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Amphetamine-Haloperidol Discrimination: Effects of Chronic Drug Treatment[1]

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HAENLEIN, M., W. F. CAUL AND R. J. BARRETT. *Amphetamine-haloperidol discrimination: Effects of chronic drug treatment.* PHARMACOL BIOCHEM BEHAV **23**(6) 949–952, 1985.—Rats responding for food reinforcement were trained in a 2-lever drug discrimination task. Groups of rats were trained to discriminate one of four doses of amphetamine (0.0, 0.1, 0.3, or 0.5 mg/kg) from haloperidol (0.02 mg/kg). Both the rate of acquisition and level of discrimination at asymptote were a function of amphetamine training dose. Following acquisition of this discrimination, choice behavior was assessed in the absence of drug during two test sessions. Twenty-four hours following the second drug-free test session, chronic drug treatment commenced. Half of the animals received 10 mg/kg amphetamine for 10 consecutive days while the other half received 1 mg/kg haloperidol during the same period. Choice behavior was assessed during three 2.5-minute unreinforced drug-free test sessions 24, 48, and 72 hours following the chronic drug regimen. Following chronic haloperidol, animals responded as though a small dose of amphetamine had been administered, while following chronic amphetamine, they responded as though a small dose of haloperidol had been administered. Collectively, these results suggest that animals trained to discriminate amphetamine from haloperidol respond on the basis of a continuum of dopaminergic function. Further, this continuum can be used to elucidate the net effect of pharmacologically-induced alterations in dopaminergic function, as well as the effect of nonpharmacological manipulations that may result in dopaminergic changes.

Drug discrimination Amphetamine Haloperidol Chronic drug treatment

BASED on the opponent-process theory of motivation [10, 11, 12], Barrett [3] has recently presented evidence suggesting that the behavioral and biological effects accompanying repeated psychoactive drug use might best be understood in terms of temporally distinct primary and compensatory processes. Briefly, it is proposed that adaptive changes are induced by a drug's primary effect. These adaptive changes may be produced by several different mechanisms such as changes in receptor affinity, number and density, alterations in receptor coupling to adenylate cyclase, and/or alterations in postreceptor-coupled phenomena. In response to the drug's disruption of steady-state equilibrium, normal homeostatic, regulatory mechanisms cause a compensatory, opponent state that is opposite to the primary effect of the drug. According to this proposal, the net effect of drug administration prior to the return of steady-state levels would be diminished. This diminished drug effect is an example of pharmacodynamic tolerance. Further, the behavioral signs of withdrawal that develop following abrupt termination of drug administration reflect the opponent processes unopposed by the drug's primary effect. Drug dependence thus refers to the dependence of a system on an amount of drug sufficient to balance the compensatory processes and prevent withdrawal following chronic use of that drug. In this way, the opponent process theory accounts for drug tolerance, dependence, and withdrawal.

Using the drug discrimination procedure, it has been possible to demonstrate the opponent process following chronic administration of either haloperidol or amphetamine. The opponent effect of chronic haloperidol on dopaminergically (DA) mediated choice behavior has been demonstrated in both an amphetamine-saline [2,3] and apomorphine-saline [13] discrimination task. In both tasks, animals were trained to discriminate between the cues associated with enhanced DA function produced by either amphetamine or apomorphine and steady-state levels of DA function present following saline administration. Following chronic haloperidol treatment, both increased choice responding on the amphetamine lever during a drug-free test session and increased choice responding on the apomorphine lever during a test session immediately following an ED_{50} dose of apomorphine suggest a compensatory increase in DA function. Conversely, following chronic amphetamine treatment, decreased choice responding on the amphetamine-lever was observed during an amphetamine test session [1].

While amphetamine-saline and apomorphine-saline choice behavior have been used to assess opponent processes following both chronic haloperidol and amphetamine treatment, this procedure allows the assessment of only enhanced DA function. This is because responding on the saline lever may reflect either suppressed or unaltered DA function [5]. Thus, the evidence of potential opponent processes following chronic DA-agonists, such as amphetamine is limited within existing drug-saline discrimination proce-

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dures. Given that haloperidol and amphetamine both produce DA based discriminative cues capable of mediating choice behavior in a two-lever drug discrimination task [3, 4, 7], and that changes in these cues may be assessed independently of changes in response rate, choice behavior between the discriminative cues produced by amphetamine and the discriminative cues produced by haloperidol should provide a useful behavioral measure capable of reflecting a continuum of DA function. Thus, the purpose of the present study is first, to provide a behavioral technique whereby alterations in DA function may be assessed along a continuum of both enhanced and suppressed DA function, and second, to evaluate proposed opponent processes following chronic amphetamine and haloperidol treatment using this behavioral tool.

