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Pharmacology, Biochemistry and Behavior 78 (2004) 811–819

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Evaluation of the motor initiation hypothesis of APD-induced conditioned avoidance decreases

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Received 30 September 2003; received in revised form 19 April 2004; accepted 27 May 2004
Available online 17 July 2004

Abstract

Antipsychotic drugs (APDs) selectively disrupt conditioned avoidance responding (CAR)—a feature that distinguishes them from all other psychotropics. It is thought that this effect reflects their effect on motor initiation; however, this conclusion is questionable because most studies it relies on have often examined avoidance responding under APD treatment, and tested animals with preshock stimuli followed by the footshock. APD-induced CAR effects are confounded by APDs' motor effects and by the presence of footshock. The objective of this study was to evaluate the motor initiation hypothesis by testing animals without drug and under extinction conditions. In Experiment 1, we administered haloperidol, clozapine or chlordiazepoxide (an anxiolytic as a pharmacological control) during the acquisition phase of CAR, but tested animals 2 days later. The APD-induced CAR disruption was present even in the absence of the drug and footshock. In Experiment 2, we first trained rats to a learning criterion, and then subjected them to 4 days of CAR extinction training under drug or vehicle. In the subsequent CAR extinction tests, the rats previously treated with APDs still showed significantly lower avoidance responses. In both experiments, the effects of haloperidol and clozapine were distinct from those of chlordiazepoxide. These data suggest that APD-induced CAR decreases cannot be explained as the unconditioned motor impairment effects of APDs, but probably reflect a dopamine-blockade-mediated change in incentive motivation.

Keywords: Haloperidol; Clozapine; Chlordiazepoxide; Conditioned avoidance response; Rat

1. Introduction

It is well established that animals treated with low doses (noncataleptic) of antipsychotic drugs (APDs) fail to acquire or perform avoidance responses to an aversive stimulus [unconditioned stimulus (US), usually footshock], whereas their escape responses to that stimulus are not affected (Ader and Clink, 1957; Arnt, 1982; Cook and Catania, 1964; Cook and Weidley, 1957; Courvoisier, 1956; Davidson and Weidley, 1976; Ponsluns, 1962). This selective disruption of avoidance but not escape responses is a characteristic pharmacologic effect of all APDs, including new atypical APDs, such as clozapine, olanzapine, risperidone and others (Moore et al., 1992; Sanger, 1985; Taboada et al., 1979; Wadenberg et al., 2001; White et al., 1992). This feature has been effectively used to differentiate APDs from other classes of psychotropic drugs (e.g., anxiolytics and antidepressants, which lack this selectivity on avoidance versus escape responses); to predict APDs' clinical potencies [potencies in the conditioned avoidance responding (CAR) test correlate with clinical potencies]; and to identify potential APDs (Arnt, 1982; Bignami, 1978; Cook and Davidson, 1978; Janssen et al., 1965; Kuribara and Tadokoro, 1981; Shannon et al., 1999; van der Heyden and Bradford, 1988; Wadenberg and Hicks, 1999). Therefore, understanding the nature of APD-induced avoidance decreases may shed light on the behavioral mechanism of antipsychotic action in the treatment of psychosis in schizophrenic patients.

One commonly invoked explanation suggests that APDs disrupt animals' ability to *initiate* voluntary motor responses

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doi:10.1016/j.pbb.2004.05.023

to the conditioned stimuli (CS) signaling shock (Aguilar et al., 2000; Anisman et al., 1982; Beninger et al., 1980c; Fibiger et al., 1975; Ogren and Archer, 1994; Ponsluns, 1962). According to this account, animals treated with APDs fail to avoid because they are deficient in their ability to initiate responses to CS. However, they still can escape the electric footshock because the shock induces unconditioned innate reflexive motor responses which are sufficient for them to run. Thus, Ponsluns (1962) found that lengthening the preshock interval from 5 to 15 s increased the number of avoidance responses made in chlorpromazine-treated rats. He also found that animals treated with chlorpromazine for 2 days failed to acquire CAR. However, when these animals were subsequently tested without chlorpromazine, on the first test day, they performed as well as animals given saline throughout the training period and significantly better than naïve saline-treated animals on the first day of training. A similar finding was reported by Fibiger et al. (1975) with haloperidol. In addition, Fibiger et al. (1975), Beninger et al. (1983) and Anisman et al. (1982) all reported that prior avoidance training significantly attenuated the disruptive effect of APDs, possibly reflecting a lack of effect of APDs on a more reflexive, automatic, synthesized type avoidance responding in well-trained animals.

