Neuropsychopharmacology (2006), 1–10

www.neuropsychopharmacology.org

Time Course of the Antipsychotic Effect and the Underlying Behavioral Mechanisms

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Antipsychotic drugs work for patients only when given repeatedly. The overall temporal pattern of symptom improvement is not clear. Some recent data question the traditional 'delayed-onset' hypothesis and suggest that the onset of antipsychotic response may be relatively early, and the improvement may grow with repeated treatment. The present study systematically examined the time course of the antipsychotic effect and the underlying behavioral mechanisms using a conditioned avoidance response (CAR) model. Rats repeatedly treated with either typical (haloperidol) or atypical (olanzapine, risperidone) antipsychotics, but not anxiolytics (chlordiazepoxide), show an early-onset, progressive across-session decline in avoidance responding, which re-emerges when the treatment is stopped. This effect is dose-dependent, transferable between antipsychotics, and cannot be attributed to simple sedation or motor side effects. Furthermore, we found that the pattern of this drug-induced decline depends on the number of exposures to the conditioned stimulus in the presence of the drug, and is best understood as the result of drug-induced attenuation of the reinforcing effectiveness of the conditioned stimulus. We also found that repeated drug exposure can create a drug interoceptive state that allows the attenuated reinforcing property of the stimulus to be maintained over time. Together, these data provide preclinical support for the newly postulated 'early-onset' hypothesis, and suggest that the repeated antipsychotic CAR model may be useful for understanding the neurochemical and behavioral mechanisms underlying the clinical effects of antipsychotics in patients with schizophrenia.
Neuropsychopharmacology advance online publication, 31 May 2006; doi:10.1038/sj.npp.1301110

Keywords: haloperidol; olanzapine; risperidone; conditioned avoidance response; time course of antipsychotic effect; drug interoceptive state

INTRODUCTION

Antipsychotics have now been in clinical use for over half a century, and their clinical potencies correlate with their ability to block dopamine D_2 receptors (Seeman, 2000). One interesting phenomenon is that although the stable dopamine D_2 receptor blockade can be achieved within hours after drug administration (Nordstrom *et al*, 1992; Tauscher *et al*, 2002), substantial improvement of symptoms is usually seen 2–3 weeks later. This apparent lag in the manifestation of symptom improvement is perplexing. Traditionally, it has been thought that the onset of antipsychotic response is delayed for 2–3 weeks after beginning drug treatment (Gelder *et al*, 2000), so priority is given to studying neurobiological changes that emerged after a delay (Bunney and Grace, 1978). This has led to a focus on various late-onset phenomena such as delayed depolarization (Grace, 1992), delayed onset of neuroplasticity (Konradi and Heckers, 2001), and others (Stein and Wise, 1971; Knight, 1982).

Recently, this long-held idea of delayed onset has been questioned by several converging clinical observations (Agid *et al*, 2003; Kapur *et al*, 2005; Leucht *et al*, 2005). Agid *et al* (2003) examined 42 double-blind, comparator-controlled studies (>7000 patients) using a meta-analysis technique, and found that psychotic symptoms improved within the first week of treatment and showed a progressive improvement over subsequent weeks, with the overall pattern of improvement approximating an exponential curve. Other studies show that the onset occurs within the first day, contemporaneous with the blockade of dopamine receptors (Kapur *et al*, 2005), and that more improvement occurs in the first few days than in any other later period of equal duration (Leucht *et al*, 2005). The time course of the antipsychotic action is thus still an unsettled central issue in psychiatry, which warrants further investigation because of

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Received 6 February 2006; revised 23 March 2006; accepted 25 April 2006
Online publication: 28 April 2006 at http://www.acnp.org/citations/Npp042806060074/default.pdf

its widespread scientific and clinical implications. The present study was designed to investigate this issue using a well-established preclinical animal model of antipsychotics—conditioned avoidance response (CAR) model.

We chose the CAR model because it shows high predictive validity for antipsychotic activity (Wadenberg and Hicks, 1999). All currently available antipsychotics selectively disrupt avoidance responding without altering unconditioned escape response and their effects in this test correlate positively with their clinical potencies (Arnt, 1982; Wadenberg *et al*, 2001). To better model clinical condition of antipsychotic treatment, which requires medications to be taken repeatedly for a prolonged period of time, in the present study, we used a repeated antipsychotic treatment regimen and tested animals throughout the entire course of treatment. In a series of experiments reported here, we first demonstrated that a repeated-treatment conditioned avoidance response model can be used to examine the time course of the antipsychotic effect (when the antipsychotic effect starts, what the overall pattern of this effect looks like, and when relapse occurs after drug withdrawal) (Experiments 1 and 2) and then used this repeated treatment model to identify the behavioral mechanisms underlying this pattern of antipsychotic response (Experiments 3–6). Our results suggest that antipsychotics may suppress avoidance responding by (a) decreasing the reinforcing property of stimuli and (b) providing an internal drug cue that allows the decreased reinforcing property of stimuli to be maintained over time. Correspondingly, we speculate that antipsychotics may exert their therapeutic effects in the clinic through a dual action: (a) selectively attenuating the reinforcing property of psychotic thoughts or perceptions and (b) creating a drug interoceptive state that allows the attenuated reinforcement of psychotic thoughts or perceptions to be maintained over the treatment period.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats, weighing 250–325 g upon arrival (Charles River, Montréal, Canada), were housed two per cage, in $48.3 \times 26.7 \times 20.3$ cm transparent polycarbonate cages (Lab Products Inc., Seaforth, DE, USA) under 12-h light/dark conditions with light on at 2000 hours. Room temperature was maintained at $21 \pm 1^{\circ}$C with a relative humidity of 55–60%. Food and water were available *ad libitum*. Rats were allowed at least 1 week of habituation to the animal facility before being used in experiments. All procedures were performed during the dark phase of the light–dark cycle and were approved by the animal care committee at the Centre for Addiction and Mental Health, Canada.

