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Parametric studies of antipsychotic-induced sensitization in the conditioned avoidance response model: roles of number of drug exposure, drug dose, and test–retest interval

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Abstract

Repeated haloperidol and olanzapine treatment produces an enhanced disruption of avoidance responding, a validated measure of antipsychotic activity. Experimental parameters affecting this sensitization-like effect have not been thoroughly examined. The present study investigated the role of three parameters (number of injections, dose, and interval between initial exposure and challenge) in antipsychotic sensitization in the conditioned avoidance response paradigm. Well-trained Sprague–Dawley rats received different numbers of drug treatment (1–5 days) or different doses of haloperidol (0.025–0.10 mg/kg, subcutaneously) or olanzapine (0.5–2.0 mg/kg, subcutaneously). After certain time intervals (4, 10 or 17 days), they were tested for the expression of haloperidol or olanzapine sensitization in a challenge test in which all rats were injected with a lower dose of haloperidol (0.025 mg/kg) or olanzapine (0.5 mg/kg). Throughout the drug-treatment period, both haloperidol and olanzapine dose-dependently enhanced their disruption of avoidance responding. Three days later, the sensitization induced by a low dose of haloperidol (0.025 mg/kg) or olanzapine (0.5 mg/kg) was only apparent in rats that received treatment for 5 days, but not in those that received treatment for 1–4 days. The sensitization induced by the medium and high doses of haloperidol (0.05 and 0.10 mg/kg) or olanzapine (1.0 and 2.0 mg/kg) was still robust even with only 3 days of treatment. The sensitization induced by a 3-day haloperidol (0.10 mg/kg) and olanzapine (2.0 mg/kg) treatment was long-lasting, still detectable 17 days after the last drug treatment. This study suggests that antipsychotic sensitization is a robust behavioral phenomenon. Its induction and expression are strongly influenced by parameters such as number of drug exposures, drug dose, and test–retest interval. Given the importance of antipsychotic sensitization in the maintenance of antipsychotic effects in the clinic, this study introduces a paradigm that can be used to investigate the behavioral and neurobiological mechanisms underlying antipsychotic sensitization.

Keywords

conditioned avoidance response; haloperidol; olanzapine; parameters; rat; sensitization

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Conflicts of interest

There are no conflicts of interest.

Introduction

Sensitization is a phenomenon often associated with repeated exposure to many psychoactive drugs, such as cocaine, amphetamine, ethanol, nicotine, phencyclidine, and morphine (Robinson and Becker, 1986; Kalivas and Stewart, 1991; Stewart *et al.*, 1993; Vanderschuren and Kalivas, 2000; Siuciak *et al.*, 2006; Richtand *et al.*, 2007; Vezina, 2007). Sensitization is commonly described as an increase in the behavioral response to a subsequent administration of a drug following a previous exposure to that particular drug (Meririnne *et al.*, 2001). Antipsychotic drugs, as medications for the treatment of schizophrenia, can also induce various clinically-relevant sensitization effects, therapeutic as well as side-effects (Emmett-Oglesby and Goudie, 1989), as a result of the brain's adaptive responses to long-term antipsychotic drug treatment (Konradi and Heckers, 2001; Schmitt *et al.*, 2004). Supersensitivity psychosis, tardive dyskinesia, and time-dependent sensitization induced by antipsychotic treatment are some better-known clinical examples (Antelman *et al.*, 2000; Fallon and Dursun, 2011).

Although extensive researches have been carried out on sensitization induced by psychotomimetic drugs, the behavioral and neurobiological mechanisms involved in antipsychotic-induced sensitization are less understood. This lack of research on the properties of antipsychotic sensitization is unexpected, given the fact that antipsychotics, such as drugs of abuse, are often taken repeatedly for a prolonged period of time, and antipsychotic sensitization is believed to be an important mechanism supporting the maintenance of antipsychotic efficacy (Remington and Kapur, 2010).

In recent years, we have used the conditioned avoidance response (CAR) model, a well-established animal model with high predictive validity for antipsychotic action (Wadenberg and Hicks, 1999), to investigate the long-term effects of repeated antipsychotic treatment (Li *et al.*, 2004b, 2007, 2009a, 2009b, 2010; Mead and Li, 2010). We showed that repeated treatment with haloperidol (HAL; a typical antipsychotic), olanzapine (OLZ), and risperidone (atypical antipsychotics) produces a progressively-enhanced disruption of avoidance responding over 7 days (Li *et al.*, 2007). This sensitization effect can also be demonstrated in a subsequent challenge test (i.e. re-exposure to the drug) in which antipsychotic-treated animals exhibit a stronger response (i.e. lower avoidance) to the drug compared with the drug-naïve animals (Mead and Li, 2010; Zhang and Li, 2012). It is also long-lasting, producing an effect that can be seen up to 3 weeks later (Mead and Li, 2010) and is subject to contextual and behavioral controls (Zhang and Li, 2012).

Despite the well-documented antipsychotic sensitization in the CAR model, the parametric conditions in which sensitization occurs have not been clearly delineated. Several factors, including the schedule of administration, drug doses, and the interval between the initial injection and subsequent tests are known to have an impact on the magnitude of psychomotor sensitization to drugs of abuse (Carey and DeVaugh-Geiss, 1984; Robinson, 1984; Robinson and Becker, 1986; Kline *et al.*, 1998; Antelman *et al.*, 2000; Vezina, 2004). How these parameters affect the strength of antipsychotic sensitization has not been systematically investigated. Previous studies indicate that the drug-treatment schedule does affect the long-term behavioral effects of a drug. For example, Samaha *et al.* (2007) reported

that the continuous administration of HAL through minipumps induces behavioral tolerance in the CAR model, whereas our laboratory found that an intermittent administration of HAL through daily injections produces sensitization (Mead and Li, 2010). Our own work also suggests that the strength of OLZ-induced sensitization may depend on the number of prior injections. In one study (Li *et al.*, 2010), we found that rats that were treated with OLZ (1.0 mg/kg, subcutaneously) for 3 days showed a relatively less robust sensitization effect compared to those who were treated with the drug for 5–7 days in other studies (Li *et al.*, 2007, 2009a; Mead and Li, 2010).

This study represents the first attempt to comprehensively assess in a single study how different experimental parameters affect antipsychotic sensitization in the CAR model, by determining the number of exposures necessary to develop sensitization (experiments 1 and 2), the possible dose-dependent nature of this effect (experiments 3 and 4), and the length of sensitization after the final drug administration (experiments 5 and 6). We also examined the similarities and differences between typical (e.g. HAL) and atypical antipsychotics (e.g. OLZ), which have different neuroreceptor binding profiles (Miyamoto *et al.*, 2005), as potential parallels or discrepancies between these two drug types could lead to an insight into the mechanisms behind antipsychotic sensitization. On the basis of a review of the literature on psychomotor sensitization, we hypothesized that HAL and OLZ sensitization would be more prominent following a greater number of drug exposures, higher doses of the drugs, and a shorter time interval. With regard to the time-interval effect, it is also possible that antipsychotic sensitization would be stronger with a longer test–retest interval because of the influence of ‘time-dependent sensitization’, referring to the observation that a brief exposure to an antipsychotic drug induces a clinical effect that grows with the passage of time (Antelman *et al.*, 2000).

