model not only supports the usefulness of this paradigm in the study of the long-term antipsychotic treatment effect, regardless of any particular class of drugs; but also reveals the generality of asenapine sensitization, independent of any particular behavioral test (avoidance and motor activity). Furthermore, the cross-sensitization to olanzapine supports the notion that asenapine possesses an olanzapine-like therapeutic profile insofar as the inhibition of PCP-induced hyperlocomotion reflects this property.

One surprising finding was that asenapine at a low dose (i.e. 0.05 mg/kg) actually increased the PCP-induced hyperlocomotion, an effect opposite to those of 0.10 and 0.20 mg/kg asenapine. Other asenapine studies have also suggested that low doses of asenapine have different behavioral effects than high doses. For example, Marston et al. (2009) found that asenapine at high doses (>0.10 mg/kg) failed to improve performance in delayed non-match to place (DNMTP) and five-choice serial reaction (5-CSR) tasks – cognitive tasks designed to assess effects on short-term spatial memory and attention. At 0.30 mg/kg, it even impaired 5-CSR accuracy. In contrast, asenapine at low doses (<0.075 mg/kg) is shown to improve various cognitive deficits (e.g. reversal learning, cognitive flexibility, object recognition, etc.) induced by PCP (acute or subchronic) treatment or brain lesions (McLean et al., 2010; Snigdha et al., 2011; Tait et al., 2009). Therefore, it is possible that asenapine at low doses exhibits a procognitive effect while at high doses it exhibits an antipsychotic effect. At even higher doses (>0.50 mg/kg), asenapine causes catalepsy and sedation (Franberg et al., 2008). Furthermore, because several antidepressants are known to increase the PCP-induced hyperlocomotion (Redmond et al., 1999), it is possible that low doses of asenapine may even possess an antidepressant property. This dose-dependent behavioral feature of asenapine has apparent implications for clinical practice. For example, if the

negative symptoms and cognitive deficits are the primary targets, a lower dose of asenapine may be preferred.

At the behavioral level, the finding that repeated asenapine treatment progressively increased its disruption of avoidance response and PCP-induced hyperlocomotion could be explained by two previously identified mechanisms: attenuation of motivational salience of the CS and interoceptive drug state-mediated drug memory (Feng et al., 2012; Li et al., 2007, 2009a, b; Mead and Li, 2010; Zhang et al., 2011). At the neuroreceptor level, previous work has suggested that dopamine D<sub>2/3</sub> and serotonin 5-HT<sub>2A/2C</sub> receptors systems may play important roles in antipsychotic sensitization and tolerance (Li et al., 2012, 2010). In the CAR test, we show that olanzapine sensitization may be mediated by D<sub>2</sub> and 5-HT<sub>2A</sub> blockade-initiated neuroplasticity (e.g. down-regulating 5-HT<sub>2A</sub> receptor), whereas clozapine tolerance may be mediated by D<sub>2</sub> blockade-initiated neuroplasticity (Atkins et al., 1999; Kapur et al., 2003; Moran-Gates et al., 2006). In the PCP model, it has been shown that acute atypical drugs such as clozapine and olanzapine inhibit PCP-induced hyperlocomotion primarily due to their multiple actions on D<sub>2</sub> and 5-HT<sub>2A</sub> receptors (Gleason and Shannon, 1997; Maurel-Remy et al., 1995; Millan et al., 1999). 5-HT<sub>2A</sub> receptors may be even more important than D<sub>2</sub> receptors due to the fact that other 5-HT<sub>2A</sub> antagonists such as LY53857, ritanserin, ketanserin, fananserin, and MDL 100, 907 are more effective than dopamine antagonists in suppressing PCP-induced hyperlocomotion (Gleason and Shannon, 1997; Millan et al., 1999); and repeated administration of clozapine causes a down-regulation of 5-HT<sub>2A</sub> receptors in the prefrontal cortex (Doat-Meyerhoefer et al., 2005). Because PCP also enhances serotonergic, dopaminergic and glutamatergic neurotransmission in the nucleus accumbens and prefrontal cortex (Abekawa et al., 2007; Maurel-Remy et al., 1995; Millan et al., 1999), we have proposed that *antipsychotic sensitization in the PCP-induced hyperlocomotion test is mediated by down-regulating 5-HT<sub>2A</sub> receptors and concomitantly decreasing PCP-induced dopamine and 5-HT increases in the prefrontal cortex* (Sun et al., 2009).