Apparatus

Six 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 $\times$ 35.56 cm D $\times$ 55.88–63.5 cm H). Each box was 64 cm long, 30 cm high (from grid floor), and 24 cm wide, and was divided into two equal-sized compartments by a white PVC partition with an arch style doorway (15 cm high $\times$ 9 cm wide at base). A 4 cm high barrier was placed between the two compartments, so the rats had to jump from one compartment to the other. The grid floor consisted of 40 stainless-steel rods with a diameter of 0.48 cm, spaced 1.6 cm apart center to center, through which scrambled footshock (US, 0.8 mA) was delivered by a constant current shock generator (Model ENV-410B) and scrambler (Model ENV-412). The rat location was detected by activation of microswitches affixed at the corner of the box. Illumination was provided by a houselight (28 V) mounted at the top of right compartment. 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.

General Training/Testing Procedure

A regular training session consisted of 30 trials. Every trial started by presenting the white noise (CS) for 10 s, followed by a continuous scrambled footshock (0.8 mA, US) on the grid floor. If a subject moved from one compartment to 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 rat did not respond during the entire 20 s presentation of the shock, the trial was terminated and *escape failure* was recorded. Intertrial intervals varied randomly between 30 and 60 s.

Drugs

The injection solutions of haloperidol (5 mg/ml ampoules, Sabex Inc., Boucheville, Quebec, Canada) and chlordiazepoxide (Sigma-Aldrich, St Louis, MO) were obtained by mixing drugs with sterile water. Olanzapine (gift from Eli Lilly & Co., Indianapolis, IN) and risperidone (Sigma-Aldrich, St Louis, MO) were dissolved in 2% glacial acetic acid in distilled water. Haloperidol, olanzapine, and risperidone were administered subcutaneously (s.c.), 1 h before testing, whereas chlordiazepoxide was administered intraperitoneally, 0.5 h before testing. PET studies in human patients have suggested that a reliable antipsychotic effect of most antipsychotic drugs requires at least 65% of D_2 occupancy (Farde *et al*, 1992; Kapur *et al*, 1999, 2000). Animal research also find that D_2 occupancy at around 70% elicits CAR deficits (an indication of antipsychotic effect) (Wadenberg *et al*, 2000). The doses of drugs were thus chosen based upon rat brain D_2 receptor occupancy data (Kapur *et al*, 2003) showing that at the doses tested in this study the drugs give rise to 50–80% D_2 occupancy. The dose of chlordiazepoxide (10 mg/kg) was chosen on the basis that it is an effective dose in other aversively conditioned paradigms, such as Pavlovian fear conditioning, and passive avoidance responding (Klint, 1991; Joordens *et al*, 1998).

Experiment 1: Effects of Repeated Haloperidol Treatment (0.025 and 0.05 mg/kg) on Avoidance Responding

This experiment was designed to examine when the antipsychotic effect on the CAR starts, whether repeated haloperidol treatment could dose-dependently disrupt avoidance responding progressively across sessions, and whether a relapse-like avoidance responding recovery could be observed after the discontinuation of the drug treatment. Twenty-one rats were trained for conditioned avoidance responding for a total of 11 sessions ($\sim$2 weeks period). At the end of the training session, 16 rats reached training criterion ($>$70% avoidance in each of the last two sessions). They were randomly assigned to two groups ($n=8$) and repeatedly tested daily for 7 days. Exactly the same procedure as that used during the CAR training was employed during testing, except that 1 h before each testing session haloperidol 0.05 mg/kg or vehicle (water) was administered s.c. One day after the end of the seventh test, the vehicle group was switched to haloperidol (0.025 mg/kg), whereas the previous haloperidol 0.05 mg/kg group was tested drug-free and under the CS-only condition (no shock was presented) for another seven sessions. The CS-only condition was used to exclude any possible relearning effect caused by the presence of the US, so any recovery of avoidance responding could only be attributed to the persistence nature of this CS-elicited behavior.

Experiment 2: Effects of Repeated Olanzapine (1.0 mg/kg), Risperidone (0.2 mg/kg), and Chlordiazepoxide (10 mg/kg) Treatment on Avoidance Responding

This experiment examined whether the effects observed in Experiment 1 with haloperidol can generalize to atypical antipsychotics, but not to other psychotropic drugs such as anxiolytics. Forty-two rats were trained for conditioned avoidance responding for a total of 11 sessions. At the end of the training session, 29 rats reached training criterion ($>$70% avoidance in each of the last two sessions). They were then randomly assigned to one of four groups—risperidone 0.2 mg/kg ($n=8$), olanzapine 1.0 mg/kg ($n=6$), chlordiazepoxide 10 mg/kg ($n=7$), and vehicle ($n=8$)—and tested daily for 7 days after receiving the corresponding drug or vehicle treatment. Risperidone and olanzapine rats and half of vehicle rats received their treatments 1 h before testing, whereas the chlordiazepoxide rats and another half of vehicle rats received their treatments 0.5 h before testing. One day after the seventh drug test, all rats were tested drug-free and under the CS-only (10 s white noise) condition for 2 consecutive days to assess the re-emergence of avoidance responding.

Experiment 3: Non-Consecutive Haloperidol Treatment Intermixed with Drug-Free Re-Trainings on Avoidance Responding Decline across Sessions

This experiment examined whether simple drug accumulation across sessions contributed to the progressive effect of repeated antipsychotic treatment on avoidance responding. A periodic drug treatment regimen intermixed with several drug-free re-training sessions was used to ensure no drug accumulation. Twenty-four rats were randomly assigned to one of the three groups, each group being trained with a different CS–US interval, 6 s ($n=8$), 12 s ($n=7$), and 24 s ($n=9$), for 11 sessions. Three CS–US intervals were used to examine whether the effect of haloperidol was restrained by any specific CS–US interval. By the end of the last training session, all rats showed $>$70% avoidance criterion, except one rat in the 6 s group, which was dropped from the experiment. Four days after the last day of training, the drug testing phase started. Exactly the same procedure was employed during testing, except that 1 h before each testing session, one of three doses of haloperidol was administered, 0.03, 0.05, and 0.07 mg/kg, to all the subjects in such an order (a within-subject design). At least 4 days were allowed to elapse between each drug session, and at least one vehicle re-training session was given during that interval to maintain a high level of avoidance responding. Each dose of haloperidol (0.03, 0.05, and 0.07 mg/kg) was tested twice in two rounds (separated by two vehicle re-training sessions) with the same drug test sequence in each round. Because there was no statistical difference among any of the three CS–US interval groups, to simplify the presentation, all three groups were combined into one single group.

