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Time-Dependence of Risperidone and Asenapine Sensitization and Associated D₂ receptor Mechanism

Jun Gao and Ming Li*

Department of Psychology, University of Nebraska-Lincoln, USA

Abstract

When an antipsychotic drug is given repeatedly and intermittently, there is often a long-term increase in its behavioral efficacy, termed antipsychotic sensitization. With the passage of time, the magnitude of antipsychotic sensitization may increase or decrease depending on the principle of Time-Dependent Sensitization (TDS) or memory decay, respectively. In the present study, we examined the time-dependent feature and possible dopamine D₂ receptor mechanism of sensitization induced by the antipsychotics risperidone and asenapine in the conditioned avoidance response test. Well-trained male adult Sprague-Dawley rats were first repeatedly treated with risperidone (1.0 mg/kg) or asenapine (0.2 mg/kg) and tested for avoidance response daily for 5 consecutive days. Eight, 18 or 38 days after the 5th drug treatment, all rats were retested drug-free to assess the residual impact of prior risperidone or asenapine treatment. Drug-pretreated rats had significantly lower avoidance than vehicle-pretreated ones on this test, and the group differences increased with the passage of time. In the subsequent drug challenge test at 10, 20 or 40 days after the 5th drug treatment, all rats were injected with a low dose of risperidone (0.3 mg/kg) or asenapine (0.1 mg/kg). Drug-pretreated rats again made significantly less avoidances than controls, confirming the drug-induced sensitization effect. Finally, in the quinpirole (a D_{2/3} receptor agonist, 1.0 mg/kg, sc)-induced hyperlocomotion test, risperidone-pretreated rats exhibited a significantly higher level of motor activity than the vehicle-pretreated ones. These findings suggest that risperidone and asenapine sensitization is long-lasting, follows the TDS principle, and is likely mediated by D₂ receptor supersensitivity.

Keywords

Time-Dependent Sensitization (TDS); Asenapine; Risperidone; Conditioned avoidance response; Quinpirole; Locomotor activity

1. Introduction

It is well documented that repeated and intermittent administrations of most antipsychotic drugs often cause an increase in the behavioral responsiveness to these drugs. This phenomenon is termed antipsychotic sensitization. Supersensitivity psychosis, tardive dyskinesia, and time-dependent increase in antipsychotic response are several well-known

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*Corresponding author: 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..

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clinical examples of antipsychotic sensitization [1-3]. In preclinical studies, antipsychotic sensitization has been demonstrated in several different behavioral models [4-7]. It is often measured using two different methods[8] similar to those for assessing psychostimulant-induced behavioral sensitization[9]. The first index of antipsychotic sensitization is revealed through a within-subjects comparison, in which the behavioral effect of a drug treatment is stronger on the last treatment day than the first day (*i.e.*, a comparison between days 1 and 5). The second index of antipsychotic sensitization is provided by a between-subjects comparison, in which the behavioral response of drug-pretreated animals to a challenge dose of an antipsychotic drug is compared to the response of vehicle-pretreated control animals. Here, antipsychotic sensitization is demonstrated by an increased sensitivity to the drug challenge in drug-pretreated animals relative to those pretreated with vehicle.

The conditioned avoidance response (CAR) model is an aversion motivated instrumental conditioning model, which is traditionally used in the preclinical study of antipsychotic drugs (APDs) [10, 11]. In this model, animals can be trained to prevent the occurrence of an aversive stimulation (e.g. electric footshock) by performing a specific response to a conditioned stimulus (e.g. tone). This response is thought to reflect a persecutory delusion [12]. The treatment of antipsychotic drugs selectively disrupts avoidance responding without altering unconditioned escape response [13, 14], and thus this test has high predictive validity for antipsychotic efficacy [15]. This feature has been effectively used to identify potential antipsychotic drugs, to differentiate antipsychotic drugs from other classes of psychotropic drugs, and to predict the clinical potency of antipsychotic drugs [11, 14, 16-18]. Our work focuses on behavioral characteristics and neurobiological mechanisms of antipsychotic sensitization in the conditioned avoidance response (CAR) and phencyclidine (PCP)-induced hyperlocomotion models, two animal behavioral tests sensitive to antipsychotic activity [8, 19-23]. We have shown that repeated administration of haloperidol, olanzapine, asenapine or risperidone daily for 5-7 days tends to cause a progressively increased inhibition of avoidance responding and PCP-induced hyperlocomotion over days (a within-subjects sign of sensitization). A few days later, when all rats are given a challenge dose of these drugs, 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 (a between-subjects sign of sensitization). In addition, our previous studies also indicate that repeated administration of haloperidol and olanzapine causes a sensitization effect that can last up to 17 days [8], and are likely mediated by dopamine D₂ and 5-HT_{2A} receptor-related neural plasticity [24]. Recently, we further show that olanzapine sensitization can be induced in adolescent rats and this effect can last up to 45 days and persist into adulthood [21].

Antipsychotic sensitization likely reflects a composite impact from two sources. One is the relatively specific pharmacological actions of a given antipsychotic drug. As mentioned before, this is likely mediated by a drug's actions on its immediate neuroreceptor targets (e.g. D₂ and 5-HT_{2A} receptors) [24] and should follow the basic principles of learning and memory, as antipsychotic sensitization represents a non-associative form of learning and memory. Under this principle, the magnitude of sensitization should decrease with the passage of time due to a memory trace decay process (similar to forgetting). Another source is the ubiquitous adaptive response to the foreign aspect of the drug (any drug is an exogenous agent to an organism), which tends to follow the "Time-dependent Sensitization (TDS)" principle. This principle, first articulated by Antelman and his colleagues [25], suggests that a drug effect often grows (*i.e.* sensitized or strengthened) with the passage of time upon acute exposure to the drug. In Antelman's earlier work, he found that a single exposure to a clinically low dose of antipsychotic produced changes that were shown up to 8 weeks later [6]. In our previous study on the time course of antipsychotic sensitization [8], we assessed the magnitude of haloperidol and olanzapine sensitization in the conditioned

avoidance response test at 4, 10, or 17 days after the last drug treatment. We did not find that haloperidol or olanzapine sensitization changed its magnitude over time, but maintained at a high level throughout the post-injection period. One limitation of the study was shorter time courses tested. It is possible that with a prolonged period of testing, the time-dependent feature of antipsychotic sensitization could be revealed.

