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The Effects of *d*-Amphetamine and Diazepam on Schedule-Induced Defecation in Rats

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LESAGE, M., M. MAKHAY, I. DELEON AND A. POLING. *The effects of* d-*amphetamine and diazepam on schedule-induced defecation in rats.* PHARMACOL BIOCHEM BEHAV **48**(3) 787-790, 1994.—The effects of *d*-amphetamine (0.32 to 5.6 mg/kg) and diazepam (0.10 to 5.6 mg/kg) on defecation were examined in two groups of rats. One group was exposed to a fixed-time 60-s (FT 60-s) schedule of food delivery and the other group was exposed to massed-food sessions. During vehicle control sessions, rats exposed to the FT 60-s schedule excreted a significantly higher number of fecal boli than rats exposed to massed-food sessions. *d*-Amphetamine, at doses above 0.56 mg/kg, significantly reduced defecation (boli produced) in both groups, although the magnitude of the drug's effect was larger in the group exposed to the FT 60-s schedule. For both groups, diazepam only produced a significant decrease in defecation at the highest dose (5.6 mg/kg). These results appear to be inconsistent with interpretations of adjunctive behavior that emphasize arousal or emotion as mechanisms.

d-Amphetamine Diazepam Schedule-induced defection Rats

WHEN food-deprived nonhumans are exposed to intermittent schedules of food delivery, they drink copious amounts of water (4). This behavior, termed schedule-induced polydipsia, is the prototype of a class of behaviors called adjunctive (5). Other putative adjunctive behaviors include pica, attack, wheel running, biting, chewing, grooming, locomotion, chain pulling, and pecking (6,10,15,16,24).

Sanger and Blackman (18) identified three reasons why the analysis of drug effects on adjunctive behavior is important. First, such an analysis may indicate the extent to which general principles of drug action found with operant behavior hold with other types of behavior. Second, the study of drug effects on adjunctive behavior may provide a better understanding of adjunctive behavior in its own right. Third, given that the majority of drug research has examined drinking as the adjunctive response, further studies of drug effects on other adjunctive behaviors may help to define similarities and differences between those behaviors.

Several studies have examined the effects of drugs on adjunctive drinking. For example, schedule-induced polydipsia has been shown to be attenuated by pentobarbital (20), atropine and scopolamine (21), and chlorpromazine (1). Two studies found that amphetamine reduced schedule-induced drinking (11,20); a third (12) reported little or no effect at low doses and decreases at a high dose. Diazepam has been reported to increase adjunctive drinking at low doses and to decrease it at high doses (12,17). Diazepam-induced increases in drinking may not be limited to schedule-induced intake, however, because benzodiazepines at certain doses generally increase fluid consumption in water-deprived rats [e.g., (2)].

To examine drug effects on a schedule-induced behavior other than polydipsia, the present study examined the effects of *d*-amphetamine and diazepam on schedule-induced defecation in rats exposed to a fixed-time 60-s (FT 60-s) schedule of food delivery. Three previous studies have shown that intermittent food delivery substantially increases the number of fecal boli produced by rats (14,25,26), but drug effects were not examined. To demonstrate that defecation actually was schedule induced in the present study, and to evaluate the specificity of drug effects on schedule-induced defecation, defecation was also examined in rats that received in bulk each session an amount of food equal to that delivered under the FT schedule.

METHOD

Subjects

Twelve male Sprague-Dawley rats, maintained at 80% of their free-feeding weights, served as subjects. Subjects were approximately 120 days old and had been used in an introduc-

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tory learning course prior to the present experiment. They were individually housed with unlimited access to water in a room with controlled lighting (12L : 12D cycle), temperature (22–24°C), and humidity (60–70%). The study was approved by the Institutional Animal Care and Use Committee at Western Michigan University.

Apparatus

Three aluminum operant conditioning chambers, measuring 23 cm long, 21 cm wide, and 17 cm high, were used. Each chamber had a metal grid floor, below which was located an aluminum pan in which fecal boli were collected. An aperture located on the bottom left side of the front wall allowed for presentation of 45 mg Noyes food pellets (P. J. Noyes, Lancaster, NH) and a white light (houselight) on the ceiling provided ambient illumination. Pellet delivery was controlled by an ALR Flyer 32DT computer (Advanced Logic Research, Irvine, CA) using MED PC Software (MED Associates, St. Albans, VT).