Experiment 4: Functional Equivalence between Haloperidol Treatment and 'Reinforcement Attenuation' on Avoidance Responding

This experiment examined whether reinforcement attenuation contributes to the antipsychotic-induced avoidance responding decline by comparing the effect of haloperidol with a behavioral technique that is known to attenuate reinforcement of the stimulus in this model (Bolles *et al*, 1971). Forty-two rats were initially trained for conditioned avoidance responding for a total of 12 sessions. Among 30 rats that reached training criterion ($>$70% avoidance in each of the last two sessions), 22 were randomly assigned to one of three groups: haloperidol 0.05 mg/kg ($n=7$), chlordiazepoxide 10 mg/kg ($n=8$), and 'reinforcement attenuation' ($n=7$, injected with water). All rats were repeatedly tested for 5 consecutive days. The haloperidol and chlordiazepoxide groups were tested in a typical training procedure (CS–US pairing), whereas the 'reinforcement attenuation' group was tested in the condition in which a brief 0.1 s shock was presented at the end of each trial regardless of whether a rat made an avoidance response or not. One day after the fifth test, all rats were tested again under the training condition for 7 days without drug (all rats were injected with the vehicle) to assess the re-emergence of avoidance responding.

Experiment 5: Effects of Number of CS Trials per Session on the Haloperidol-Induced Avoidance Responding Decline

This experiment further examined the reinforcement attenuation mechanism identified in the last two experiments. We examined whether the speed of avoidance responding decline is dependent on the number of stimulus exposures in the presence of the drug. Forty-eight rats were initially trained for conditioned avoidance responding for

a total of 12 sessions. Of 40 rats that reached training criterion (>70% avoidance in each of the last two sessions), 32 were randomly assigned to one of four groups: haloperidol-3 trials ($n=8$), haloperidol-10 trials ($n=8$), haloperidol-40 trials ($n=8$), and vehicle-40 trials groups. One day after the last training session, all rats were first tested under the CS-only condition (no shock) to assess their baseline avoidance responding (40 trials of the CS presentations). Then, they were tested under drug or vehicle for 3 consecutive days, then 48 h later (to eliminate drug accumulation), tested for another 3 consecutive days. The three haloperidol groups received the same haloperidol treatment (0.025 mg/kg, s.c., −60 min); the only difference was the number of CS trials per session (three, 10, or 40 CS presentations per session). The vehicle-40 trial groups were tested after receiving vehicle treatment. At 48 h after the last drug test (a total of six drug tests was given to assess the across-session decline effect), all groups were tested after receiving 0.025 mg/kg haloperidol treatment in a 40 trials CS-only session.

Experiment 6: Effects of Prior Haloperidol Treatment on Avoidance Responding under Olanzapine, Chlordiazepoxide, or Vehicle

This experiment examined whether repeated antipsychotic treatment produces a drug internal state that allows animals to 'remember' the attenuated reinforcing property of the stimulus in the CAR model. Fifty-four rats were initially trained for conditioned avoidance responding for a total of 11 sessions, of which 44 reached training criterion (>70% avoidance in each of the last two sessions). They were then randomly assigned to one of two groups, haloperidol ($n=30$) and vehicle ($n=14$), and tested daily after receiving either haloperidol (0.03 mg/kg) or vehicle treatment for 7 consecutive days. At the end of this first test phase, the haloperidol group was then randomly subdivided into four groups: haloperidol–vehicle (water, $n=7$), haloperidol–haloperidol (0.03 mg/kg, $n=7$), haloperidol–olanzapine (1.0 mg/kg, $n=8$), or haloperidol–chlordiazepoxide (10 mg/kg, $n=8$). All groups were then tested daily for 5 consecutive days under the new drug treatment regimens. The vehicle group was subdivided into two groups that either continued on the vehicle treatment (vehicle–vehicle, $n=8$) or switched to olanzapine (vehicle–olanzapine, 1.0 mg/kg, $n=6$). Haloperidol and olanzapine rats and half of vehicle rats received their treatments 60 min before testing, whereas chlordiazepoxide rats and another half of vehicle rats received their treatments 30 min before testing.

Statistical Analysis

The percent of avoidance responding trials (number of avoidance responses/30 × 100%) was calculated as the main dependent variable. Data 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 ('Drug') and the within-subject factor being the test sessions ('Session'). Two-group comparisons were tested using *post hoc* LSD tests. To determine the temporal course of the drug effect and to pinpoint when significant differences appeared, one-way ANOVAs (>3 groups) or independent-samples *t*-tests (two groups) were conducted for each test time point, followed by *post hoc* LSD tests to compare the group differences if necessary. Once significant interaction between 'Drug' and 'Session' was found, paired-sample *t*-tests were used to determine across-session differences within a group. A conventional two-tailed level of significance at the 5% level was required.

RESULTS

Experiment 1: Effects of Repeated Haloperidol Treatment on Avoidance Responding

Haloperidol dose-dependently disrupted avoidance responding starting on the first day of treatment and this effect increased across test sessions (Figure 1a and b). For the first eight sessions (one pre-drug and seven drug test sessions), a repeated measure ANOVA showed a significant effect of 'Drug' ($F_{(1,14)}=72.356$, $p=0.000$), 'Session' ($F_{(7,98)}=23.251$, $p=0.000$), and a significant 'Drug' × 'Session' interaction ($F_{(7,98)}=19.862$, $p=0.000$). Figure 1b also indicates that avoidance responding re-emerged when the haloperidol treatment was stopped, even though there was no shock (only the white noise) present at this stage. A repeated measure ANOVA confirmed this observation (for the main effect of 'Drug', $F_{(1,14)}=11.628$, $p=0.004$; the main effect of 'Session', $F_{(6,84)}=2.465$, $p=0.030$; a 'Drug' × 'Session' interaction, $F_{(6,84)}=13.129$, $p=0.000$).

