Pharmacology, Biochemistry and Behavior 90 (2008) 551–562

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Clozapine and olanzapine exhibit an intrinsic anxiolytic property in two conditioned fear paradigms: Contrast with haloperidol and chlordiazepoxide

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ARTICLE INFO

Article history:
Received 27 February 2008
Received in revised form 14 April 2008
Accepted 24 April 2008
Available online 2 May 2008

Keywords:
Haloperidol
Clozapine
Chlordiazepoxide
Passive avoidance
Conditioned place aversion
Conditioned avoidance response
Ultrasonic vocalizations

ABSTRACT

Psychotic fear and anxiety disturbances are seen at a relatively high frequency in patients with schizophrenia. Atypical anti-psychotics are believed to show superior efficacy in reducing these symptoms. However, clinical and preclinical evidence regarding their anxiolytic efficacy has been mixed. In this study, we evaluated the possible anxiolytic property of two atypicals clozapine and olanzapine and compared them with typical haloperidol and chlordiazepoxide (a prototype of sedative-anxiolytic drug) in two preclinical models of fear. In Experiment 1, we used a fear-induced passive avoidance and conditioned place aversion paradigm and examined the effects of clozapine (20 mg/kg, sc), haloperidol (0.05 mg/kg, sc) and chlordiazepoxide (10 mg/kg, ip). In Experiments 2 and 3, we used a two-way active avoidance conditioning paradigm and further compared the effects of clozapine (20 mg/kg, sc), haloperidol (0.05 mg/kg, sc), chlordiazepoxide (10 mg/kg, ip) and three doses of olanzapine (0.5, 1.0, and 2.0 mg/kg, sc). Results show that clozapine and chlordiazepoxide, but not haloperidol, significantly attenuated the shock conditioning-induced place aversion, decreased the amount of defecations and the number of the 22-kHz vocalizations. Clozapine also reduced the shock conditioning-induced hyperthermia. Similar to clozapine, olanzapine also significantly decreased the amount of defecations and reduced the shock conditioning-induced hyperthermia, but it did not inhibit the 22-kHz vocalizations. This study demonstrates that clozapine and olanzapine possess an intrinsic anxiolytic property, which is not attributable to its superior anti-"psychotic" effect or its favorable effects on motor functions or learning and memory processes. These findings also suggest that the combined use of passive avoidance and active avoidance conditioning models can be useful in better differentiating typical and atypical anti-psychotics as well as anxiolytics.

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1. Introduction

In recent years, atypical anti-psychotic drugs (APDs) such as risperidone, olanzapine and quetiapine have been increasingly used to treat anxiety-related disorders in addition to their use in the treatment of psychosis. The results have been mixed (Carson et al., 2004). Some case reports suggest that atypicals improve symptoms of obsessive–compulsive disorder and panic disorder, while an equal number of reports indicate the worsening effects on these disorders (Brooke et al., 2005). Preclinical evidence is also inconclusive. Some studies have demonstrated an anxiolytic-like activity with the atypicals. For example, *clozapine* is reported to be effective in attenuating shock-induced conditioned freezing (Inoue et al., 1996; Ishida-Tokuda et al., 1996), footshock-induced ultrasonic vocalization (De Vry et al., 1993), passive avoidance response (Rasmussen et al., 2001) and increasing time spent in the central area of an open field (Bruhwyler et al., 1990b). It has also been found that clozapine can produce a significant increase in responding during the conflict component of a modified Geller–Seifter operant procedure, while typicals such as haloperidol, chlorpromazine, and thioridazine did not (Wiley et al., 1993). The ability of clozapine to increase punished responding has also been found in pigeons (Mansbach et al., 1988), mice and squirrel monkeys (Spealman and Katz, 1980), suggesting it may possess some anxiolytic activity. *Olanzapine* is found to be effective in reducing conditioned freezing, in increasing time spent on the open arms of the elevated plus maze as well as that in social interaction (Frye and Seliga, 2003) and in decreasing stress-induced ultrasonic vocalizations (Siemiatkowski et al., 2001). Both olanzapine and clozapine have been found to increase water licking of an electrified water bottle in the Geller–Seifter conflict task (Moore et al., 1992), demonstrating that these atypical anti-psychotics may possess anxiolytic activity. However, there are other reports that fail to confirm this anxiolytic activity (Cao and Rodgers, 1997; Fernandez-Tome et al., 1979; Masson et al., 2003; Shadach et al., 1999). Still others even suggest an anxiogenic activity (Karl et al., 2006; Manzaneque et al., 2002). This issue is further complicated by other studies showing

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doi:10.1016/j.pbb.2008.04.014

that even typical APDs also display an anxiolytic property in the same behavioral paradigms mentioned above (Allen et al., 1974; Greba et al., 2001; Guarraci et al., 2000; Inoue et al., 2005; Inoue et al., 1996; Johnson, 1970a,b; Joordens et al., 1998; Olivier et al., 2003; Ponnusamy et al., 2005; Taukulis et al., 1992).

Other than the procedural differences (species, drug doses, timing of drug administrations, etc.), several factors may also account for this rather confusing group of studies. First, a wide variety of behavioral models including both *unconditioned* (e.g., elevated plus maze, open field) and *conditioned* fear paradigms (e.g., conditioned freezing, fear-potentiated startle) have been used, which may not measure the same aspects of fear/anxiety responses and may not provide the same assessment of the drug effects. Furthermore, most studies often employ just one behavioral task or measure, rather than a series of convergent tasks and measurements to cross-validate the findings. Second, all APDs have multiple behavioral actions (e.g., motor, emotion, motivation or even cognition), the interactions among which may mask the effect size of one specific effect and influence the way we interpret the data. Finally, the intrinsic anti-psychotic effect of the drugs may also impact the measurements of anxiolytic activity, and most previous studies have not carefully controlled this influence. Therefore, clinical and preclinical evidence accumulated so far is inadequate to assess the intrinsic anxiolytic property of both typical and atypical APDs, and the possible advantages of atypicals over typicals in the treatment of anxiety disorders or anxiety in schizophrenia have not been determined.

In light of these observations, the present study further investigated the possible anxiolytic property of typical and atypical APDs using a preclinical approach. Specifically, in Experiment 1, we used a composite *passive* avoidance and conditioned place aversion task and examined the effects of *acute* treatment with clozapine, haloperidol or chlordiazepoxide (one single injection) on various conditioned fear responses (e.g. inhibitory passive avoidance, ratio of time spent in the shock compartment relative to time in the safe compartment, etc.). Haloperidol and clozapine were chosen as the representatives of typical and atypical APDs. Chlordiazepoxide was chosen as the representative of classic benzodiazepine anxiolytics. In Experiment 2, using an *active* avoidance conditioning model (CAR), we compared the effects of repeated treatment with these drugs (7 daily repeated injections) on the conditioned active avoidance response as well as other associated conditioned fear responses (amount of defecation, ultrasonic vocalization, change in body temperature) to cross-validate the results from Experiment 1 and to extend them to a repeated treatment regimen. The CAR model was carefully selected because it is a well-established model for the study of anti-psychotic activity (Wadenberg and Hicks, 1999). All currently used APDs selectively inhibit conditioned avoidance responding but not escape, whereas anxiolytics or antidepressants do not have such selectivity (Arnt, 1982). More importantly, in addition to robust avoidance responding, animals tested in this model also show various signs of fear and anxiety, such as increased body temperature, emission of ultrasonic vocalization (termed 22-kHz calls), and defecation and urination, which have been routinely used as reliable measures of conditioned reactive fear as well as to assess anxiolytic properties of psychotropic drugs (De Vry et al., 1993; Fanselow, 1986; Godsil et al., 2000; Sanchez, 2003). Thus we were able to use this single behavioral paradigm to compare the anxiolytic property of typical and atypical APDs (as indexed by their action on various fear responses), while properly matching their anti-psychotic property (as indexed by their action on active avoidance responding). This latter point is *particularly* important because one of the problems in previous drug comparison studies is that the typical APDs and atypicals often were not compared under the same conditions, with the doses for the typicals substantially higher than those of atypicals (Siemiatkowski et al., 2001) in terms of their efficacy to produce a clinically comparable level of D2 receptor occupancy (60–80%) (Kapur et al., 2003b) and to disrupt avoidance responding to the same extent (Li et al., 2007); thus, any beneficial effect resulting from the atypicals may simply be attributed to the dose differences. In Experiment 3, using the same paradigm and fear response measures as used in Experiment 2, we investigated the potential anxiolytic property of olanzapine, another atypical APD with a similar profile to clozapine, but much more widely used than clozapine, to further examine this issue. We also employed 3 different doses of olanzapine to examine any dose-dependent effects of the most clinically relevant atypical.