In the case of asenapine, we would speculate that similar to olanzapine sensitization, asenapine sensitization in both the CAR and PCP models may also be mediated by its actions on D<sub>2</sub> (up-regulation) and 5-HT<sub>2A</sub> receptor (down-regulation) systems. This speculation is supported by the findings that asenapine has a relatively high occupancy of 5-HT<sub>2A</sub> compared with the D<sub>2</sub> receptor, a profile similar to that of olanzapine (Meltzer et al., 2009); and chronic asenapine treatment increases D<sub>2</sub> receptor and decreases 5-HT<sub>2A</sub> receptor binding in the medial prefrontal cortex (Tarazi et al., 2008, 2010), an effect also shared by olanzapine (Tarazi et al., 2001); and also by the findings that asenapine has a local 5-HT<sub>2A</sub> receptor antagonistic activity in the medial prefrontal cortex and can dose-dependently increase extracellular dopamine release in this area (Franberg et al., 2012, 2008). Because chronic treatment of asenapine also alters other receptor systems, notably 5-HT<sub>1A</sub>, D<sub>4</sub>, NMDA, and adrenergic  $\alpha_1$  and  $\alpha_2$  receptors (Choi et al., 2010; Franberg et al., 2012; Shahid et al., 2009; Tarazi and Neill, 2012), their involvement in asenapine sensitization also needs to be examined; and the two behavioral paradigms introduced in this study provide a valid approach in addressing this issue.

One limitation of the present study is that the clozapine challenge test was always conducted after the olanzapine test. There was a possibility that the clozapine's effect on avoidance response and PCP-induced hyperlocomotion had been altered by olanzapine exposure; thus, it would be problematic if we only rely on the behavioral profile in the challenge tests to make comparison between asenapine and clozapine. Another limitation is that we did not directly examine the brain mechanisms of asenapine sensitization and did not examine parameters (e.g. number of drug injections, interval between initial drug exposure and challenge test, etc.) that influence the development and expression of asenapine

sensitization. Future studies should examine these issues to enhance our understanding of the repeated effects of asenapine.

In summary, asenapine is a new antipsychotic drug which shares a similar behavioral profile with olanzapine but differs from clozapine on the basis of the findings that its repeated treatment caused an olanzapine-like sensitization effect in both the CAR and PCP-induced hyperlocomotion tests. Future work on the neurobiological mechanisms of asenapine sensitization may further enhance our understanding of antipsychotic sensitization in general and asenapine sensitization in particular. Such knowledge may also be useful for future drug discovery.

## Acknowledgments

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## References

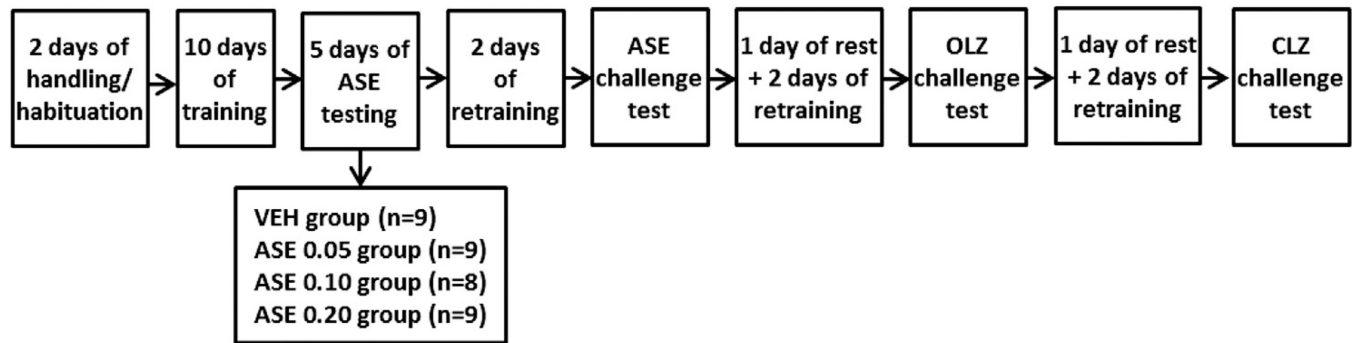
- Abekawa T, Ito K, Koyama T. Different effects of a single and repeated administration of clozapine on phencyclidine-induced hyperlocomotion and glutamate releases in the rat medial prefrontal cortex at short- and long-term withdrawal from this antipsychotic. *Naunyn Schmiedeberg Arch. Pharmacol.* 2007; 375:261–271. [PubMed: 17458542]
- Arnt J. Differential effects of classical and newer antipsychotics on the hypermotility induced by two dose levels of D-amphetamine. *Eur. J. Pharmacol.* 1995; 283:55–62. [PubMed: 7498321]
- Atkins JB, Chlan-Fourney J, Nye HE, Hiroi N, Carlezon WA Jr, Nestler EJ. Region-specific induction of deltaFosB by repeated administration of typical versus atypical antipsychotic drugs. *Synapse.* 1999; 33:118–128. [PubMed: 10400890]
- Choi YK, Wong EH, Henry B, Shahid M, Tarazi FI. Repeated effects of asenapine on adrenergic and cholinergic muscarinic receptors. *Int. J. Neuropsychopharmacol.* 2010; 13:405–410. [PubMed: 19835670]
- Doat-Meyerhoefer MM, Hard R, Winter JC, Rabin RA. Effects of clozapine and 2,5-dimethoxy-4-methylamphetamine [DOM] on 5-HT<sub>2A</sub> receptor expression in discrete brain areas. *Pharmacol. Biochem. Behav.* 2005; 81:750–757. [PubMed: 15972234]
- Elsworth JD, Groman SM, Jentsch JD, Valles R, Shahid M, Wong E, Marston H, Roth RH. Asenapine effects on cognitive and monoamine dysfunction elicited by subchronic phencyclidine administration. *Neuropharmacology.* 2012; 62:1442–1452. [PubMed: 21875607]
- Feng M, Sui N, Li M. Avoidance disruptive effect of clozapine and olanzapine is potentiated by increasing the test trials: further test of the motivational salience hypothesis. *Pharmacol. Biochem. Behav.* 2012; 103:467–473. [PubMed: 23026062]
- 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]
- Franberg O, Marcus MM, Svensson TH. Involvement of 5-HT<sub>2A</sub> receptor and alpha(2)-adrenoceptor blockade in the asenapine-induced elevation of prefrontal cortical monoamine outflow. *Synapse.* 2012; 66:650–660. [PubMed: 22362425]
- Franberg O, Wiker C, Marcus MM, Konradsson A, Jardemark K, Schilström B, Shahid M, Wong EH, Svensson TH. Asenapine, a novel psychopharmacologic agent: preclinical evidence for clinical effects in schizophrenia. *Psychopharmacology (Berl.).* 2008; 196:417–429. [PubMed: 17940749]
- Gleason SD, Shannon HE. Blockade of phencyclidine-induced hyperlocomotion by olanzapine, clozapine and serotonin receptor subtype selective antagonists in mice. *Psychopharmacology (Berl.).* 1997; 129:79–84. [PubMed: 9122367]
- Howland RH. Update on newer antipsychotic drugs. *J. Psychosoc Nurs. Ment. Health Serv.* 2011; 49:13–15.