However, these results are difficult to reconcile with other observations. For example, Davis et al. (1961) first trained rats to escape footshock in the shuttle box, then confined them in one side of the box and exposed them to 15 trials of the buzzer–footshock pairing after a treatment with either chlorpromazine or saline. When tested without drug, the previously chlorpromazine-treated rats made significantly less avoidance responses to the buzzer (not followed by shock) than the saline rats (Davis et al., 1961). Beninger et al. (1980a,b) demonstrated that when animals were pretrained in the avoidance task before being tested with pimozide, the drugs failed to disrupt avoidance responses, at least on the first administration. Avoidance responding showed a gradual decline with repeated drug testing. Clearly, these observations suggest that the motor impairment is not the only, and perhaps also not the *critical* action of APDs on avoidance.

The motor initiation hypothesis is largely based upon the traditional ways of studying APDs in CAR, which have several problems. One problem is that the majority of the studies have examined CAR effects when the APDs were still in the system (Ader and Clink, 1957; Wadenberg and Hicks, 1999), making it difficult to separate the unconditioned motor effects of APDs from their effects on other processes, such as learning or motivational processes. All of these studies tested animals in a session in which CS was followed by the footshock, which allows continued CAR learning, obscuring the APD effects on the initial acquisition of CAR and making it difficult to pinpoint the effects of APDs on avoidance responding.

The primary purpose of the present study was to further investigate the motor initiation hypothesis using a two-way CAR task by studying the effects of APDs on the acquisition (Experiment 1) and extinction (Experiment 2) of CAR. We employed a design in which the training (including both acquisition training and extinction training) and testing phases were separate. In Experiment 1, three groups of animals were trained under haloperidol (typical APD), clozapine (atypical APD) or chlordiazepoxide (an anxiolytic) treatment and one group without drug for the acquisition of CAR, then their avoidance responding was compared in a later undrugged and extinction test session, i.e., shocks were no longer presented. In Experiment 2, we examined the effects of APDs on CAR extinction. Animals were first trained to a learning criterion (70% CAR in last two consecutive training sessions). Then, they were exposed to four CAR extinction training sessions under either drug or vehicle and later tested without drug in six additional extinction sessions. Because animals were tested in the absence of drug, if animals previously treated with APDs exhibited impaired CAR during test, it would suggest that APD-induced CAR decreases cannot be attributed to motor impairments induced by APD. To differentiate the specific antipsychotic effects from those of sedation/anxiolysis, and to better understand the exact behavioral processes uniquely affected by APDs but not by anxiolytics, in the present study, we included a typical and atypical antipsychotic (haloperidol and clozapine) as well as an anxiolytic (chlordiazepoxide).

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats, weighing 275–325 g upon arrival (Charles River, Montréal, Canada) were housed two per cage, in 48.3 × 26.7 × 20.3 cm transparent polycarbonate cages (Lab Products, Seaforth, DE, USA) under 12-h light/dark conditions with lights on at 8:00 p.m., and off at 8:00 a.m. Room temperature was maintained at 21 ± 1 °C with a relative humidity of 55–60%. Food and water was available ad libitum. Animals 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.

2.2. 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 × 35.56 cm D × 55.88–63.5 cm H). Each box was 64 cm long, 24 cm high (from grid floor) and 30 cm wide, and divided into two equal-sized compartments by a white PVC partition with an arch-style doorway (15 cm high × 9 cm wide at

base). The grid floor consisted of 40 stainless steel rods with a diameter of 0.48 cm, spaced 1.5 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.

2.3. Drugs

Haloperidol, 5 mg/ml ampoules (Sabex Boucheville, Quebec, Canada), clozapine (Anawa Biomedical Services and Products, Zurich, Switzerland), and chlordiazepoxide (Sigma, St. Louis, MO) were used. Haloperidol and clozapine were administered subcutaneously, and chlordiazepoxide was administered intraperitoneally. The injection solutions of haloperidol and chlordiazepoxide were obtained by mixing drugs with sterile water. Clozapine was dissolved in 1% glacial acetic acid in sterile water. The doses of haloperidol (0.05 mg/kg) and clozapine (20 mg/kg) were chosen based upon a pilot study that found that these doses of haloperidol and clozapine selectively disrupted the avoidance responses, but had no effect on escape responses. 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. Experiment 1: Effects of haloperidol, clozapine and chlordiazepoxide on the acquisition of CAR