The present study addressed this limitation by examining the potential time-dependent feature of antipsychotic sensitization induced by risperidone and asenapine, which represent the earliest and latest atypical antipsychotic drugs in a broader time frame. Both drugs are medically approved for the treatment of schizophrenia and bipolar disorders, with a multiple receptor binding profile targeting dopamine D₂ and D₁ receptors, serotonin receptors (5-HT_{2A}, 5-HT_{2C}, 5-HT₇), adrenergic α_1 and α_2 receptors, histamine H₁ and H₂ receptors, etc. [26, 27]. Both drugs have shown to give rise to a sensitization effect in animal tests of antipsychotic activity, including conditioned avoidance test and PCP-induced hyperlocomotion. We also investigated the involvement of dopamine D₂ receptor as a biological mechanism underlying antipsychotic sensitization by comparing the quinpirole-induced increase in motor activity in drug-pretreated animals to vehicle-pretreated animals. Because quinpirole is a selective D_{2/3} receptor agonist, a higher level of motor activity under quinpirole challenge presumably reflects an upregulation of D₂ receptor function [28, 29]. Quinpirole-induced hyperlocomotion is also thought to be mediated through an increase in the efficacy of the post-synaptic D_{2/3} transductional mechanisms [30, 31], and has been widely used to assess drug or non-drug induced changes in D_{2/3} functions [32, 33].

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (226-250g 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 6:30am and 6:30pm). 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. All experimental treatment and procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

2.2. Drugs and choice of doses

Risperidone (RIS) (a gift from the NIMH drug supply program) was dissolved in distilled sterile water with 1.0% glacial acetic acid. Asenapine Maleate (ASE) (a gift from the NIMH drug supply program) and quinpirole hydrochloride (Tocris Bioscience, Bristol, UK) were dissolved in 0.9% saline. All drugs were administered subcutaneously in a volume of 1.0 ml/kg body weight. Doses of RIS (1.0 and 0.3 mg/kg, for drug test and challenge respectively) and ASE (0.2 and 0.1 mg/kg, for drug test and challenge respectively) were chosen on the basis of literature review showing that these doses produce a reliable disruption of avoidance responding and cause a sensitization effect [23, 34-39]. The chosen quinpirole dose (1.0 mg/kg) targets post-synaptic D₂ receptors and causes an increase in motor activity [29, 40-42].

2.3. Two-way avoidance conditioning apparatus

Ten 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 (as an unconditioned stimulus, US; 0.8mA, 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 conditioned stimulus (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

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.

2.5. Experiment 1: Effect of time interval between initial drug exposure and subsequent drug challenge on the strength of risperidone sensitization

This experiment examined the time course of the risperidone sensitization effect in the CAR model. The experiment was comprised of the following three phases: Avoidance training, five days of repeated risperidone testing and risperidone challenge test. Figure 1 depicts the experimental procedure and groups at different phases of this experiment.

Avoidance training—Sixty-four rats were first habituated to the CAR boxes for 2 days (20 min/day). They were then trained for conditioned avoidance responding for 10 sessions (1 session/day). Each session consisted of 30 trials. Every trial started by presenting a white noise (CS) for 10 s, followed by a continuous scrambled foot shock (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 (before the shock). 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 60s.

Five days of repeated risperidone testing—At the end of the training session, 51 rats acquired a robust avoidance responding (> 70% avoidance in each of the last 2 sessions). They were matched on the level of avoidance and then randomly assigned into 6 groups (n = 8-9/group): 2 ten-day groups (VEH D10 and RIS D10), 2 twenty-day groups (VEH D20 and RIS D20) and 2 forty-day groups (VEH D40 and RIS D40). They were then tested daily under the CS-only (no shock, 30 trials/session) condition for 5 consecutive days, following the same procedure as employed before [8, 19, 22, 43]. On each test day, rats were first injected with vehicle (VEH; sterile water with 1.0% glacial acetic acid) or RIS (1.0 mg/kg) sc and tested in the CAR boxes 1 h later.

Risperidone challenge test—After the last (5th) drug test session, rats were undisturbed in their home cages for either 7 (for the VEH D10 and RIS D10 groups), 17 (for the VEH D20 and RIS D20 groups) or 37 (for the VEH D40 and RIS D40 groups) days. They were then given 2 drug-free avoidance retraining sessions (one CS-only session and one CS-US session) to bring their avoidance responses back to the pre-drug level before the challenge test. These retraining sessions also served as a test for the residual impact of prior RIS treatment on avoidance responding. One day later, rats in each group pair (e.g. VEH D10 and RIS D10) were injected with RIS at 0.3 mg/kg and tested for avoidance performance in the CS-only condition (30 trials) 1 h later.

2.6. Experiment 2: Effect of prior risperidone treatment on quinpirole-induced hyperlocomotion

Forty-eight rats randomly selected from 51 used in Experiment 1 were used in this experiment. They included 8 rats from each of the 6 groups: RIS D10, VEH D10, RIS D20, VEH D20, RIS D40, and VEH D40 ($n = 8/\text{group}$). As shown in Figure 1, 48 days after the last (5th) RIS test, all rats were first habituated to the locomotor activity apparatus for 2 days (30 min/day), on the second day, all rats were injected with saline before putting into the test chambers. One day later, they were injected with quinpirole (1.0 mg/kg, sc) and immediately placed in the test apparatus for 120 min. Locomotor activity (number of photobeam breaks) was measured in 10-min blocks throughout the entire 120-min test period.