- Huang M, Li Z, Dai J, Shahid M, Wong EH, Meltzer HY. Asenapine increases dopamine, norepinephrine, and acetylcholine efflux in the rat medial prefrontal cortex and hippocampus. *Neuropsychopharmacology*. 2008; 33:2934–2945. [PubMed: 18418367]
- Kalinichev M, Robbins MJ, Hartfield EM, Maycox PR, Moore SH, Savage KM, Austin NE, Jones DN. Comparison between intraperitoneal and subcutaneous phencyclidine administration in Sprague–Dawley rats: a locomotor activity and gene induction study. *Prog. Neuropsychopharmacol. Biol. Psychiatry*. 2008; 32:414–422. [PubMed: 17945407]
- 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]
- 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.* 2009a; 20:184–194. [PubMed: 19322074]
- Li M, He W, Mead A. Olanzapine and risperidone disrupt conditioned avoidance responding in phencyclidine-pretreated or amphetamine-pretreated rats by selectively weakening motivational salience of conditioned stimulus. *Behav. Pharmacol.* 2009b; 20:84–98. [PubMed: 19179852]
- Li M, Sun T, Mead A. Clozapine, but not olanzapine, disrupts conditioned avoidance response in rats by antagonizing 5-HT(2A/2C) receptors. *J. Neural Transm.* 2012; 119:497–505. [PubMed: 21986871]
- 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]
- Marston HM, Young JW, Martin FD, Serpa KA, Moore CL, Wong EH, Gold L, Meltzer LT, Azar MR, Geyer MA, Shahid M. Asenapine effects in animal models of psychosis and cognitive function. *Psychopharmacology (Berl.)*. 2009; 206:699–714. [PubMed: 19462162]
- Maurel-Remy S, Bervoets K, Millan MJ. Blockade of phencyclidine-induced hyperlocomotion by clozapine and MDL 100,907 in rats reflects antagonism of 5-HT<sub>2A</sub> receptors. *Eur. J. Pharmacol.* 1995; 280:R9–R11. [PubMed: 7589172]
- McLean SL, Neill JC, Idris NF, Marston HM, Wong EH, Shahid M. Effects of asenapine, olanzapine, and risperidone on psychotomimetic-induced reversal-learning deficits in the rat. *Behav. Brain Res.* 2010; 214:240–247. [PubMed: 20561963]
- 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]
- Meltzer HY. Clozapine: balancing safety with superior antipsychotic efficacy. *Clin. Schizophr. Relat. Psychoses*. 2012; 6:134–144. [PubMed: 23006238]
- Meltzer HY, Dritselis A, Yasothan U, Kirkpatrick P. Asenapine. *Nat. Rev. Drug Discov.* 2009; 8:843–844. [PubMed: 19876039]
- Millan MJ, Brocco M, Gobert A, Joly F, Bervoets K, Rivet J, Newman-Tancredi A, Audinot V, Maurel S. Contrasting mechanisms of action and sensitivity to antipsychotics of phencyclidine versus amphetamine: importance of nucleus accumbens 5-HT<sub>2A</sub> sites for PCP-induced locomotion in the rat. *Eur. J. Neurosci.* 1999; 11:4419–4432. [PubMed: 10594669]
- Moran-Gates T, Gan L, Park YS, Zhang K, Baldessarini RJ, Tarazi FI. Repeated antipsychotic drug exposure in developing rats: dopamine receptor effects. *Synapse*. 2006; 59:92–100. [PubMed: 16270300]
- Qiao J, Li H, Li M. Olanzapine sensitization and clozapine tolerance: from adolescence to adulthood in the conditioned avoidance response model. *Neuropsychopharmacology*. 2013; 38:513–524. [PubMed: 23132270]
- 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]
- Shahid M, Walker GB, Zorn SH, Wong EH. Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. *J. Psychopharmacol.* 2009; 23:65–73. [PubMed: 18308814]