Methods

Subjects

Male Sprague–Dawley rats (226–250 g upon arrival; Charles River, Portage, Michigan, USA) were pair-housed in 48.3 × 26.7 × 20.3 cm transparent polycarbonate cages under 12-h light/dark conditions (lights off between 18:00 and 06:00 h). All tests were carried out during the light cycle. Room temperature was maintained at 22±1° with a relative humidity of 45–60%. Food and water were freely available. Animals were habituated to the animal facility for 5 days. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

Avoidance conditioning apparatus

Eight identical two-way shuttle boxes custom designed and manufactured by Med Associates (St Albans, Vermont, USA) were used as testing apparatuses. Each box was housed in a ventilated, sound-insulated isolation cubicle (96.5 cm width × 35.6 cm depth × 63.5 cm height). 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. 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 [unconditioned stimulus (US) 0.8 mA, maximum duration 5 s] was delivered by a constant-current shock generator (model ENV-410B; Med Associates Inc., St Albans, Vermont, USA) and scrambler (model ENV-412; Med Associates Inc.). The location of the rat, motor activity (photobeam breaks), and crossings between compartments were monitored constantly by a set of 16 photobeams (ENV-256-8P; Med Associates Inc.) at the bottom of the box (3.5 cm above the grid floor). Illumination was provided by two house lights fixed to the top of each compartment. The conditioned stimulus (CS, 76 dB white noise) was produced by a speaker (ENV-224 AMX; Med Associates Inc.) mounted on the ceiling of the cubicle, centered above the shuttle box. Background noise (~74 dB) was provided by a ventilation fan fixed at the top corner of each isolation cubicle. All training, habituation, and testing procedures were controlled by a personal computer with MED-PC interfacing software (Med Associates Inc.).

Experiment 1: Effect of number of injections on haloperidol sensitization—

Experiment 1 was designed to test the number of drug exposures necessary to induce robust sensitization (Fig. 1 for the experimental design). Sixty-four rats (divided into two batches) were used. First, all rats were handled for 2 min daily and habituated to the testing chamber for a total of 2 days (30 min/day, habituation program). After habituation, they were trained to acquire CAR daily for 10 days. Each training session consisted of 30 trials (intertrial intervals on a variable interval schedule between 30 and 60 s). Each trial began with a presentation of white noise (CS) for 10 s, followed by a continuous scrambled footshock (0.8mA, US, maximum duration 5 s) on the grid floor where the rat was located. If the rat crossed the compartments within the 10-s CS presentation, it would avoid the shock (recorded as an avoidance). If the rat crossed only after receiving the shock presentation, this was recorded as an escape. If the rat did not switch compartments during the presentation of the shock (5 s), shock was discontinued, and an escape failure was recorded.

At the end of the training session, 48 rats had reached the training criterion (at least 70% avoidance on day 10 of training) and were used in the subsequent drug tests. They were matched for performance level (number of avoidances on day 10) and then semirandomly assigned (randomly assigned based on the matched groups) into one of the six treatment groups ($n=8$ /group) based on the number of days of HAL exposure: vehicle (VEH) or HAL for 1–5 days (HAL1, HAL2, HAL3, HAL4, and HAL5). In the next 5 days, the rats were repeatedly tested in the CAR apparatus under a CS-only condition (presentation of tone not followed by shock, 30 trials/session). The CS-only condition was used as a way to prevent relearning and eliminating the potential confound of number of shocks received. On each day, the groups received an injection of either HAL (0.025 mg/kg) or VEH (sterile water) and were placed in the boxes and tested 1 h later. The HAL5 group received an injection of HAL on each of the 5 days, whereas the HAL1, HAL2, HAL3, and HAL4 groups received the corresponding number of drug exposures; for example, the HAL2 group was injected with VEH for the first 3 days of exposure and HAL on the fourth and fifth day.

One day after the last drug test, all rats were tested drug-free for one session under the CS-only condition and retrained for one session under the CS–US condition to bring their avoidance back to predrug level. The first CS-only session was used to examine any

potential carryover effects of the drug. A final drug-challenge test was conducted 24 h after the retraining session to assess sensitization. The test days were identical to the exposure days, with an injection of 0.025 mg/kg HAL 1 h before a CS-only 30-trial session.

Experiment 2: Effect of number of injections on olanzapine sensitization—

Experiment 2 was identical to experiment 1, except that HAL (0.025 mg/kg) was replaced by OLZ (0.5 mg/kg). Sixty-four rats were trained to the criterion level, of which 48 rats were used. Following the group-assigning procedure as described in experiment 1, they were assigned to six groups ($n=8/\text{group}$): VEH, and OLZ for 1–5 days (OLZ1, OLZ2, OLZ3, OLZ4, and OLZ5), and were subjected to five sessions of drug testing and two sessions of drug-free testing/retraining and a final drug-challenge test. During the challenge test, all rats were tested with OLZ (0.5 mg/kg, subcutaneously).

Experiment 3: Effect of haloperidol dose on the strength of antipsychotic sensitization effect—

Experiment 3 was conducted to determine whether the HAL sensitization effect was dose-dependent (Fig. 2). The procedure used in this experiment was similar to that of experiment 1. Forty rats were handled and habituated for 2 days and trained for 10 days in the CAR apparatus. Thirty-two rats reached the training level and were matched and assigned into four groups: VEH, low-dose HAL group (0.025 mg/kg), medium-dose HAL group (0.05 mg/kg), and a high-dose HAL group (0.1 mg/kg). We tested three doses of HAL, which covered subclinical, clinical, and superclinical doses in terms of dopamine D_2 receptor occupancy (50–80%; Kapur *et al.*, 2003). Rats were then injected with VEH or HAL 1 h before being tested in the CAR box under the CS-only condition for a total of 3 days. This was followed by 2 days of rest when the rats remained in the home cage, followed by one session of drug-free testing and one session of retraining. After the retraining session, all rats went through a final challenge session and were injected with a low dose of HAL (0.025 mg/kg).

Experiment 4: Effect of olanzapine dose on the strength of antipsychotic sensitization effect—

In this experiment, OLZ was tested. Forty Sprague–Dawley rats were handled and habituated for 2 days (30min/day). Four groups ($n=8/\text{group}$) of rats (VEH, low-dose OLZ 0.5mg/kg, medium-dose OLZ 1.0 mg/kg, and high-dose OLZ 2.0 mg/kg) were tested. These doses of OLZ have been shown to produce disrupted avoidance measures (Li *et al.*, 2004b) and give rise to 50–80% dopamine D_2 receptor occupancy (Kapur *et al.*, 2003). During the drug-challenge test, all rats were injected with OLZ at 0.5mg/kg.