The first experiment examined the effects of APDs on the acquisition of CAR. After 2 days (15 min/day) of habituation to the CAR boxes, four groups of rats were trained in a 40-trial CAR session/day for 3 days. Before each daily training session, animals were injected with haloperidol ($n=20$, 0.05 mg/kg), clozapine ($n=20$, 20 mg/kg), chlordiazepoxide ($n=20$, 10 mg/kg) or vehicle ($n=45$, water). Forty-eight hours later, they were tested for their avoidance responses without drug and shock. A total of 105 rats were used in this study, they were tested in six batches, with a requirement that each batch contained at least four randomly assigned vehicle controls. The motor initiation hypothesis predicts that haloperidol- and clozapine-treated rats would show impaired CAR during the three training sessions, but not during the test when the drugs are no longer present.

2.4.1. CAR training (under drug)

Every trial started by presenting the CS for 10 s, followed by a continuous scrambled footshock (0.8 mA, US, max. 20 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 20-s presentation of the shock, the trial was terminated and an *escape failure* was recorded. Intertrial intervals varied randomly between 30 and 60 s. Each training session lasted about 30–40 min with a total of 40 trials presented.

2.4.2. CAR test (without drug)

Forty-eight hours after the last CAR training, all rats were tested for their avoidance response to the CS under extinction condition (i.e., without shock presentations). The CS was presented as 15 s of white noise. The number of trials was 40. Once again, an avoidance response was recorded when a subject moved from one compartment into the other within the first 10 s of CS presentation.

It is conceivable that if a rat failed to escape during the training phase, it might develop "learned helplessness" which may have "antipsychotic-like" effect on avoidance response (Friedhoff et al., 1995). To control for this factor, it is critical that rats with substantial escape failures (>5%) be excluded from analysis. Only 4 out of 20 rats in the haloperidol group and none from other groups merited exclusion and their avoidance data on the testing day were not analyzed.

2.5. Experiment 2: Effects of haloperidol, clozapine and chlordiazepoxide on the extinction of CAR

The second experiment examined the effects of APDs on the extinction of an established CAR. The basic procedure was adopted from Miller et al. (1957b). All rats were first trained for 10 consecutive days (20 trials/day). Those which attained at least 70% of avoidance responses in the last two training days were further subjected to 4 days of extinction training under drug or vehicle. Then, their avoidance responses were examined in six additional extinction test sessions without drug. A total of 108 rats was used in this study. They were run in three replications (each $n=36$). The motor initiation hypothesis predicts that haloperidol- and clozapine-treated rats would show impaired CAR during the four extinction training sessions, but not during the extinction test sessions when the drugs are no longer present.

2.5.1. CAR training

Each daily CAR training session included 20 trials. The CS and US were the same as used in Experiment 1, but a progressive reduction of the CS duration and the intertrial

interval during training phase was employed. This method has been shown to produce a rapid and stable avoidance response which is highly resistant to extinction (Miller et al., 1957b). The white noise (CS) was decreased from 10 s (Days 1–2), to 8 s (Days 3–4), and to 5 s (Days 5–10). The intertrial interval was decreased from 45 s (30–60 s, Days 1–2) to 40 s (25–55 s, Days 3–4), then to 30 s (20–40 s, Days 5–6), and to 20 s (10–30 s, Day 7–10). At the end of 10 days of training, only those rats which displayed avoidance responses on 70% of trials in the last two training days were included in the remaining experiment. A total of 57 rats achieved this criterion, and they were randomly assigned to five groups: haloperidol—0.05 mg/kg ($n=12$), clozapine—20.0 mg/kg ($n=12$), chlordiazepoxide—10.0 mg/kg ($n=12$), VEH-ext ($n=12$), and VEH-no-ext ($n=9$). The first four groups underwent 4 days extinction training under drug or vehicle, whereas the VEH-no-ext group was returned to the home cage after 4 daily vehicle injections.

2.5.2. CAR extinction training (under drug)

Beginning 1 day after the last CAR training session, rats received 4 daily sessions of 20 extinction trials in which the 5-s CS only was presented. Sessions began 90 min after haloperidol or vehicle injection, or 30 min after clozapine, chlordiazepoxide or vehicle injection. Half of the rats in VEH group underwent extinction training 90 min after vehicle injection, and another half 30 min after injection. All extinction trials were run with an average intertrial interval of 20 s (10–30 s).

2.5.3. CAR extinction test (without drug)

Twenty-four hours later, all rats (including VEH-no-ext rats) were given 20 extinction trials every day for six consecutive days. The procedure was the same as in the previous extinction training.