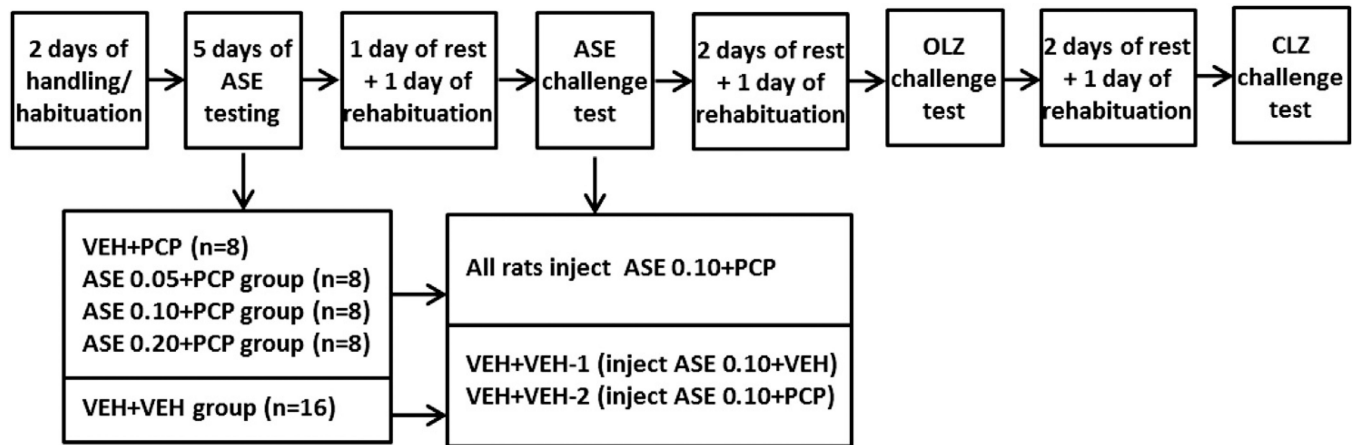
- Snigdha S, Idris N, Grayson B, Shahid M, Neill JC. Asenapine improves phencyclidine-induced object recognition deficits in the rat: evidence for engagement of a dopamine D1 receptor mechanism. *Psychopharmacology (Berl)*. 2011; 214:843–853. [PubMed: 21088957]
- Sparkman NL, Li M. Drug-drug conditioning between citalopram and haloperidol or olanzapine in a conditioned avoidance response model: implications for polypharmacy in schizophrenia. *Behav. Pharmacol.* 2012; 23:658–668. [PubMed: 22903071]
- 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]
- Swalve N, Li M. Parametric studies of antipsychotic-induced sensitization in the conditioned avoidance response model: roles of number of drug exposure, drug dose, and test-retest interval. *Behav. Pharmacol.* 2012; 23:380–391. [PubMed: 22732209]
- Tait DS, Marston HM, Shahid M, Brown VJ. Asenapine restores cognitive flexibility in rats with medial prefrontal cortex lesions. *Psychopharmacology (Berl)*. 2009; 202:295–306. [PubMed: 18925388]
- Tarazi FI, Moran-Gates T, Wong EH, Henry B, Shahid M. Differential regional and dose-related effects of asenapine on dopamine receptor subtypes. *Psychopharmacology (Berl)*. 2008; 198:103–111. [PubMed: 18297468]
- Tarazi FI, Moran-Gates T, Wong EH, Henry B, Shahid M. Asenapine induces differential regional effects on serotonin receptor subtypes. *J. Psychopharmacol.* 2010; 24:341–348. [PubMed: 18719049]
- Tarazi FI, Neill JC. The preclinical profile of asenapine: clinical relevance for the treatment of schizophrenia and bipolar mania. *Expert Opin. Drug Discov.* 2012
- Tarazi FI, Shahid M. Asenapine maleate: a new drug for the treatment of schizophrenia and bipolar mania. *Drugs Today (Barc)*. 2009; 45:865–876. [PubMed: 20135021]
- Tarazi FI, Zhang K, Baldessarini RJ. Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: implications for antipsychotic drug treatment. *J. Pharmacol. Exp. Ther.* 2001; 297:711–717. [PubMed: 11303062]
- 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]
- 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.* 2012; 38:175–182. [PubMed: 22476004]