2. Materials and methods

2.1. Subjects

Sixty male Sprague–Dawley rats (275–325 g upon arrival, Charles River, Montréal, Canada) were used in Experiment 1, 44 male rats (250–275 g upon arrival, Charles River, Potage, MI) were used in Experiment 2, and 45 male rats (250–275 g upon arrival, Charles River, Potage, MI) were used in Experiment 3. They 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 8:00 pm and 8:00 am in Experiment 1, and between 6:00 am and 6:00 pm in Experiments 2 and 3). Room temperature was maintained at 21±1 ° with a relative humidity of 55–60%. Food and water was available *ad libitum*. Animals were allowed at least one week of habituation to the animal facility before being used in experiments. All procedures were approved by the animal care committees at either the Centre for Addiction and Mental Health (for Experiment 1) or the University of Nebraska-Lincoln (for Experiments 2 and 3).

2.2. Apparatus

Two 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 divided into two equal-sized compartments by an automatic guillotine door (ENV-010B, Experiment 1) or a white PVC partition with an arch style doorway (15 cm high×9 cm wide at base, Experiments 2 and 3). 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 scrambled footshock (US, 0.8 mA) was delivered by a constant current shock generator (Model ENV-410B) and scrambler (Model ENV-412). For Experiments 2 and 3, illumination was provided by two house-lights (28 V) mounted at the top of each compartment. An ultrasonic vocalization detector (ANL-937A) was situated on the right side wall of each box. The rat location and locomotor activity was detected by a set of 16 photobeams (ENV-256-8P) affixed at the bottom of the box (3.5 cm above the grid floor). The CS was a 74-dB white noise produced by a speaker (ENV 224AMX) mounted on the ceiling of the cubicle, centered above the shuttle box. All the training and testing procedures were controlled by Med Associates programs running on a computer. Background noise (approximately 68 dB) was provided by a ventilation fan affixed at the top corner of each isolation cubicle.

2.3. Drugs and choice of doses

Haloperidol (HAL), 5 mg/ml ampoules (Sabex Inc. Boucheville, Quebec, Canada), clozapine (CLZ, gift from National Institute of Mental Health's Chemical Synthesis and Drug Supply Program or from Anawa Biomedical Services and Products, Zurich, Switzerland), olanzapine (OLZ, purchased from Toronto Chemicals Inc, Ontario, Canada), and chlordiazepoxide (CDP, Sigma Chemical, St. Louis, MO) were used. The injection solutions of haloperidol and chlordiazepoxide were obtained by mixing the drugs with sterile water. Clozapine and olanzapine were

dissolved in 1–2% glacial acetic acid in sterile water. The doses of haloperidol (0.05 mg/kg), clozapine (20 mg/kg) and olanzapine (0.5, 1.0, and 2.0 mg/kg) were chosen based on the following considerations: (1) Our previous report shows that at these doses, haloperidol, clozapine and olanzapine produced a comparable level of disruption on the acquisition and extinction of avoidance responding, a validated behavioral index of anti-psychotic activity, but had no effect on escape (Li et al., 2007; Li et al., 2004); (2) all three drugs at these doses also gave rise to 50%–70% striatal dopamine D_2 occupancy in rats comparable to those observed in schizophrenic patients (Kapur et al., 2003a), so these doses were considered clinically relevant. The dose of chlordiazepoxide (10 mg/kg) was chosen on the basis of the fact that it is an effective dose in other aversively conditioned paradigms, such as Pavlovian fear conditioning and passive avoidance responding (Joordens et al., 1998; Klint, 1991; Nabeshima et al., 1990; Sanger and Joly, 1985; Tohyama et al., 1991).

2.4. Statistical analysis

Passive avoidance latency data and the relative time ratio data were expressed as median ± interquartile ranges because they were not normally distributed due to ceiling effects, and thus could not be analyzed using parametric tests. These data were analyzed using the nonparametric Kruskal–Wallis test (>3 groups) or Mann–Whitney *U* test (Decker et al., 1990). Within-group comparisons across days were performed using Friedman Test (for more than 3 related samples) or Wilcoxon Signed Ranks Test (for 2 related samples).

Parametric data such as number of avoidance responses, amount of defecations, and body temperature change were expressed as mean values ± SEM and were analyzed using a factorial repeated measures analysis of variance (ANOVA) with the between-subjects factor being treatment condition ("Treatment", e.g. haloperidol, clozapine, etc.) and the within-subject factor being the test sessions ("Session", e.g. day 1 test, day 2 test, etc.) and Post hoc Tukey HSD tests were used to identify the overall group differences. If necessary, one-way ANOVAs were used to identify the group differences on each test session. A conventional two-tailed level of significance at the 5% level was required.

2.5. Experiment 1: effects of acute haloperidol, clozapine or chlordiazepoxide treatment on the acquisition of passive avoidance and conditioned place aversion

This experiment was aimed to characterize the behavioral effects of acute treatment with haloperidol, clozapine and chlordiazepoxide treatment on various conditioned fear responses in a composite passive avoidance and conditioned place aversion task, two traditional behavioral tests that have been widely used to assess anxiolytic activity of a drug (Papp, 1988; Sanger and Joly, 1985). This task encompasses multiple measures indicative of conditioned fear, including passive avoidance response (e.g. passive avoidance latency and number of entries to the shock compartment), conditioned place aversion (e.g. the ratio of time spent in the shock compartment relative to the time spent in the safe compartment), and defecations.

2.5.1. Procedure

A total of 60 rats were randomly assigned to the following 4 groups: haloperidol, clozapine, chlordiazepoxide or vehicle groups. There were 15 rats in each group.

2.5.1.1. Baseline test (habituation). After receiving 5 days of handling (approximately 1 min/rat), subjects were placed into the shuttle boxes for the baseline test and habituation. The left compartment of the shuttle box was decorated with 2-cm horizontal tape stripes on the back and front walls and the ceiling, while the right compartment was decorated with vertical stripes, with an automatic guillotine door (ENV-010B) sitting in between. The left compartment also had a light bulb (28 V) in the middle of the side wall providing illumination. A subject was first placed in the left compartment ("safe") at the beginning of the test. Thirty seconds later, the door was lifted and the subject was allowed to enter the right compartment and explore the whole apparatus for 10 min. The latency to enter the right compartment, time spent in each compartment, number of entries to the right compartment, and numbers of defecations were recorded.

2.5.1.2. Conditioning (under drug). On the next day, the subject was placed in the left compartment of the shuttle box after receiving HAL (0.05 mg/kg, −90 min), CLZ (20 mg/kg, −30 min), CDP (10 mg/kg, −30 min) or vehicle injection (distilled water, −90 min). Thirty seconds later, the door was lifted and the subject was allowed to enter the right compartment. Once entered, the door was immediately closed and 10 trials of CS–US were given. The CS was 11 s, 10 kHz, 85 dB pure tone, while the US was 1 s, 0.8 mA footshock. The onset of the US occurred 10 s after the onset of the CS and co-terminated with the CS. The mean intertrial interval was 60 s (40–80 s).

2.5.1.3. Post-conditioning test (without drug). Two days after the conditioning, the test was conducted. The basic procedure was exactly the same as the baseline test (see Baseline test above) and lasted 10 min. Similarly, the latency to enter the shock compartment (passive avoidance latency), time spent in each compartment (max 600 s), number of entries to the shock compartment, and the numbers of defecations were recorded. If the subject failed to enter the right compartment within 10 min, a latency of 600 s was assigned.

2.6. Experiment 2: effects of repeated haloperidol, clozapine or chlordiazepoxide treatment on conditioned active avoidance responding and various conditioned fear responses

To cross-validate the anxiolytic effects of clozapine and chlordiazepoxide observed in Experiment 1, this second experiment used an *active* avoidance conditioning model and examined how repeated haloperidol, clozapine or chlordiazepoxide treatment (7 consecutive days) differentially affected various conditioned fear responses in this model, such as body temperature change (before and after conditioning), 22-kHz USVs, and amount of defections, along with their effects on active avoidance responding, an index of an anti-psychotic property. This model allowed us to *concurrently* evaluate and compare the anti-psychotic and potential anxiolytic activities of haloperidol and clozapine.

2.6.1. Procedure

In order to adapt rats to the body temperature measuring procedure and injection procedure and to minimize the associated stress, all rats were first handled daily and habituated to the body temperature measuring procedure (twice daily, 20 min interval) and injection procedure for 6 successive days, which was sufficient to obtain a stable baseline body temperature (Godsil et al., 2000). Following this adaptation phase, all rats were habituated to the CAR boxes for 2 days (20 min/day) and their body temperatures (twice: before and after habituation) and amount of defecations (in mg) were recorded. In addition, the "22-kHz" ultrasonic vocalizations (USVs) were also recorded. After the habituation, the rats were randomly assigned to 5 groups: haloperidol (HAL, $n=9$), clozapine (CLZ, $n=10$), chlordiazepoxide (CDP, $n=10$), vehicle (VEH, $n=9$) and control (CON, $n=6$). In the subsequent 7 days, the first 4 groups were trained in a 20-trial CAR session/day, whereas the control group continued on the 20 min habituation procedure (no CS or US). Before each daily session, the rats were injected with HAL (0.05 mg/kg, sc), CLZ (20 mg/kg, sc), CDP (10 mg/kg, ip) or vehicle (sterile water for both VEH and CON groups, ip or sc), and their body temperatures were recorded before being placed in the boxes. HAL and CLZ were administered 1 h before

Table 1
The stages of the experimental design for Experiment 2

Stage 1	Stage 2	Stage 3	Stage 4
6 days of handling and habituation	2 days of CAR boxes habituation	7 days CAR training (under drug)	3 days of CAR CS-alone and drug-free tests

the training, whereas CDP was administered 0.5 h before. Body temperatures were taken again immediately after each test. Following the 7 daily drug tests, all rats were continuously tested drug-free for an additional 3 sessions under the CS-only condition (no US) to assess the long-term drug effects not contaminated by the presence of the shock or during the extinction phase. Table 1 summarizes the experimental procedure.

