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Interaction of Food Deprivation with Different Measures of Amphetamine Effects

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COLE, S. O. *Interaction of food deprivation with different measures of amphetamine effects.* PHARMAC. BIOCHEM. BEHAV. **10**(2) 235–238, 1979.—The effects of d-amphetamine (0.0, 0.5, 1.0 mg/kg) on feeding, activity, and food-dish contact time of male Holtzman rats were investigated under 4 different levels of food deprivation (0, 12, 24, 36 hr). Differences in the amount of food deprivation significantly influenced the drug's effect on feeding and food-dish contact time, but not on activity. Also, differences in the amount of food deprivation significantly influenced the interrelationship (correlation) of amphetamine's effects on activity and food-dish contact time and on food-dish contact time and feeding, but not so much the interrelationship of the drug's effects on feeding and activity. These findings suggest that the amount of food deprivation differentially influences different general measures of amphetamine effects as well as differentially affecting the interrelationship of amphetamine effects. The importance of the correlation data to the potential incompatibility of the drug's effects as well as to an interpretation of food-dish contact time is briefly considered.

d-Amphetamine Food deprivation Anorexia Activity Food-dish contact time Interrelationship of drug effects

WHILE the feeding-depressant and motor-stimulating effects of amphetamine are well documented [4,9], the importance of various experimental parameters to the measurement of these effects remains, to a large degree, unclear. Yet, the critical role experimental parameters play in the determination of the drug's effects has long been recognized [4].

Previous research has demonstrated that differences in the size of an open-field test arena differentially influence the effects of amphetamine on concurrent measures of feeding and activity [5], and that differences in the amount of food deprivation alter the effects of the drug on independent measures of food-motivated behavior [7]. However, the latter conclusion may depend upon how one determines drug dosage in feeding studies; i.e., as absolute dosage or mg/kg dosage [8,11]. While the specific role that food deprivation plays in determining the effects of amphetamine on independent measures of food-motivated behavior is not fully resolved, the importance of food deprivation to the concurrent assessment of the drug's effects on feeding and activity is even less clearly understood. The present study addresses itself to this particular issue.

Specifically, the objectives of the present study are: (a) to determine the importance of differences in food deprivation to the general effects of amphetamine on concurrent measures of feeding, activity, and food-dish contact time; and (b) to further determine the importance of differences in food deprivation to the interrelationship (correlation) of the drug's effects on these behaviors. In addition to assessing the relationship of food-dish contact time with feeding and activity, the second of these objectives assesses the potential for amphetamine's depression of feeding being due to the drug's incompatible (competing) hypermotility action. Such a competing-response hypothesis has been proposed by Carlton [2] and by Lyon and Robbins [10] and has important implications concerning the interpretation of the drug's action.

METHOD

Animals

Forty-eight adult male Holtzman rats, weighing 250–300 g, were subjects. They were housed under standard laboratory conditions and permitted ad lib access to Purina laboratory chow and water in the home cages, except when otherwise specified in the procedure.

Apparatus

The 30×45×30 cm test apparatus, which was designed to provide a concurrent assessment of feeding-directed behavior and activity, has been described in detail elsewhere [3]. Briefly, the floor of the apparatus served as an activity platform, with the movement of an animal between the 4 quadrants of the platform automatically recorded by frequency counters. A food dish, with the front partially cut down for easier access to food, was mounted on a fulcrumed lever approximately 10 cm above the platform floor at one end of the apparatus. The slightly elevated position of the food dish assured that feeding was contingent upon the animal making forelimb contact with the food dish, thereby vertically displacing it and activating a timer. Such an arrangement provided a measure of cumulative contact time with the food dish as well as food consumption. Water was available to

subject through a nozzle adjacent to the food dish, and fluorescent lighting directly above the testing area provided uniform illumination of the apparatus.

Procedure

Initially, all animals were administered two 1-hr adaptation sessions in the apparatus following approximately 18 hr food deprivation, during which time they were permitted to eat freely 97 mg precision food pellets (Noyes) placed in the food dish.

Following adaptation sessions, subjects were assigned randomly to one of 3 d-amphetamine conditions (0.0, 0.5, 1.0 mg/kg d-amphetamine SO_4 in 1 ml/kg 0.9% NaCl) and to one of 4 food deprivation conditions (0, 12, 24, 36 hr) according to a 2-factor (levels of drug dose and levels of food deprivation) factorial design [1]. All subjects were then administered a single 1-hr test in the apparatus under the appropriate d-amphetamine and food deprivation condition, during which time food consumption, activity, and food-dish contact time were recorded. While activity and food-dish contact time were automatically recorded, food consumption was determined by placing a specified number of 97 mg precision pellets (200) in the food dish at the beginning of the test, counting the number remaining at the end of the test, and taking the difference (corrected for spillage) as the number of pellets eaten. Water consumption was not measured, although water was available to subject throughout the test period.