2.7. Experiment 3: Effect of time interval between initial drug exposure and subsequent drug challenge on the strength of asenapine sensitization

This experiment examined the time course of asenapine sensitization using an identical procedure as that in Experiment 1. Fifty-three rats that had met the training criterion (out of an initial 60 rats that were trained) were matched and assigned into 6 groups ($n = 8-9/\text{group}$): VEH D10, ASE D10, VEH D20, ASE D20, VEH D40, and ASE D40. All rats were first repeatedly tested for avoidance performance 30 min after VEH (saline) or ASE (0.2 mg/kg) injection for 5 consecutive days. After 7, 17 or 37 days of home cage rest, the rats were given 2 retraining sessions and 1 challenge test in 3 consecutive days. On the challenge day, the rats were injected with ASE (0.1 mg/kg) and tested for avoidance response in a 30-trial CS-only session 30 min later. One additional ASE challenge test was conducted in all rats at 50 days after the last (5th) ASE treatment to evaluate possible group differences under the same drug challenge condition. This test was conducted following 2 drug-free avoidance retraining sessions (see Figure 1 for the experimental procedure and groups in details).

2.8. Statistical Analysis

Data (mean + SEM) from the 5 drug test sessions (e.g. avoidance response) were analyzed using a split-plot repeated measures analysis of variance (ANOVA) with the between-subjects factor being drug group and the within-subjects factor being drug-test session. Differences between groups on the specific drug test days were analyzed using one-way ANOVAs followed by post hoc Tukey's HSD tests. Repeated measures ANOVA was also used for analyzing locomotor activity test, with drug group and time-block as between- or within-subject factor respectively. Data from the challenge tests were analyzed using a two-way ANOVA, with treatment (RIS and VEH) and interval (10-, 20-, and 40-day) as two between-subjects factors, followed by post hoc Tukey's HSD tests. Significance was predetermined at $p < 0.05$. All data was analyzed using SPSS Version 21.

3. Results

3.1. Experiment 1: Effect of time interval between initial drug exposure and subsequent drug challenge on the strength of risperidone sensitization

Avoidance response during the repeated RIS test period—No significant group differences were found on the predrug day [$F(5,45) = 0.549, p = 0.738$]. Throughout the 5 days of RIS testing, avoidance response was suppressed by repeated RIS treatment. Repeated measures ANOVA revealed a significant main effect of *group* [$F(5,45) = 391.892, p = 0.000$], *session* [$F(4,180) = 7.778, p = 0.000$], but no significant interaction between the two [$F(20,180) = 0.855, p = 0.644$] (Figure 2a).

Avoidance response on the retraining sessions—On the 1st retraining day (30 trials of CS-only session), RIS-pretreated rats in the 20-day and 40-day groups, but not 10-day group, had lower avoidances than their respective control groups. Independent samples *t* tests showed that the RIS D20 differed significantly from VEH D20 [$t(15) = 2.221, p = 0.042$], and RIS D40 from VEH D40 [$t(15) = 3.084, p = 0.008$] (Figure 2b). The difference between the 2 10-day groups was not significant [$t(15) = 0.473, p = 0.643$]. To further illustrate this point, we calculated the group percentage of avoidance response by dividing the group mean number of avoidance in the RIS-pretreated group by the mean number of avoidance in its corresponding vehicle group (Figure 2c). Using this calculation, a lower percentage of avoidance response indicates a stronger persistent impact of prior RIS treatment. As Figure 2c shows, the group mean avoidance percentages were lower at 40 and 20 days than at 10 days, suggesting that the residual impact of prior RIS treatment increased with the passage of time. No significant group difference was found on the 2nd retraining day ($ps > 0.083$).

Avoidance response on the challenge test—On the 3 challenge tests at the 10-, 20- and 40-day intervals, the 3 RIS groups all had lower avoidances than their VEH control groups under the same RIS challenge [10-day groups, $t(15) = 5.946, p = 0.002$; 20-day groups, $t(15) = 5.319, p = 0.000$; 40-day groups, $t(15) = 3.723, p = 0.002$; Figure 2d]. Two-way ANOVA revealed a main effect of *group* [$F(1, 45) = 72.392, p = 0.000$] and *interval* [$F(2,45) = 4.360, p = 0.019$], but no interaction between the two [$F(2,45) = 1.869, p = 0.166$]. Post hoc Tukey's HSD tests comparing the interval differences found a significantly lower avoidance at the 40-day interval than at the 10-day interval ($p = 0.006$). The RIS sensitization effect appeared to be increased with the passage of time, as the relative group avoidance percentage was lower in the RIS D40 group than other groups (Figure 2e).

3.2. Experiment 2: Effect of prior risperidone treatment on quinpirole-induced hyperlocomotion

To test whether the D_2 receptor system was involved in RIS sensitization, we tested quinpirole-induced locomotor activity in rats that were pretreated with RIS (3 RIS groups) or vehicle (3 VEH groups). This test was conducted at 50 days after the last (5th) RIS or vehicle treatment. As shown in Figure 3, quinpirole at 1.0 mg/kg increased motor activity during the 120-min test period, with an even higher motor activity found the RIS pretreated rats than the VEH pretreated ones. Repeated measures ANOVA revealed a significant main effect of *time-block* [$F(11,506) = 114.795, p = 0.000$], a significant *block* \times *group* interaction [$F(11,506) = 2.473, p = 0.005$], but no main effect of *group* [$F(1,46) = 3.798, p = 0.057$]. Independent samples *t* test at each block showed that the RIS group had significantly higher motor activity than the VEH group on the last three 10-min blocks [100 min, $t(46) = -2.279, p = 0.027$; 110 min, $t(46) = -2.176, p = 0.035$; 120 min, $t(46) = -2.135, p = 0.038$]. These data suggest that prior RIS treatment induced a long-lasting supersensitivity of $D_{2/3}$ receptors, which may serve as a mechanism underlying RIS sensitization.

3.3. Experiment 3: Effect of time interval between initial drug exposure and subsequent drug challenge on the strength of asenapine sensitization

Avoidance response during the repeated ASE test period—During the initial 5 days drug testing, repeated ASE treatment suppressed avoidance response. Repeated-measures ANOVA revealed a main effect of *group* [$F(5,47) = 546.703, p = 0.000$], *session* [$F(4,188) = 13.914, p = 0.000$] and a significant interaction between the two [$F(20,188) = 2.383, p = 0.001$]. Inspection of Figure 4a reveals that the suppressive effect of ASE increased across the 5 test sessions.