Procedure

Subjects were randomly assigned to one of two equal-sized groups. Members of one group were exposed to an FT 60-s schedule of food delivery. Under this schedule, during daily 30-min sessions, a 45 mg food pellet was delivered every 60 s to individual rats, regardless of the subject's behavior. Members of the massed-food group received 30 food pellets at the beginning of daily 30-min sessions. For both groups, the chamber was lighted throughout the experimental session.

Immediately following each session, subjects were removed from the experimental chambers and the bolus collection pans were removed. The fecal boli were counted and the chambers were examined for fecal boli that did not drop into the collection pans; these were also counted. The pans were cleaned after each session and replaced.

Dose–response determinations began when there was no visually evident trend in rates of defecation for 10 consecutive sessions. The acute effects of five doses of *d*-amphetamine (0.32, 0.56, 1.0, 3.2, and 5.6 mg/kg) and diazepam (0.10, 0.32, 1.0, 3.2, and 5.6 mg/kg) were then determined. *d*-Amphetamine was evaluated initially. Following completion of the dose–response determinations for *d*-amphetamine, subjects were exposed to 10 consecutive baseline sessions, during which no injections were given, followed by dose–response determinations for diazepam.

d-Amphetamine (Sigma, St. Louis, MO) was dissolved in isotonic saline solution and injected intraperitoneally (IP) at an injection volume of 1 ml/kg. Diazepam (Sigma) was injected IP as a suspension in isotonic saline with Tween-80 (Sigma) added (1 drop every 2 ml), prepared at an injection volume of 1 ml/kg and dispersed by ultrasound prior to injection. All drug injections were given according to a repeating BBCD design, where B represents baseline (no injections), C represents vehicle, and D represents drug sessions. Vehicle and drug were administered 15 min prior to behavioral testing. All subjects received one administration of each dose of each drug. The order in which the various doses of each drug were administered was determined randomly for each subject.

For the primary statistical analyses, a two-factor ANOVA with one repeated and one nonrepeated factor was used. The ANOVA was followed by simple main effects tests on mean differences in rates of defecation (mean boli excreted) at each dose of drug. Multiple comparisons of the means attributable to the main effects of the drugs were done using Tukey HSD tests. Results were considered as significant if p was less than 0.05.

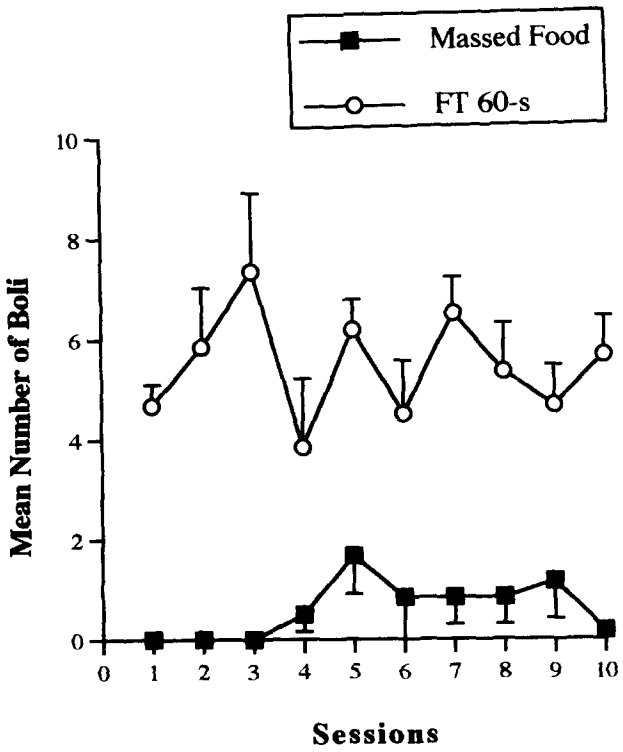

FIG. 1. Mean number of fecal boli (plus or minus one standard deviation) produced by rats exposed in 30-min daily sessions to an FT 60-s schedule of food delivery, or to 30 food pellets presented in mass at the beginning of the session. The data shown represent performance during 10 consecutive sessions immediately prior to drug evaluation. Each group comprised six rats.