2.6.1.1. Two-way active avoidance training (under drug). Every trial started by presenting the CS for 10 s, followed by a continuous scrambled footshock (0.8 mA, US, maximum 5 s) on the grid floor. If a subject moved from one compartment into the other within the 10 s of CS presentation, it avoided the shock, and this shuttling response was recorded as *avoidance*. If the rat remained in the same compartment for more than 10 s and made a crossing upon receiving the footshock, this response was recorded as *escape*. If the animal did not respond during the entire 5 s presentation of the shock, the trial was terminated and *escape failure* was recorded. Intertrial intervals varied randomly between 30 and 60 s. Each training session lasted about 20 min with a total of 20 trials presented. The number of avoidance responses (max: 20) was calculated as the main dependent variable for avoidance responding. Fecal matter was collected and weighed on a Mettler Toledo scale (<0.1 mg). An ultrasonic vocalization detector (model number: ANL-937-1) recorded the ultrasonic events throughout each session. This detector scans the environment every 30 ms and counts any vocalization call with a minimum duration of 30 ms. We chose to record "22-kHz" calls occurring between 20 kHz and 32 kHz and above 50 dB because vocalizations within this range are often found in rats that are exposed to fearful stimuli (Wohr et al., 2005) and are sensitive to anxiolytic treatments (Sanchez, 2003). The rat's body temperature was taken using a probe (lubricated with mineral oil) inserted in the rectum (Thermalert TH-5, Physitemp, Clifton, NJ, USA) before and after the behavioral training/testing and the difference was calculated. The thermistor is accurate to 0.1 °C.

2.6.1.2. Two-way active avoidance test (drug-free). One day after the last training session, all rats were continuously tested drug-free for an additional 3 sessions under the CS-alone (no shock) condition. The exact same procedure was employed except that the footshock was omitted.

2.7. Experiment 3: effects of repeated olanzapine or chlordiazepoxide treatment on conditioned active avoidance responding and various conditioned fear responses

The purpose of this experiment was to determine the potential anxiolytic effect of olanzapine, a much more widely used drug in the clinic. This experiment also used the *active* avoidance conditioning model to examine how repeated olanzapine or chlordiazepoxide treatment differentially affected various conditioned fear responses in this model. We employed three doses of olanzapine which covered subclinical, clinical and superclinical doses of olanzapine in terms of D_2 receptor occupancy (50%–80%) to explore its dose-dependent effect (Kapur et al., 2003b).

2.7.1. Procedure

The exact same procedure as used in Experiment 2 was used. After the habituation, the rats were randomly assigned to 5 groups: vehicle (VEH, $n=9$), olanzapine 0.5 mg/kg (OLZ 0.5, $n=9$), olanzapine 1.0 mg/kg (OLZ 1.0, $n=9$), olanzapine 2.0 mg/kg (OLZ 2.0, $n=9$) and chlordiazepoxide 10 mg/kg (CDP, $n=9$) and tested for 7 days under drug and 3 days under drug-free.

3. Results

3.1. Experiment 1: effects of acute haloperidol, clozapine or chlordiazepoxide treatment on the acquisition of passive avoidance and conditioned place aversion

Haloperidol significantly increased, while chlordiazepoxide significantly decreased the passive avoidance latency. On the baseline test day and the conditioning day, the latencies to enter the right ("to be shocked") compartment were similar among the 4 groups (all $ps>0.056$, data not shown). However, the passive avoidance latencies on the test day were significantly different among the haloperidol, clozapine, chlordiazepoxide and vehicle-treated groups (Kruskal–Wallis tests, $p=0.004$). In comparison to the vehicle, chlordiazepoxide significantly decreased the passive avoidance latency (Mann–Whitney U test, $U=65.50$, $p=0.050$), whereas haloperidol significantly increased it ($U=65.50$, $p=0.050$). Clozapine had no significant effect on this measure ($U=99.00$, $p>0.59$). As can be seen in Fig. 1, when compared to the baseline measures, the passive avoidance latencies in both the haloperidol and vehicle groups showed a significant increase from the baseline day to the test day (Wilcoxon Signed Ranks Test, all $ps<0.013$). In contrast, the clozapine and chlordiazepoxide groups did not show such a significant change ($p=0.069$ for CLZ and $p=0.91$ for CDP), indicating that the inhibitory passive avoidance to the shock compartment was attenuated by chlordiazepoxide and, to some extent, by clozapine.

Clozapine and chlordiazepoxide, but not haloperidol, significantly attenuated the shock conditioning-induced place aversion. Fig. 2 shows the median ratios of the time spent in the shock compartment relative to the time spent in the safe compartment (Fig. 2A) and the mean numbers of entries into the shock compartment (Fig. 2B) on both the baseline habituation day and the post-conditioning test day. These two behaviors provide reliable indices of the shock-induced conditioned place aversion effect (Di Scala and Sandner, 1989; Holahan and White, 2004). Clozapine and chlordiazepoxide exhibited a very similar

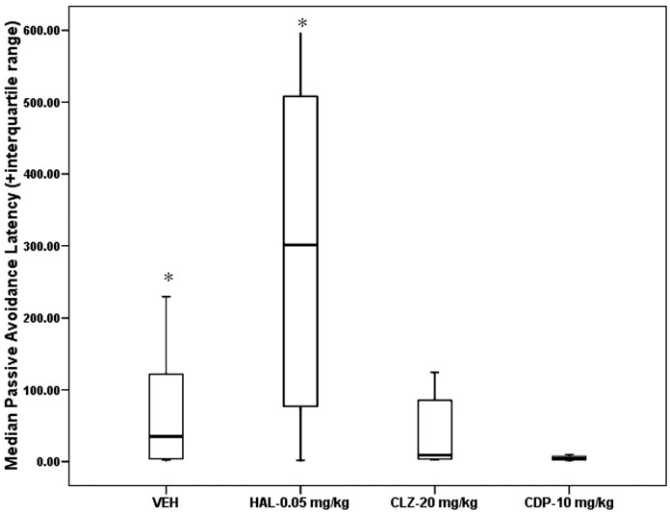

Fig. 1. Passive avoidance latencies during the retention test conducted 2 days after the passive avoidance training under the influence of haloperidol, clozapine, chlordiazepoxide or vehicle. The box represents the interquartile range. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. The line across the box indicates the median. *$p<0.05$ significantly different from the baseline day to test day.

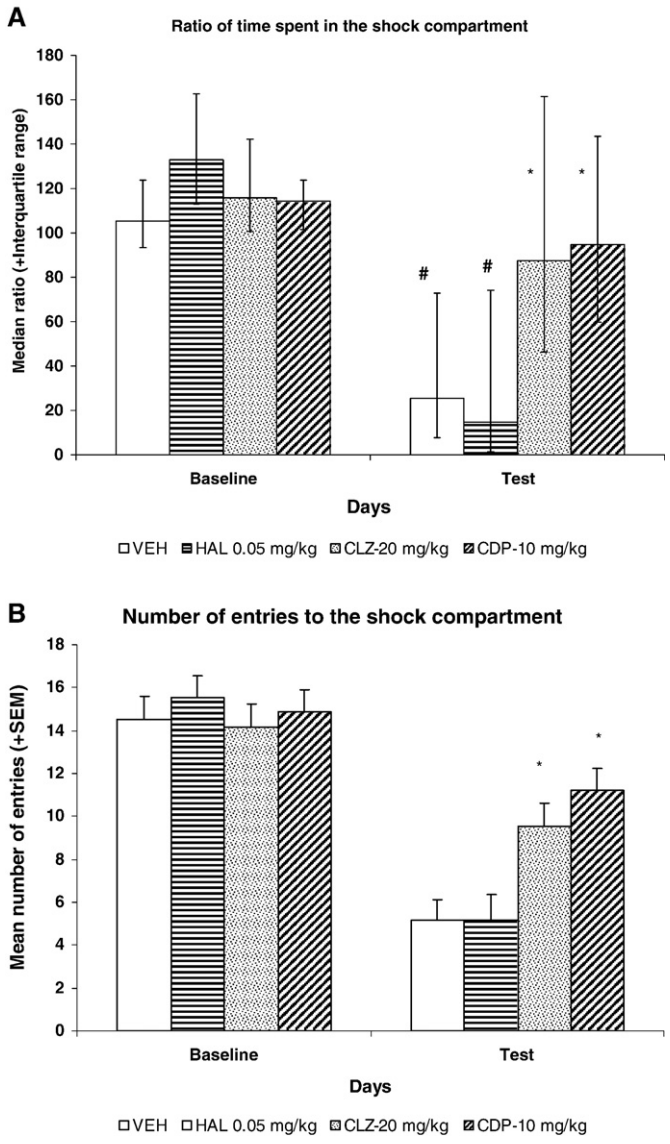

Fig. 2. Median ratio of the time spent in the right (shock) compartment vs. the time spent in the left compartment apparatus and interquartile ranges (A) and the mean (+SEM) number of entries to the shock compartment (B) on both the baseline habituation day and the post-conditioning test day. *$p<0.05$ significantly different from the vehicle group; #$p<0.05$ significantly different from the baseline.