2.6. Statistical analysis

The avoidance response and escape failure were computed and presented as a median percentage of the total trials in each session. Because a decrease in avoidance performance was always accompanied by a corresponding increase in escapes, the percent escape response was omitted. The median escape latencies for each group were computed instead. The overall group comparisons were analyzed using the nonparametric Kruskal–Wallis tests because the Levene test suggested nonhomogeneity of variance between groups. Where overall significant effects were found, two group comparisons between the drug group and vehicle group or between two drug groups were performed using the Mann–Whitney U test. Within-group comparisons across days were performed using Friedman test (for more than three related samples) or Wilcoxon Signed Ranks Test (for two related samples). Significance was accepted at $P<.05$.

3. Results

3.1. Experiment 1: Effects of haloperidol, clozapine and chlordiazepoxide on the acquisition of CAR

3.1.1. Avoidance

Fig. 1A shows the median percentage of avoidance responses in the four groups trained under the drug or vehicle on the three acquisition days. As can be seen, there were significant differences among the groups across three training days ($P<.001$). Compared to vehicle, haloperidol and clozapine *inhibited* avoidance ($P<.001$), whereas chlordiazepoxide *enhanced* avoidance ($P<.004$). Fig. 1B presents the median percentage of avoidance responses in the four groups tested without drug and in the absence of footshock 48 h after the last training session. Once again, the groups treated with haloperidol and clozapine exhibited a significantly lower percentage of avoidances in comparison to the vehicle (P=.008 and 0.024 for haloperidol and clozapine, respectively), whereas the chlordiazepoxide group did not differ from the vehicle group (P=.83).

3.1.2. Escape

Table 1 shows the median group escape latencies. All the drugs examined in the present study significantly increased the escape latencies, with haloperidol showing the largest effect. Specifically, rats treated with haloperidol had significantly longer escape latencies than the vehicle rats on every

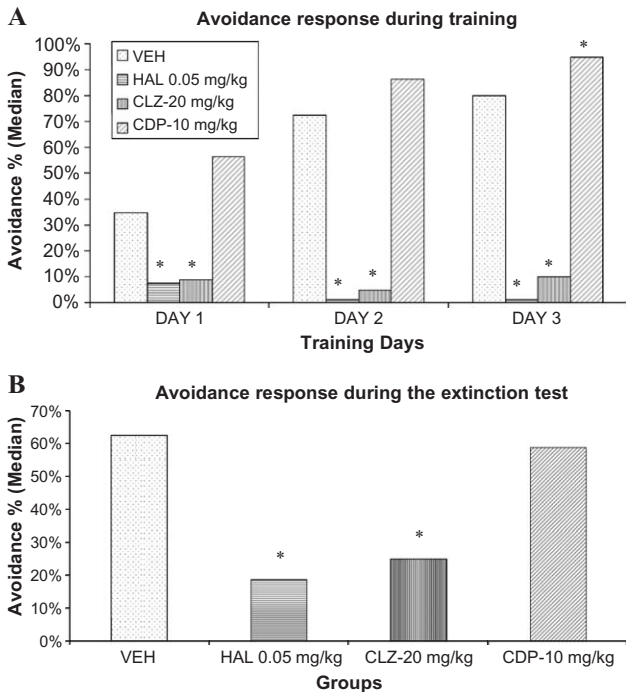

Fig. 1. Median percentage of avoidance responses in four groups of rats trained under the haloperidol, clozapine, chlordiazepoxide or vehicle treatment on three acquisition days (A) and on the undrugged test day (B) in Experiment 1. * $P<.05$ compared with the vehicle group.

Table 1
Effects of haloperidol, clozapine, chlordiazepoxide, or vehicle treatments on the escape response latency

Escape latency (second + interquartile range)	Vehicle	HAL-0.05 mg/kg	CLZ-20 mg/kg	CDP-10 mg/kg
Day 1	0.92 (0.51)	1.10* (1.73)	1.07 (0.60)	1.14* (0.50)
Day 2	0.65 (0.60)	1.41* (1.55)	1.06* (0.67)	0.91* (0.47)
Day 3	0.66 (0.64)	2.02* (3.16)	0.88* (0.86)	0.76 (0.37)

Median escape latency (± interquartile range) in four groups of rats trained under the haloperidol, clozapine, chlordiazepoxide or vehicle treatment on three acquisition days in Experiment 1. * $P < .05$ compared with the vehicle group.