RESULTS

Figure 1 shows the daily rates of defecation for each group across the 10 consecutive sessions of stable performance prior to drug administration. Prior to initial dose–response determinations, mean rates of defecation were significantly higher in the FT 60-s group than in the massed-food group, $F = 49.95$, $p < 0.01$, demonstrating the development of schedule-induced defecation in the former animals.

Figure 2 shows mean rates of defecation for both the massed-food and FT 60-s groups as a function of *d*-amphetamine dose. Data shown are the average number of fecal boli across the six subjects in each group during vehicle control sessions and exposure to each dose of *d*-amphetamine. In the absence of drug, rats exposed to the FT 60-s schedule excreted a significantly higher number of fecal boli than rats exposed to massed-food sessions, $F = 5.21$, $p < 0.05$. Tukey tests showed mean rates of defecation were significantly below the vehicle control level at doses above 0.56 mg/kg. Simple main effects tests showed that defecation rates did not differ between groups at doses of 0.56 mg/kg and above. That is, convergence in rates of defecation between groups was observed, with the schedule by drug interaction approaching significance, $F = 1.97$, $p = 0.1$.

Figure 3 shows mean rates of defecation for both the massed-food and FT 60-s groups as a function of dose of

diazepam. In the absence of drug, rats exposed to the FT 60-s schedule excreted a significantly higher number of fecal boli than rats exposed to massed-food sessions, $F = 0.37$, $p < 0.05$. Although there was a significant overall drug effect, $F = 2.97$, $p < 0.05$, Tukey tests showed that the the mean rate of defecation was significantly below the vehicle control level only at the highest dose (5.6 mg/kg). Simple main effects tests showed that mean group differences in rate of defecation were significant at every dose except 0.32 and 3.2 mg/kg, and the schedule by drug interaction did not approach significance, $F = 0.25$, $p = 0.94$.

DISCUSSION

As in previous investigations (14,25,26), schedule-induced defecation occurred in the present study. Schedule-induced defecation is a candidate for inclusion in a class of behaviors known as adjunctive, of which the best-studied member is schedule-induced polydipsia. One way to determine the degree of commonality among behaviors that may be so classified is to examine their sensitivity to drugs (18), and this tack was taken in the present investigation.

As in prior studies of the effects of amphetamine on schedule-induced polydipsia (11,12,20), this drug only reduced schedule-induced defecation in the present investigation. Thus, with respect to the effects of amphetamine, schedule-induced drinking and schedule-induced defecating are similar.

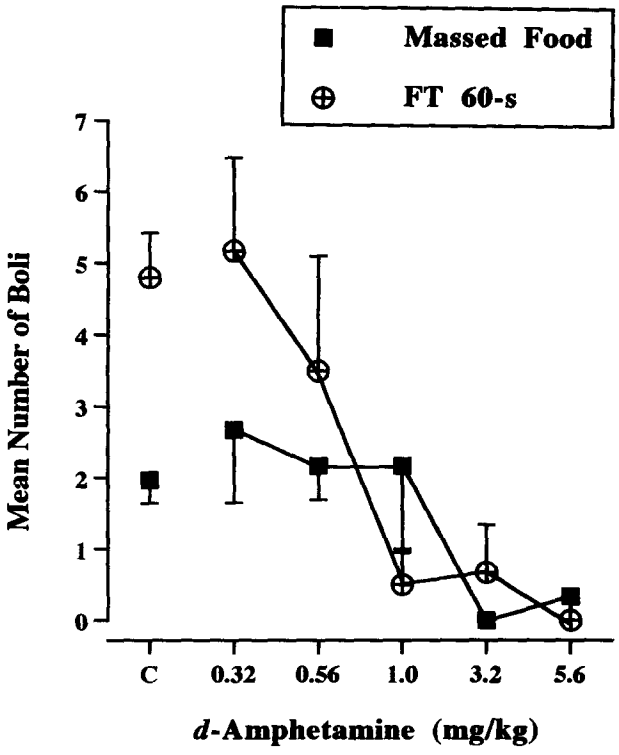

FIG. 2. Effects of *d*-amphetamine on the mean number of fecal boli (plus or minus one standard deviation) produced by rats exposed in 30-min daily sessions to an FT 60-s schedule of food delivery, or to 30 food pellets presented in mass at the beginning of the session. Each drug data point represents the performance of six rats during a single exposure to the listed dose. Each control data point represents performance during the six vehicle control sessions that preceded *d*-amphetamine administration.