Avoidance response on the retraining sessions—On the 1st retraining day (30 trials of CS-only session), only ASE-pretreated rats in the 40-day group had lower avoidance than their vehicle controls [$t(16) = 3.613, p = 0.002$, Figure 4b]. The differences between the two 10-day [$t(15) = 1.964, p = 0.068$] and 20-day groups [$t(16) = 1.042, p = 0.313$] were not significant. The mean group avoidance percentage also showed that the residual impact of prior ASE treatment on avoidance response was greater at the 40-day interval than at other intervals (Figure 4c). No significant group difference was found on the 2nd retraining day ($ps > 0.095$).

Avoidance response on the challenge test—On the challenge days, all 3 ASE groups had lower avoidances than their respective VEH groups. Two-way ANOVA showed a main effect of *group* [Figure 4d, $F(1, 47) = 67.486, p = 0.000$] and a significant interaction between *group* and *interval* [$F(2,47) = 4.050, p = 0.024$], suggesting that the RIS sensitization effect varied across the 3 test intervals (Figure 4e). Independent samples t-test confirmed that the 3 ASE groups all had significantly lower avoidances than their VEH control groups [10-day groups, $t(15) = 4.681, p = 0.000$; 20-day groups, $t(16) = 3.660, p = 0.002$; 40-day groups, $t(16) = 5.669, p = 0.000$; Figure 4d].

Avoidance response on the final challenge test—To evaluate possible group differences under the same condition, all 6 groups of rats were challenged with ASE at 0.1 mg/kg on the 50th day after the last ASE treatment. Before the challenge, there was no group difference ($> 70\%$ avoidances, data not shown). On the challenge day, ASE-pretreated rats had lower avoidance than the vehicle-pretreated ones (Figure 5). There was a main effect of *group* [$F(1, 47) = 18.094, p = 0.000$], but no main effect of *interval* [$F(2,47) = 0.589, p = 0.559$], nor a *group* \times *interval* interaction [$F(2,47) = 0.538, p = 0.588$]. Independent samples t tests found a significant group difference between the ASE D10 and VEH D10 groups [$t(15) = 2.358, p = 0.032$] and between the ASE D40 and VEH D40 groups [$t(16) = 3.848, p = 0.001$], but not between the ASE D20 and VEH D20 [$t(16) = 1.806, p = 0.090$].

4. Discussion

Although antipsychotic sensitization effects have been demonstrated in the CAR model [8, 22, 24, 38] and other behavioral models [4, 5, 44-47], little research is devoted to documenting the temporal feature of its development and related behavioral and receptor mechanisms, especially those induced by newer atypical antipsychotic drugs (e.g. asenapine, aripiprazole). The present study investigated the time-dependent feature of antipsychotic sensitization in the CAR model and found: 1) both risperidone (1.0 mg/kg) and asenapine (0.2 mg/kg) induced a long-lasting sensitization effect, detectable even 40 or 50 days after the initial drug exposure; 2) the time-dependence of risperidone and asenapine sensitization manifested mainly in the drug-free retraining session, less obvious under the drug challenge test session due to the persistently lower avoidance responding in the 3 drug pre-treated groups at the 3 test intervals; 3) risperidone-pretreated rats showed enhanced motor activity under quinpirole challenge, suggesting that repeated risperidone treatment (and possibly

asenapine, too) may induce a relatively permanent $D_{2/3}$ receptor supersensitivity that supports the long-lasting sensitization. Because suppression of avoidance response is thought to be a behavioral measure of antipsychotic activity, the time-dependent feature of enhanced efficacy due to past drug history may have clinical implications regarding the therapeutic management of schizophrenia.

In many previous antipsychotic sensitization studies, the magnitude of sensitization is often assessed at one single time point, several days or weeks after the initial drug treatment. Thus, these studies were unable to provide much information regarding the changes in sensitization with the passage of time. As mentioned in the Introduction, two basic principles (TDS and forgetting) could govern the time-dependence of antipsychotic sensitization, which give rise to two different predictions. According to the forgetting principle, antipsychotic sensitization, as a basic form of non-associative learning and memory, will decrease when the interval between the initial drug exposure (the induction/learning phase) and the challenge test (the expression/memory phase) is prolonged. In contrast, sensitization will increase with the passage of time on the basis of the TDS principle due to the body's adaptive and compensatory responses to a foreign agent. The ultimate intensity of antipsychotic sensitization at any given time point likely reflects the consequence of a joint action from these two forces. In our previous CAR study of antipsychotic sensitization, we assessed haloperidol sensitization and olanzapine sensitization at 3 time intervals (i.e., 4, 10, or 17 days), but did not find much change across the 3 time points [8]. The present study extended the test time intervals up to 50 days after the last drug treatment. We not only replicated risperidone [23, 38] and asenapine sensitization [48], but also found their time-dependent feature. This feature was mainly observed in the drug-free CS-only retraining/retesting sessions and was less obvious under the drug challenge test sessions, consistent with our previous findings [8]. The risperidone and asenapine sensitization effects, indexed by a suppression of avoidances, on the drug-free retraining days increased with the passage of time. The D40 drug groups had significantly fewer avoidance responses than the D40 vehicle groups on the 1st retraining day, whereas the D10 groups did not differ from its vehicle controls (Fig. 2b and 4b). This increase in antipsychotic sensitization was also observed in the group avoidance percentage measure (Fig. 2c and 4c). Thus, it appears that the current test conditions were in favor of the manifestation of TDS as opposed to forgetting. We do not know why we failed to demonstrate the time-dependence of risperidone and asenapine sensitization in the drug challenge tests. One possibility is that the initial tested dose and challenge dose of risperidone and asenapine were still too high, causing a floor effect (i.e. a high dose causes a large disruption of avoidance responding, leaving no room to show the residual impact of prior risperidone or asenapine treatment), especially in the drug pretreated groups. As can be seen in Figures 2a and 4a, risperidone and asenapine at the chosen doses suppressed avoidance responding to the near bottom level even on the 1st test day. Similarly, on the challenge tests (Figure 2d and 4d), the drug pretreated groups at the 3 test intervals all displayed rather low levels of avoidance responding, causing difficulties in detecting their potential differences. One important future study is to further manipulate experimental conditions, including the utilization of different challenge doses, to delineate the exact experimental conditions that would allow TDS to mediate time-dependent alterations of antipsychotic sensitization.