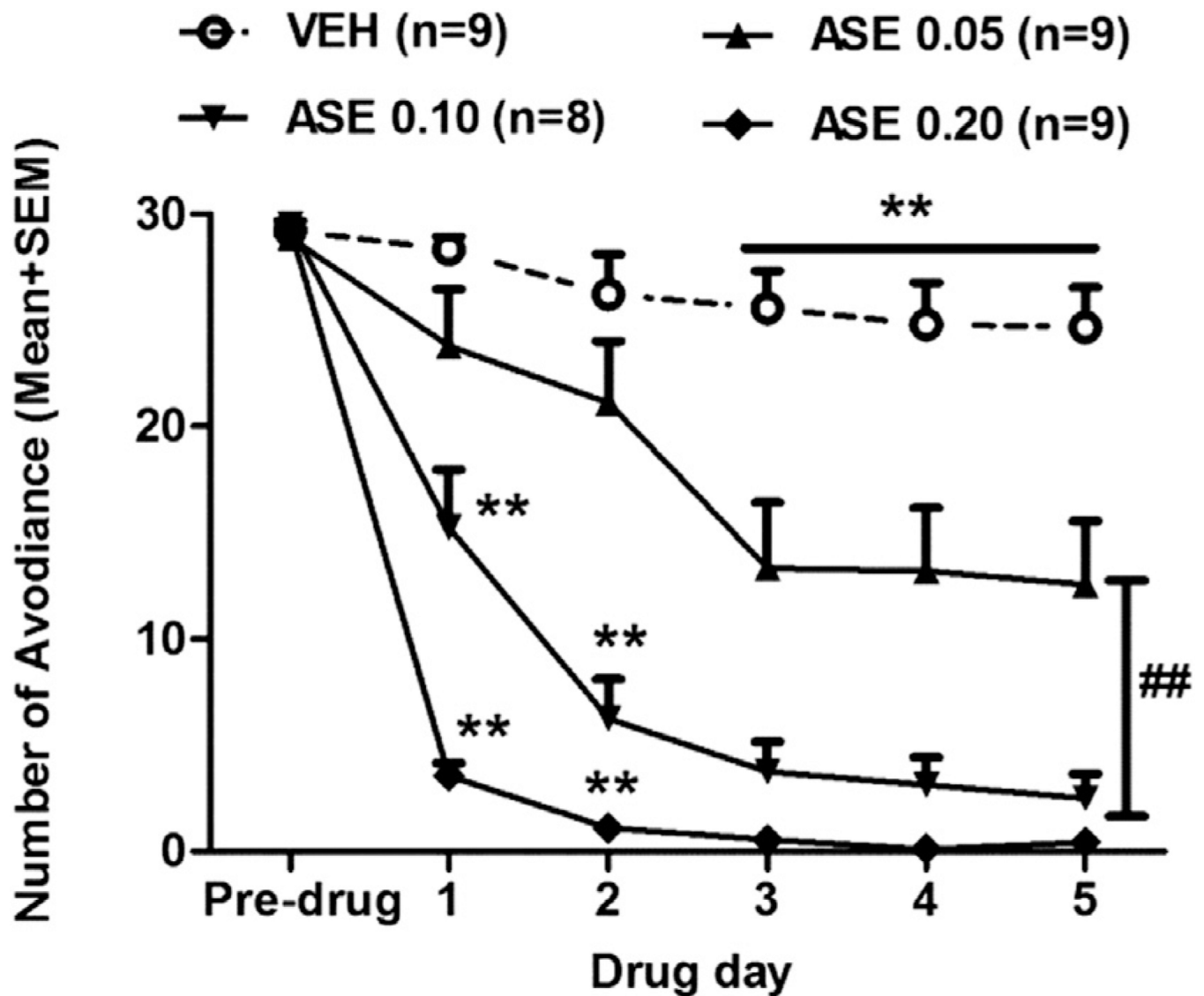
**Fig. 1. A schematic illustration of the experimental procedure and groups in Experiment 1 (CAR model)**

ASE: asenapine; OLZ: olanzapine; CLZ: clozapine; VEH: vehicle.