METHOD

Subjects

Subjects were 36 male rats bred from Sprague-Dawley (Madison, WI) stock in our laboratory. At weaning, animals were housed individually in standard metal cages with free access to lab chow and water. They were maintained on a 12 hour light-dark cycle (6 a.m.–6 p.m. light).

Apparatus

Four operant boxes (25×25×25 cm) were used. Mounted on the front panel of each box were two response levers, one to the right and a second to the left of a centrally placed pellet dispenser. Each operant box was housed within a sound attenuating chamber. White noise (72 dB S.P.L.) was present throughout testing to mask external auditory stimuli. The operant boxes were located in a room adjacent to electromechanical programming and recording equipment.

Procedure

At 73±5 days of age animals were weighed and started on a food deprivation regimen designed to reduce their weight to 85% of their starting weight. During the entire study, animals were maintained at their deprivation weights by being given powdered lab chow immediately following testing. The animals were tested at the same time on consecutive days during the light phase of the cycle.

Daily 20 minute training sessions for food reinforcement (Noyes pellets, 45 mg) began on the eleventh day after initiation of food deprivation. With only one of the two levers present, animals were trained using a schedule of continuous reinforcement to a criterion of 100 responses. After meeting this criterion on each lever, two additional sessions, one with each lever were conducted using a variable interval-20 second (VI-20) schedule of reinforcement. Nine animals were then randomly assigned to each of four training-dose groups (0.0, 0.1, 0.3, or 0.5 mg/kg d-amphetamine sulfate vs. 0.02 mg/kg haloperidol). Thirty minutes prior to each training session each animal received a subcutaneous (SC) injection of either the appropriate amphetamine dose or 0.02 mg/kg haloperidol. Animals were housed in an environment other than their home cage from the time of drug injection until testing 30 minutes later. Responses during the first 2.5 minutes of each session were unreinforced to allow assessment of discrimination behavior unconfounded by reinforcement. Correct responses were reinforced on a VI-20 schedule during the remaining 17.5 minutes of each session. Drug-appropriate lever position was counterbalanced across both drug condition and amphetamine training dose. Thirty-six training sessions were run, eighteen each of amphetamine and haloperidol. A double-alternation schedule of amphetamine-haloperidol injections was used.

Following these 36 training sessions, animals received two drug-free test sessions on consecutive days. During the first of these drug-free test sessions, animals were injected with distilled water, housed for 30 minutes in the non-home-cage environment used during training, and tested in a 2.5 minute unreinforced test session. During the second drug-free test session, animals received no injection. They were taken directly from their home cages and tested in a 2.5 minute unreinforced test session. Chronic drug treatment began 24 hours later. Half the animals were randomly assigned to be injected SC with haloperidol (1.0 mg/kg) for 10 consecutive days while the remaining animals were injected SC with amphetamine (10.0 mg/kg) for 10 consecutive days. Animals were not run in the behavioral task during chronic drug treatment. All subjects received a 2.5-minute unreinforced test session 24, 48 and 72 hours following the final chronic drug injection. No drug was given prior to these 2.5 minute unreinforced test sessions. The testing procedure on these three days was precisely the same as the drug-free testing procedure used during the test session immediately preceding chronic drug treatment. That is, animals were taken directly from their home cages and immediately tested. Choice behavior during all 2.5 minute unreinforced test sessions was determined by calculating the percent amphetamine lever responses based on a minimum of three responses.

Drugs

The drugs used in this experiment were d-amphetamine sulfate purchased from Sigma Chemical Co. and haloperidol (Haldol), in solution, purchased from McNeil Laboratories, Fort Washington, PA. Amphetamine was dissolved and haloperidol diluted in distilled water. Both amphetamine and haloperidol were administered in 1 ml/kg volume SC. The dose of d-amphetamine refers to the salt.

RESULTS

Acquisition of the d-Amphetamine-Haloperidol Discrimination

Figure 1 shows the acquisition of amphetamine-haloperidol choice behavior, expressed in terms of the mean percent amphetamine lever responding during the initial 2.5 minute unreinforced period of two consecutive amphetamine or haloperidol test sessions. As can be seen in this figure, animals learned to reliably discriminate amphetamine from haloperidol, $F(1, 32)=470.81$, $p<0.001$. Further, a significant Drug × Dose × Days interaction, $F(17,57)=32.82$, $p<0.001$, was observed indicating that the rate of acquisition of amphetamine-haloperidol discrimination was a function of amphetamine training dose. As the training dose of amphetamine increased, the rate of acquisition of the amphetamine-haloperidol discrimination also increased. Further, analysis of the percentage correct responses on individual amphetamine and haloperidol training days revealed that the four training doses of amphetamine used in this study affected not only the rate of acquisition of drug-appropriate discrimination behavior on amphetamine training days, $F(51,542)=1.96$, $p<0.001$, but also the acquisition of drug-appropriate responding on haloperidol training days, $F(51,519)=1.54$, $p<0.05$. Discrimination behavior at asymp-

