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Haloperidol Prevents the Reinstatement of Amphetamine-Rewarded Runway Responding in Rats

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ETTENBERG, A. *Haloperidol prevents the reinstatement of amphetamine-rewarded runway responding in rats.* PHARMACOL BIOCHEM BEHAV **36**(3) 635–638, 1990.—Animals were trained to traverse a straight-alley once each day for a reward of 1.0 mg/kg SC d-amphetamine sulfate. After 14 days of acquisition, extinction trials were initiated in which the amphetamine reward was replaced by injections of physiological saline. After running speeds had decreased to less than one third those of preextinction values, rats received a single amphetamine-rewarded trial either in the absence or presence of haloperidol (0.075, 0.15 or 0.3 mg/kg IP). Twenty-four hours later, animals were tested for reinstatement of operant running in a single drug-free Test trial. Animals that were nondrugged during the amphetamine-rewarded trial demonstrated a statistically reliable increase in running speed on the Test trial relative to extinction baseline speeds. In contrast, animals that were under the influence of medium or high doses of haloperidol during the amphetamine-rewarded trial failed to show Test day increases in running speed. This result did not stem from some residual sedative or performance impairing quality of the drug since a "motor control group" administered a high dose of haloperidol shortly *after* a rewarded trial, was able to demonstrate unimpaired reinstatement of operant running on Test day (i.e., 24 hr later). These findings support the view that dopamine systems play a role in the neural substrates underlying the incentive motivational properties of amphetamine reinforcement.

d-Amphetamine Dopamine Drug reinforcement Haloperidol Incentive motivation Operant behavior

FOR many years the primary explanation for drug-taking behavior postulated the presence of an internal aversive "drive" or "need" state whose intensity was reduced by drug self-administration. In such theories, humans and animals were thought to self-administer drugs only to avoid or terminate the aversive consequences of withdrawal. Although this remains a popular notion [e.g., (18)], more recent research has demonstrated that animals will readily initiate and maintain drug self-administration in the absence of any measurable internal "aversive" state [e.g., (17,24)]. Indeed, most contemporary theories of drug self-administration have replaced "need" and "drive" principles with the view that drugs can in and of themselves generate a motivational state through their inherent positive (i.e., incentive) properties (22). Central to this "incentive motivation" view of drug-reinforced behavior are studies of the effects of reward "priming" on subsequent operant behavior (22). For example, drug-seeking behavior can be reinitiated after a long period of abstinence by a single administration of a rewarding "prime" (7, 8, 10). Indeed, this animal model appears to be particularly comparable to the human condition where relapse probability is extremely high even after an extended period of abstinence if the individual is reexposed to the drug or even the environmental stimuli previously associated with the drug (12,22). In such cases, it is presumed that the powerful positive incentive properties of the drug (or drug-associated stimuli) serve to activate a central motivational state which is responsible for reinstating the previously "extinguished" self-administration behavior. For some [e.g., (22)], this "central motive state" involves the same neural mechanisms that mediate the rewarding properties of self-administered drugs.

We have previously demonstrated that the response-reinstating (i.e., incentive) properties of a single food-reinforced trial delivered after a period of nonreinforced responding, is dose-dependently inhibited by pretreatment with the dopamine receptor antagonist, haloperidol (9,11). Such work suggests an important role of central dopaminergic pathways in mediating the incentive or rewarding properties of food reinforcement. In the present study, the effects of haloperidol were examined in a comparable test procedure devised to assess the role of dopamine elements as a substrate for the incentive-motivational properties of d-amphetamine.

METHOD

Subjects

The subjects consisted of 56 male Sprague-Dawley rats purchased from Charles River Laboratories and weighing between 300–350 grams at the start of the experiment. Each animal was individually housed in metal wire hanging cages located within a temperature-controlled (23°C) 12-hr light-dark vivarium environment (lights on at 0700 hr). Throughout the course of the study the animals were provided ad lib access to food and water in their home cages. The subjects were given two weeks to adapt to the

vivarium environment prior to the start of the experiment during which time they were individually handled and weighed once each day.

Apparatus

All trials were conducted in a wooden straight-arm runway (measuring 155 cm in length by 15 cm wide by 20 cm high) located within a small sound-attenuated room. A white start box ($24 \times 25 \times 20$ cm) was attached at one end of the runway and a black goal box of the same dimensions was attached at the opposite end. The floor of the apparatus consisted of wire mesh as did the removable top (put in place to prevent animals from leaving the runway). A guillotine door provided access from the start box to the runway. Lifting this door initiated the start of a trial the timing of which terminated when the animal interrupted an infrared photocell beam detecting the animal's presence in the goal box. The data for each animal on each trial therefore consisted of the time required to leave the start box, traverse the runway, and enter the goal box.