behavioral profile on these two measures. In comparison to vehicle treatment, both clozapine and chlordiazepoxide significantly attenuated the shock conditioning-induced *decrease* on the time spent in the shock compartment ($U=43.00$, $p=0.003$ for CLZ; $U=42.00$, $p=0.003$ for CDP) and the number of entries into the shock compartment on the test day (Tukey Post hoc $p=0.031$ and 0.001 for CLZ and CDP). Haloperidol had little effect (all $ps>0.36$). Compared to the baseline measures, although the vehicle and haloperidol-treated rats decreased their time spent in the shock compartment after conditioning (Wilcoxon Signed Ranks Test, $p=0.011$ for the vehicle and $p=0.002$ for the haloperidol), the clozapine and chlordiazepoxide rats did not (all $ps>0.06$).

Clozapine and chlordiazepoxide, but not haloperidol, significantly attenuated the shock conditioning-induced increase in defecations. Fig. 3 shows the number of defecations across the baseline, conditioning and test days. In comparison to the baseline, all four groups showed a significant increase in defecations on the test day (Paired samples *T*-Tests, all $ps<0.015$), suggesting that rats did acquire conditioned fear after conditioning. In comparison to the vehicle group, defecations in both the clozapine and chlordiazepoxide groups were significantly decreased on the conditioning day (Tukey Post hoc, $p=0.000$ for CLZ and 0.041 for CDP), and chlordiazepoxide also decreased defecations on the test day ($p=0.016$).

3.2. Experiment 2: effects of repeated haloperidol, clozapine or chlordiazepoxide treatment on active avoidance responding and various conditioned fear responses

Repeated haloperidol and clozapine, but not chlordiazepoxide treatment significantly inhibited avoidance responding during the CAR training phase. Fig. 4A shows the number of conditioned avoidance responses in the four groups trained under the drug or vehicle over the 7 training days. Both the vehicle and chlordiazepoxide-treated rats, but not the haloperidol or clozapine rats, showed a progressive across-session increase in avoidance responding, indicating a clear learning effect (Repeated Measures ANOVAs: a significant main effect of "Treatment": $F(3, 34)=16.011$, $p<0.001$; "Sessions": $F(6, 204)=16.822$, $p<0.001$; and "Treatment"×"Sessions" interaction, $F(18, 204)=4.761$, $p<0.001$). In comparison to the vehicle treatment, haloperidol and clozapine, but not chlordiazepoxide, significantly inhibited the acquisition of avoidance responding (Tukey Post hoc Tests: HAL vs. VEH, $p<0.001$; CLZ vs. VEH, $p=0.001$; CDP vs. VEH, $p=0.818$). Most importantly, both drugs did not differ in the magnitude of their inhibition during the drug test days (Tukey HSD, $p=0.950$), suggesting that at the chosen doses, they exhibited a very similar level of anti-psychotic efficacy as this measure is a reliable and sensitive predictor of anti-psychotic efficacy (Wadenberg and Hicks, 1999).

Fig. 4B shows the number of conditioned avoidance responses in the three drug-free and CS-alone test days (i.e. extinction). The haloperidol group still exhibited significantly lower numbers of avoidances than the vehicle group on day 1 ($p=0.008$), whereas the clozapine group did not ($p>0.270$). Data from the clozapine group in both the drug training days and the drug-free test days suggest that clozapine did not impair the animals' ability to learn how to actively respond to the aversive CS, but only inhibited its expression.

Clozapine and chlordiazepoxide, but not haloperidol, inhibited the expression of 22-kHz ultrasonic vocalizations. As can be seen from Fig. 5, during the 2 habituation days, there were few vocalizations recorded. During the 7 CAR training days, only the chlordiazepoxide group showed a progressive decrease in the number of 22-kHz calls. The haloperidol group had consistently high levels of 22-kHz calls, whereas the clozapine group showed consistently lower levels of 22-kHz calls

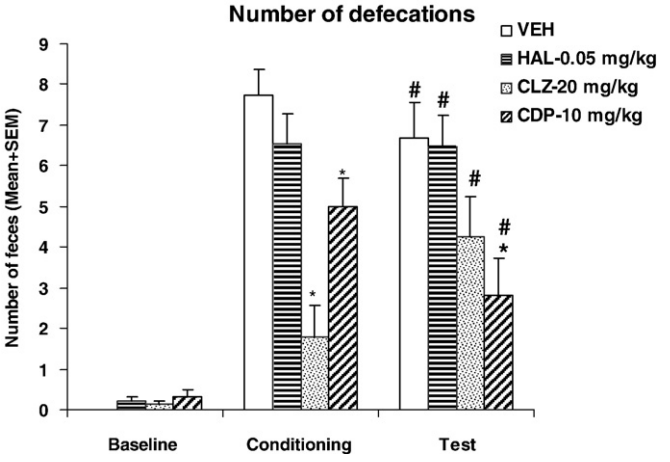

Fig. 3. Mean (+SEM) number of defecations on the baseline habituation day, conditioning day (under drugs) and the retention test day. *$p<0.05$ significantly different from the vehicle group; #$p<0.05$ significantly different from the baseline.

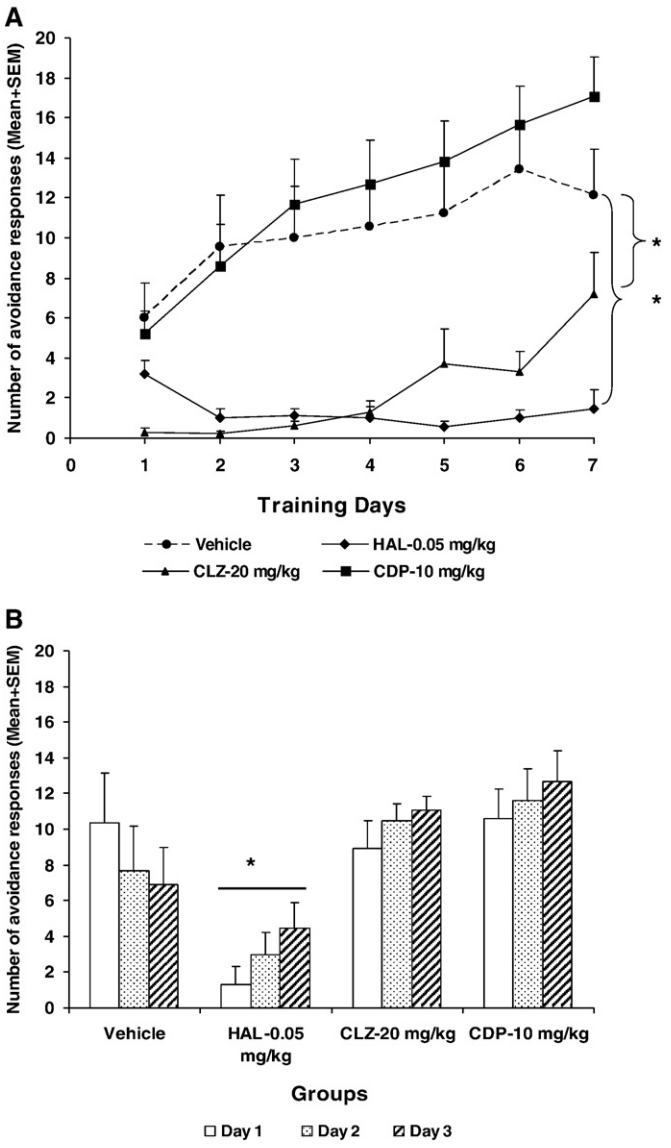

Fig. 4. Mean (+SEM) numbers of avoidance responses of the four groups of rats that were trained under the haloperidol, clozapine, chlordiazepoxide or vehicle treatment over the 7 avoidance conditioning days (A) and on the three undrugged test days (B). *$p<0.05$ significantly different from the vehicle group.