Experiment 5: Effect of time interval between initial haloperidol exposure and re-exposure on the strength of haloperidol sensitization—

Experiment 5 examined the longevity of HAL sensitization. The interval between the initial drug treatment sessions and the challenge days was manipulated (e.g. 4, 10, and 17 days) while maintaining the dose and number of the HAL treatment constant (Fig. 3). Forty-eight rats that had fulfilled the criterion previously (in two batches, out of an initial 64 that were trained) were divided into six groups ($n=8/\text{group}$): two 4-day groups (VEH–HAL4 and HAL–HAL4), two 10-day groups (VEH–HAL10 and HAL–HAL10) and two 17-day groups (VEH–HAL17 and HAL–HAL17). All rats were first tested under VEH (sterile water) or HAL (0.1 mg/kg) for 3 days.

They then went through 1, 7, or 14 days of rest, followed by 1 day of retesting in a CS-only condition and 1 day of retraining. This was followed by a challenge day when all rats were injected with HAL (0.025 mg/kg) 1 h before sensitization testing. During the rest interval, rats remained in their home cages.

Experiment 6: Effect of time interval between initial olanzapine exposure and re-exposure on the strength of olanzapine sensitization—This experiment tested the longevity of OLZ (2.0 mg/kg) sensitization using an identical procedure to experiment 5. Forty-eight rats that had previously met criterion (in two batches, out of an initial 64 that were trained) were chosen, matched, and assigned into six groups ($n=8$ /group): VEH–OLZ4, VEH–OLZ10, VEH–OLZ17, OLZ–OLZ4, OLZ–OLZ10, and OLZ–OLZ17. On the challenge day, all rats were injected with OLZ (0.5mg/kg) 1 h before sensitization testing.

Drugs

The injection solution of HAL (5.0 mg/ml Ampoules, Shanghai Xudong Haipu Pharmaceutical Co. Ltd, Shanghai, China) was obtained by mixing drugs with sterile water. OLZ (a gift from the NIMH drug supply program) was dissolved in 1.0% glacial acetic acid in distilled water. Both drugs were administered subcutaneously in a volume of 1.0 ml/kg body weight. Drug doses for HAL and OLZ were based on previous studies showing that at the chosen doses, both drugs produce a reliable disruption of avoidance responding while not producing significant motor impairment (Li *et al.*, 2004a, 2009a, 2009b; Mead and Li, 2010; Zhang and Li, 2012).

Statistical analysis

All data are expressed as mean \pm SEM. Data from the drug exposure sessions were analyzed using a split-plot repeated-measures analysis of variance (ANOVA) with the between-subjects factor drug group and the within-subjects factor test session. Data from the challenge sessions were analyzed using either a one-way or a two-way ANOVA. Planned comparisons were used to determine specific group differences. Block data were also analyzed using either a one-way or two-way ANOVA (daily trials were split into three batches and analyzed by 10-trial blocks), followed by least significant difference (LSD) post-hoc tests. A conventional α value of 0.05 was used to determine significance. All data were analyzed using SPSS version 17 (SPSS Inc., Armonk, New York, USA).

Results

Experiment 1: Effect of number of injections on haloperidol sensitization

As Figure 4a shows, although the VEH group maintained a high level of avoidances (>85% avoidances over the 5 days of VEH treatment), those that had been exposed to HAL showed lower levels of avoidances. A repeated-measures ANOVA revealed significant main effects of Drug Group [$F(5,50)=23.40$, $P<0.001$] and Session [$F(4,200)=102.734$ $P<0.001$] and a significant Group \times Session interaction [$F(20,200)=7.33$, $P<0.001$], suggesting that the groups that had more than one exposure to the drug (HAL2, HAL3, HAL4, and HAL5) displayed a progressive across-session decrease in avoidance levels (Fig. 4a). During the two drug-free testing/retraining days, the avoidance levels returned to predrug levels (>90%

avoidances, Fig. 4a). The one-way ANOVA revealed a significant main effect of Drug Group on avoidance levels on the first day of testing/retraining [$F(5,50)=4.65$, $P<0.001$]. Multiple group comparisons revealed that the HAL4 and HAL5 groups had a significantly lower number of avoidances compared with the VEH group (LSD tests, all P values <0.05). This potential carryover effect appears to be driven by two rats and was not seen in any other experiment. There was no significant difference between groups on the second day of retesting [$F(5,50)=0.77$, NS].

On the challenge day when HAL sensitization was assessed, only the HAL5 group had lower avoidance than the VEH group. One-way ANOVA showed the main effect of Drug Group was marginally significant [$F(5,50)=2.18$, $P=0.071$]. Independent-samples t -tests found that the HAL5 group had significantly lower avoidance levels compared with the VEH group ($P=0.015$, Fig. 4b). These findings suggest that repeated HAL treatment at a relatively low dose induced a significantly enhanced response to HAL during the induction phase. This enhanced sensitivity to HAL was still detectable 3 days later in rats that received 5 days of HAL injections.

Experiment 2: Effect of number of injections on olanzapine sensitization

Figure 5a shows that the VEH group displayed a high level of avoidances over the 5 days of testing, whereas rats injected with OLZ showed decreased avoidance levels after OLZ injections. A repeated-measures ANOVA revealed significant main effects of Drug Group [$F(5,50)=19.09$, $P<0.001$] and Session [$F(4,200)=101.73$, $P<0.001$] and a significant Group \times Session interaction [$F(20,200)=10.75$, $P<0.001$], suggesting that the groups that had more than one exposure to the drug (OLZ2, OLZ3, OLZ4, and OLZ5) showed a progressive across-session decrease in avoidance levels (Fig. 5a). Avoidances recovered back to predrug levels after 2 days of drug-free testing/retraining ($>90\%$ avoidances, Fig. 5a). There was no significant difference on either day [day 1: $F(5,50)=1.20$, NS; day 2: $F(5,50)=0.59$, NS].

On the challenge day when OLZ sensitization was assessed, once again, only the OLZ5 group appeared to have lower avoidance than the VEH group. One-way ANOVA showed a significant main effect of Drug Group on the number of avoidances [$F(5,50)=3.42$, $P<0.01$] and two-group comparisons showed that the OLZ5 group had significantly lower avoidances than the VEH group ($P<0.001$, Fig. 5b). These findings suggest that repeated OLZ treatment at a relatively low dose induced a significantly enhanced response to OLZ during the induction phase. This enhanced sensitivity to OLZ was still detectable 3 days later in rats that received 5 days of OLZ injections.

Experiment 3: Dose-dependent effect of haloperidol on the strength of antipsychotic sensitization effect

Although the VEH group maintained a high level of avoidances ($>85\%$ avoidances over 3 days of VEH exposure), those that were treated with HAL had lower avoidance levels during the drug-treatment period (Fig. 6a). A repeated-measures ANOVA revealed significant main effects of Drug Group [$F(3,28)=67.95$, $P<0.001$] and Session [$F(2,56)=27.76$, $P<0.001$] and a significant Group \times Session interaction [$F(6,56)=3.04$, $P<0.02$], suggesting that the HAL groups showed a progressive across-session decrease in

avoidance levels (Fig. 6a). During the two drug-free testing/retraining days, the avoidance levels returned to predrug levels (>90% avoidances, Fig. 6a). There was no significant difference on the first day of retesting [$F(3,28)=2.79$, $P=0.059$] or the second day of retesting [$F(3,28)=1.56$, NS].