The phenomenon of antipsychotic sensitization supports the notion that once an organism is exposed to an antipsychotic drug, this drug experience stays with it for a long period of time and may undergo a time-dependent change. Behaviorally, an antipsychotic drug may act as an occasion-setter [49], which sets the condition in which rats behave on the basis of their previous drug experience in the avoidance testing context. Neurobiologically, the antipsychotic drug may imprint the brain to create a drug “memory trace” of avoidance

responding under drug treatment by altering the functions of the neuroreceptors it targets. Risperidone has a relatively high affinity for dopamine D₂, serotonin 5-HT_{2A}, 5-HT₇, and adrenergic α_1 receptors [27] and asenapine has a high affinity for 5-HT_{2A}, 5-HT_{2C}, 5-HT₆, 5-HT₇, adrenergic α_1 and α_2 , and histamine H₁ and H₂ receptors, and a relatively low action on D₂ and D₁ receptors [26]. It is conceivable that 5 days of repeated risperidone and asenapine treatment altered the functions of these receptors. Indeed, substantial evidence suggests that chronic antipsychotic treatment does modify these neurochemical systems and induces various forms of neuroplasticity [50-53]. For example, it has been shown that the density and sensitivity of dopamine D₂ receptors is upregulated after chronic antipsychotic treatment, including risperidone and asenapine in both animals [54, 55] and humans [56]. In the present study, using a behavioral assay of D₂-mediated neurotransmission, we also obtained evidence of a functional upregulation of D₂ receptors caused by prior risperidone exposure, as risperidone-pretreated rats had significantly higher motor activity than the corresponding vehicle rats under quinpirole challenge. We hypothesized that D₂ receptor supersensitivity is one critical mechanism underlying antipsychotic sensitization (including, olanzapine, risperidone and asenapine sensitization), and this conjecture is strengthened by the following three observations. First, all antipsychotic drugs antagonize D₂ receptors and their chronic use causes a long-lasting change in this receptor system [57]. Second, olanzapine sensitization in the CAR model also relies on D₂ receptors. Li et al. (2010) showed that pretreatment of quinpirole during the induction phase attenuated the expression of olanzapine sensitization in the challenge test. Third, there exists a cross-sensitization between risperidone and olanzapine [38] as well as asenapine and olanzapine [48], as rats previously treated with risperidone or asenapine show enhanced responsiveness to olanzapine in the suppression of avoidance responding. This hypothesis may warrant further investigation. Because risperidone and asenapine also have relatively high 5-HT_{2A} receptor occupancies [58], and chronic risperidone and asenapine treatments decrease 5-HT_{2A} receptor binding in the medial prefrontal cortex [55, 59, 60], 5HT_{2A}'s involvement in risperidone and asenapine sensitization also needs to be examined. Other receptors such as 5-HT_{1A}, D₄, NMDA, and adrenergic α_1 and α_2 receptors could also play a role in antipsychotic sensitization [26, 61-63].

Antelman et al. (2000) has argued that TDS is a useful principle for the explanation of clinical improvement which grows with the passage of time [64]. One direct implication is that "instead of managing disorders such as depression by multiple daily drug treatments, it may be possible to accomplish the same ends by treating once every few weeks." (page 354). In the case of clinical treatment of schizophrenia, psychotic symptoms do improve exponentially with the passage of time and with the increase of treatment duration [3, 65], but the relative contributions from each factor (i.e. time vs. treatment duration) on symptom improvement has not been investigated. Currently, the most common practice in the clinic is to treat schizophrenic patients with antipsychotic drugs daily to achieve approximately 60% to 80% of dopamine D₂ receptor occupancy [66]. If we do not need to maintain a daily treatment schedule, it would avoid many side effects, including extrapyramidal symptoms (EPS) and excess weight gain. Recent studies showing that dosing every 2-3 days is sufficient to maintain antipsychotic efficacy in schizophrenic patients is in support of this practice and TDS principle [67, 68]. This finding suggests that upon initial exposure, physiological events initiated by a drug enhance the antipsychotic's effects beyond its presence at the receptor, thereby inducing efficacy without requiring constant receptor binding. This idea is strongly supported by our current findings that risperidone and asenapine sensitization persisted and even increased to some degree with the passage of time. Previous studies in the literature generally suggest that continuous antipsychotic treatment is more effective than interment antipsychotic regimen in preventing relapse in people with schizophrenia [67, 68]. One problem with the previous intermittent approach is that it allowed for lengthy "time off" and re-administration based on the re-appearance of

symptoms. The current effective approach differs from the previous ones in which it requires an intermittent but regular dosing regardless of symptom status [68]. Our findings provide a preclinical support for this approach. Of course more preclinical and clinical research is needed to address the potential benefits or harms of these two different drug treatment regimens.

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1. Repeated risperidone and asenapine treatment enhanced acute effect of these drugs in the conditioned avoidance model;
2. Risperidone and asenapine sensitization lasted up to 40 days;
3. Risperidone and asenapine sensitization increased with the passage of time;
4. Repeated risperidone treatment caused a functional upregulation of dopamine D_{2/3} receptors.