("Treatment": $F(4, 39)=11.371$, $p<0.001$; "Sessions": $F(6, 234)=2.657$, $p=0.016$; "Treatment"×"Sessions" interaction, $F(24, 234)=3.745$, $p<0.001$). On the first drug test day, clozapine significantly decreased the 22-kHz calls ($p=0.035$ vs. VEH). In comparison to the "unconditioned" control group, all conditioned groups showed significantly higher numbers of 22-kHz calls during certain drug test days (Individual Repeated Measures ANOVAs, all $ps<0.01$), suggesting that the footshock did cause fear or anxiety in these groups (Wohr et al., 2005). Importantly, during the subsequent 3 drug-free test days, rats that were previously treated with clozapine and chlordiazepoxide still made significantly less 22-kHz calls than did the vehicle rats (CLZ: $F(1, 17)=7.039$, $p=0.017$; CDP: $F(1, 17)=8.114$, $p=0.011$), and their numbers of the 22-kHz calls were not significantly different from the "unconditioned" controls (all $ps>0.756$). Haloperidol rats, like the vehicle controls, still exhibited more 22-kHz calls than the "unconditioned" controls (day 1: $ps<0.031$; day 2: $ps<0.018$; day 3: $p=0.42$ for VEH and 0.58 for HAL). Because no shock was ever presented at this test stage, these 22-kHz calls could be considered as an acquired "conditioned fear response" to the CS and/or to the environment. These results suggest that clozapine and chlordiazepoxide, but not haloperidol, do possess an anxiolytic property in decreasing this particular conditioned fear response.

Clozapine and chlordiazepoxide inhibited the physiological fear responses (e.g. body temperature increase and defecations). Stress-induced hyperthermia and defecation is an integral part of an individual's physiological response to threatening situations and have been used as valid tools to screen chemical compounds with anxiolytic property (Bruhwyler et al., 1990a; Olivier et al., 2003). Fig. 6A and B depicts the body temperature changes (before and after testing) and the amounts of defecation that the rats made throughout the habituation, CAR training and drug-free test phases. During the habituation period, both measures remained low, and no significant group difference was detected (all $ps>0.05$). During the CAR training phase, the clozapine and "unconditioned" control groups did not show much change in either measure from the habituation days, whereas the other three groups increased their body temperatures and amount of defecations. Throughout the training sessions, all groups except the clozapine group showed a relatively stable level of hyperthermia. On the measure of defecations, the haloperidol group appeared to defecate more over the sessions, whereas the vehicle and chlordiazepoxide groups defecated progressively less. Repeated Measures ANOVAs indicated a significant effect of "Treatment" (body temperature: $F(4, 39)=18.334$, $p<0.001$; defecations: $F(4, 39)=27.033$, $p<0.001$), "Sessions" (body temperature: $F(6, 234)=3.861$, $p=0.001$; defecations: $F(6, 234)=3.977$, $p=0.001$) and "Treatment"×"Sessions" interactions (body temperature: $F(24, 234)=3.618$, $p<0.001$; defecations:

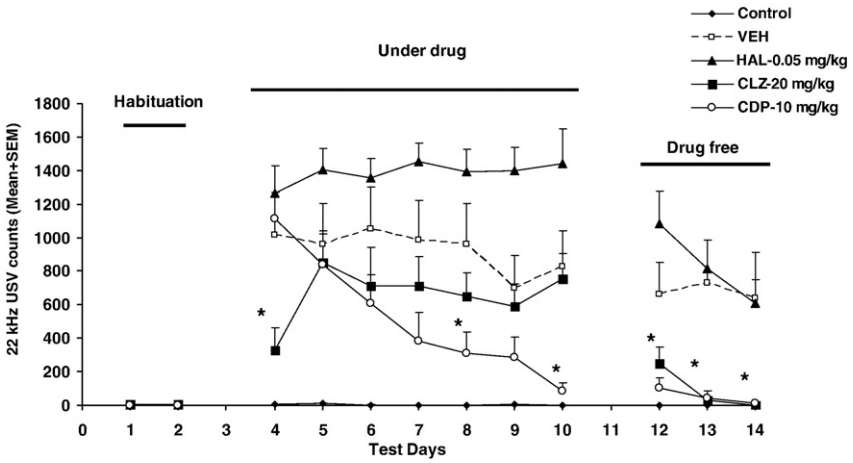

Fig. 5. Mean (+SEM) numbers of the 22-kHz USV counts of the four groups of rats that were trained under the haloperidol, clozapine, chlordiazepoxide or vehicle treatment over the 7 avoidance conditioning days and on the three undrugged test days. *$p<0.05$ significantly different from the vehicle group.

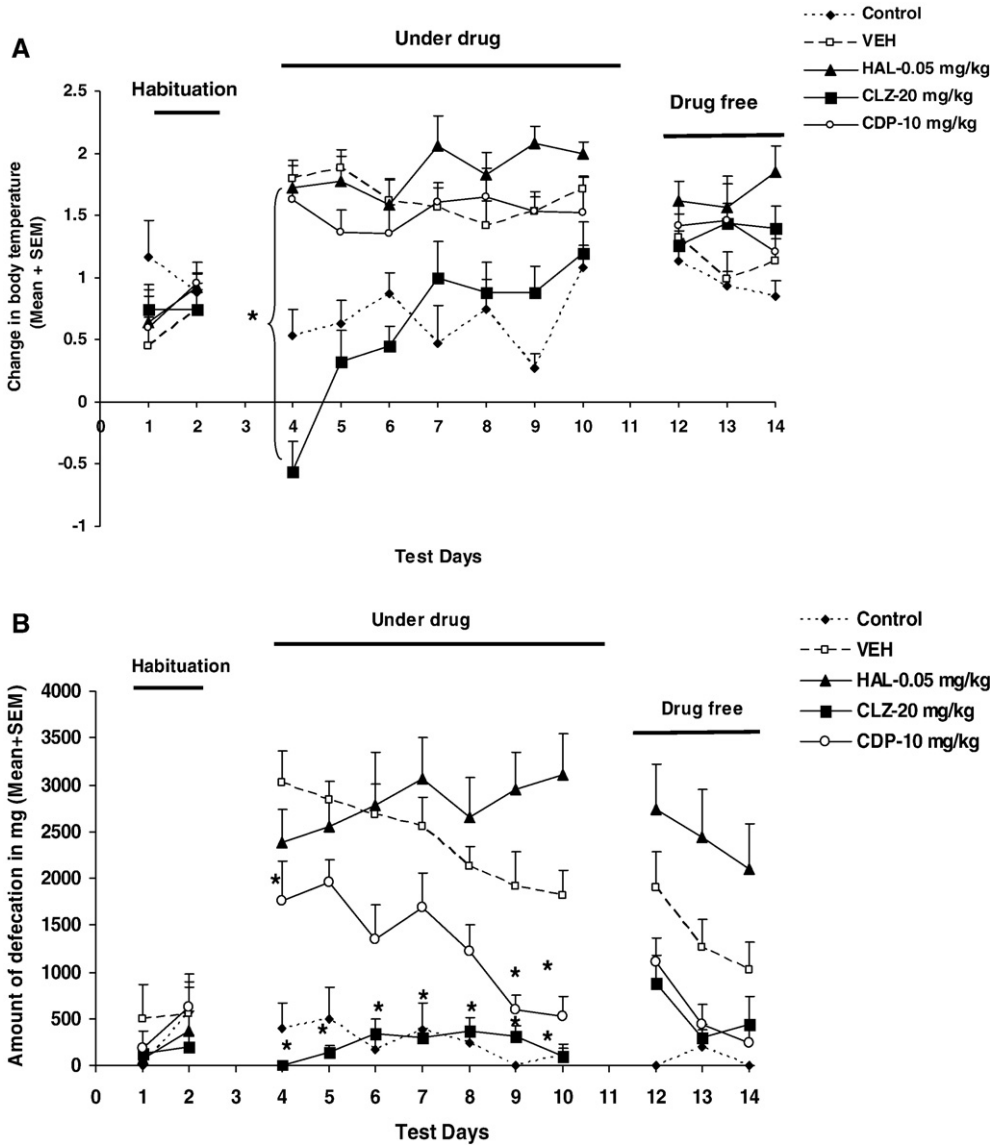

Fig. 6. Mean (+SEM) change of the body temperature (body temperature taken *after* conditioning − body temperature taken *before* conditioning) (A) and amount of defecations (B) of the four groups of rats that were trained under the haloperidol, clozapine, chlordiazepoxide or vehicle treatment over the 7 avoidance conditioning days and on the three undrugged test days. *$p<0.05$ significantly different from the vehicle group.

$F(24, 234)=2.653$, $p<0.001$). In comparison to the vehicle treatment, clozapine treatment significantly inhibited hyperthermia ($p<0.001$), and both clozapine ($p<0.001$) and chlordiazepoxide treatment ($p=0.007$) also significantly inhibited defecations. In contrast, haloperidol treatment did not affect hyperthermia, but did *increase* the amount of defecation on the last training day compared to the vehicle group ($p=0.010$).

During the drug-free test phase, although both clozapine and chlordiazepoxide-treated rats seemed to defecate less, while the haloperidol-treated rats defecated more in comparison to the vehicle rats, these effects failed to reach a significant level (all $ps>0.05$).