* Significantly different compared to the vehicle group ($P < .05$) using Mann–Whitney U test.

training day ($P < .025$). Clozapine and chlordiazepoxide also significantly slowed down the escape responses, but only on Days 2 and 3, and Days 1 and 2, respectively.

3.2. Experiment 2: Effects of haloperidol, clozapine and chlordiazepoxide on the extinction of CAR

3.2.1. Avoidance responses during CAR extinction training under drug

At the end of 10 training sessions, the median avoidance percentages for haloperidol, clozapine, chlordiazepoxide, VEH-ext, and VEH-no-ext groups were 95%, 90%, 92.5%, 95%, and 95%, respectively, and they did not differ from each other ($P = .38$). On the subsequent four extinction training days when drug was administered, as shown in Fig. 2, significant differences between the groups emerged. Both haloperidol and clozapine significantly decreased avoidance responses compared with the vehicle treatment, whereas chlordiazepoxide had little effect. Specifically, haloperidol decreased avoidance responses significantly on the second, third, and fourth days (all $Ps < .02$), whereas clozapine significantly decreased avoidances on every extinction day (all $Ps < .02$). In contrast, chlordiazepoxide had little effect on the avoidance responses except on the second day ($P = .017$), when the vehicle group showed a greater improvement on CAR.

3.2.2. Avoidance responses during CAR extinction testing without drug

Fig. 2 also presents the avoidance percentages in the five groups on the six extinction test days. First, the VEH-ext group and VEH-no-ext group were compared to see whether the previous 4 days of extinction training had any effect on the performance of avoidance responses. Results show that the VEH-ext group had significantly lower percentages of avoidance responses than the VEH-no-ext group on the fifth and sixth days ($Ps < .042$), suggesting that prior extinction training did facilitate to some extent the extinction of avoidance responses.

Secondly, the VEH-ext group was compared to each drug group to examine how prior drug treatments affect animals' avoidance responses when tested without drug. This showed that both haloperidol and clozapine suppressed avoidances, reaching significant levels on the first 2 days (all $Ps < .04$). The haloperidol and clozapine rats still showed lower avoidances on last four testing days; however, the differences were not statistically significant. Chlordiazepoxide had no effect on any of the days (all $Ps > .21$).

Thirdly, the VEH-no-ext group was compared to each drug group to examine the combined effects of prior extinction training and drug treatment on avoidance

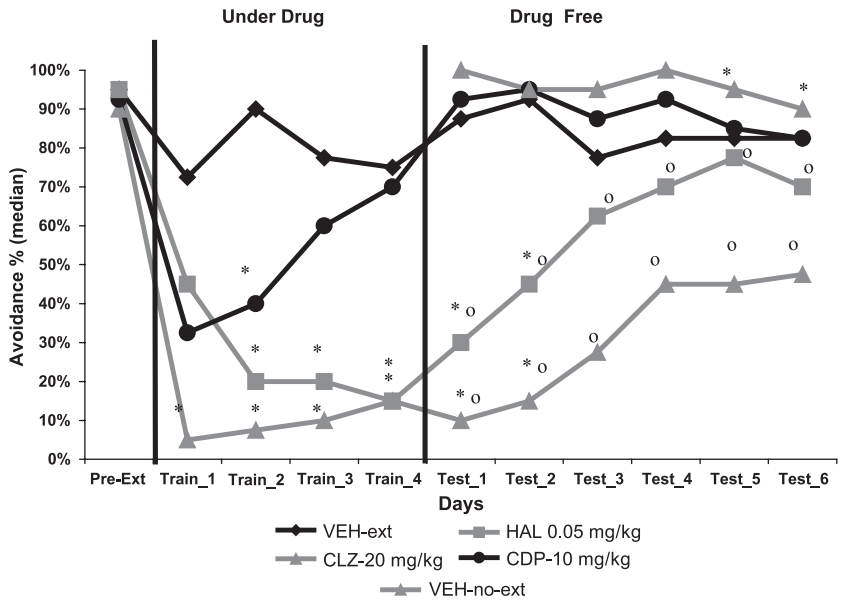

Fig. 2. Median percentage of avoidance responses on the last CAR acquisition training day (pre-ext), during four CAR extinction training days (Train 1 to Train 4), and during the six extinction test days (Test 1 to Test 6) in Experiment 2. * $P < .05$ compared with the vehicle group; $^{o}P < .05$ compared with the vehicle-no-ext group.

responses. The haloperidol and clozapine groups, but not the chlordiazepoxide group, had significantly lower avoidance responses on every extinction test day (for haloperidol, all Ps < .019; for clozapine, all Ps < .01; for chlordiazepoxide, all Ps > .11).