Experiment 2: Effects of Repeated Olanzapine or Risperidone Treatment on Avoidance Responding

Rats repeatedly treated with either olanzapine (1.0 mg/kg) or risperidone (0.2 mg/kg) showed a progressive, across-session decline in avoidance responding. In contrast, rats treated with chlordiazepoxide (10 mg/kg) or vehicle maintained a high level of avoidance responding throughout the entire testing period (Figure 1d). An ANOVA using 'Drug' as a between-subjects factor and 'Session' as a within-subjects factor showed a significant main effect of 'Drug' ($F_{(3,25)}=15.008$, $p=0.000$), 'Session' ($F_{(7,175)}=21.904$, $p=0.000$), and a significant 'Drug' × 'Session' interaction ($F_{(21,175)}=8.799$, $p=0.000$). *Post hoc* two-group comparisons showed that the olanzapine and risperidone groups were significantly different from the vehicle and chlordiazepoxide groups (all p's <0.015), which did not differ from each other ($p=0.937$). Similar to the haloperidol-treated rats in Experiment 1, rats that were treated with olanzapine and risperidone reinstated their avoidance responding in just two sessions when the drug treatments were stopped (Figure 1c and d). Paired samples *t*-tests indicated that for both olanzapine and risperidone groups, avoidance responding percentages on the second drug-free test day were not significantly different from their pre-drug levels ($p=0.064$ and 0.081, respectively).

Experiment 3: Non-Consecutive Haloperidol Treatment Intermixed with Drug-Free Re-Trainings on Avoidance Responding Decline across Sessions

The data from the three CS groups were combined into one group because this factor was not statistically significant on

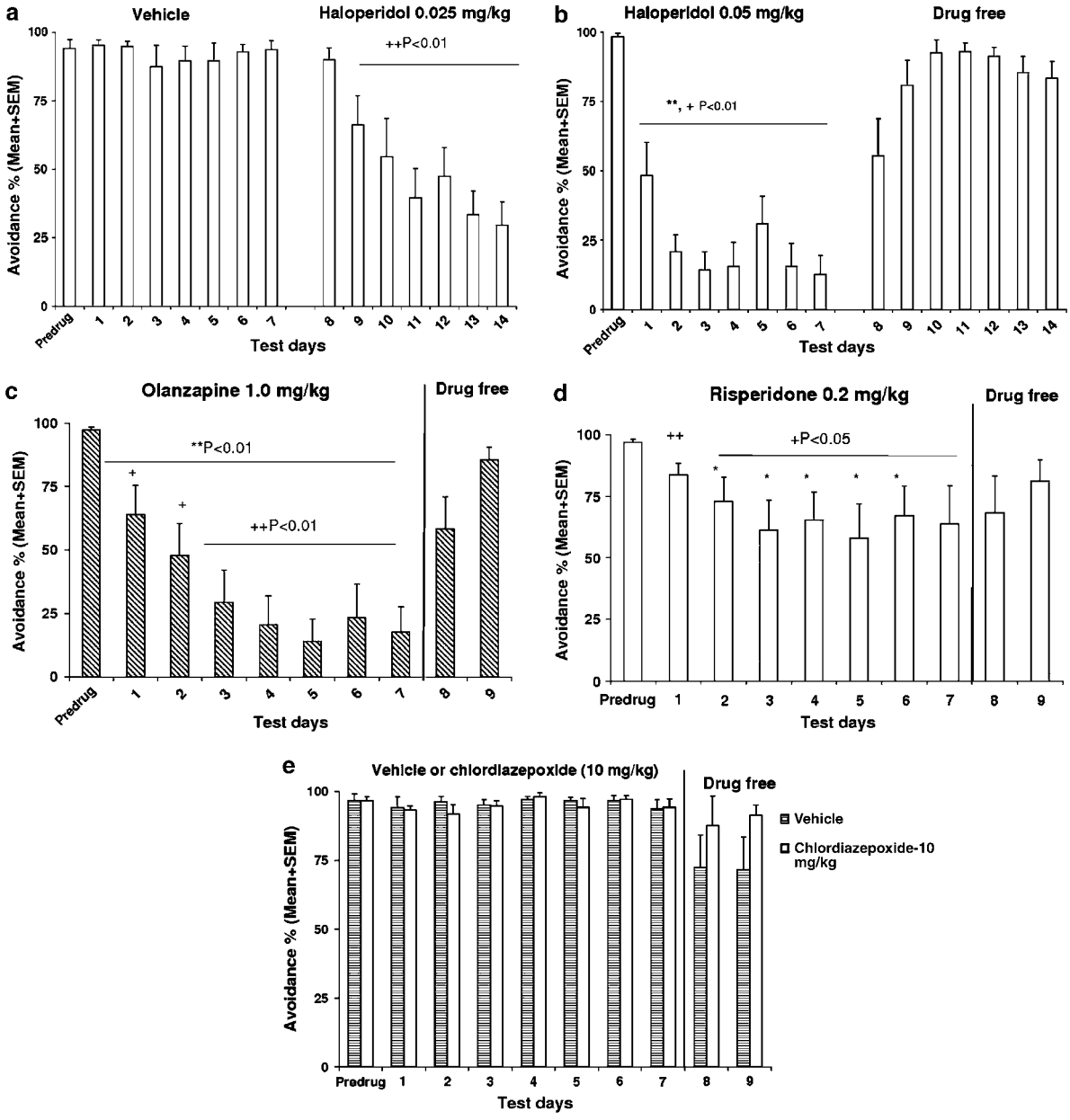

Figure 1 Effects of repeated antipsychotic treatments on conditioned avoidance responding. Each point represents mean avoidance percent + SEM. Repeated haloperidol (0.025 and 0.05 mg/kg, s.c., −60 min) (a and b) or olanzapine (1.0 mg/kg, s.c., −60 min) (c), or risperidone (0.2 mg/kg, s.c., −60 min) (d) treatment significantly disrupted avoidance responding across the seven daily test sessions. Throughout the sessions, the disruptive effect was enhanced. Avoidance responding re-emerged once the treatment was stopped, even when no shock was presented. Repeated chlordiazepoxide treatment had little effect on avoidance responding (e). $^{*}p<0.05$, $^{**}p<0.01$ for comparisons between the vehicle (or no treatment group) and antipsychotic treatment groups (haloperidol, olanzapine, or risperidone). $^{+}p<0.05$, $^{++}p<0.01$ for comparisons between the pre-drug (baseline) and each drug test session.