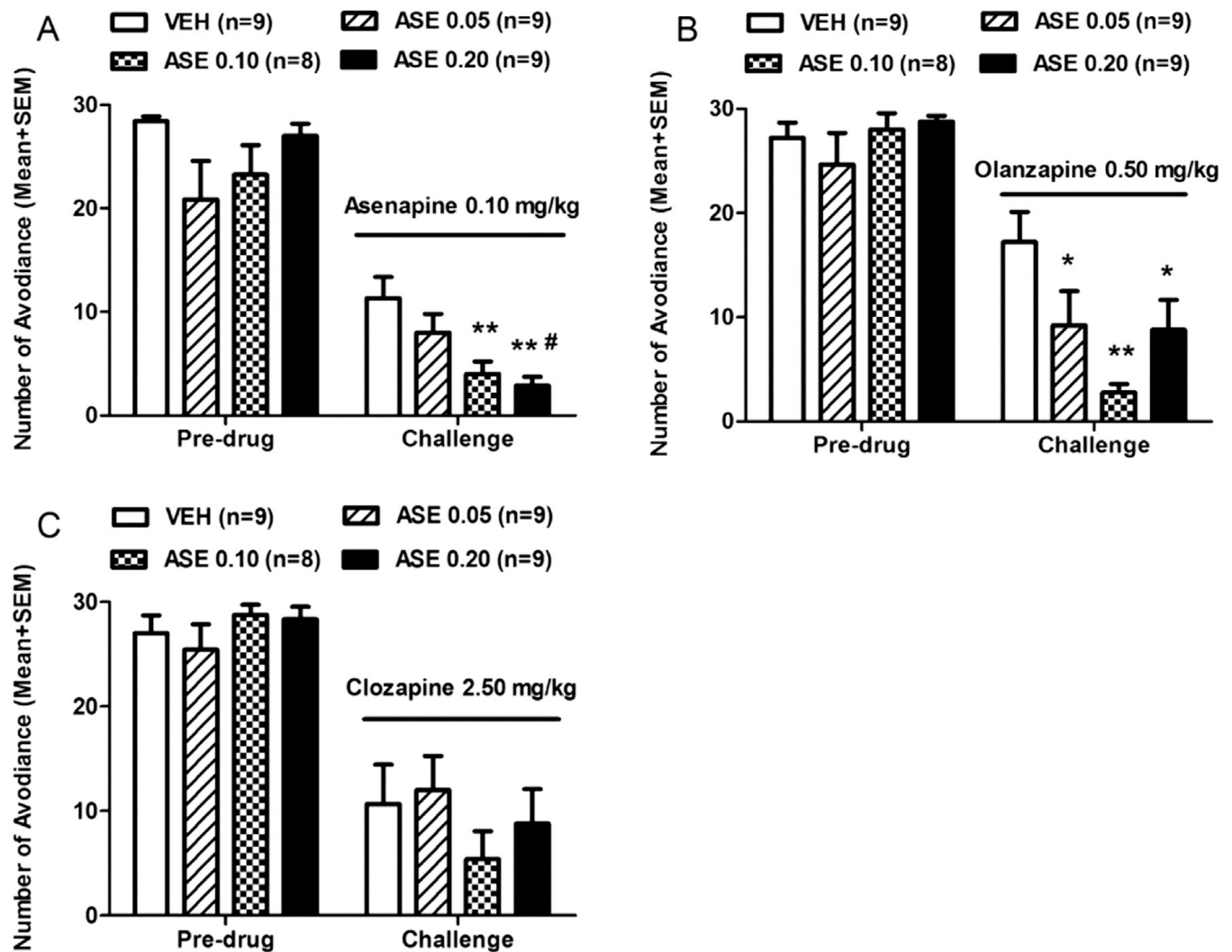
**Fig. 2.** A schematic illustration of the experimental procedure and groups in Experiment 2 (PCP model)

ASE: asenapine; PCP: phencyclidine; OLZ: olanzapine; CLZ: clozapine; VEH: vehicle.