Finally, within-group comparisons over the course of the six extinction test sessions for each group were performed. Results indicated that only the clozapine group showed a significant improvement in avoidance responses (P=.007). Although the haloperidol group also showed some improvement, it failed to reach a significant level (P=.082).

4. Discussion

4.1. The motor initiation hypothesis and other hypotheses

The present results confirmed the selective antiavoidance property of APDs when administered during CAR acquisition or extinction training. The results further show that haloperidol and clozapine significantly impaired CAR even when animals were tested 2 days after the last training session without drug and under extinction conditions. This suggests that the actions of APDs on CAR cannot be accounted for by an effect of APDs on motor initiation or general motor function. The motor initiation explanation of CAR effects also has difficulty in accounting for why the chlordiazepoxide-treated rats, similarly impaired in the escape running speed as the haloperidol- and clozapine-treated rats, still displayed much higher avoidance responses than the antipsychotics-treated rats, and even higher than the vehicle controls. The opposite result should be expected according to the motor initiation hypothesis. This finding further reinforces that not all CNS chemicals with motor impairment properties have this selective antiavoidance feature. Miller et al. (1957b) found that 40 mg/kg of phenobarbital sodium impaired the motor movement to the same extent as 1.2 mg/kg of chlorpromazine did; however, the level of avoidance behaviors in the chlorpromazine-treated rats was markedly depressed, while that of the phenobarbital group was unaffected (Miller et al., 1957a). It should be noted that these findings can only be viewed as indirect evidence arguing against the motor initiation hypothesis because the motor impairment induced by chlordiazepoxide or phenobarbital sodium is a different type than those produced by haloperidol and clozapine, and this difference on motor function may explain their differences on CAR. Nevertheless, these findings show that the simple motor hypothesis cannot adequately account for the CAR decreases produced by APDs.

Few studies in the literature have used experimental conditions similar to ours—but comparisons are still possible. Beninger et al. (1980a) and Aguilar et al. (2000) found that rats trained under pimozide or clozapine, respectively, when tested later undrugged, avoided shock significantly less than the vehicle-treated ones. These findings are similar in principle to what we observed in Experiment 1, although they tested rats in a session in which CS was followed by the footshock; thus, their results were confounded by the CS–US relearning effect. Using a similar paradigm as those used in our second experiment, Miller et al. (1957b) found that chlorpromazine significantly facilitated the extinction of avoidance response; we found that this facilitative effect with haloperidol and clozapine lasted only 2 days. This difference might be drug specific (chlorpromazine vs. haloperidol and clozapine) or might be due to the differences in experimental procedures (e.g., 20 trials/day vs. 15 trials/day, etc.). It should be noted that our results are consistent with the well-known fact that this avoidance behavior, once acquired, is very resistant to extinction and can persist for a prolonged period of time, even years (Rescorla and Solomon, 1967; Seligman and Johnston, 1973).

As mentioned in the Introduction, Ponsluns (1962) found that animals treated with chlorpromazine for 2 days failed to acquire CAR. However, when these animals were subsequently tested without chlorpromazine, on the first day, they performed as well as animals given saline throughout the training period and significantly better than naïve saline-treated animals on the first day of training. A similar finding was reported by Fibiger et al. (1975) with haloperidol. This kind of results was not observed in the present study (Fig. 1B). Our results indicated that haloperidol- and clozapine-treated rats had significantly lower CAR percentages than the vehicle ones on the test day. When the avoidance data of the haloperidol and clozapine rats on the test day were compared to those of the vehicle rats on their first CAR training day, the haloperidol rats still showed significantly lower avoidance responses (P=.006), whereas the clozapine rats showed marginally lower CAR (P=.054, one tailed). Ponsluns (1962) also found that lengthening the preshock interval from 5 to 15 s increased the number of avoidance responses made in chlorpromazine-treated rats and he used this finding as supporting evidence for the motor initiation hypothesis. In a recent unpublished study, we trained rats on either 5 s CS or 15 s CS and we did not see that lengthening the CS from 5 to 15 s had any beneficial effect on avoidance responding. Haloperidol (0.05 mg/kg) had a similar effect on both groups of rats. It should be noted that both Ponsluns (1962) and Fibiger et al. (1975) used a one-way CAR paradigm, in which one compartment always serves as the safe area and the other as the danger area. The two-way CAR as used in our study requires animals to run from one compartment to another on one trial, and back to the previous danger compartment on the next trial. Because it involves an ‘‘emotional conflict’’: animals are required to make a directional response incompatible with one that previously proved successful, the acquisition of the two-way CAR is much slower than in the one way, but once acquired, it tends to slower to extinguish too (Anisman, 1978). This paradigm difference may explain the different results in these reports.