On the challenge day when HAL sensitization was assessed, one-way ANOVA showed a significant main effect of Drug Group on the number of avoidances [$F(2,21)=7.98$, $P<0.005$]. Post-hoc tests showed that the HAL 0.1 mg/kg group had significantly lower levels of avoidance compared with the VEH group (Fig. 6b and c, $P<0.05$). The difference between the HAL 0.05 mg/kg group and the VEH group was marginally significant ($P=0.051$). To further examine the temporal course of this difference, we examined the 10-trial block data. One-way ANOVAs showed significant group differences in all three blocks (P values <0.05). Post-hoc tests showed that the HAL 0.1 mg/kg group had significantly lower avoidances than the VEH group in all three blocks, whereas the HAL 0.05 mg/kg group was significantly lower than the VEH group only on the block 3 (all P values <0.05). These results suggest that HAL sensitization is dose-dependent, with the high dose and the medium dose (to a lesser extent) inducing a strong sensitization, whereas the low dose does not.

Experiment 4: Effect of olanzapine dose on the strength of antipsychotic sensitization effect

Although the VEH group maintained a high level of avoidances (>70% avoidances over 3 days of VEH exposure), those that were treated with OLZ had lower avoidance levels during the drug treatment period (Fig. 7a). A repeated-measures ANOVA revealed significant main effects of Drug Group [$F(3,28)=73.38$, $P<0.001$] and Session [$F(2,56)=62.40$, $P<0.001$] and a significant Group \times Session interaction [$F(6,56)=45.913$, $P=0.001$], showing that the OLZ groups showed a progressive across-session decrease in avoidance levels. One-way ANOVAs showed that there was no significant difference among groups on either drug-free testing/retraining day [day 1: $F(3,28)=1.83$, NS; day 2: $F(3,28)=0.14$, NS]. All groups returned to predrug levels (>90% avoidances) by the second day of retesting.

On the challenge day when OLZ sensitization was assessed, both OLZ 2.0mg/kg and 1.0 mg/kg groups had lower avoidances than the VEH group. This observation was confirmed by the statistical analysis. One-way ANOVA showed a significant main effect of Drug Group [$F(2,21)=8.86$, $P<0.002$, Fig. 7b], and two-group comparisons indicated that both the OLZ 2.0 mg/kg group and the OLZ 1.0 mg/kg group were significantly different from the VEH group (P values <0.01). These results suggest that OLZ sensitization is dose-dependent, with the medium and high doses inducing a robust sensitization, whereas the low dose fails to do so.

Experiment 5: Effect of time interval between initial haloperidol exposure and re-exposure on the strength of haloperidol sensitization

During the initial drug-treatment period, the VEH group maintained a high level of avoidances (>80% avoidances over 3 days of exposure), whereas the HAL groups had reduced avoidance levels. A repeated-measures ANOVA revealed significant main effects of

Drug Group [$F(5,42)=1794.09$, $P<0.001$] and Session [$F(2,84)=22.53$, $P<0.001$] and a significant Group \times Session interaction [$F(10,84)=2.70$, $P<0.01$], suggesting that the HAL groups showed a progressive across-session decrease in avoidance levels (Fig. 8a). There was no significant difference between the VEH and HAL groups on the first or second retesting day for any of the 4-, 10- or 17-day groups (all P values >0.05). All groups returned to predrug levels by the retraining day ($>90\%$ avoidances, Fig. 8a).

On the challenge test, there was a significant main effect of Drug Group (Fig. 8b and c, $F(1,42)=9.83$, $P<0.005$), and a significant main effect of Interval [$F(1,42)=6.30$, $P<0.005$], but no significant Group \times Interval interaction [$F(3,42)=0.008$, NS]. Planned comparisons showed that the HAL–HAL4 group had a significantly lower avoidance level than the VEH–HAL4 group ($P<0.05$, Fig. 8b). However, the HAL–HAL10 group did not differ significantly from the VEH–HAL10 group, whereas the difference between the VEH–HAL17 and HAL–HAL17 groups approached significance ($P=0.050$). Further analysis revealed that the two 17-day groups had significantly lower avoidance levels on the challenge day than the 4-day and 10-day groups (all P values <0.05).

These results show that the time interval between the drug treatment and challenge test (test–retest interval) plays a role in HAL sensitization. However, since there was no significant interaction between interval and HAL, it appears that HAL sensitization did not decrease over time and was maintained throughout the 17-day period.

Experiment 6: Effect of time interval between initial olanzapine exposure and re-exposure on the strength of olanzapine sensitization

During the initial drug-treatment period, the VEH group maintained a high level of avoidances ($>70\%$ avoidances over 3 days of exposure), whereas the groups receiving OLZ had lower avoidance levels. A repeated-measures ANOVA revealed significant main effects of Drug Group [$F(5,40)=797.76$, $P<0.000$] and Session [$F(2,80)=31.69$, $P<0.000$], but no significant Group \times Interval interaction [$F(10,80)=1.74$, $P=0.087$]. The OLZ groups did show a progressive across-session decrease in avoidance levels (Fig. 9a). There was no significant difference between the VEH and OLZ groups on either the first or second retesting day (all P values >0.05). All groups returned to predrug levels by the retraining day ($>90\%$ avoidances, Fig. 9a).

On the challenge day, there was a significant main effect of Drug Group [$F(1,40)=25.47$, $P<0.001$], but no significant main effect of Interval [$F(2,40)=1.28$, NS] or Group \times Interval interaction [$F(2,40)=1.62$, NS]. Further analysis using planned comparisons showed that there was a significant difference between the VEH–OLZ4 and OLZ–OLZ4 groups ($P<0.05$, Fig. 9b). There was no significant difference between the VEH–OLZ10 and OLZ–OLZ10 groups, although it approached significance ($P=0.055$), whereas the difference between the VEH–OLZ17 and OLZ17 groups was significant ($P<0.01$). These results show that avoidance responding as well as OLZ sensitization did not change over time and was maintained after 17 days.

Discussion

In the series of experiments, we investigated the impact of three parameters previously implicated in the development of psychomotor sensitization of drugs of abuse (i.e. number of exposures, length of interval, drug dose) on the induction and maintenance of antipsychotic sensitization in the CAR model. We found that the three tested doses of HAL and OLZ produced the classic sensitization pattern of progressively increased behavioral effect during the induction phase, as characterized by a progressively-enhanced disruption of conditioned avoidance responding across sessions. However, the sensitization effect during the expression/maintenance phase (i.e. the drug-challenge test) was strongly affected by various parameters. Sensitization induced by a relatively low dose of antipsychotics (0.025mg/kg HAL and 0.5 mg/kg OLZ) was only apparent in rats that received treatment for 5 days. The groups that had 1–4 days of exposure did not have even slightly lower avoidance levels on the challenge day; instead, their levels were no different from that of the VEH group. These results suggest that the induction and maintenance of antipsychotic sensitization may have different temporal courses. In the dose-dependence experiments, we replicated the finding that a low dose of HAL or OLZ injected for 3 days was unable to induce a long-term sensitization as assessed in the expression phase. In contrast, the medium or high doses of HAL or OLZ were able to induce robust sensitization with just 3 days of drug treatment. Both drug-induced sensitizations were dose-dependent, with higher doses inducing stronger sensitization. Finally, the HAL (0.10 mg/kg) and OLZ (2.0 mg/kg) sensitization effect was long-lasting: it was still apparent even 17 days after the last drug treatment. The selected intervals (i.e. 4, 10, and 17) were not as critical a factor as drug dose and number of exposures. It should be noted that although we examined these three parameters in separate experiments, they do not operate in isolation. It is likely that these parameters interact in determining the magnitude and strength of antipsychotic sensitization. The above findings, when viewed together, are in support of this point.