Drugs

Haloperidol (0.075, 0.15 and 0.3 mg/kg) was dissolved in a vehicle solution of 0.002 M lactic acid. Intraperitoneal (IP) injections of haloperidol and vehicle were delivered in a volume of 1.0 ml/kg of body weight 45 min prior to testing (unless stated otherwise). d-Amphetamine sulfate (1.0 mg/kg) was dissolved in a vehicle solution of 0.9% physiological saline and injected subcutaneously (SC) behind the neck in a volume of 1.0 ml/kg of body weight.

Procedure

As described for our previous work with food reward (11), the experiment was conducted in five consecutive phases: Acquisition, Extinction, Baseline trial, Treatment trial and Test trial. During all these phases running times were recorded for every animal on every trial. Since only a single runway apparatus was employed in this experiment, the work was necessarily completed over a period of several months. Sample sizes were increased gradually in a balanced fashion to ensure that all groups were represented during all phases of the study. Furthermore, subsequent analyses of the data revealed no differences in the performance of animals run "early" from those run "late" in the experiment.

Acquisition. Animals received one runway trial per day. On a given day, the animal was placed into the white start box and, after 5 sec, the start door was raised thereby initiating a trial. Once the animal reached the black goal box, the goal door was lowered (to prevent retracing), the animal was picked up, injected with d-amphetamine (1.0 mg/kg SC), and then replaced in the goal box for 30 minutes. Subjects were then returned to their home cages. This procedure was repeated one trial each day for 14 consecutive days.

The dose and route of amphetamine administration employed here were chosen because they have been repeatedly demonstrated to produce robust and reliable Conditioned Place Preferences [for a review of this literature see Carr *et al.* (6)]. To ensure that the drug was in fact rewarding, a control group of 8 Ss was treated precisely as described above except that they received SC injections of saline rather than amphetamine on each trial. After the 14th trial, the performance of each amphetamine subject was compared to an arbitrary acquisition criterion (based on pilot data) requiring that running time be under 15 sec on three of the last four consecutive trials. This was implemented to ensure that animals for whom the amphetamine did not serve as a reinforcer were not included in the study. A total of 5 animals failed to meet this criterion and were removed from the study at this point, thereby reducing the total number of subjects to 51 (eight of which were in the no-reward control group). The control subjects were only tested during the acquisition phase of the experiment; none of these 8 Ss met the acquisition criterion.

Extinction. Extinction trials began on Day 15 of the experiment. These trials were identical to those of the acquisition phase with the sole exception that animals earned no amphetamine reward upon entering the goal box. Instead, SC injections of saline were administered followed by 30 min in the goal box. Extinction trials continued for a given animal until its running behavior slowed to a point where its "goal response" was three times slower than its average goal response during the last three reinforced acquisition trials. This is comparable to the "extinction criteria" that we have already successfully employed in previous work (11). The next phase of the experiment began for an individual animal upon its meeting this arbitrary criterion on three of four consecutive trials (this required an average of 15 days/trials per subject).

Baseline trial. The Baseline trial merely represented an additional and final extinction trial from which data were used for comparison with those obtained during the next two phases of the experiment.

Treatment trial. Upon reaching the extinction criterion, animals were assigned to one of six different treatment groups: a *Vehicle-Saline Group* (VEH/SAL; $n=7$) in which animals were pretreated with the lactic acid vehicle solution 45 minutes prior to a single additional extinction (saline in goal box) trial; a *Vehicle-Amphetamine Group* (VEH/AMPH; $n=8$) where subjects were similarly pretreated with the lactic acid vehicle solution but experienced an amphetamine injection in the goal box; three *Haloperidol-Amphetamine Groups* each of which was pretreated with either 0.075 (HAL.075/AMPH; $n=7$), 0.15 (HAL.15/AMPH; $n=7$), or 0.3 mg/kg (HAL.3/AMPH; $n=7$) of haloperidol 45 min prior to a single amphetamine-rewarded trial; and an *Amphetamine-Haloperidol* "motor control" group that received the highest dose of haloperidol (0.3 mg/kg) 45 minutes *after* an amphetamine-rewarded runway trial (AMPH/HAL.3; $n=7$).

Test trial. Twenty-four hours following the Treatment trial, the time required to reach the goal box was again determined in one final extinction trial for each animal. No pre- or posttreatments were administered on test day.