The testing procedure for any one animal was as follows. After being food deprived for the appropriate number of hours in the home cage, the subject was removed, weighed, injected IP with the appropriate dose of d-amphetamine, and returned to the home cage. Thirty minutes later, the subject was placed in the apparatus to begin the 1-hr test.

RESULTS

The food consumption of animals under the combined d-amphetamine and food deprivation conditions of the study is summarized in Fig. 1. Analysis (ANOVA) of these data demonstrated a highly significant Drug Dose effect, $F(2/36)=44.47$, $p<0.01$, a significant Deprivation effect, $F(3/36)=9.63$, $p<0.01$, and a significant Drug Dose × Deprivation Interaction, $F(6/36)=2.94$, $p<0.05$. Further analysis (t-tests following ANOVA) of drug dose data indicated that, under 0 hr food deprivation, only the 1.0 mg/kg d-amphetamine group differed significantly ($p<0.05$) from the vehicle (0.0 mg/kg) group, whereas, under 12 hr food deprivation, both drug groups differed significantly from the vehicle group ($p<0.05$ for both groups) but did not differ significantly from each other. Under 24 hr food deprivation, both drug groups differed significantly from the vehicle group ($p<0.05$ and $p<0.01$ for 0.5 and 1.0 mg/kg d-amphetamine groups, respectively) as well as differing significantly from each other ($p<0.05$), while under 36 hr food deprivation, both drug groups differed significantly from the vehicle group ($p<0.05$ and $p<0.01$ for 0.5 and 1.0 mg/kg d-amphetamine groups, respectively), but did not differ significantly from each other.

The activity of animals under the combined d-amphetamine and food deprivation conditions of the study is summarized in Fig. 2. Analysis of these data demonstrated a highly significant Drug Dose effect, $F(2/36)=73.91$, $p<0.01$, but no additional significant sources of variance. Further

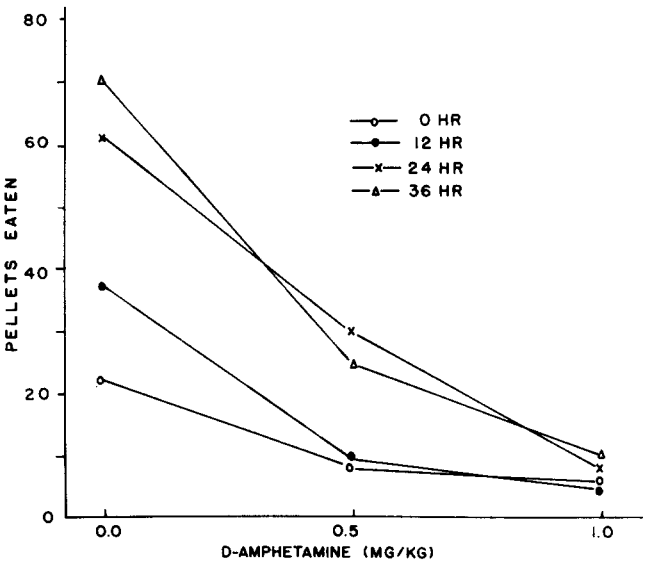

FIG. 1. Mean number of 97 mg pellets eaten by independent groups of animals under combined d-amphetamine (0.0, 0.5, 1.0 mg/kg) and food deprivation (0, 12, 24, 36 hr) conditions during a 1-hr test period.

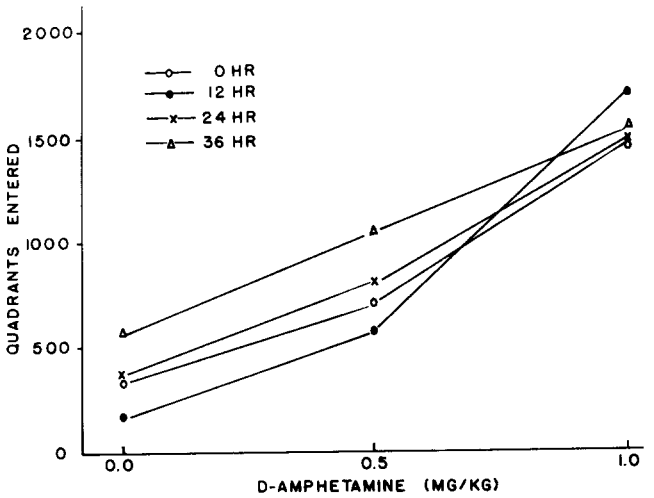

FIG. 2. Mean number of platform quadrants entered by independent groups of animals under combined d-amphetamine (0.0, 0.5, 1.0 mg/kg) and food deprivation (0, 12, 24, 36 hr) conditions during a 1-hr test period.

