Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 79 (2004) 325–330
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effects of amphetamine, dexfenfluramine, and diazepam on responding during extinction in nonhuman primates

Richard W. Foltin*

Division on Substance Abuse, New York State Psychiatric Institute and Department of Psychiatry, College of Physicians and Surgeons of Columbia University, 1051 Riverside Drive, Unit 120, New York, NY 10032, USA

Received 11 May 2004; received in revised form 2 August 2004; accepted 4 August 2004
Available online 29 September 2004

Abstract

The effects of pharmacological manipulations on responding under extinction conditions were determined in baboons using a schedule of reinforcement that modeled food acquisition and food consumption. Responding during the initial acquisition component was reinforced by stimuli paired with food, while responding during the latter consumption component was reinforced with food. Certain sessions began with a 7-h extinction phase, where responding in both components produced only the paired stimuli. Dexfenfluramine (DFEN) decreased responding during extinction. Diazepam (DZP) increased responding during extinction. Low doses of amphetamine (AMPH) increased responding during extinction. Thus, DZP and AMPH increased and DFEN decreased the conditioned reinforcing effects of stimuli paired with food.

Keywords: Food intake; Baboon; Motivation; Deprivation; Amphetamine; Dexfenfluramine; Diazepam; Extinction

1. Introduction

Recently, much experimental attention has focused on the role that environmental stimuli paired with primary reinforcement play in modulating appetitive behavior (e.g., Haracz et al., 1999; Schultz et al., 1997). We have developed a procedure for studying the appetitive effects of stimuli paired with food in baboons using a schedule of reinforcement that models food acquisition and food consumption (e.g., Collier, 1983; Collier et al., 1977). Responding during the acquisition component, reinforced by stimuli paired with food using a second-order schedule (Kelleher, 1966), provided a measure of incentive value (Bindra, 1978; Berridge and Robinson, 1998). Using variants of this procedure, we have reported that (1) dexfenfluramine (DFEN) decreased both food acquisition and food consumption; (2) D-amphetamine (AMPH) increased food acquisition, but decreased food consumption; and (3) diazepam (DZP) increased both food acquisition and food consumption (Foltin, 2001, 2004). The findings obtained with DFEN and AMPH replicate findings in laboratory rodents (Fletcher, 1995, 1996; Files et al., 1989; Kelley and Delfs, 1991; Robbins, 1978; Taylor and Robbins, 1984).

A procedure commonly used with laboratory rodents to demonstrate the conditioned effects of stimuli paired with primary reinforcement is to train the animals to associate the stimulus cues with primary reinforcement, then allow the animals to respond in an operant chamber to receive only the paired cues (Sutton and Beninger, 1999). One problem with the previous studies (Foltin, 2001, 2004) was that the delivery of food during the sessions may have altered the effects of the conditioned reinforcers. The purpose of the present study was to confirm the results of our earlier studies (Foltin, 2001, 2004) by testing the effects of the experimental manipulations under conditions where responding was not reinforced by the primary commodity, i.e., extinction.

* Tel.: +1 212 543 5717; fax: +1 212 543 5991.
E-mail address: rwf2@columbia.edu.

0091-3057/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.08.010

2. Methods

2.1. Animals

Seven adult male baboons (*Papio cynocephalus anubis*), weighing 26.5 to 31.4 kg at the start of the study, were individually housed in standard nonhuman primate cages (0.94×1.21×1.52 m high) at The New York State Psychiatric Institute. Body weights remained stable, or increased slightly over the study. The room was illuminated with fluorescent lighting from 0700 to 1900 h. daily. In addition to food earned during experimental sessions, two chewable vitamins, two pieces of fresh fruit, and a dog biscuit were also given daily. Water was available ad libitum from a spout located at the back of each cage.