either drug or vehicle test sessions, nor was its interaction with other factors significant (all p's >0.1). As can be seen from Figure 2a, avoidance responding was dose-dependently decreased by haloperidol and the mean percent avoidance was also decreased progressively across the test sessions. These observations were confirmed statistically in that the effect of drug dosage was highly significant, $F_{(2, 40)} = 175.14$, $p<0.001$, as was the effect of repeated drug testing sessions, $F_{(2, 40)} = 134.10$, $p<0.001$. Within each haloperidol dose, the group difference between the two rounds of drug tests was also significant (all p's <0.05). During the intervening vehicle days, the high avoidance performance was maintained (ranging from the lowest 88% to the highest 96%). Statistically, avoidance performance during these days was not significantly different from that of the pre-drug day (Figure 2a, inset), except on the post-0.05 and post-0.07 days in the first round of haloperidol testing (paired sample t-tests, $p=0.011$ and 0.003, respectively).

The mean numbers of avoidance in each 10-trial block on the drug test days are shown in Figure 2b. First, it is evident that haloperidol had a much stronger effect on the last block than on the first, and this within-session deterioration of avoidance responding was apparent in both rounds of the

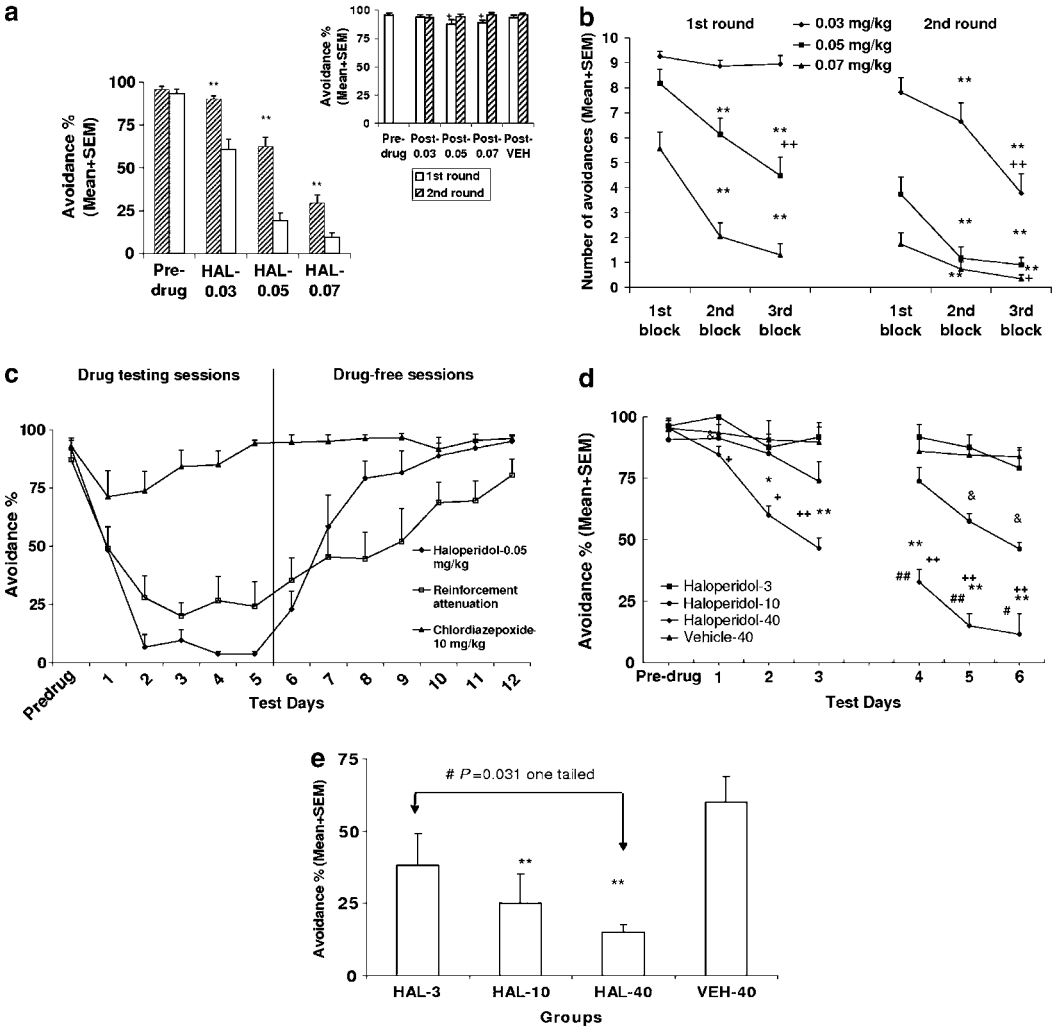

Figure 2 Haloperidol attenuates the reinforcing property of the CS. (a) Avoidance responding was dose-dependently decreased by repeated haloperidol treatment. The magnitude of disruption was always larger in the second test than in the first one. Inset: avoidance % during the drug-free sessions. $^{*}p<0.05$, $^{**}p<0.01$: first round *vs* second round. Inset: avoidance performance during the intervening vehicle test days. $^{+}p<0.05$ for comparisons between the pre-drug (baseline) and each vehicle test session. (b) Under each dose of haloperidol, a clear dose-dependent within-session decline was seen. $^{*}p<0.05$, $^{**}p<0.01$: the first block *vs* other blocks. $^{+}p<0.05$, $^{++}p<0.01$: the second *vs* third block. (c) Both haloperidol and 'reinforcement attenuation' treatment, but not chlordiazepoxide, produced a similar pattern of avoidance responding decline, as well as avoidance responding re-emergence. (d) Haloperidol (0.025 mg/kg, s.c., −60 min) had differential effects on the speed of avoidance responding decline, depending on the number of the CS exposures per session. The 40-trial haloperidol group showed a faster decline than other haloperidol groups. (e) The 40-trial haloperidol group (HAL-40) still showed significantly lower number of avoidance responses than the 3-trial group (HAL-3) when they were tested 48 h later in a 40-trial CS session. $^{*}p<0.05$, $^{**}p<0.01$ for between-group comparisons. $^{+}p<0.05$, $^{++}p<0.01$ for baseline comparisons. $^{\#}p<0.05$, $^{\#\#}p<0.01$ for comparisons between the haloperidol-40 and haloperidol-3 group. $^{\&}p<0.05$ for comparisons between the haloperidol-10 and haloperidol-3 groups.

drug treatment. Second, at each dose level, the avoidance performance at the beginning of the second test (eg first 10-trial block in the second test) was very similar to the performance at the end of the previous test (eg last 10-trial block in the first test), even though there were intervening non-drug sessions (Figure 2b). Statistical analysis confirmed these observations. We subjected these data to a four-way ANOVA with repeated measures on the drug *doses*, *blocks*, and treatment *rounds* variables and a between-subjects factor on the *groups* variable. The groups factor was not significant, $F_{(2,20)}=0.797$, $p=0.465$. However, all three within-subjects factors were: doses, $F_{(2,40)}=179.55$, $p=0.000$, blocks, $F_{(2,40)}=58.26$, $p=0.000$, and rounds, $F_{(1,20)}=136.07$, $p=0.000$. There was also an interaction between drug doses and blocks, $F_{(4,80)}=5.303$, $p=0.001$, an interaction between drug doses and rounds, $F_{(2,40)}=6.357$, $p=0.004$, and an interaction among drug doses, blocks, and round, $F_{(4,80)}=13.159$, $p=0.000$. Thus, the magnitude of haloperidol-induced avoidance decreases depended on the drug doses, trial blocks, and the treatment rounds.