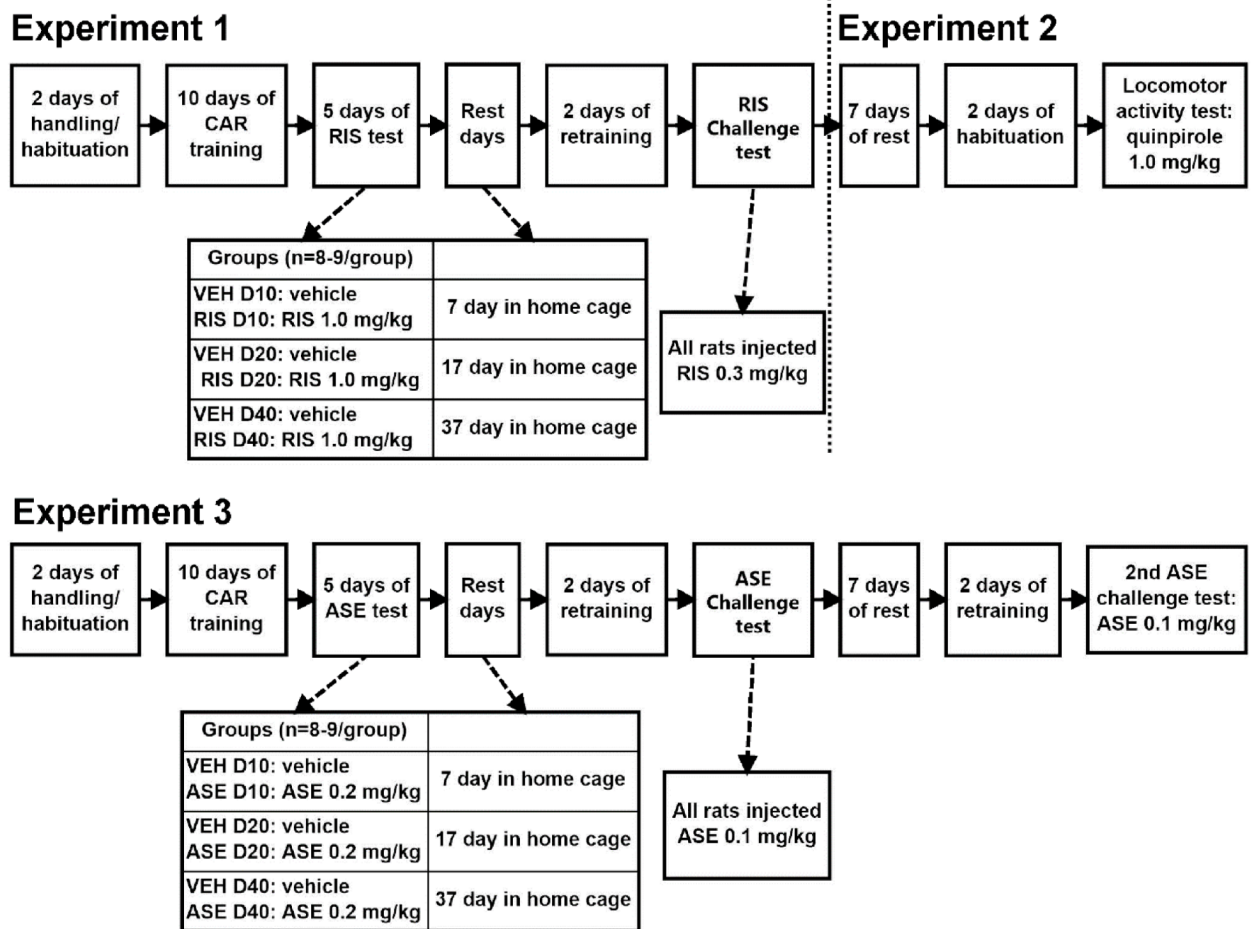


Figure 1.

A schematic depiction of the experimental procedures and groups in Experiment 1, 2 and 3. CAR: conditioned avoidance response; VEH: vehicle; RIS: risperidone; ASE: asenapine.