Some other experimental results are also pertinent to the discounting the motor initiation hypothesis. Amalric and

Koob (1987) examined the performance of rats trained in an operant reaction-time task and found that disruption of dopamine activity in the nucleus accumbens did not affect the performance, whereas lesions of the dopamine terminals of the nigrostriatal pathway in the corpus striatum did. These results suggest that dopamine in the corpus striatum has a role in the *initiation* of complex goal-directed responses (Amalric and Koob, 1987). However, the corpus striatum is not a critical brain site for APD-induced CAR suppression, whereas the nucleus accumbens is (Wadenberg et al., 1990). These findings also cast doubt on the simple motor initiation explanation.

The second experiment added another piece of evidence which was incompatible with the motor initiation hypothesis. It also served as a test of other possible explanations that could be used to account for the results from Experiment 1. For example, it is plausible that haloperidol and clozapine might have changed animals' perception of the white noise and footshock during the training phase, such that animals under these drugs had learned CAR less efficiently (impairment in learning the CS–US association). In Experiment 1, animals treated with haloperidol and clozapine made less avoidance responses during the training so they experienced less CS–response pairings, leading to the possibility that the drugs' effects may be related to their effects on CS–response association (impairment in learning the CS–response association). Results of the second experiment refuted these possibilities. Haloperidol and clozapine impaired CAR even in well-trained animals, after they had acquired the CS–US and CS–response associations. This clearly shows that APDs' effects on CAR cannot be accounted for solely in terms of the impairments in learning the CS–US or CS–response associations. Because animals never experienced APDs together with the footshock, it also cannot be said that APDs disrupted CAR by changing animals' perception of the footshock.

Similar findings that APDs do not impair learning the CS–US association or change the perception of the US have been reported before (Anisman et al., 1982; Beninger et al., 1980a,b,c; Harvey and Gormezano, 1981). Beninger et al. (1980b) reported that rats treated with pimozide failed to acquire the avoidance response, but they showed a significant decrease in their bar pressing for food when the tone (previously used in the avoidance training) was presented, suggesting that they learned the CS–shock relation. Anisman et al. (1982) found that prior treatment with pimozide during the CS–shock training did not influence the performance enhancement on avoidance responding to the CS in a later undrugged test, thus the formation of the CS–shock association seems not be disrupted by pimozide. Harvey and Gormezano (1981) found that haloperidol retarded the acquisition of a classical conditioning of the rabbit nictitating membrane response (NMR) and suggested that its effect was specific on the performance, not on learning the CS–shock association because this effect was evident even in well-trained animals. They also found that haloperidol had no effect on the amplitude of the NMR elicited by the 3-mA shock, and no effect on the amplitude of the unconditioned NMR elicited by various intensities of the shock, suggesting that the haloperidol's effect on the acquisition of conditioned NMR could not be attributed to either an alternation in the sensory processing of the US or in the motor response system of the NMR.

4.2. Incentive motivation account of APD-induced CAR decreases

If APD-induced CAR decreases cannot be attributed to impairments in the animals' sensory, motor ability, or learning ability to acquire the CS–US, or CS–response association, to what can they be attributed? One possibility is that animals' incentive motivation to actively respond to the CS is dampened by APDs. This idea is consistent with the incentive motivational theory of dopamine (Beninger, 1989a,b; Berridge and Robinson, 1998; Kapur, 2003; Salamone and Correa, 2002). According to this theory, DA is centrally involved in attributing incentive salience to objects or behavioral acts. This attributed salience then facilitates approach (to positive reinforcers) or avoidance (to negative reinforcers) responses. Accordingly, antagonism of DA receptor function not only influences the assignment of incentive salience but also inhibits the use of this salience to elicit actions (Ikemoto and Panksepp, 1999; Salamone and Correa, 2002). In CAR, it is possible that a previously neutral stimulus (white noise in this study) that is presented in close temporal contiguity with the US acquires the motivational property of US through an incentive salience attribution process and becomes a CS. As a CS, it can activate the motor response that is usually elicited by the US. As DA antagonists, APDs may dampen the incentive salience of the CS, i.e., decrease the ability of the CS to elicit active motor response to terminate the CS and avoid the US (Beninger, 1989b; Berridge and Robinson, 1998). Thus, it seems that an incentive motivation account may provide a better explanation for the CAR effect.