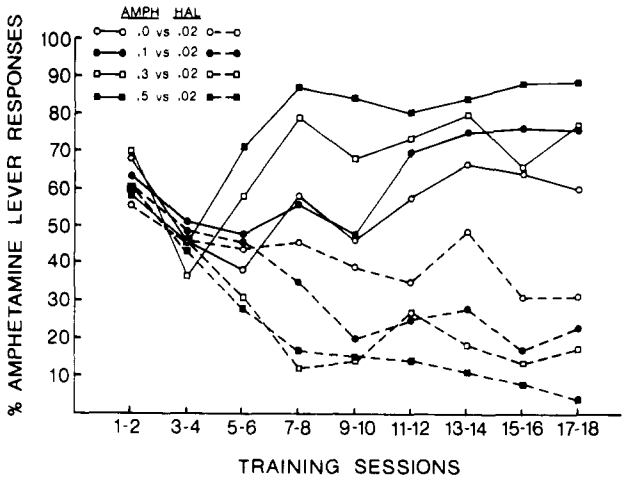

FIG. 1. Percent responding on the amphetamine lever during the unreinforced 2.5 minutes of training sessions.

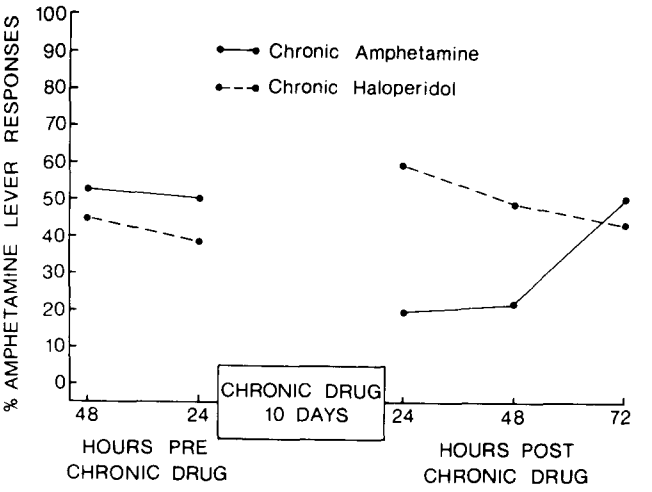

FIG. 2. Percent responding on the amphetamine lever during test sessions prior to chronic drug treatment (PRE) and after chronic drug treatment (POST).

totic levels was assessed by analyzing the percentage of drug-appropriate lever responses during the last four amphetamine and haloperidol training days. This analysis indicated that final levels of correct responding on amphetamine days, $F(3,32)=3.37$, $p<0.05$, as well as on haloperidol days, $F(3,32)=3.45$, $p<0.05$, were also dependent upon the training dose of amphetamine.

Throughout testing, response rates following amphetamine administration were higher than rates following haloperidol administration in both the unreinforced, $F(1,32)=146.45$, $p<0.001$, and reinforced, $F(1,32)=233.32$, $p<0.001$, periods of each session. Further, the magnitudes of the differences between amphetamine and haloperidol response rates were a function of amphetamine training dose, $F(3,32)=2.97$, $p<0.05$. The mean difference between amphetamine and haloperidol response rates during the 2.5 minute unreinforced period initiating each training session, was 7.0 for group 0 mg/kg, 14.0 for group 0.1 mg/kg, 20.0 for group 0.3 mg/kg and 26.0 for group 0.5 mg/kg.

Drug-Free Test Sessions Prior to Chronic Drug Treatment

Following acquisition of the amphetamine-haloperidol discrimination, all animals received two drug-free test sessions. The critical difference between these two test sessions was that animals were injected with distilled water 30 minutes prior to the first session, but, received no injection prior to the second test session. Thus, the influence of the vehicle injection procedure on choice responding during the drug-free test session could be evaluated. The mean percentage amphetamine-appropriate responses during the first of these test sessions, which occurred 48 hours prior to chronic drug treatment, was 48%, while the mean percentage amphetamine-appropriate responses during the second drug-free test session, which occurred 24 hours prior to chronic drug treatment, was 44%. There was no significant difference in the percentage of amphetamine-appropriate responses between these two drug-free test sessions, $F(1,22)<1$, and percentage of amphetamine-appropriate responses was unaffected by amphetamine training dose, $F(3,27)<0.1$.

The mean number of responses during the first 2.5 minute drug-free test session was significantly greater than the mean number of responses during the second drug-free test session, $F(1,28)=45.91$, $p<0.01$, reflecting the effects of extinction. Given that each test session represented a 2.5 minute unreinforced session, some response extinction is expected. This reduction in response rate, however, was not a function of amphetamine training dose, $F(3,28)=2.43$, $p=0.09$, nor did it differ between groups assigned to the chronic amphetamine vs. chronic haloperidol treatment, $F(1,28)=2.28$, $p0.09$.