2.2. Schedule of reinforcement

Responding under each component of a two-component chain schedule of reinforcement was on a separate response manipulandum. (See Foltin, 2001, 2004 for description of response panels.) The session began with the illumination of a single light above the acquisition lever. The first response on the acquisition lever began a 30-min timer and illuminated a second light over the acquisition lever, i.e., the 30-min acquisition component was indicated by the illumination of two lights above the acquisition lever. The acquisition component was an FI 30 min schedule, with a FR 10 second-order component [FI 30′ (FR 10:S)]. Thus, after every 10th response during the FI component, the stimuli associated with reinforcer delivery during the second component were presented. There was a 10-min limited hold for the acquisition component, such that after the expiry of the 30-min FI, the next FR 10 had to be completed within 10 min. Failure to complete an FR 10 within 10 min canceled that acquisition component, and extinguished one light over the acquisition lever such that only a single light was illuminated over the acquisition lever. The first FR 10 completed after 30 min resulted in the two lights above the left lever being extinguished and a single light above the right lever being illuminated, signalling the availability of reinforcement under the FR consumption component of the chain schedule. The consumption component of the chain schedule was maintained under an FR 10 schedule of food reinforcement (1 grain-based "dustless" banana-flavored 1-g food pellet; 3.34 kcal/g: 20.1% protein, 3.3% fat, 55.3% carbohydrate, 3.3% ash, <5% moisture and 4.0% fiber; Bio-serv, Frenchtown, NJ). After a 10-min interval in which no responses occurred, the consumption component terminated; that is, the duration of each consumption component was determined by each baboon. The single light above the right consumption lever was then extinguished, and the single light above the left acquisition lever was again illuminated. In order to initiate another eating occasion, the baboon was required to start another 30-min acquisition component by pulling on the left lever. The initiation and termination of all components, and the interval between the end of a consumption component and the beginning of the next acquisition component were determined by the baboon.

Once or twice each week, the session began with a 7-h extinction condition. At 0800 h, a research assistant disconnected the cable that operated the feeder, such that food pellets would not be delivered, and at 1500 h, a research assistant reconnected the cable that operated the feeder, such that food pellets could be delivered. During these 7-h extinction conditions, all other operant schedule conditions remained in effect, i.e., the stimulus lights flashed during acquisition and consumption components. On the remaining weekdays, a research assistant disconnected and reconnected the cable that operated the feeder at 0800 and 1500 h so that the assistants did not become discriminative stimuli for extinction sessions.

2.3. Procedure and drugs

Four manipulations were accomplished in the following order: DFEN hydrochloride (0.12–1.0 mg/kg, Sigma, St. Louis, MO), D-AMPH sulfate (0.06–0.50 mg/kg, Sigma), DZP (0.25–2.0 mg/kg, courtesy of Hoffman LaRoche, Nutley, NJ), and AMPH sulfate (0.015–0.03 mg/kg, Sigma). The decision to test the two lowest doses of AMPH was made partway through the study, such that all of the AMPH doses were not tested in close temporal proximity. Sequential drug doses varied by 0.30 log units. Drug doses are expressed as total weight of the salt or base.

Drugs were given intramuscularly (i.m.) in a thigh muscle (location varying among sessions) on Tuesday and/or Friday of each week at 0800 h prior to an extinction session, with a matching number of placebo injections given on Monday and/or Thursday of the weeks that each was tested. Doses were administered only when responding on the two previous days was stable. Dose order for DFEN, DZP, and the four largest doses of AMPH was systematically varied within and between baboons such that all possible dosing orders were tested for each drug. Because the two smallest AMPH doses were tested after the original four doses, half of the baboons received the 0.015-mg/kg dose first and half received the 0.03-mg/kg dose first.

2.4. Data analysis

Data for each drug were summarized using two-factor repeated-measures analyses of variance (ANOVAs): the first factor was drug condition (placebo vs. active; there was one placebo session for each active dose session), and the second factor was dose (four to six doses). For all analyses, the ANOVAs provided the error terms needed to calculate the planned comparisons that were used to analyze the data. There were four planned comparisons for DFEN and DZP:

each of the active drug doses was compared to the placebo doses. There was a shift in baseline responding during extinction phases over time with baboons responding significantly less as the study progressed. The baseline data for DFEN and DZP were collected over a 2- to 3-week interval, which allowed the placebo data to be pooled in the analyses. Because the baseline data for the four largest doses of AMPH were collected early in the study and the baseline data for the two smallest doses of AMPH were collected at the end of the study, two different baselines had to be used for the AMPH data; that is, the two lowest doses were compared to the placebo data collected at the end of the study, and the other doses were compared to the placebo data collected at the middle of the study. Data were considered significantly different at $P<0.05$, using Huynh–Feldt corrections.