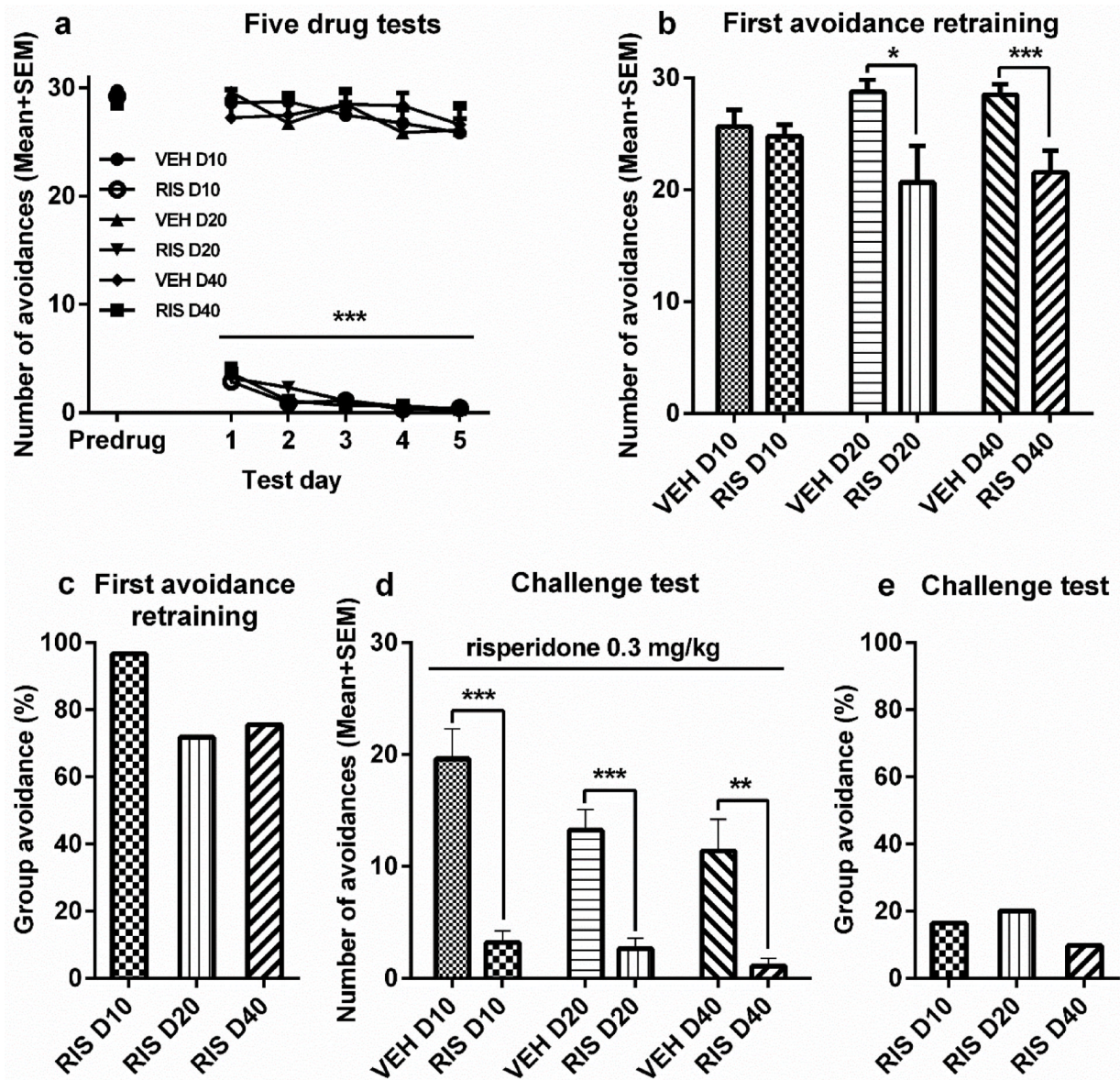


Figure 2.

The induction of risperidone (RIS) sensitization and its expression assessed at 3 time intervals. Number of avoidance responses made by the rats on the final training (predrug) day, five drug test days (a), and the first drug-free retraining day (b). Rats were injected with RIS (1.0 mg/kg, sc, -60 min) or vehicle (VEH) and tested for avoidance responses for 5 daily sessions. D10, D20, or D40 groups (n = 8-9/group) received either 7, 17, or 37 days of resting, and were then retrained in the conditioned avoidance response boxes for two sessions. (c) The mean group avoidance percentage calculated by dividing the mean group number of avoidance in the RIS group by that in its corresponding VEH group. (d, e) RIS sensitization tested at 10-, 20- and 40-day intervals. All groups were injected with RIS (0.3 mg/kg, sc) and avoidance responses were measured 60 min later. All data are expressed as mean + SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ for comparison to the respective VEH group.

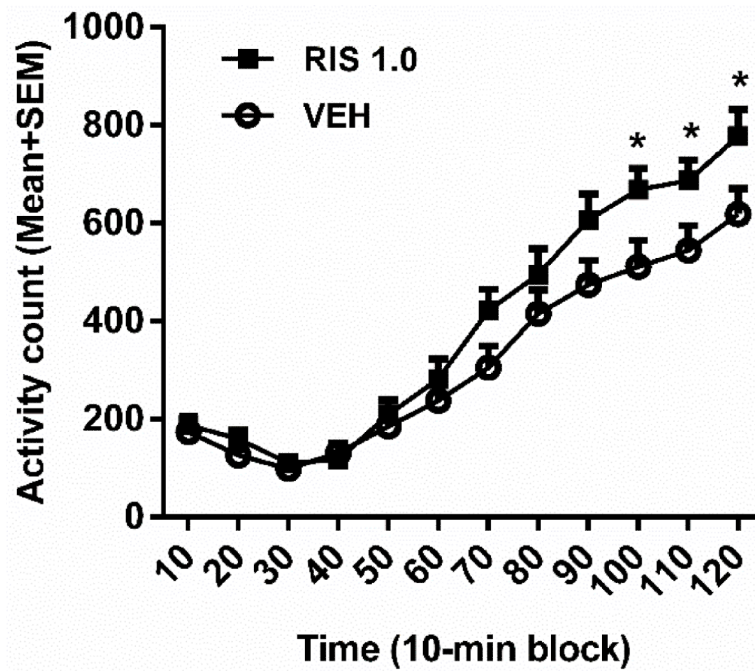


Figure 3. Quinpirole-induced locomotor activity in rats that were pretreated with risperidone (RIS, 1.0 mg/kg) for 5 days. The test was conducted 50 days after the last RIS (1.0 mg/kg) treatment. All rats were injected with quinpirole (1.0 mg/kg, sc) and then measured for motor activity for 120 min. All data are expressed as mean + SEM in 12 10-min blocks. * $p < 0.05$ for comparison to the respective VEH group.