Drug-Free Test Sessions Following Chronic Drug Treatment

Figure 2 shows the percentage amphetamine-appropriate lever responses during the 2.5 min unreinforced test sessions 48 and 24 hours prior to, and 24, 48 and 72 hours following, chronic drug treatment. Because choice behavior following chronic drug treatment was assessed using the same test procedure as the one used 24 hours prior to chronic drug treatment, the effect of chronic drug treatment on choice behavior was evaluated by comparing choice behavior during the 24 hour pre-chronic drug test session to that following chronic drug treatment. As can be seen in Fig. 2, 24 hours following chronic haloperidol, the percentage of amphetamine-appropriate responses was enhanced, $F(1,9)=31.89$, $p<0.001$, while 24 hours following chronic amphetamine the percentage of amphetamine-appropriate responses was suppressed, $F(1,15)=7.78$, $p<0.05$, compared to the 24 hour pre-chronic drug test session. As seen in Fig. 2, these effects observed 24 hours following chronic drug treatment dissipated over the subsequent two test sessions (Chronic drug × Hours Post-Chronic Drug interaction, $F(2,40)=3.95$, $p<0.05$).

Response rates also differed significantly as a function of chronic drug treatment across the three post-chronic drug test sessions, $F(2,56)=4.10$, $p<0.05$. The mean response rates 24, 48 and 72 hours following chronic amphetamine were 12.3, 15.8 and 10.5, respectively, and following haloperidol were 20.5, 37.2 and 24.0, respectively. While response rates during acquisition were higher during amphetamine compared to haloperidol sessions, response rates 48 and 72 hours following chronic amphetamine were significantly lower than response rates following chronic haloperi-

dol, $F(1,34)=9.81$, $p<0.005$; $F(1,34)=6.03$, $p<0.05$, respectively.

DISCUSSION

The present study clearly demonstrates that rats can learn to discriminate amphetamine from haloperidol. Given that haloperidol can be discriminated from saline [5], and that acute haloperidol antagonizes DA function [4], the cue state underlying haloperidol choice behavior is likely reflecting suppressed DA function. Amphetamine also can be discriminated from saline and the discriminative cue mediating amphetamine choice behavior has been shown to be enhanced DA function [7,14]. Thus, it seems reasonable to suggest that amphetamine-haloperidol choice behavior reflects a continuum of DA mediated cues.

Consistent with this proposal, animals tested in the current study following acquisition responded equally on the amphetamine and haloperidol levers during a drug-free test session when steady-state levels of DA function would be expected. Further, if amphetamine-haloperidol discrimination is based on such a continuum, increasing the training dose of amphetamine, while holding the training dose of haloperidol constant, should increase the net difference between the cue saliency of amphetamine and haloperidol, thereby resulting in more rapid acquisition and possibly higher asymptotic levels of both amphetamine-appropriate and haloperidol-appropriate choice behavior. The rate of acquisition of amphetamine-haloperidol choice behavior was in fact observed to be dependent upon the training dose of amphetamine in the current study. The training dose of amphetamine also affected asymptotic levels of both amphetamine and haloperidol choice behavior. Although these findings alone do not prove that amphetamine-haloperidol choice behavior reflects a continuum of DA mediated cues, they are certainly consistent with this proposal. Further converging evidence may be provided by utilizing phenomena such as stimulus generalization and peak shift [6].

Using this behavioral baseline, compensatory opponent processes following chronic haloperidol and amphetamine were demonstrated. Following chronic haloperidol, animals responded during a drug-free test session as though they had received a small dose of amphetamine. Conversely, following chronic amphetamine, animals responded during a drug-free test session as though they had received a small dose of haloperidol. This interpretation of the choice behavior in the current study is supported by Barrett and Steranka's [2] demonstration that, as calculated from dose-response functions, the theoretical dose of amphetamine necessary to produce the levels of choice behavior observed following chronic haloperidol in two different training dose groups, were nearly identical. The gradual return to steady-state levels of the dopaminergic alterations observed in the current study are also consistent with the opponent process theory [3].

Thus, the behavior reported in the present study suggests first, that animals can be trained to discriminate amphetamine from haloperidol and that responding in this task likely reflects a continuum of dopaminergic function. This task is particularly useful in that the limitations of interpreting saline choice behavior are eliminated and both increases and decreases in dopaminergic function can be monitored uncontaminated by acute drug challenge. Second, the present study provides further evidence of dopaminergic opponent processes that may underlie tolerance, withdrawal, and dependence. These opponent-processes may also explain why initial reductions in activity in hyperactive boys receiving stimulant medication are followed by "rebound" increases in activity [9].

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