3. Results

Under baseline conditions, baboons began the first consumption component about 120 min after the start of the session. During the 7-h extinction phase (0–7 h), baboons earned about 50 conditioned reinforces during acquisition components, and about 30 conditioned reinforcers during consumption components. Over the remainder of the session (8–24 h), baboons earned about 30 conditioned reinforcers during acquisition components, and about 300 conditioned (and primary) reinforcers during consumption components.

Fig. 1 compares the effects of the pharmacological manipulations on the daily total number of acquisition and consumption conditioned reinforcers delivered during the 7-h extinction phase and during the remainder of the

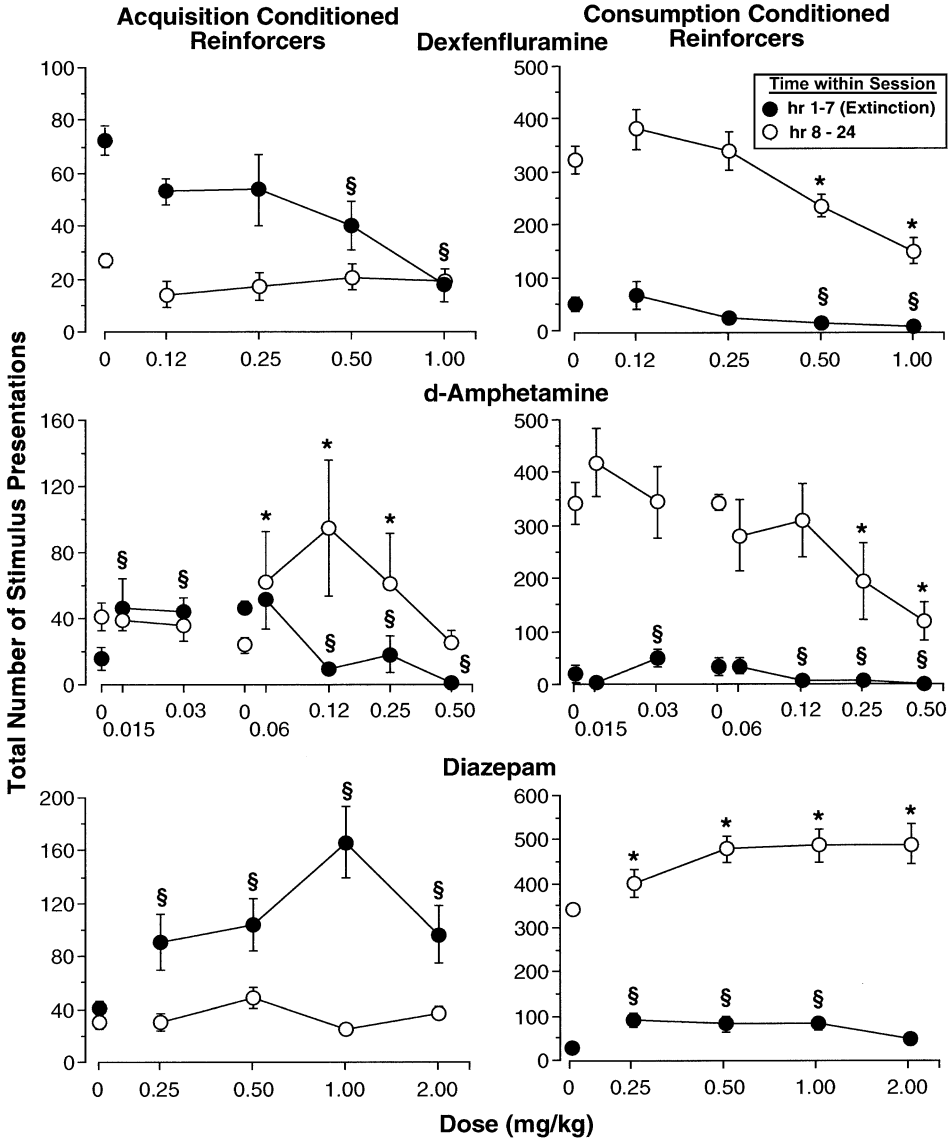

Fig. 1. Total daily number of acquisition and consumption conditioned reinforcers delivered during the 7-h extinction phase and the remainder of the experimental day (8–24 h) as a function of drug and dose. An § or * indicates a significant difference between that dose of drug and its placebo condition ($P<0.05$). Error bars represent ±1 S.E.M.

session. DFEN (top left panels) produced dose-dependent decreases in the number of conditioned reinforcers earned in acquisition components during extinction without affecting the acquisition responding during the remainder of the session. By contrast, the two lowest AMPH doses (middle left panels) increased the number of conditioned reinforcers earned in acquisition components during extinction. AMPH produced an inverted-U-shaped dose–response function for the total