The present study demonstrated antipsychotic sensitization using a test paradigm that is commonly used in psychomotor sensitization (Anagnostaras and Robinson, 1996; Pierce and Kalivas, 1997; Robinson *et al.*, 1998). It consists of an induction phase (i.e. repeated drug treatment period) and an expression phase (i.e. challenge test) during which all animals are tested under the same antipsychotic drug treatment. Antipsychotic-induced sensitization is indicated by the higher inhibition of avoidance in the HAL-treated or OLZ-treated group than the VEH group. From a learning and memory perspective, these two phases can be characterized as the training (i.e. acquisition) and memory testing phases. Furthermore, similar to a typical learning task (e.g. Morris water maze), the number of drug injections, drug dose, and test–retest interval can be conceptualized as the number of learning trials (sessions), learning intensity, and time interval between learning and memory tests in a learning task (Domjan, 2005). Their impacts on the induction and maintenance of antipsychotic sensitization can thus be understood, as all the three factors are known to affect learning and memory. From this perspective, antipsychotics drugs can be viewed as exogenous stimuli that impact on brain structure, brain functions, and behavior. The fact that antipsychotic treatment itself induces a long-term potentiation, a molecular mechanism of learning and memory is in support of this view (Centonze *et al.*, 2004). Similar to other

discrete stimuli, they can function as conditioned or unconditioned stimuli in an associative learning paradigm. Indeed, our recent work provided evidence that HAL and OLZ can be used as unconditioned stimuli in a drug–drug conditioning paradigm to alter behavioral effects of other psychoactive drugs (Li *et al.*, 2009a).

In experiments 5 and 6, we showed that sensitization was present after 17 days for both HAL and OLZ, which is consistent with previous work showing that sensitization was seen up to 22 days later in a catalepsy test (Barnes *et al.*, 1990) and even 3 weeks after the last drug treatment (Mead and Li, 2010). In the field of psychostimulant sensitization, it has been shown that sensitization to a single dose of amphetamine can last as long as 12 weeks (Robinson, 1984). Future research using longer intervals is needed to determine how long antipsychotic sensitization can last.

Antipsychotic sensitization appears to be a universal phenomenon associated with repeated drug treatment. Besides the CAR model, it has been reported in a catalepsy test (Lanis and Schmidt, 2001; Amtage and Schmidt, 2003; Klein and Schmidt, 2003), a phencyclidine-induced hyperlocomotion test (Sun *et al.*, 2009; Zhang and Li, 2012), a prepulse inhibition of acoustic startle procedure (Li *et al.*, 2011), and an operant responding procedure (Varvel *et al.*, 2002), as well as for the metabolic effect (Boyda *et al.*, 2012). This is not to suggest that all antipsychotic drugs will induce a sensitization effect after repeated drug administration. One exception is clozapine. For example, although repeated HAL and OLZ induce sensitization in the CAR test, repeated clozapine induces tolerance (Li *et al.*, 2010). Clozapine-induced tolerance has also been observed in a drug discrimination task (Goudie *et al.*, 2007a, 2007b). Therefore, different antipsychotic drugs may have different intrinsic properties linked to their unique receptor binding profiles and clinical effects.

There appear to be a number of similarities between sensitization observed with drugs of abuse and that in antipsychotic drugs. Behavioral sensitization, a widely studied phenomenon associated with psychostimulants, is characterized by an augmented motor-stimulant response to stimulants seen after repeated, intermittent treatment to a specific compound (Paulson and Robinson, 1995). Historically, the term ‘behavioral sensitization’ has been associated with drugs of abuse; here we use ‘antipsychotic sensitization’ to refer to the observation that (a) during repeated drug treatment, avoidance disruptive effects of HAL and OLZ actually increase in magnitude (a within-group sensitization); and (b) during the challenge test, there is an enhanced behavioral response to a drug due to prior exposure to that drug (a between-group sensitization). Behavioral sensitization induced by drugs of abuse is dependent upon a number of factors including interval, dose, and number of exposures (Post and Contel, 1983), which were shown in this study also to be important to antipsychotic sensitization. In this study, we found that antipsychotic sensitization, as assessed in the CAR model required 5 days of treatment at a low dose to produce sensitization, which contrasts with results from the behavioral sensitization literature, in which behavioral sensitization could be seen after only one prior dose of the drug (Robinson *et al.*, 1982; Kalivas and Alesdatter, 1993; Vanderschuren *et al.*, 1999; Grignaschi *et al.*, 2004). Although the sensitization seen with drugs of abuse may share similar properties to sensitization seen with antipsychotics, such as dependency on specific parameters, antipsychotic sensitization may be less robust than that seen with addictive substances.

So what are the possible neurobiological mechanisms involved in antipsychotic sensitization? We speculate that it may have something to do with the drug-induced brain changes (e.g. neuroplasticity) because of its learning-like and memory-like characteristics (Konradi and Heckers, 2001). These changes thus might include elevations in the number and sensitivity of neuroreceptors (e.g. D₂ receptors; Samaha *et al.*, 2007, 2008), changes in immediate early gene expressions (e.g. *c-fos*, *zif268*, *FosB*, Nguyen *et al.*, 1992; Robertson and Fibiger, 1992; Robertson *et al.*, 1994; Grande *et al.*, 2004), and associated intracellular-signaling pathways (e.g. DARPP-32, cAMP, and PKA phosphorylation in the striatopallidal neurons, Bateup *et al.*, 2008), or even adult neurogenesis (Kippin *et al.*, 2005). Other parametric work with antipsychotics also shows that dopamine turnover may play a role in the development of sensitization (Csernansky *et al.*, 1990). We also recently examined the neurochemical basis of the antipsychotic sensitization induction in the CAR model and found that induction of HAL sensitization may be directly mediated by 5-HT_{2A/2C} blockade-initiated neuroplasticity, whereas the induction of OLZ sensitization may be directly mediated by D_{2/3} blockade-initiated neuroplasticity (Li *et al.*, 2010). One important line of research is to delineate how antipsychotics induce brain changes through these and other receptor systems (e.g. 5-HT_{1A}, D₁, D₄, etc.) and where these changes take place.