RESULTS

To control for heterogeneity of variance (common when employing response-latency measures) the raw data were converted from running times (x sec) to their reciprocals, speeds (1/x sec) (5). All statistical analyses were computed on the speed data.

Figure 1 illustrates the mean ($\pm$ S.E.M.) running speed of each group across the final three consecutive days: Baseline, Treatment and Test trials. A two-factor analysis of variance (with repeated measures on one factor) was computed on the data depicted in the figure. This analysis revealed statistically reliable effects for Group, $F(5,37)=3.65$, $p<0.01$, indicating a difference in mean running speed across treatment conditions; Trials, $F(2,74)=3.21$, $p<0.05$, reflecting the general tendency of subjects to increase running speed across the final three trials; and most importantly a Group $\times$ Trial interaction, $F(10,74)=2.59$, $p<0.01$, confirming that the tendency to increase running speeds across trials differed for different treatment groups. Indeed, post hoc analyses confirmed that only the VEH/AMP, the HAL.075/AMP and the AMP/HAL.3 ("motor control") groups ran reliably faster on Test Trial compared to the extinction Baseline Trial (Tukey post hoc tests; $p<0.05$). This result can be clearly seen in Fig. 1, which

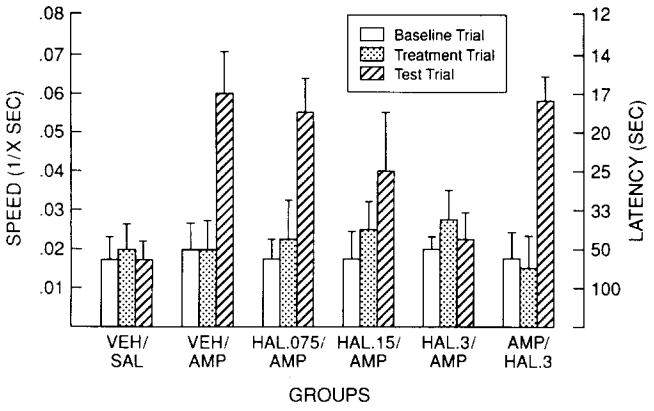

FIG. 1. Mean running speeds (±S.E.M.) for each of the six groups of rats on the final Extinction trial (i.e., Baseline), the Treatment trial and the drug-free Test trial. Note that the data are expressed as "running speeds"; hence, faster speeds are represented as higher points on the ordinate. As a frame of reference, the vertical access on the right provides the actual "running times" for each point on the ordinate. The figure illustrates that for animals whose running behavior has been extinguished, a single amphetamine-rewarded trial is sufficient to reinstate operant behavior the very next day (i.e., the VEH/AMP group). Animals that continue to experience extinction (i.e., the VEH/SAL group) continue to run slowly on Test day. Finally, rats that earn amphetamine in the presence of the haloperidol (the HAL/AMP groups) approach nonreward performance in a dose-dependent manner.

shows that the presentation of a single amphetamine-rewarded trial during extinction resulted in a reinstatement of operant running in the VEH/AMP group. Animals that continued to experience extinction conditions (the VEH/SAL group) did not demonstrate any such reinstatement in operant behavior. In addition, pretreatment with the dopamine antagonist, haloperidol (the three HAL/AMP groups), dose-dependently reversed the response-reinstating effects of amphetamine reward. The low dose (0.075 mg/kg) had virtually no antagonist effect, the high dose (0.3 mg/kg) completely reversed the effects of amphetamine, and the intermediate dose (0.15 mg/kg) produced intermediate results. Finally, the AMP/HAL.3 "motor control" group demonstrated no residual motoric or sedative effects of haloperidol and performed equivalently to nonhaloperidol animals.

DISCUSSION

The primary concerns of this experiment were to determine whether a) a single amphetamine-trial during extinction would be sufficient to reinstate operant running on the next trial (i.e., 24 hr later); b) whether pretreatments with the dopamine antagonist, haloperidol, would prevent the predicted response reinstatement (as measured the next day in nondrugged animals); and c) whether any observed effects of haloperidol in this experiment could be accounted for by some residual sedative or performance incapacity present on the Test trial 24 hr postinjection. Together, the results of this study support the hypothesis that the incentive motivational properties of amphetamine are prevented by antagonism at dopaminergic postsynaptic receptors.