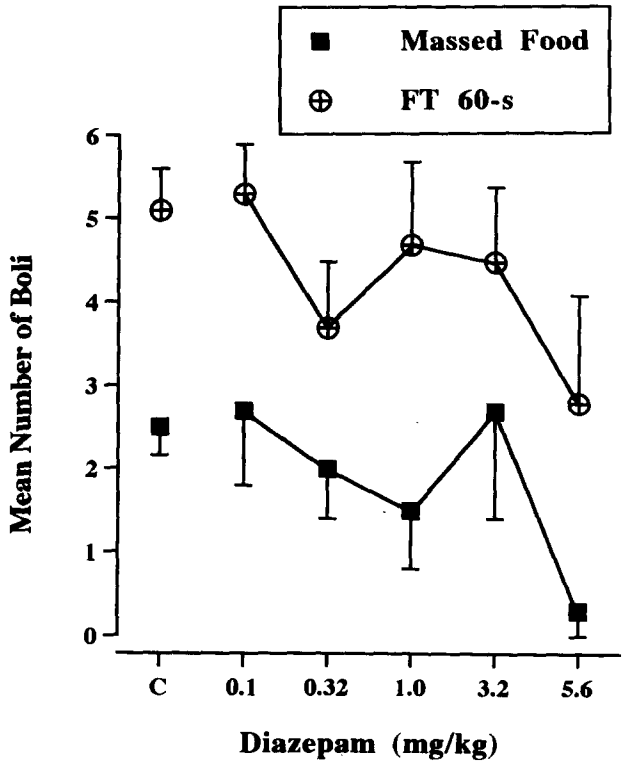

FIG. 3. Effects of diazepam on the mean number of fecal boli (plus or minus one standard deviation) produced by rats exposed in 30-min daily sessions to an FT 60-s schedule of food delivery, or to 30 food pellets presented in mass at the beginning of the session. Each drug data point represents the performance of six rats during a single exposure to the listed dose. Each control data point represents performance during the six vehicle control sessions that preceded diazepam administration.

In contrast, the effects of diazepam on schedule-induced defecation differed somewhat from those reported in two previous studies of schedule-induced polydipsia (12,17). In those studies, the drug increased drinking at low doses and decreased drinking at high doses. No increases in schedule-induced defecation were observed in the present study, regardless of the dose of diazepam administered. Thus, schedule-induced drinking and defecation appear to be affected differently by diazepam. Although this finding is not a sufficient reason for excluding schedule-induced defecation from the class of adjunctive behaviors, it does suggest that these behaviors should be grouped together with some caution.

The neurochemical mechanisms that underlie adjunctive behaviors are unknown, and the behavioral mechanisms that produce it are speculative. Several interpretations of schedule-induced behavior have been offered and none are universally accepted (18). One that may be relevant to the present study was proposed independently by Killeen (9) and Wayner (22, 23). They suggested that intermittent schedules of reinforcement produce a state of arousal or activation which, in turn, produces adjunctive behavior. Similarly, Segal and Oden (19) proposed that the conditions of food deprivation and intermittent reinforcement affect emotional substrates of behavior which, in turn, somehow produce polydipsia.

A plausible case can be made for the role of increased arousal in schedule-induced defecation. Increased defecation

by rats has been considered as a collateral response with anxiety or stress (3). This contention is supported by data from open-field studies (7) and conditioned emotional response studies (3), two assays that arrange stressful conditions and produce copious defecation by rats. High rates of defecation have also been observed under intermittent schedules of food delivery (14,25,26), which may also be stressful and increase arousal or emotion (9,20,22,23).

If this is the case, drugs that increase arousal, including *d*-amphetamine (8), might be expected to increase schedule-induced defecation, whereas drugs that attenuate arousal, including diazepam (13), should decrease it. Such effects were not observed in the present study; depending on dose, both *d*-amphetamine and diazepam either had no effect on or attenuated schedule-induced defecation. At certain doses, each drug also reduced the number of fecal boli produced by animals in the massed-food group, which were not exposed to obviously stressful conditions. Given these findings, general arousal models do not appear especially useful in predicting drug effects on schedule-induced defecation.