In this account, we emphasize the effect of APDs on the incentive salience of the CS, its ability to facilitate active motor approach (to positive reinforcers) or avoidance (to negative reinforcers) responses. One remaining issue is whether APDs also disrupt the process of attribution of incentive salience itself in CAR. Results from Experiment 1 cannot give us a definite answer because the CAR decreases by haloperidol and clozapine can be explained by suggesting that they inhibited the attribution of incentive salience, resulting lower incentive attributed to the CS. However, in Experiment 2, all rats were well trained before they were given APDs; presumably, the attribution process of incentive salience had finished before the administration of APDs. Still, the APD-treated rats showed lower avoidance responding, suggesting that an APD does not have to affect the attribution of incentive salience per se to produce its effect on CAR. It is, in our opinion, still an open question

whether APDs do disrupt the attribution process in the CAR paradigm.

In our account, the concept of ''incentive salience'' refers to the ability of a stimulus to elicit active, voluntary motor responses, while the ''incentive motivation'' refers to the motivation instigated by a salient stimulus that drive organisms to actively pursue a goal or goals (reward or safety). Thus, the incentive motivation inherently encompasses a complex motor component which is context-dependent or stimulus-evoked response initiation. This conceptualization of incentive motivation is very similar to that of Salamone and Correa (2002), who suggest that low doses of DA antagonists do impair activational aspects of motivation, making animals less likely to engage in instrumental responses to obtain rewards. This idea that APDs specifically dampen incentive motivation is also consistent with evidence showing that APDs have no effect on passive avoidance, in which animals are not required to make an overt motivated response (Bignami, 1978).

The incentive motivation account not only can explain the APD-induced CAR decreases but also can be extended to explain the APD effects on Pavolvian conditioning. For example, Harvey and Gormezano (1981) found that haloperidol retarded the acquisition of a classical conditioning of the rabbit NMR and they suggested that its effect was largely due to the drug-induced suppression in the excitatory or arousal (energizing) property of a stimulus being employed as a CS. According to Schultz (1992), a salient stimulus has perceptual properties that are alerting, arousing and attention grabbing (Schultz, 1992); therefore, the CS property that Harvey and Gormezano (1981) referred to, can be viewed as the perceptual aspect of incentive salience. In this sense, the APDs' effects on Pavlovian conditioning may also be attributed to their actions on the incentive salience of a stimulus.

4.3. Methodological consideration

The present study used a consecutive treatment schedule of repeated daily drug injection. This schedule could lead to presence of drug in the organism even after cessation of treatment, and might be responsible for the decreased avoidance responses observed on the testing days without drug. This possibility is discounted by the following observations: First, several studies have consistently found that drug accumulation is not an important factor for inducing an avoidance decrease—even up to 4 days of treatment (Beninger et al., 1983; Miller et al., 1957b; Sanger, 1985). If anything, it has been shown that tolerance develops with repeated injections of clozapine (Sanger, 1985), so that the disruption of avoidance responding produced by clozapine was significantly attenuated after just three prior treatments. If drug accumulation is a factor, the clozapine-treated rats should show continued deterioration in avoidance responding. Second, we recently have shown that even just 24 h following a 7 daily repeated haloperidol (0.05 mg/kg) or clozapine (15 mg/kg) injection, the brain DA D_2 receptor occupancy was minimal, suggesting no evidence of drug accumulation (Kapur et al., 2003). D_2 receptor occupancy has been shown to be the critical underlying mechanism for APD-induced CAR decreases (Wadenberg et al., 2001); therefore, the CAR decrease observed without drug is very unlikely due to the drug accumulation.

Taken together, the results indicate that APD-induced CAR decreases, especially when tested without drug, cannot be simply accounted for by the motor initiation explanation, or the deficit in learning the CS–US or CS–response association, but is more consistent with the incentive motivation hypothesis of APDs. Yet, the effects of the drugs are transient—they do not permanently alter avoidance behaviors, just suppress them for an extended period.

Acknowledgements

This study was funded by the Ontario Mental Health Foundation Postdoctoral Fellowship to ML and a support from Canada Research Chair program to SK. We also thank three anonymous reviewers for their comments on the previous draft of this manuscript.

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