3.3. Experiment 3: effects of repeated dose-dependent olanzapine or chlordiazepoxide treatment on conditioned active avoidance responding and various conditioned fear responses

Repeated olanzapine treatment, but not chlordiazepoxide, significantly inhibited avoidance responding during the CAR training phase and drug-free test phase. Fig. 7A shows the number of conditioned avoidance responses in the five groups for the 7 training days. Both the vehicle and chlordiazepoxide-treated rats, but not the olanzapine-treated rats, showed a progressive across-session increase in avoidance responding, indicating a clear learning effect (Repeated Measures ANOVAs: a significant main effect of "Treatment": $F(4, 40)=15.489$, $p<0.001$; "Sessions": $F(6, 240)=3.506$, $p=0.002$; and "Treatment" × "Sessions" interaction, $F(24, 240)=5.745$, $p<0.001$). In comparison to the vehicle treatment, all three doses of olanzapine, but not chlordiazepoxide, significantly inhibited the acquisition of avoidance responding (Tukey Post hoc Tests: VEH vs. OLZ 0.5, 1.0, and 2.0 mg/kg, $ps<0.001$; VEH vs. CDP, $p=0.853$). The three doses of olanzapine did not differ in their magnitude of inhibition during the drug test days (Tukey Post hoc Tests: OLZ 0.5 vs. OLZ 1.0, $p=0.988$, OLZ 1.0 vs. OLZ 2.0, $p=1.000$, OLZ 0.5 vs. OLZ 2.0, $p=0.973$), indicating a very similar level of anti-"psychotic" efficacy at these doses. Fig. 7B shows the number of conditioned avoidance responses in the three drug-free and CS-alone test days. The three olanzapine-treated groups still exhibited significantly lower numbers of avoidances than the vehicle group (VEH vs. OLZ 2.0, $p=0.032$, vs. OLZ 1.0, $p=0.023$, vs. OLZ 0.5, $p=0.002$) and the chlordiazepoxide group (CDP vs. OLZ 2.0, $p=0.001$, vs. OLZ 1.0, $p=0.001$, and vs. OLZ 0.5, $p<0.001$).

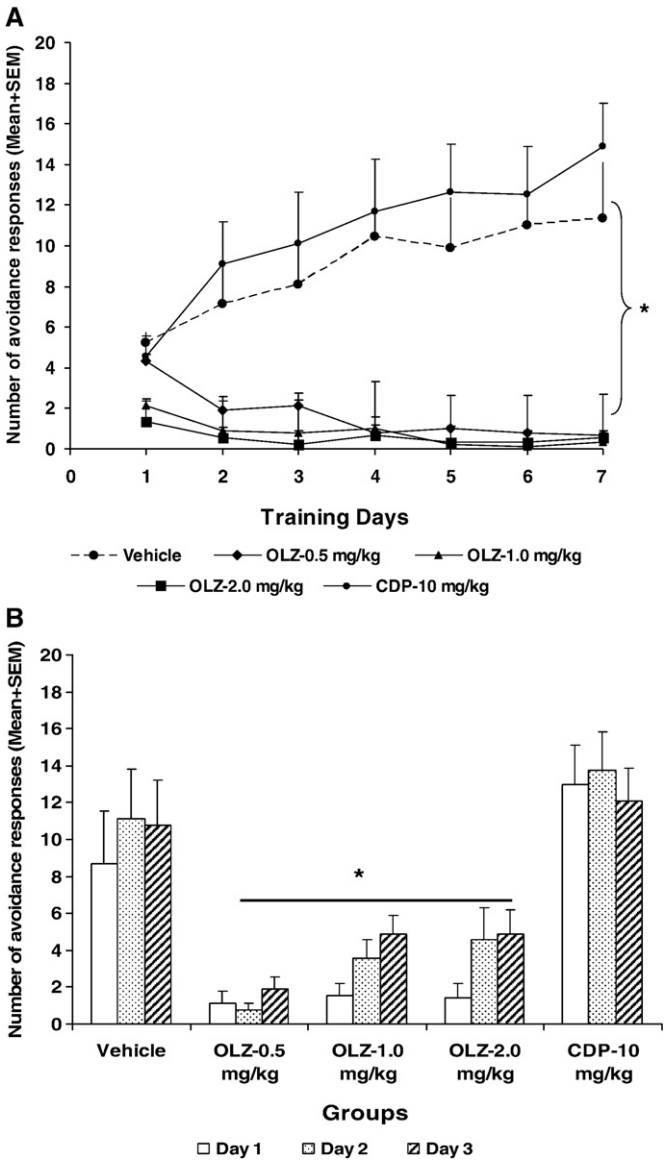

Fig. 7. Mean (+SEM) numbers of avoidance responses of the five groups of rats that were trained under olanzapine (0.5, 1.0 and 2.0 mg/kg), chlordiazepoxide (1.0 mg/kg) or vehicle treatment over the 7 avoidance conditioning days (A) and on the three undrugged test days (B). *$p<0.05$ significantly different from the vehicle group.

Olanzapine did not affect the expression of 22-kHz ultrasonic vocalizations. As seen in Fig. 8, few vocalizations were recorded during the habituation days. During the 7 CAR training days, only the chlordiazepoxide group showed a progressive decrease on the number of 22-kHz calls, consistent with the finding from Experiment 2. However, the difference between the vehicle and the chlordiazepoxide groups was not as large as seen in Experiment 2. Importantly, none of the three doses of olanzapine inhibited the ultrasonic vocalizations. Overall the olanzapine groups actually emitted more 22-kHz vocalization calls, a profile similar to that of the haloperidol group, but dissimilar to that of clozapine, as seen in Experiment 2 (Repeated Measures ANOVAs: a significant main effect of "Treatment": $F(4, 40)=3.429$, $p=0.017$; "Sessions": $F(6, 240)=2.970$, $p=0.008$; and "Treatment"×"Sessions" interaction, $F(24, 240)=1.946$, $p=0.007$). In the subsequent 3 drug-free test days, although the chlordiazepoxide rats showed less 22-kHz calls relative to the vehicle controls, the difference was not significant ($ps>0.05$). However, the chlordiazepoxide rats did show less 22-kHz calls than the olanzapine 0.5 mg/kg (day 1, $p=0.011$; day 2: $p=0.014$) and olanzapine 2.0 mg/kg rats (day 1: $p=0.040$).

Olanzapine and chlordiazepoxide inhibited the physiological fear responses (e.g. body temperature increase or defecations). Fig. 9A and B depicts the body temperature changes and amount of defecation during the habituation phase, the training phase and the drug-free test phase. Both measures were low during the habituation phase, with no significant group difference detected (all $ps>0.05$). During the CAR training phase, there was a clear dose-dependent effect of olanzapine treatment on body temperature change. Repeated Measures ANOVAs revealed a significant main effect of "Treatment" ($F(4, 40)=7.439$, $p<0.001$) and a significant main effect of "Sessions" ($F(6, 240)=3.50$, $p=0.002$), but no significant "Treatment"×"Sessions" interaction ($F(24, 240)=0.854$, $p=0.665$). Post hoc tests showed that only the high dose of olanzapine significantly inhibited the body temperature increase in comparison to vehicle (OLZ 2.0 vs. VEH: $p=0.002$). Consistent with the result from Experiment 2, chlordiazepoxide did not significantly decrease this measure (Tukey Post hoc, CDP vs. VEH, $p=0.07$). Low and medium doses of olanzapine were also not effective (OLZ 0.5 vs. VEH, $p=0.999$; OLZ 1.0 vs. VEH, $p=1.00$). No group difference was found during the three drug-free CS-only test sessions (all $ps>0.05$), suggesting that prior olanzapine treatment did not have a long-lasting effect on stress-induced hyperthermia.

All three doses of olanzapine, as well as chlordiazepoxide, significantly decreased the amount of defecations across the 7 testing days (Tukey Post hoc tests: VEH vs. OLZ 0.5, 1.0, and 2.0 mg/kg, $p<0.019$, 0.002 and 0.001 respectively, VEH vs. CDP, $p<0.035$, one-tailed). Once again, no group difference was found during the three drug-free test CS-only test sessions (all $ps>0.05$). These results suggest that olanzapine, like chlordiazepoxide, does possess an anxiolytic property in decreasing these particular conditioned fear responses.

4. Discussion

The present study used two distinct behavioral models and evaluated the potential anxiolytic-like activity of typical antipsychotic haloperidol and atypicals clozapine and olanzapine, and compared them with that of a classical anxiolytic chlordiazepoxide. Table 2 summarizes the results from all experiments. Inspection of this table reveals that clozapine and olanzapine show some similarities to chlordiazepoxide in terms of their effects on a variety of fear measures indicative of anxiolytic property. Specifically, clozapine and chlordiazepoxide, but not haloperidol, significantly attenuated the shock conditioning-induced place aversion, decreased the amount of defecations and the number of 22-kHz vocalizations. Clozapine also reduced shock conditioning-induced hyperthermia. Similar to clozapine, olanzapine also significantly decreased the amount of defecations and reduced shock conditioning-induced hyperthermia, but it did not inhibit 22-kHz vocalizations. Although it can be said that clozapine, olanzapine and chlordiazepoxide all show some anxiolytic effects in the fear/anxiety measures in this study, some differences did exist. Clozapine significantly decreased the shock conditioning-induced hyperthermia but had no effect on the passive avoidance latency, whereas chlordiazepoxide significantly decreased the passive avoidance latency but had no effect on body temperature change. Olanzapine differed from clozapine and chlordiazepoxide in that it had no effect on 22-kHz ultrasonic vocalizations at the three doses tested. Olanzapine's anxiolytic property was mainly manifested in its effect on hyperthermia and defecations, and this effect seems limited to the drug test phase.