Experiment 4: Functional Equivalence between Haloperidol Treatment and 'Reinforcement Attenuation' on Avoidance Responding

Figure 2c shows that both haloperidol and the 'reinforcement attenuation' groups, but not the chlordiazepoxide

group, produced a very similar pattern of avoidance responding decline, as well as re-emergence of responding when the drug, or the attenuation condition, was stopped. For the drug (or reinforcement attenuation) test sessions, a repeated measure ANOVA showed a significant main effect of 'Drug' ($F_{(2,19)} = 31.476$, $p = 0.000$), 'Session' ($F_{(5,95)} = 40.976$, $p = 0.000$), and a significant 'Drug' × 'Session' interaction ($F_{(10,95)} = 11.298$, $p = 0.000$). *Post hoc* LSD two-group comparisons showed that the haloperidol and the 'reinforcement attenuation' groups did not differ from each other ($p = 0.116$), but were significantly different from the chlordiazepoxide group ($p = 0.000$). During the drug-free test sessions, both haloperidol and 'reinforcement attenuation' groups gradually reinstated their avoidance responding. They did not differ significantly from each other ($p = 0.099$).

Experiment 5: Effects of Number of CS Trials per Session on the Haloperidol-Induced Avoidance Responding Decline

As can be seen from Figure 2d, despite their identical drug histories, the 40-trial haloperidol group showed a faster decline than other haloperidol groups, and the 10-trial group declined faster than the 3-trial group. A repeated measure ANOVA (4 × 7) using 'Drug' (4 : 1 vehicle + three levels of CS trials) as a between-subjects factor and 'Session' as a within-subjects factor showed a significant main effect of 'Drug' ($F_{(3,28)} = 14.574$, $p = 0.000$), 'Session' ($F_{(6,168)} = 29.127$, $p = 0.000$), and a significant 'Drug' × 'Session' interaction ($F_{(18,175)} = 6.058$, $p = 0.000$). *Post hoc* two-group comparisons showed that the haloperidol-40 trial group was significantly different from all other groups (all p's < 0.003), and the haloperidol-10 trial group was significantly different from the haloperidol-3 trial and vehicle groups (all p's < 0.044), which did not differ from each other ($p = 0.831$).

After 48 h, all groups were tested again in a 40-trial session after being injected with haloperidol 0.025 mg/kg. Once again, the 40-trial haloperidol had significantly lower avoidance responding than the 3-trial group ($p = 0.037$, one-tail; Figure 2e).

Experiment 6: Effects of Prior Haloperidol Treatment on Avoidance Responding under Olanzapine, Chlordiazepoxide, or Vehicle

In the first phase, well-trained rats first received either repeated haloperidol or vehicle treatment and were tested for 7 consecutive days. The haloperidol group showed an orderly decline in avoidance responding, whereas the vehicle showed no change (a significant 'Drug' × 'Session' interaction, $F_{(1,42)} = 207.996$, $p = 0.000$). The groups with previous haloperidol experience (in the first phase) continued to show the suppressed avoidance responding when switched to olanzapine or continued on haloperidol in the second phase (Figure 3a). In contrast, the haloperidol-treated group that was switched to chlordiazepoxide showed re-emergence of avoidance responding (Figure 3b) as did the haloperidol subgroup switched to the vehicle. A repeated measure ANOVA showed a significant main effect of 'Drug' ($F_{(5,38)} = 11.419$, $p = 0.000$) and a significant