Though our model uses a preclinical approach to determine the parameters of the sensitization effect of antipsychotics, this model has the potential to be translated into clinical research. The ability to generalize such an effect from preclinical to clinical models has been validated in the past and allows for the possibility of exploring a lower dosage regimen for patients in the future (Antelman and Gershon, 1998). The conventional clinical approach to a dosing regimen is daily administration of a typically high dose of antipsychotics to produce the dopamine D₂ receptor occupancy that has been believed to be necessary to diminish symptoms. Recent advances in pharmacology have shown that continuous high D₂ receptor occupancy from antipsychotics is not as critical as once thought (Remington and Kapur, 2010). Drugs that produce only transient binding to the receptor have been shown to be clinically effective in treating schizophrenia and the same behavioral response can be maintained at lower levels of occupancy (Rabin and Siegel, 2010; Kapur *et al.*, 2000). The present study could be used to determine possible parameters of clinical treatment to create dosing regimens that would lower the adverse side-effects of antipsychotics as well as produce necessary symptom alleviation.

Conclusion

Our study not only details the exact parameters involved in the antipsychotic sensitization effect, but also chronicles some unexpected findings. The development of sensitization to HAL and OLZ appears to be as robust as sensitization seen with drugs of abuse such as cocaine and it is maintained over a long period of time. Antipsychotics require at least 5 days at a low dose to see a significant difference on the challenge day. However, at more salient dosages, sensitization is present at even shorter intervals. Intriguingly, there seemed to be no significant change over time in the strength of antipsychotic sensitization induced by a high dose of HAL or OLZ. This study provides valuable information for future studies on the mechanisms involved in the sensitization effect of antipsychotics but also has clinical implications that extend beyond the preclinical realm.

Acknowledgments

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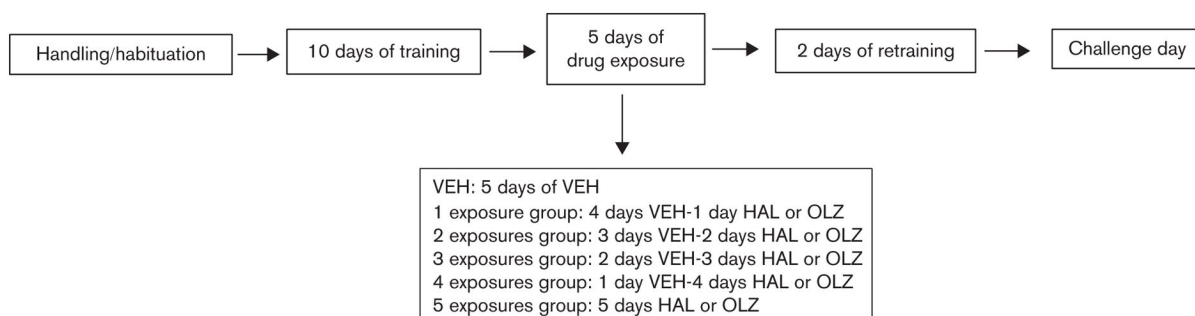
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**Fig. 1.**

A schematic representation of the experimental procedure in experiments 1 and 2. HAL, haloperidol; OLZ, olanzapine; VEH, vehicle.

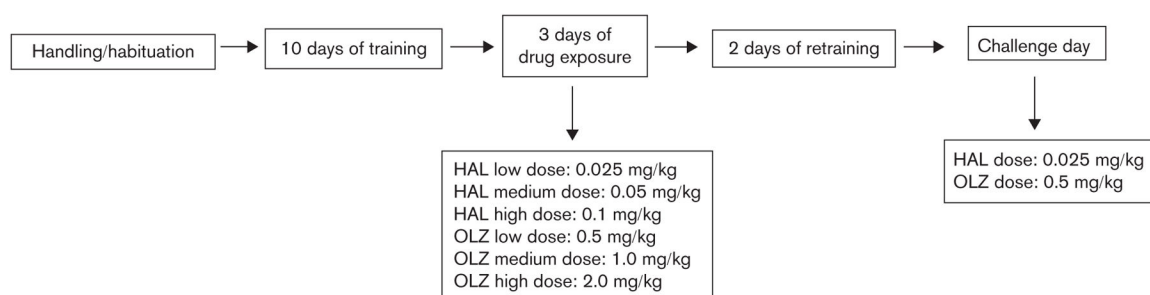


Fig. 2.

A schematic representation of the experimental procedure in experiments 3 and 4. HAL, haloperidol; OLZ, olanzapine.

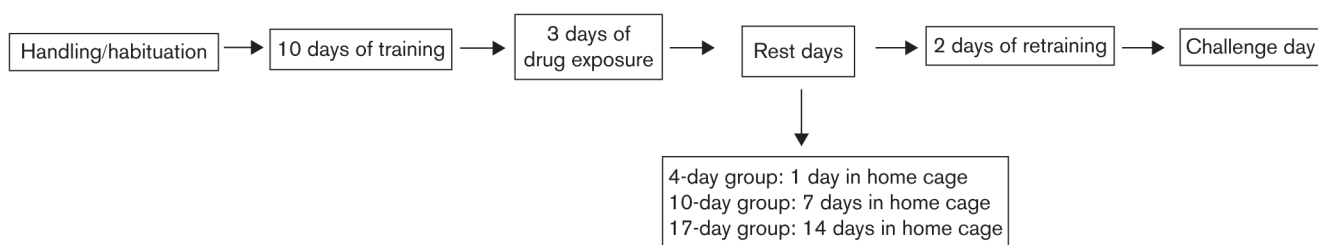
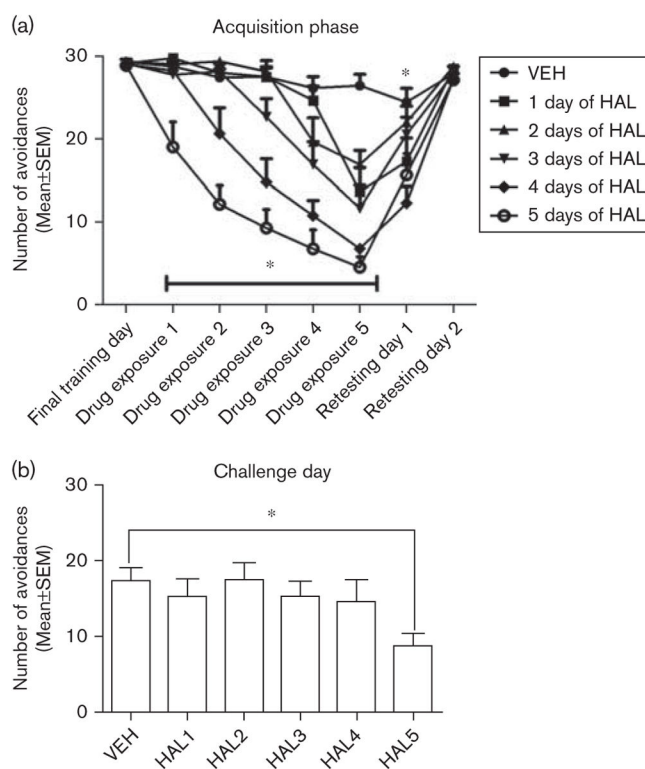
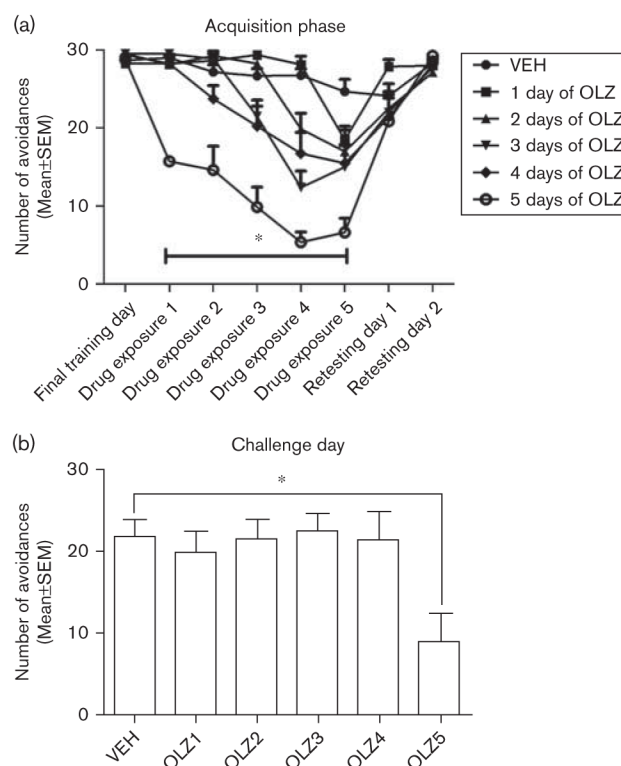