Our results also indicate that haloperidol does not seem to possess any anxiolytic-like property, as tested in these models, but may instead possess an anxiogenic-like activity as it increased the passive avoidance latency and the amount of defecations.

As mentioned in the Introduction section, evidence so far is variable in regards to the potential anxiolytic property of both typical and atypical APDs. Behavioral studies have reported anxiolytic-like, anxiogenic-like and lack of effects with the use of typical or atypical

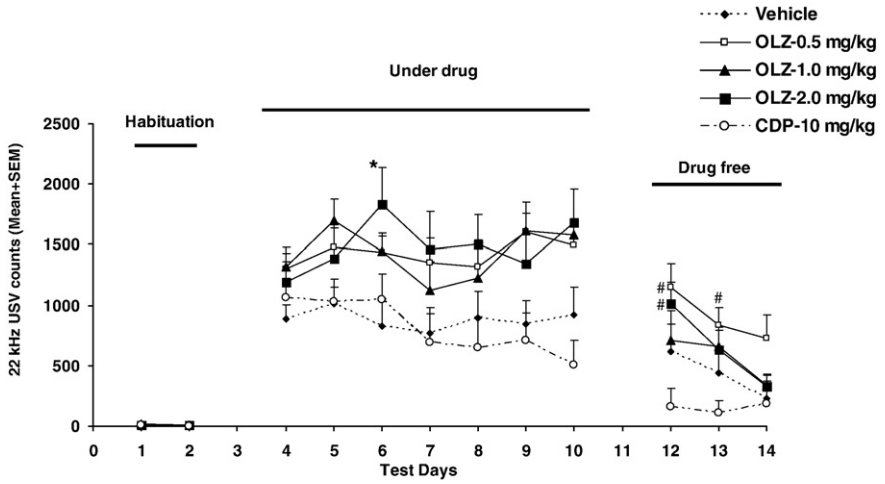

Fig. 8. Mean (+SEM) numbers of the 22-kHz USV counts of the five groups of rats that were trained under olanzapine (0.5, 1.0 and 2.0 mg/kg), chlordiazepoxide (1.0 mg/kg) or vehicle treatment over the 7 avoidance conditioning days and on the three undrugged test days. *$p<0.05$ significantly different from the vehicle group. #$p<0.05$ significantly different from the chlordiazepoxide group.

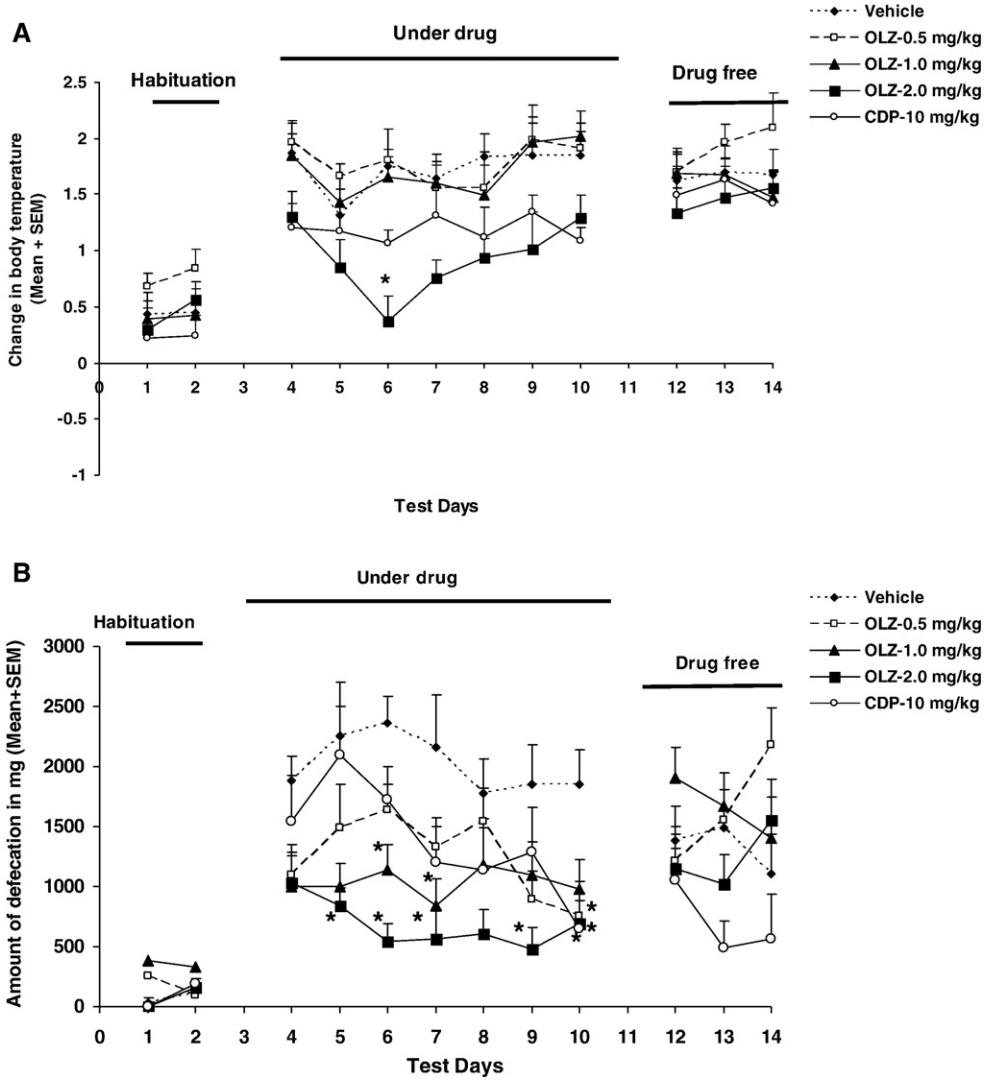

Fig. 9. Mean (+SEM) change of the body temperature (body temperature taken *after* conditioning − body temperature taken *before* conditioning) (A) and amount of defecations (B) of the five groups of rats that were trained under the olanzapine (0.5, 1.0 and 2.0 mg/kg), chlordiazepoxide (1.0 mg/kg) or vehicle treatment over the 7 avoidance conditioning days and on the three undrugged test days. *$p<0.05$ significantly different from the vehicle group.

Table 2
Summary of the effects of haloperidol, clozapine, olanzapine, and chlordiazepoxide treatment on various fear responses (indices of an "anxiolytic" property) and on conditioned avoidance response (an index of an "anti-psychotic" property)

Drugs	Behavioral and physiological measures of fear (anxiolytic or anxiogenic property)					Active avoidance responding (anti-"psychotic" property)
	Passive avoidance latency	Place aversion	Defecations	22-kHz USVs	Body temperature increase	Active avoidance responding
Haloperidol	↑	–	↑	–	–	↓
Clozapine	–	↓	↑	↓	↓	↓
Olanzapine	–	–	↑	–	–	–
Chlordiazepoxide	↓	↓	↑	↓	↓	↓

"↑": denotes a significant increasing effect. "↓": denotes a significant decreasing effect.
"–" denotes no effect, and ⇑ ⇓ denote a slight increasing or decreasing effect.