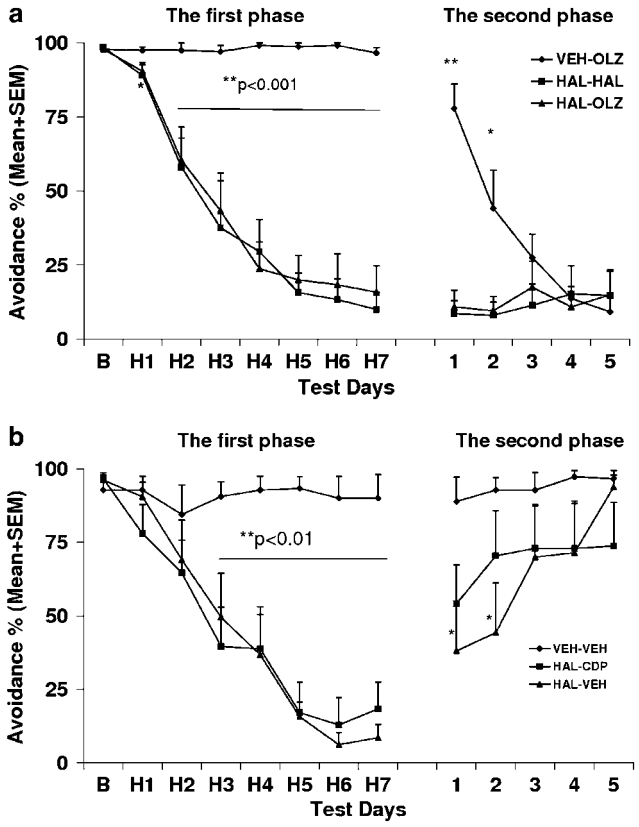

Figure 3 Prior antipsychotic experience influences subsequent antipsychotic experience. In the first phase, avoidance responding was progressively decreased by repeated haloperidol treatment. In the second phase, olanzapine (1.0 mg/kg, s.c., −60 min) disrupted avoidance responding significantly more in the rats with previous haloperidol experience than those without (a). In the second phase, the previous haloperidol-treated group still showed decreased avoidance responding even 2 days after the last drug treatment (b). 'HAL', haloperidol; 'OLZ', olanzapine; 'VEH', vehicle; 'CDP', chlordiazepoxide. *$p < 0.05$, **$p < 0.01$ for comparisons between the vehicle group and antipsychotic treatment groups (haloperidol or olanzapine) on the basis of independent-samples *t*-test.

'Drug' × 'Session' interaction ($F_{(20,152)} = 11.744$, $p = 0.000$), but not a significant main effect of 'Session' ($F_{(4,152)} = 1.163$, $p = 0.330$). *Post hoc* two-group comparisons showed that the haloperidol–olanzapine group did not differ from the haloperidol–haloperidol group ($p = 0.933$), but was significantly different from the haloperidol–vehicle, haloperidol—chlordiazepoxide, and vehicle–vehicle groups (all p's < 0.002). The haloperidol–chlordiazepoxide group did not differ from the haloperidol–vehicle ($p = 0.694$) or vehicle–vehicle group ($p = 0.083$).

DISCUSSION

In this series of sequential experiments, we showed that suppression in avoidance responding by repeated antipsychotic treatment exhibits an early onset and a progressive increase. This effect is observed with both typical and atypical antipsychotics, but not with other sedatives or anxiolytics such as chlordiazepoxide. It is dose-dependent, and as in the clinical conditions, the animals 'relapse' when taken off the drug. The increase of the effect over time does

not reflect drug accumulation or a motoric fatigue, but instead is most compatible with a drug-induced facilitation on extinction of behaviors (eg the progressive enhanced decline in avoidance responding). We identified two mechanisms that contribute to this effect: a drug-induced attenuation in the (*negative*) reinforcing property of the conditioned stimulus; and a 'memory-like' mechanism that allows the attenuated reinforcing property of the stimulus to be carried over from one drug session to another.

As it has traditionally been assumed that the onset of antipsychotic action is 'delayed', the preclinical studies of antipsychotics fall into two camps. Hundreds of studies have examined the *acute* effects of antipsychotics in a number of paradigms ranging from amphetamine-induced hyperlocomotion, the catalepsy and paw test to prepulse inhibition, latent inhibition and social interaction (Arnt, 1982; Ellenbroek *et al*, 1987; Hoffman and Donovan, 1995; Sams-Dodd, 1999; Swerdlow *et al*, 2000; Weiner, 2003). In general, most of these models have high predictive validity for antipsychotic effect (eg conditioned avoidance response, catalepsy test, paw test, etc) (Arnt, 1982; Ellenbroek *et al*, 1987; Hoffman and Donovan, 1995). Some may possess certain degrees of face and neuropsychological construct validity (eg amphetamine-induced prepulse inhibition deficit and latent inhibition deficit, phencyclidine-induced social interaction deficit, etc) (Johansson *et al*, 1995; Sams-Dodd, 1998; Weiner, 2003), or neurobiological construct validity (eg neonatal hippocampal lesions, genetic models, etc) (Lipska, 2004). However, none of these models provides a good modeling of the time course of the antipsychotic effect owing to the nature of the acute single injection regimen, nor could they model the relapse. On the other hand, models that have used chronic treatment regimens such as 'depolarization block' (Grace and Bunney, 1986), antipsychotic-induced Fos or FosB expressions (Hiroi and Graybiel, 1996), chronic prepulse inhibition model (Andersen and Pouzet, 2001) have often examined behavioral or physiological changes *after* a certain period of treatment has elapsed (eg $\sim$21 days after the first drug administration), instead of *during* the chronic treatment period. Thus, they are limited in tracking the changes that occurred along the treatment period.

There are, however, a few early CAR studies in the 1980s that had used a repeated treatment schedule and tested animals throughout the treatment period. Fregnan and Chieli (1980) found that the anti-avoidance effect of haloperidol started on the first testing day and was progressively enhanced with each subsequent drug administration (across-session decline in avoidance responding). It reached a maximum level within 5–8 days (Fregnan and Chieli, 1980). Kuribara and Tadokoro (1981) and Beninger *et al* (1983) confirmed this finding and extended it to two other classes of antipsychotics, YM-08050, YM-08051 and pimizode, respectively. Using a home-cage control group injected with drugs but not tested repeatedly for avoidance responding, they also showed that the across-session decline in avoidance responding was not because of accumulation of the drugs with repeated dosing (Kuribara and Tadokoro, 1981; Beninger *et al*, 1983). The present study not only confirmed the across-session enhancement effect with typical antipsychotics, but also extended it to atypical drugs such as olanzapine and risperidone. It further demonstrated that anxiolytic chlordiazepoxide does not possess this property, and the drug-decreased avoidance responding can re-emerge if the drug treatment is discontinued, providing a novel model mimicking a relapse-like phenomenon in the clinic. More importantly, our work highlights two behavioral mechanisms that could provide a plausible link between the neurochemical effects of antipsychotics on the dopamine system, their observed behavioral effects in the animal model presented here, and their clinical effects on psychosis.