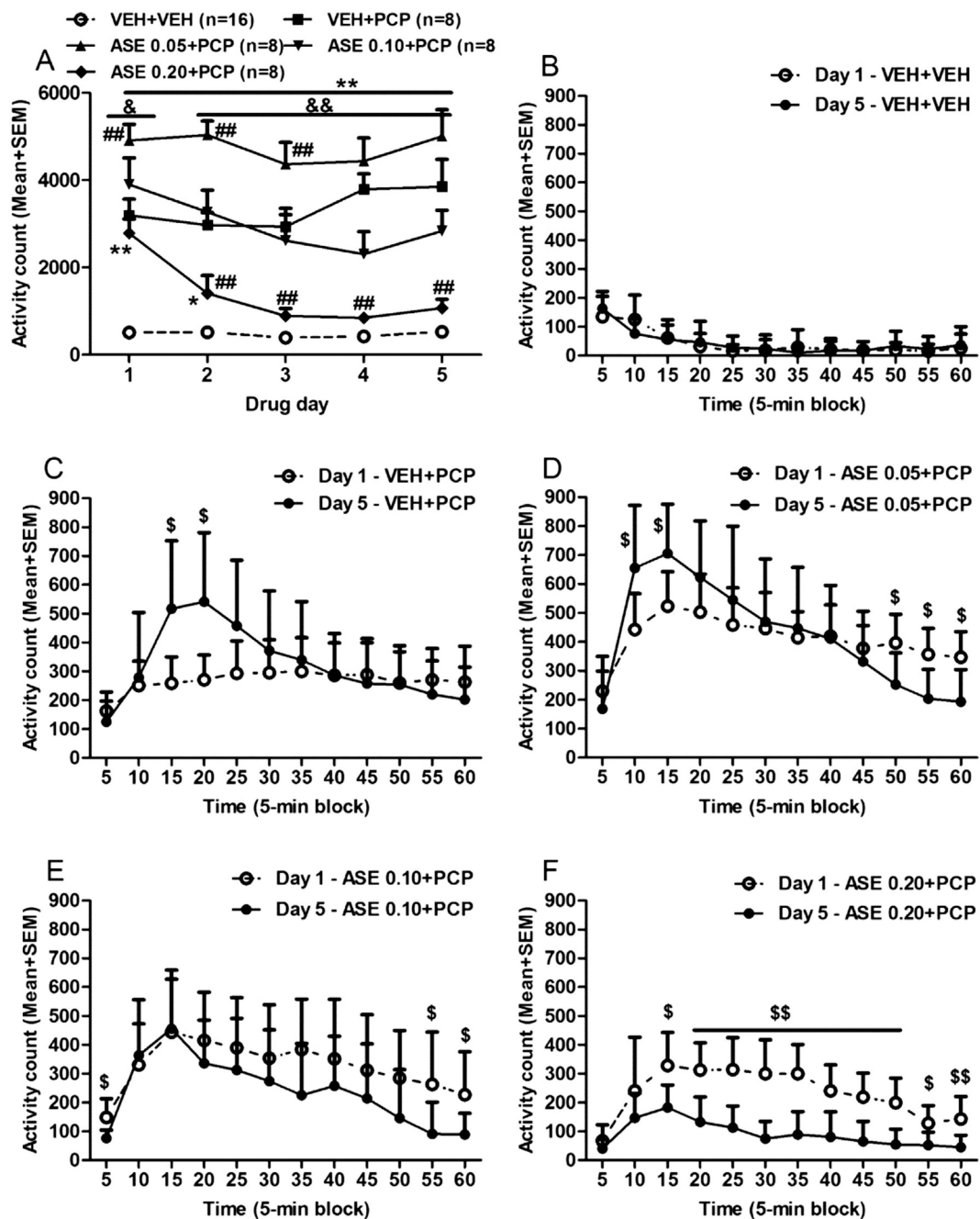
**Fig. 3. Repeated asenapine treatment increased avoidance response disruption but did not affect 22 kHz USV**

Number of avoidance responses made by the rats from the three asenapine treatment groups (ASE, 0.05, 0.10, and 2.0 mg/kg) and the vehicle group on the last training (pre-drug) day and throughout the five drug test days are expressed as mean + SEM. \*\* $p < 0.001$  relative to the VEH group; ## $p < 0.004$  the ASE 0.10 and ASE 0.20 groups relative to the ASE 0.05 group.