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REFERENCES

1. Byrd, L. D. Modification of the effects of chlorpromazine on behavior in the chimpanzee. J. Pharmacol. Exp. Ther. 189:24–32; 1974.
2. Cooper, S. J. Benzodiazepine mechanisms and drinking in the water-deprived rat. Neuropharmacology 21:775–780; 1982.
3. Estes, W. K.; Skinner, B. F. Some quantitative properties of anxiety. J. Exp. Psychol. 29:390–400; 1941.
4. Falk, J. L. Production of polydipsia in normal rats by an intermittent food schedule. Science 133:195–196; 1961.
5. Falk, J. L. The nature and determinants of adjunctive behavior. Physiol. Behav. 6:577–588; 1971.
6. Falk, J. L. The environmental generation of excessive behavior. In: Mule, S. J., ed. Behavior in excess: An examination of the volitional disorders. New York: Free Press; 1981:313–337.
7. Hall, C. S. Emotional behavior in the rat. J. Comp. Psychol. 18: 385–403; 1934.
8. Hoffman, B. B.; Lefkowitz, R. J. Catecholamines and sympathomimetic drugs. In: Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, eds. The pharmacological basis of therapeutics. New York: Pergamon Press; 1990:187–220.
9. Killeen, P. R. On the temporal control of behavior. Psychol. Rev. 82:89–115; 1975.
10. Looney, T. A.; Cohen, P. S. Aggression induced by intermittent positive reinforcement. Neurosci. Biobehav. Rev. 6:15–37; 1982.
11. Pellon, R.; Blackman, D. E. Effects of drugs on the temporal distribution of schedule-induced polydipsia in rats. Pharmacol. Biochem. Behav. 43:689–695; 1992.
12. Pellon, R.; Mas, B.; Blackman, D. E. Effects of *d*-amphetamine and diazepam on nonpunished and punished schedule-induced drinking in rats. Behav. Pharmacol. 3:75–81; 1992.
13. Rall, T. W. Hypnotics and sedatives; Ethanol. In: Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, eds. The pharmacological basis of therapeutics. New York: Pergamon Press; 1990:345–382.
14. Rayfield, F.; Segal, M.; Goldiamond, I. Schedule-induced defecation. J. Exp. Anal. Behav. 38:19–34; 1982.
15. Roper, T. J. What is meant by the term "schedule-induced," and how general is schedule induction? Anim. Learn. Behav. 9:443–440; 1981.
16. Sanger, D. J. Drug taking as adjunctive behavior. In: Goldberg, S. R.; Stolerman, I. P., eds. Behavioral analysis of drug dependence. New York: Academic Press; 1986:123–160.
17. Sanger, D. J.; Blackman, D. E. Effects of diazepam and ripazepam on two measures of adjunctive drinking in rats. Pharmacol. Biochem. Behav. 5:139–142;1976.
18. Sanger, D. J.; Blackman, D. E. The effects of drugs on adjunctive behavior. In: Blackman, D. E.; Sanger, D. J., eds. Contemporary research in behavioral pharmacology. New York: Plenum Press; 1978:239–287.
19. Segal, E. F.; Oden, D. L. Determinants of polydipsia in rats: A reply to Stein: I. Emptying the water bottle. Psychonom. Soc. 2: 201–202; 1976.
20. Segal, E. F.; Oden, D. L.; Deadwyler, S. A. Determinants of polydipsia: V. Effects of amphetamine and pentobarbital. Psychonom. Soc. 3:33–34; 1964.
21. Stien, L. Anticholinergic drugs and the central control of thirst. Science 139:46–48; 1963.
22. Wayner, M. J. Motor control functions of the lateral hypothalamus and adjunctive behavior. Physiol. Behav. 5:1319–1325; 1970.
23. Wayner, M. J. Specificity of behavioral regulation. Physiol. Behav. 12:851–869; 1974.
24. Wetherington, C. L. Is adjunctive behavior a third class of behavior? Neurosci. Biobehav. Rev. 6:329–350; 1982.
25. Wylie, A. M.; Layng, M. P.; Meyer, K. A. Schedule-induced defecation by rats during ratio and interval schedules of food reinforcement. J. Exp. Anal. Behav. 60:611–620; 1993.
26. Wylie, A. M.; Springis, R.; Johnson, K. S. Schedule-induced defecation: No-food and massed-food baselines. J. Exp. Anal. Behav. 58:389–397; 1992.