It has been shown previously that the effects of antipsychotics in the CAR model are dependent upon D_2 blockade in the nucleus accumbens (Wadenberg *et al*, 1990). One prominent function of dopaminergic transmission in this region is to mediate the reinforcing property of stimuli in the control of behavior (Berridge and Robinson, 1998). It has long been recognized that when a neutral stimulus is paired with an aversive outcome (eg shock), the stimulus itself can acquire conditioned aversive qualities—such that it now can motivate and reinforce instrumental behavior that leads to its termination (Miller, 1948; McAllister and McAllister, 1971). Several findings from our experiments suggest that the effect of antipsychotics on avoidance responding is most likely owing to attenuation of this reinforcing ability of the aversively conditioned stimulus. First, haloperidol caused a within-session decline in avoidance responding (Figure 2b), suggesting that the CS was gradually losing its reinforcing ability under the influence of drug. A within-session decline has often been used as evidence supporting the reinforcement attenuation effect of dopamine antagonists (Fouriezos *et al*, 1978; Dickinson *et al*, 2000). Second, one behavioral technique (Experiment 4; Figure 2c) that is known to attenuate reinforcement of the CS in this model (Bolles *et al*, 1971) produced a pattern of avoidance responding decline, as well as recovery, very similar to that produced by haloperidol. Other (unpublished) data from our lab indicate that this behavioral technique can even substitute for haloperidol in maintaining decreased avoidance responding, indicating a functional equivalence between haloperidol and 'reinforcement attenuation'. Finally, the haloperidol-induced avoidance responding decline was not dependent on simple drug exposure, nor on the repeated exposure to the CS, but most critically, on the number of exposures to the CS in the presence of the drug. Together, these data indicate that the progressive antipsychotic effect may reflect the ability of antipsychotics to attenuate the reinforcing ability of the CS—a position compatible with the finding in the literature that rats previously treated with antipsychotics still show significantly lower avoidance responses even in the absence of drug (Li *et al*, 2004).

However, a simple attenuation of reinforcement would not be enough to explain how avoidance responding progressively declined across sessions or how this low performance on drug survived intervening drug-free high-performance sessions. These data implicate a drug-state-dependent 'memory-like' mechanism that allows animal to 'remember' the attenuated reinforcement across multiple drug sessions. This mechanism is likely driven by the interoceptive state caused by the antipsychotics (Schechter and Cook, 1975; Overton, 1979). A similar mechanism was implicated by Wise *et al* (1978) in an appetitive condition-

ing paradigm where they found that pimozide dose-dependently decreased the lever-pressing for food progressively across four drug test sessions, despite normal responding on drug-free test days inserted between drug tests (Wise *et al*, 1978). In the context of our experiments, it can be argued that antipsychotics may provide an internal drug state that allows the animals to 'recall' (without implying any cognitive or conscious recall) the diminished reinforcing property of the CS across sessions. We would thus speculate that antipsychotic drugs, by blocking the dopamine system, may dampen the (aberrant) reinforcing effectiveness of stimuli that the patient is experiencing. This may lead to the almost immediate halt of the generation of new psychotic material, and allows for the gradually progressive extinction of the psychotic symptoms. As the diminished reinforcement of the conditioned stimuli is dependent upon the presence of the drug, so long as treatment is continued the attenuated reinforcement persists. Because antipsychotics do not eradicate the psychotic constructs from memory bank (Miller, 1987), once the drug treatment is stopped, the same psychotic symptoms return—not dissimilar to the return of the aversive conditioned responding on discontinuation of antipsychotics in this animal model.

We should point out several limitations with the current report. First, in this study, the CAR model was simply used as a behavioral preparation for the identification of antipsychotic action. Whether it can be used to identify drugs with novel mechanisms other than dopamine receptor blockade, and how avoidance responding, presumably an adaptive behavior, relates to psychotic symptoms were not addressed. Second, because our experiments designed to identify the behavioral mechanisms of antipsychotics were carried out primarily by using haloperidol, whether the same mechanisms are also responsible for the effects of atypical antipsychotics such as clozapine or quietapine has not been tested and is still an open question. Because of the unique receptor binding profile associated with each antipsychotic drug (Kapur and Remington, 2001), it is possible that some antipsychotics may work differently via different neurochemical mechanisms (eg 5-HT_{2A}, α_2 adrenoceptors). Finally, the subjects used in this study were normal rats, whereas antipsychotics are usually used to treat patients with schizophrenia. The possibility that these two functionally different populations might respond differently to antipsychotic treatment may limit the generalization of our conclusion. Future research needs to address these issues.

The present study has several scientific and practical implications. First, it provides an animal behavioral model that captures several characteristic features along the time course of antipsychotic treatment in the clinic (early-onset, progressive accumulation, asymptote and drug-discontinuation relapse). Thus, this animal preparation can be used to study the neurobiological mechanisms that underpin various stages of antipsychotic effect in patients. Second, today's antipsychotics are still less than optimal (Lieberman *et al*, 2005)—the full effect still emerges slowly and not at all for some patients. The model developed here may be of empirical use for testing add-on therapies or new drugs that may increase the speed of progression or final asymptote of antipsychotic drugs. Finally, the behavioral mechanisms identified in animals (reinforcement attenuation, and drug state acting as 'memory' cue) may provide a set of new targets for drug development and evaluation.

ACKNOWLEDGEMENTS

Dr Ming Li was supported by a fellowship from Ontario Mental Health Foundation. Dr Shitij Kapur is supported by Canada Research Chair program. We thank Jun Parkes for helping with Experiments 1 and 4.

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