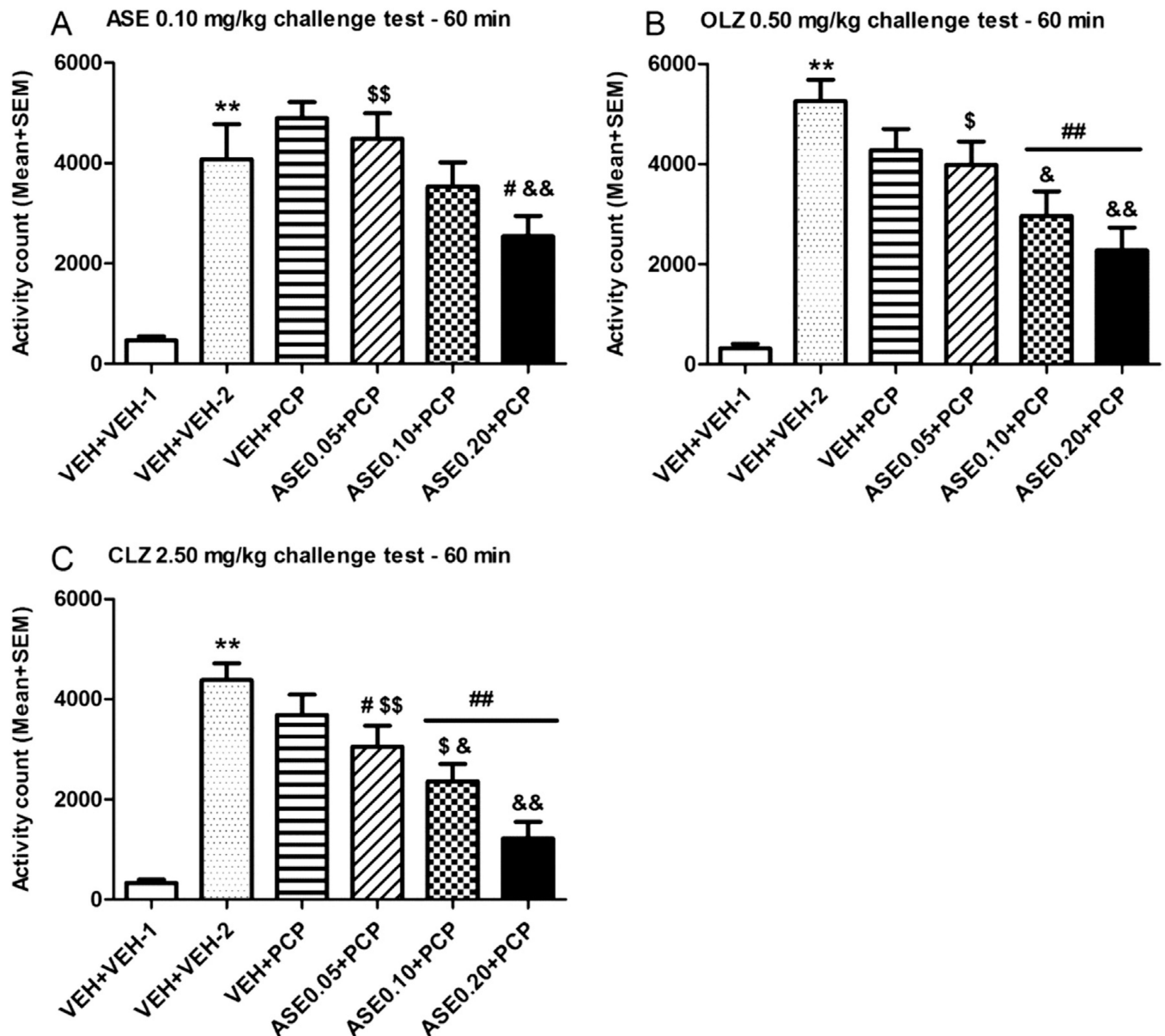
**Fig. 4. Prior asenapine treatment increased sensitivity to asenapine re-exposure and olanzapine exposure in the avoidance response**

Number of avoidance responses in the asenapine (0.10 mg/kg) challenge test (A), olanzapine (0.50 mg/kg) challenge test (B) and clozapine (2.50 mg/kg) challenge test (C) is expressed as mean + SEM. \* $p < 0.05$ , \*\* $p < 0.01$  relative to the VEH group; # $p < 0.05$  relative to the ASE 0.05 group.



**Fig. 5. Repeated asenapine treatment increased inhibition of PCP-induced hyperlocomotion throughout the 5 drug test days**

(A) Locomotor activity was measured for 60 min after vehicle or PCP injection and expressed as mean + SEM for each group. Asenapine (0.05, 0.10 and 0.20 mg/kg) was injected 30 min before the vehicle or PCP injection. \* $p < 0.05$  relative to the VEH + VEH group, \*\* $p < 0.001$  ASE 0.20 + PCP group (day 1) and VEH + PCP, ASE 0.05 + PCP and ASE 0.10 + PCP group (all 5 days) relative to the VEH + VEH group; ### $p < 0.01$  relative to the VEH + PCP group; & $p < 0.05$ , && $p < 0.01$  the 3 ASE group relative to each other. (B, C, D, E, and F) Locomotor activity in 12 5-min blocks of individual group on day 1 and day 5. \$ $p < 0.05$ , \$\$ $p < 0.001$  day 5 relative to day 1.



**Fig. 6. Prior asenapine treatment increased the inhibition of PCP-induced hyperlocomotion upon asenapine re-exposure and on olanzapine and clozapine treatment**

(A) Locomotor activity was measured for 60 min after vehicle or PCP (3.20 mg/kg, sc) injection and expressed as mean + SEM for each group. Asenapine (0.10 mg/kg, sc) was injected 30 min before the vehicle or PCP injection. (B) Locomotor activity was measured for 60 min after vehicle or PCP injection and expressed as mean + SEM for each group. Olanzapine (0.50 mg/kg, sc) was injected 30 min before the vehicle or PCP injection. (C) Locomotor activity was measured for 60 min after vehicle or PCP injection and expressed as mean + SEM for each group. Clozapine (2.50 mg/kg, sc) was injected 30 min before the vehicle or PCP injection. ( $n = 8/\text{group}$ ). \*\* $p < 0.001$  relative to VEH + VEH-1 group; # $p < 0.05$ , ## $p < 0.001$  relative to VEH + VEH-2; & $p < 0.05$ , && $p < 0.004$  relative to VEH + PCP; \$ $p < 0.05$ , \$\$ $p < 0.009$  relative to ASE 0.20 + PCP group.