Fig. 3.

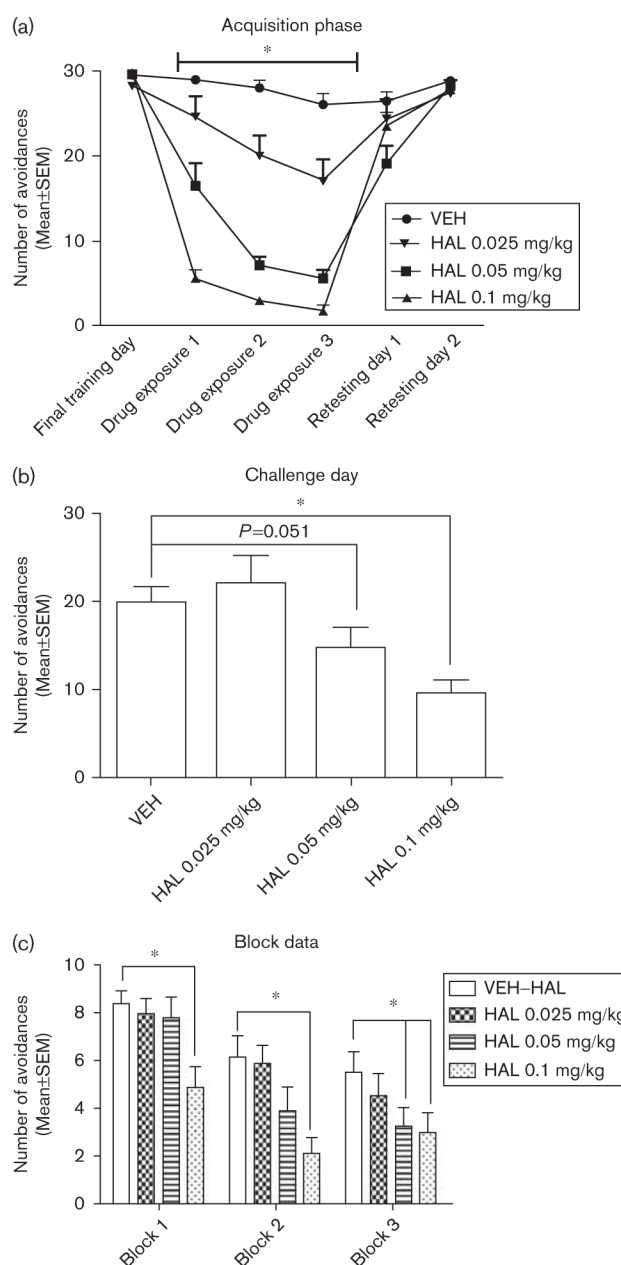
A schematic representation of the experimental procedure in experiments 5 and 6.

**Fig. 4.**

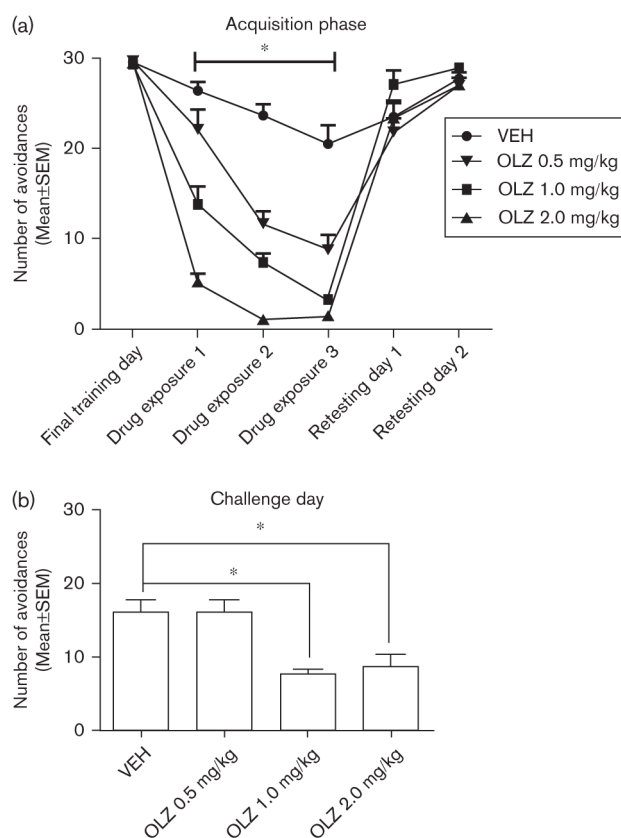
(a) Effect of repeated haloperidol (HAL) treatment (0.025 mg/kg, subcutaneously, 60 min) on conditioned avoidance responding across sessions. Number of avoidance responses made by the rats in the final training day (drug-free), 50 days of drug exposure, and two drug-free retesting sessions are expressed as mean±SEM. Different groups of rats received either 0, 1, 2, 3, 4, or 5 days of HAL (* $P<0.05$). (b) Effect of number of drug exposure days on final challenge day. All groups were injected with HAL (0.025 mg/kg) and avoidance responses were measured (* $P<0.05$). VEH, vehicle.