number of conditioned reinforcers earned in acquisition components during the remainder of the session. DZP (bottom left panels) also produced an inverted-U-shaped dose–response function for the number of conditioned reinforcers earned in acquisition components during extinction. DZP did not affect the total number of conditioned reinforcers earned in acquisition components during the remainder of the session.

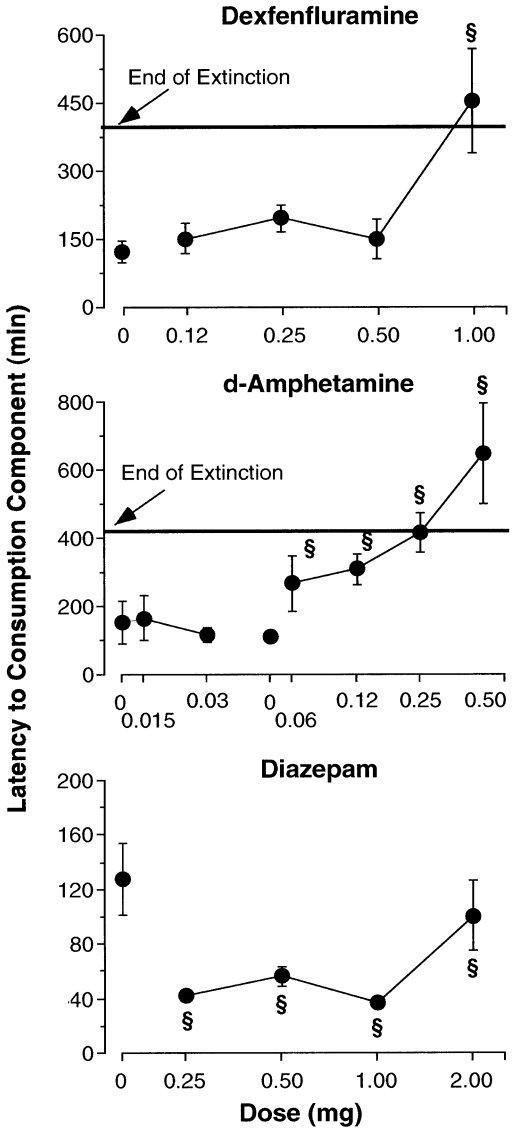

Fig. 2. Latency to the first consumption component as a function of drug and dose. An § indicates a significant difference between that dose of drug and its placebo condition ($P<0.05$). Error bars represent ±1 S.E.M.

DFEN and AMPH produced dose-dependent decreases in both the number of conditioned reinforcers earned in consumption components during extinction, and the total number of conditioned reinforcers earned in consumption components during the remainder of the session. By contrast, all doses of DZP similarly increased both the number of conditioned reinforcers earned in consumption components during extinction, and the total number of conditioned and primary reinforcers earned in consumption components during the entire session.

Only the largest dose of DFEN increased the latency to the first consumption component, while AMPH produced dose-dependent increases in the latency to the first consumption component (Fig. 2). The latencies following the highest dose of DFEN and the two highest doses of AMPH were longer than the duration of the extinction phase. All doses of DZP decreased the latency to the first consumption component.