Amphetamine administration during extinction (the VEH/AMP group) resulted in a dramatic reinstatement of operant runway behavior even when tested 24-hr posttrial. This result is consistent with lever-press studies demonstrating that even after prolonged periods of extinction, drug self-administration behavior can be readily reinstated by "priming" injections of the previously administered drug [e.g., (7, 8, 10)]. As suggested by Stewart *et al.* (22), such results indicate "that the presence of the drug in the body (not its absence) activates appetitive motivational mechanisms that are involved in the reinitiation of drug-seeking behavior" (p. 253). Of particular relevance here was the demonstration that the response-activating effects of amphetamine were dose-dependently reversed by the dopamine receptor antagonist haloperidol. The haloperidol results are particularly important in light of the performance of the "motor control" group (AMP/HAL.3). These animals were injected with haloperidol 45 min *after* the rewarded Treatment trial yet still were able to demonstrate dramatic increases in running speed during the subsequent Test trial. This result, which is precisely what we observed in our previous work (11), makes it highly unlikely that there were any significant residual performance deficits 24-hr posthaloperidol. It would seem then, that the attenuation in running speeds produced in the haloperidol *pre*treated (HAL/AMP) groups, cannot easily be accounted for by some sedative, aversive or motor-attenuating action of haloperidol. In fact, even on the Treatment trial, runway performance in the three HAL/AMP groups was slightly elevated rather than reduced in the presence of haloperidol (see Treatment trial scores in Fig. 1). Such a result should not be particularly surprising since it is well documented that neuroleptic-induced impairments in operant tasks are relatively weak at the onset of the test session and become progressively stronger as the session continues [e.g., see review by Wise (23)]. A one-trial-per-day testing protocol, as employed here and in our previous work, would therefore be expected to minimize the behavioral disruption produced by acute neuroleptic administration.

One explanation for the present haloperidol data might be that the drug produced a form of "state-dependent learning" [e.g., (15)] or perhaps that it interfered in some way with normal memory processes. Both such hypotheses presume that the animals continue to run slowly on Test trial due to a failure to retrieve their memory for the previous (rewarded) trial. However, in previous work we have directly tested the "state-dependency" hypothesis and found it lacking to account for the present data [see Horvitz and Ettenberg (11)], even when all animals are drugged on test day (i.e., the haloperidol Ss are in the "same" drug state as during treatment day) the relative performance of the various groups remains the same. While it is possible that haloperidol interferes with normal memory processing, there is virtually no information that we are aware of to suggest that this is the case. Quite to the contrary, there are numerous reports in the literature of learned associations acquired in the presence of neuroleptic drugs that are readily accessible when the animals are later tested in the nondrugged state [e.g., (1–4)].

Alternatively, one might argue that the relative reductions in running speeds on the Test trial results from some aversive consequence of the neuroleptic pretreatment. In this view, the rewarding properties of the amphetamine remain intact but are counteracted by some concurrent aversive action of haloperidol. This hypothesis seems unlikely for a variety of reasons. First, from a conceptual perspective, even if haloperidol pretreatments did result in some aversive state, such a state would have been present well before the Treatment trial began, thereby greatly reducing the likelihood that it would be associated with subsequent events in the goal box. Second, we are aware of no data demonstrating an aversive action of haloperidol. In fact, we have found that repeated taste- and place-haloperidol pairings fail to produce conditioned taste or place aversions, respectively (Ettenberg, unpublished data). Others have similarly failed to demonstrate conditioned aversions with haloperidol in doses ranging from 0.15 to 1.0 mg/kg (19–21). As we have indicated before (9,11) a haloperidol-induced aversive effect does not appear to be a likely explanation for the present results.

Previous studies have demonstrated that neuroleptic drugs can

attenuate the rewarding properties of amphetamine as measured in intravenous drug self-administration studies [e.g., (16, 25, 26)]. Similarly, haloperidol has been shown to interfere with the development of amphetamine-induced Conditioned Place Preferences [e.g., (13, 14, 19)]. In the present study a test procedure was employed that incorporates aspects of both self-administration and place preference methodologies: animals emit an operant response (alley-running) in order to approach a distinctive environment which has been paired with prior drug administration. In this situation operant behavior increases in strength (animals run faster) over trials suggesting that running speed is a useful index of drug reward. Indeed, when the drug reinforcer is removed (i.e., replaced with saline injections) the operant behavior does in fact weaken over trials. The presumed "incentive motivational" aspects of amphetamine are related to its ability to reinstate operant behavior with a single "prime" following a period of nonreward (22). In the present study, a single reinforced trial was sufficient to completely reactivate runway behavior 24 hr later. The demonstration that haloperidol can attenuate this aspect of amphetamine's action is certainly consistent with the view that dopamine elements are involved in mediating the rewarding (i.e., incentive) properties of this drug.

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