**Fig. 5.**

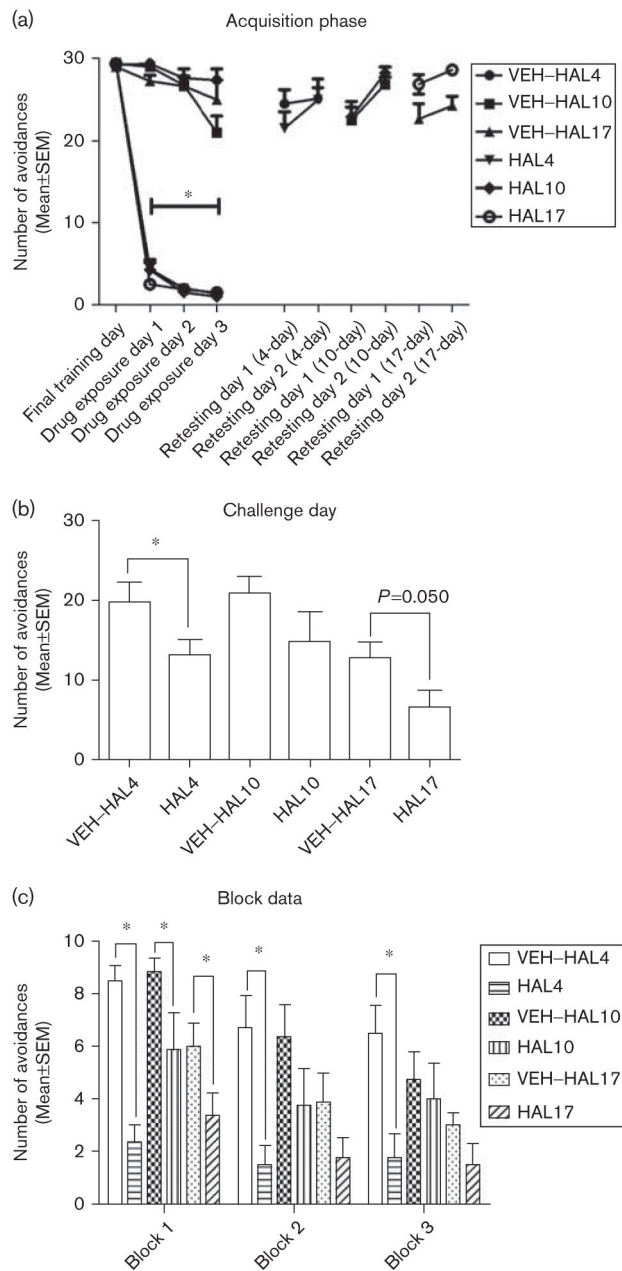
(a) Effect of repeated olanzapine (OLZ) treatment (0.5 mg/kg, subcutaneously, 60 min) on conditioned avoidance responding across sessions. Number of avoidance responses made by the rats in the final training day (drug-free), 5 days of drug exposure, and two drug-free retesting sessions are expressed as mean ± SEM. Different groups of rats received either 0, 1, 2, 3, 4, or 5 days of olanzapine (* $P < 0.05$). (b) Effect of number of drug exposure days on final challenge day. All groups were injected with OLZ (0.5 mg/kg) and avoidance responses were measured (* $P < 0.05$). VEH, vehicle.

**Fig. 6.**

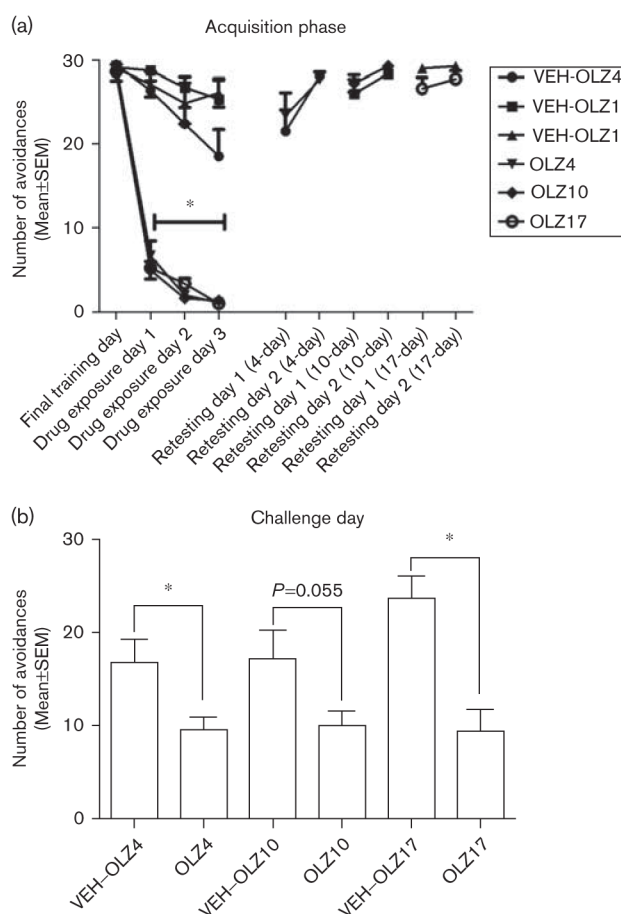
(a) Effect of repeated haloperidol (HAL) treatment (0.025, 0.05, or 0.1 mg/kg, subcutaneously, 60 min) on conditioned avoidance responding across sessions. Number of avoidance responses made by the rats on the final training day (drug-free), 3 days of drug exposure, and two drug-free retesting sessions are expressed as mean±SEM (* $P<0.05$). (b) Effect of dose on final challenge day. All groups were injected with HAL (0.025 mg/kg) and avoidance responses were measured (* $P<0.05$). (c) Effect of dose on 10-trial blocks on challenge day. VEH, vehicle.

**Fig. 7.**

(a) Effect of repeated olanzapine (OLZ) treatment (0.5, 1.0, or 2.0 mg/kg, subcutaneously, 60 min) on conditioned avoidance responding across sessions. Number of avoidance responses made by the rats on the final training day (drug-free), 3 days of drug exposure, and two drug-free retesting sessions are expressed as mean \pm SEM (* P <0.05). (b) Effect of dose on final challenge day. All groups were injected with OLZ (0.5 mg/kg) and avoidance responses were measured (* P <0.05). VEH, vehicle.

**Fig. 8.**

(a) Effect of repeated haloperidol (HAL) treatment (0.1 mg/kg, subcutaneously, 60 min) on conditioned avoidance responding across sessions. Number of avoidance responses made by the rats on the final training day (drug-free), 3 days of drug exposure, and two drug-free retesting sessions are expressed as mean±SEM. Rats received either 1, 7, or 14 days of rest according to their group and were then retested in the conditioned avoidance response boxes (* $P < 0.05$). (b) Effect of test–retest interval on final challenge day. All groups were injected with HAL (0.025 mg/kg) and avoidance responses were measured (* $P < 0.05$). (c) Effect of dose on 10-trial blocks on challenge day. VEH, vehicle.

**Fig. 9.**

(a) Effect of repeated olanzapine (OLZ) treatment (2.0 mg/kg, subcutaneously, 60 min) on conditioned avoidance responding across sessions. Number of avoidance responses made by the rats in the final training day (drug-free), 3 days of drug exposure, and two drug-free retesting sessions are expressed as mean±SEM. Rats received either 1, 7, or 14 days of rest according to their group and were then retested in the conditioned avoidance response boxes (* $P<0.05$). (b) Effect of test–retest interval on final challenge day. All groups were injected with OLZ (0.5 mg/kg) and avoidance responses were measured (* $P<0.05$).