APDs in a broad range of animal models of fear or anxiety (Ichihara et al., 1988; Ishida-Tokuda et al., 1996; Kovacs and de Wied, 1978; Thiessen and Upchurch, 1981; Timmerman et al., 1990). One contribution of the present study is that we employed two different behavioral models of fear/anxiety and multiple measures (both behavioral as well as physiological) to examine this issue to ensure that our results were not an artifact of a single model or measure. Experiment 1 uses a composite passive avoidance and conditioned place aversion task, with acute treatment commonly used in these paradigms, to evaluate various conditioned fear responses, while Experiments 2 and 3 use a two-way active avoidance model to simultaneously examine the anti-psychotic properties as well as the conditioned fear responses, using a repeated treatment regimen to best mimic clinical features (Li et al., 2007). Results from Experiments 1 and 2 were consistent in showing that clozapine, but not haloperidol, has a strong anxiolytic activity as it reduced a number of shock conditioning-induced fear responses, and results from Experiment 3 show that olanzapine possesses some anxiolytic properties as it reduced shock conditioning-induced hyperthermia and defecations. The fact that clozapine and olanzapine share certain anxiolytic effects with chlordiazepoxide further strengthens this conclusion. The anxiolytic activity of clozapine or olanzapine does not seem to be due to their effects on motor functions because rats were tested in the drug-free condition, and measurements such as the passive avoidance latency, body temperature change, and defecations are independent of the animals' motor ability. This activity could not simply be attributed to their action on constipation (Bhana et al., 2001; Sachdev and Saharov, 1998) or core body temperature (Millan et al., 1995; Salmi et al., 1994) either, because this anxiolytic effect was observed even when the treatment was stopped. We do not think the anxiolytic-like activity of clozapine, olanzapine and chlordiazepoxide can be attributed to the drug effects on associative learning and memory specifically employed in fear conditioned tasks; however, some studies have shown that anti-psychotics impair learning and memory on various other tasks in rodents. Skarsfeldt, (1996) found that several typical and atypical anti-psychotics impaired spatial learning in the Morris water maze, while other studies have found atypicals to cause significant impairment of performance on the radial arm maze and in the passive avoidance test (Ortega-Alvaro et al., 2006, Ishiyama et al., 2007). However, this memory impairment effect may not completely account for our results. Clozapine and chlordiazepoxide-treated rats acquired the CS–US association as evidenced by the fact that all groups of rats showed a significant increase in defecations on the test day when they were being placed back into the environment where they had received the shock (Experiment 1), and while haloperidol significantly impaired the acquisition of avoidance responding, it did not impair the conditioned fear responses. This conclusion is also consistent with other studies showing that APDs generally do not impair the learning or even expression of associative conditioning (Anisman et al., 1982; Beninger et al., 1980; Li et al., 2004). Li et al. (2007) also found that after repeated haloperidol or olanzapine treatment in the conditioned avoidance response paradigm animals were able to recover avoidance responding after the treatment was stopped, suggesting that both drugs do not affect the memory of responding to the CS, but merely inhibit the motivation to respond. Our results are also consistent with the finding showing that chlordiazepoxide affects declarative memory only, but does not disrupt procedural memory (Fang et al., 1987; Nissen et al., 1987). In the current study the fear conditioned tasks are concerned with emotional memory, rather than spatial learning or memory, which may explain the conflicting results that exist. Due to these dissociations found between the anxiolytic effect and memory, the anxiolytic activity of clozapine and chlordiazepoxide may be separated from any possible effect on learning and memory.

Although there is a suggestion that atypical APDs are better than typicals in alleviating psychotic fear or anxiety, evidence on this issue is still controversial. Most studies either did not directly compare atypicals to typicals (De Vry et al., 1993; Frye and Seliga, 2003) or did not find any difference between the two (Fernandez-Tome et al., 1979; Inoue et al., 1996; Rasmussen et al., 2001). Even in studies that demonstrate atypicals show a superior anxiolytic effect (Siemiatkowski et al., 2001), it is not clear whether the observed superiority is independent of their reduced liability to produce akathisia (which may look like anxiety) or the intrinsic anxiolytic effects. The second contribution of the present study is that we carefully matched the anti-psychotic efficacy of haloperidol with clozapine in Experiments 1 and 2, and with olanzapine in Experiment 3, thus ensuring that they were compared under the same conditions so that any behavioral difference between the two cannot be attributed to the influence from their intrinsic anti-psychotic effect. The way we achieved this was by using a dose for each drug that produced a comparable level of disruption on avoidance responding (Li et al., 2004). The approach is justified by the well documented fact that avoidance responding is a reliable behavioral index of anti-psychotic efficacy (Wadenberg and Hicks, 1999), and the potency of a drug in the avoidance responding test correlates well with its clinical potencies (Arnt, 1982). Our results clearly show that under the current experimental conditions, clozapine and olanzapine are indeed superior in alleviating a variety of fear-related responses when compared with haloperidol.

The 22-kHz USVs are often observed when rats are exposed to *aversive* situations (Antoniadis and McDonald, 1999; Burgdorf et al., 2001; Wohr et al., 2005). Previous studies have shown that anxiolytics such as diazepam and chlordiazepoxide reduce the number of 22-kHz calls (Vivian and Miczek, 1993). The effects of anti-psychotics on this measure are inconsistent, with some studies reporting reducing effects (De Vry et al., 1993; Molewijk et al., 1995), some reporting no change (Bartoszyk, 1998), and others reporting an enhancing effect (Siemiatkowski et al., 2001; Thiessen and Upchurch, 1981). Our clozapine and chlordiazepoxide results were consistent with the literature. The finding that olanzapine failed to decrease this measure was inconsistent with Siemiatkowski et al. (2001), who reported that acute treatment with olanzapine (1.0 mg/kg) reduced the pre-shock contextual vocalizations and tended to diminish the post-shock

ultrasonic vocalizations. The exact causes of this discrepancy are not clear, possibly due to the methodological differences (rat strains: SD vs. Wistar; behavioral tasks: active avoidance vs. contextual fear conditioning, etc.).

The neurobiological mechanism(s) of the anxiolytic action of clozapine and olanzapine is poorly understood. Little work has *directly* examined this issue. Recently, there has been indirect evidence suggesting that the effects of clozapine on allopregnanolone, a metabolite of progesterone, may be responsible for its anxiolytic effect. In 2003 and 2006, Marx et al. found that clozapine and olanzapine can dose-dependently increase allopregnanolone in the rat cerebral cortex and hippocampus (Marx et al., 2006a; Marx et al., 2006b; Marx et al., 2003). Since allopregnanolone acts as a positive modulator of the $GABA_A$ receptor (Majewska, 1990), and shows a strong anxiolytic effect in the elevated plus-maze task and the Geller–Seifter conflict test (Akwa et al., 1999; Bitran et al., 2000; Brot et al., 1997), it is therefore *possible* that clozapine or olanzapine-induced elevations in allopregnanolone may contribute to their anxiolytic-like effect. Using these models and specific pharmacological agents will allow one to parse out which of clozapine and olanzapine's properties are critical for these anxiolytic effects and whether they are completely dissociable from its anti-psychotic efficacy.

Results from the present report also suggest interesting behavioral dissociations among haloperidol, clozapine, olanzapine and chlordiazepoxide with regards to their effects on two categories of conditioned fear responses (e.g. active vs. reactive). Conditioned fear responses such as freezing, passively avoiding a "shocked" environment, or increasing the body temperature, number of 22-kHz USVs, or defecations, are innate, reflexive species-typical responses to threats and are expressed automatically in the presence of danger, thus they are classified as "conditioned reactive fear responses". Drug effects on these measures may indicate an anxiolytic-like or anxiogenic-like activity. In contrast, *active* avoidance to a fearful stimulus requires animals to make an overt motor action and is a voluntary and intentional motor response to danger, thus it is deemed as "conditioned active fear response" (Amorapanth et al., 2000). An action on the active avoidance response is a well-established indicator of anti-psychotic-like activity (Wadenberg and Hicks, 1999). Collectively, our results suggest that haloperidol, a typical APD blocking dopamine D_2 receptors, selectively disrupts *active* avoidance response but has little or even an enhancing effect on conditioned *reactive* fear. Anxiolytic chlordiazepoxide, an agonist on benzodiazepine/GABA complex receptor system, inhibits conditioned *reactive* fear at doses that have no effect on *active* avoidance consistent with its exclusive sedative hypnotic profile. Clozapine and olanzapine, the multi-receptor blocking atypical anti-psychotics, have inhibitory effects on both types of fear responses, indicating a dual efficacy against both fear and psychosis. It would be interesting to explore other APDs and anxiolytics and see whether they conform to these dissociations.

The present study has several limitations. First, we did not examine the dose–response effect of clozapine or haloperidol. Only one dose for each of these drugs was tested, though we attempted to explore this issue with olanzapine. This issue will be addressed in the next study. It is well-known that the same drug can have quite different behavioral effects at different dosage levels (Murphy and Feldon, 2000). It is thus possible that haloperidol might even show an anxiolytic activity when tested at a lower dose. However, because our haloperidol dose is considered to be clinically relevant in terms of its effect on dopamine D_2 occupancy and on avoidance responding (Kapur et al., 2003b), it could be said that at least haloperidol has little anxiolytic effect at the clinically relevant doses. Second, we did not examine how sensitive and reliable our models are in comparison to other established animal models of fear and anxiety such as elevated plus maze. Third, we only examined how anti-psychotic treatment affects the *acquisition* of conditioned fear, not its retention and extinction.

In summary, the present study demonstrates that atypical APDs such as clozapine and olanzapine do possess a certain degree of anxiolytic efficacy. This additional efficacy is not attributable to its superior anti-psychotic effect or its favorable effects on motor functions or learning and memory processes. The findings also suggest that the combined use of passive avoidance and active avoidance conditioning models can be useful in better differentiating typical, atypical anti-psychotics and anxiolytics. To some extent, this study clarifies certain confusions in the literature regarding the intrinsic anxiolytic property of both typical and atypical anti-psychotics.

Acknowledgments

This study was funded in part by a support from the Nebraska Tobacco Settlement Biomedical Research Development Funds, UNL Faculty Seed Grant, and NIMH (R21MH079894) to ML and in part by a support from Canada Research Chair program to SK. The authors wish to thank Jun Parkes for technical assistance and John Weishahn for assistance with Experiment 1.

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