4. Discussion

The results of the present study replicate our earlier findings that DFEN and AMPH have different effects on responding maintained by conditioned reinforcers (Foltin, 2001, 2004). The finding that DFEN decreased responding reinforced by conditioned reinforcers in baboons, within both acquisition and consumption components during extinction, confirms previous data obtained using laboratory rodents (Fletcher, 1995, 1996; Wilson et al., 2000). The finding that low doses of AMPH increased responding reinforced by conditioned reinforcers during acquisition components during extinction also confirms previous data obtained using laboratory rodents (e.g., Fletcher, 1995, 1996; Taylor and Robbins, 1984). The response-increasing effect of AMPH was more subtle in nonhuman primates. This most likely is due to the fact that the baboons, with 24 h a day access to food, were not food deprived or food restricted, as is common in studies with rodents. In addition, AMPH produced long latencies to initiate the first consumption component; latencies, which at the larger doses, were longer than the 7-h extinction phase.

Because AMPH increases DA, these findings support the hypotheses of Robbins (1975), Robinson and Berridge (1993), and Taylor and Robbins (1984) that drugs that increase DA increase responding that is reinforced by the presentation of stimuli paired with primary reinforcement. In addition, because DFEN increases 5HT levels, these findings support the hypothesis of Fletcher (1995) that increases in 5-HT decrease responding that is reinforced by the presentation of stimuli paired with primary reinforcement.

The paradoxical increase in food acquisition and decrease in food consumption produced by AMPH after the extinction phase ended complements other earlier studies (Cohen and Branch, 1991; Evans and Foltin, 1997; Foltin and Evans, 1999; Kornblith and Hoebel, 1976) showing that AMPH can

increase and decrease responding in the same session. This effect should be interpreted cautiously here because the reinforced-responding phase began 8 h after drug was administered, which was certainly after the duration of action of lower doses. Further evidence for the dissociation between conditioned reinforcers and primary reinforcers was presented by Grimm and See (2000), who demonstrated that reversible inactivation of the nucleus accumbens blocked primary, but not conditioned reinforcement, while reversible inactivation of the basolateral amgydala blocked conditioned, but not primary, reinforcement.

DZP increased responding reinforced by conditioned reinforcers, during both acquisition and consumption components during extinction. In rats, the anxiolytic chlordiazepoxide had no effect on responding reinforced by stimuli paired with water reinforcement under extinction conditions (Robbins et al., 1983), suggesting that the present results were not due to a specific increase in the reinforcing effects of the stimuli paired with food. For this reason, and because DZP is an efficacious appetite stimulant in nonhuman primates (Foltin, 1993), it is most likely that these effects are due to an increase in motivation to eat, increasing the incentive salience of the stimuli paired with food (Bindra, 1978; Toates, 1981).

In summary, the present study replicates previous results from this laboratory (Foltin, 2001, 2004), and extends data obtained in laboratory rodents on the effects of pharmacological manipulations on responding reinforced with conditioned reinforcers under extinction conditions. A better understanding of the variables that are involved in determining the motivation to seek a commodity may well be key in the development of better medication and enhanced behavioral therapy for the treatment of excessive appetitive behaviors.

Acknowledgments

This research was supported by DA-04130 from The National Institute on Drug Abuse, and approved by the New York State Psychiatric Institute Animal Care and Use Committee. The assistance of Julian Perez, Angel Ramirez, April Modranowski, and Drs. Suzette Evans, Margaret Haney, and Mohamed Osman is gratefully acknowledged.

References

Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 1998;28:309–69.

Bindra D. How adaptive behavior is produced: a perceptual–motivational alternative to response-reinforcement. Behav Neurosci 1978;1:41–91.

Cohen SL, Branch MN. Food-paired stimuli as conditioned reinforcers: effects of D-amphetamine. J Exp Anal Behav 1991;56:277–88.