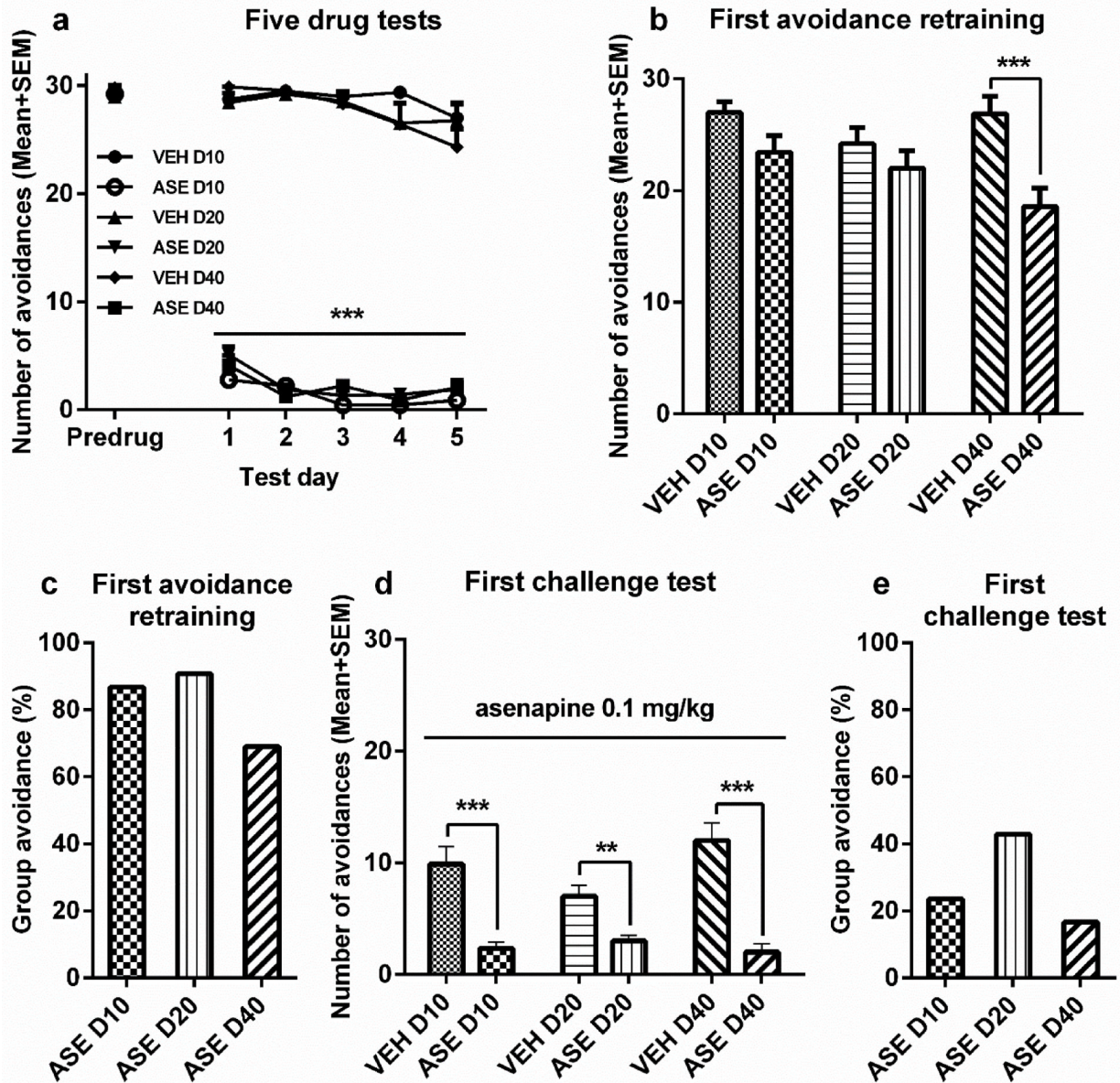


Figure 4.

The induction of Asenapine (ASE) sensitization and its expression assessed at 3 time intervals. Number of avoidance responses made by the rats on the final training (predrug) day, five drug test days (a), and the first drug-free retraining day (b). Rats were injected ASE (0.2 mg/kg, sc, -30 min) or vehicle (VEH) and tested for avoidance responses for 5 daily sessions. D10, D20, or D40 groups (n = 8-9/group) received either 7, 17, or 37 days of resting, and were then retrained in the conditioned avoidance response boxes for two sessions. (c) The mean group avoidance percentage calculated by dividing the mean group number of avoidance in the ASE group by that in its corresponding VEH group. (d, e) ASE sensitization tested at 10-, 20- and 40-day intervals. All groups were injected with ASE (0.1 mg/kg, sc) and avoidance responses were measured 30 min later. All data are expressed as mean + SEM. *** $p < 0.001$, ** $p < 0.01$ for comparison to the respective VEH group.

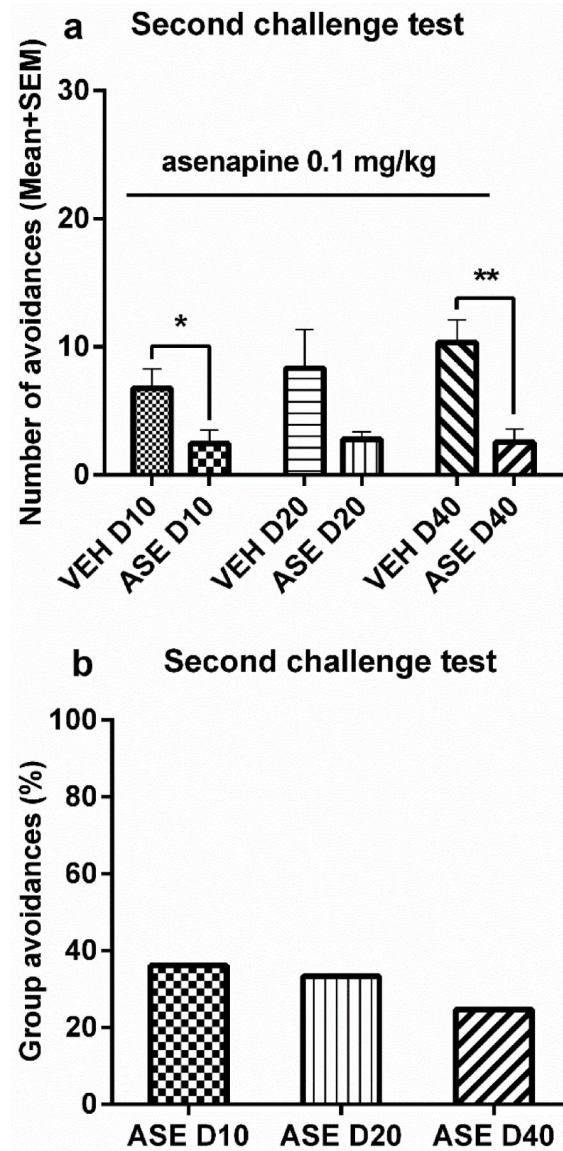


Figure 5.

The expression of Asenapine (ASE) sensitization assessed after a 50-day interval. Number of avoidance responses (a) and the mean group avoidance percentage (b) made by the rats on the challenge day. After retraining in the conditioned avoidance response boxes for two sessions, all groups were injected with ASE (0.1 mg/kg, sc) and avoidance responses were measured 30 min later on 50th day after the last drug test (ASE 0.2 mg/kg). All data are expressed as mean + SEM. ** $p < 0.01$, * $p < 0.05$ for comparison to the respective VEH group.