Collier GH. Life in a closed economy: the ecology of learning and motivation. In: Zeiler MD, Harzem P, editors. Advances in the Analysis of Behavior, vol 3. Hoboken, New Jersey: John Wiley and Sons; 1983. p. 223–74.

Collier G, Hirsch E, Kanarek R. The operant revisited. In: Honig WR, Staddon JER, editors. Handbook of operant behavior. Englewood Cliffs, New Jersey: Prentice Hall; 1977. p. 28–52.

Evans SM, Foltin RW. The effects of D-amphetamine on the reinforcing effects of food and fluid using a novel procedure combining self-administration and location preference. Behav Pharmacol 1997;8:429–41.

Files FJ, Branch MN, Clody D. Effects of methylphenidate on responding under extinction in the presence and absence of conditioned reinforcement. Behav Pharmacol 1989;1:113–21.

Fletcher PJ. Effects of D-fenfluramine and metergoline on responding for conditioned reward and the response potentiating effect of nucleus accumbens D-amphetamine. Psychopharmacology 1995;118:155–63.

Fletcher PJ. Injection of 5-HT into the nucleus accumbens reduces the effects of D-amphetamine on responding for conditioned reward. Psychopharmacology 1996;126:62–9.

Foltin RW. Effects of pharmacological manipulations on "demand" for food by baboons. Behav Pharmacol 1993;4:586–96.

Foltin RW. Effects of amphetamine, dexfenfluramine, diazepam, and other pharmacological and dietary manipulations on food "seeking" and "taking" behavior in non-human primates. Psychopharmacology 2001;158(1);28–38.

Foltin RW. Effects of amphetamine, dexfenfluramine, diazepam and dietary manipulations on responding reinforced by stimuli paired with food in non human primates. Pharmacol Biochem Behav 2004;77:471–9.

Foltin RW, Evans SM. The effects of D-amphetamine on intake of food and a sweet fluid containing cocaine. Pharmacol Biochem Behav 1999;62:457–64.

Grimm JW, See RE. Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology 2000;22:473–9.

Haracz JL, Mash DC, Sircar R. A multicomponent learning model of drug abuse Drug taking and craving may involve separate brain circuits underlying instrumental and classical conditioning, respectively. Ann NY Acad Sci 1999;877:811–9.

Kelleher R. Conditioned reinforcement in second-order schedules. J Exp Anal Behav 1966;9:475–85.

Kelley AE, Delfs JM. Dopamine and conditioned reinforcement: I. Differential effects of amphetamine microinjections into striatal subregions. Psychopharmacology 1991;103(2);187–96.

Kornblith CL, Hoebel BG. A dose–response study of anorectic drug effects on food intake, self-stimulation, and stimulation-escape. Pharmacol Biochem Behav 1976;5:215–8.

Robbins TW. The potentiation of conditioned reinforcement by psychomotor stimulant drugs A test of Hill's hypothesis. Psychopharmacologia 1975;45:103–14.

Robbins TW. The aquisition of responding with conditioned reinforcement: Effects of pipradrol, methylphenidate, D amphetamine, and nomifensine. Psychopharmacology 1978;58:79–87.

Robbins TW, Watson BA, Gaskin M, Ennis C. Contrasting interaction of pripradol, D-amphetamine, cocaine, cocaine analogues, apomorphine and other drugs with conditioned reinforcement. Psychopharmacology 1983;80:113–9.

Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 1993;18:247–291.

Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. Science 1997;275:1593–9.

Sutton MA, Beninger RJ. Psychopharmacology of conditioned reward: evidence for a rewarding signal at D1-like dopamine receptors. Psychopharmacology 1999;144:95–110.

Taylor JR, Robbins TW. Enhanced behavioural control by conditioned reinforcers following microinjections of d amphetamine into the nucleus accumbens. Psychopharmacology 1984;84:4-5–12.

Toates FM. The control of ingestive behaviour by internal and external stimuli—a theoretical review. Appetite 1981;2:35–50.

Wilson AW, Costall B, Neill JC. Manipulation of operant responding for an ethanol-paired conditioned stimulus in the rat by pharmacological alteration of the serotonergic system. J Psychopharmacology 2000; 14:340–6.