analysis (t-tests following ANOVA) of drug dose data indicated that, under all of the food deprivation conditions, both drug groups differed significantly from the vehicle group ($p<0.05$ and <0.01 for 0.5 and 1.0 mg/kg d-amphetamine groups, respectively) and differed significantly from each other ($p<0.05$ under 0, 24, and 36 hr food deprivation and $p<0.01$ under 12 hr food deprivation).

The food-dish contact time of subjects under the combined d-amphetamine and food deprivation conditions of the study is summarized in Fig. 3. Analysis of these data demonstrated a significant Drug Dose effect, $F(2/36)=6.09$,

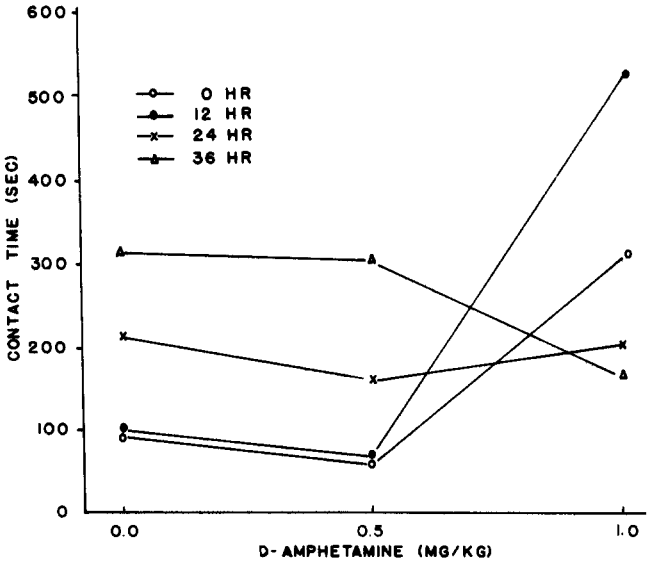

FIG. 3. Mean seconds of food-dish contact time by independent groups of animals under combined d-amphetamine (0.0, 0.5, 1.0 mg/kg) and food deprivation (0, 12, 24, 36 hr) conditions during a 1-hr test period.

$p<0.01$, and a significant Drug Dose × Deprivation Interaction, $F(6/36)=5.24$, $p<0.01$. Further analysis (t-tests following ANOVA) of drug dose data indicated that, under 0 hr food deprivation, the 1.0 mg/kg d-amphetamine group differed significantly from the vehicle group ($p<0.05$) and from the 0.5 mg/kg d-amphetamine group ($p<0.05$). While under 12 hr food deprivation, the 1.0 mg/kg d-amphetamine group also differed significantly from the vehicle group ($p<0.01$) and from the 0.5 mg/kg d-amphetamine group ($p<0.01$), under 24 and 36 hr food deprivation, none of the groups differed significantly from each other.

In order to determine the interrelationship of the effects of d-amphetamine on behavioral measures, drug dose correlations of feeding with activity, activity with food-dish contact time, and food-dish contact time with feeding were calculated for each of the food deprivation conditions. These results are summarized in Table 1. As is quite apparent, the drug dose effects of d-amphetamine on feeding demonstrated a significant negative correlation with the effects of the drug on activity under all food deprivation conditions except 0 hr. However, additional interrelationships of drug dose effects appeared to depend upon differences in the amount of food deprivation. With 0 and 12 hr food deprivation, activity and food-dish contact time demonstrated a significant positive correlation, whereas, with the highest level of food deprivation (36 hr), food-dish contact time and feeding demonstrated a significant positive correlation.

TABLE 1

SUMMARY OF DRUG DOSE CORRELATIONS (PEARSON R) OF FEEDING WITH ACTIVITY (F-A), ACTIVITY WITH FOOD-DISH CONTACT TIME (A-C), AND FOOD-DISH CONTACT TIME WITH FEEDING (C-F) UNDER 0, 12,24, AND 36 HR FOOD DEPRIVATION

Conditions	Hr Food Deprivation 0	12	24	36
F-A	−0.51	−0.57*	−0.86†	−0.78†
A-C	+0.69†	+0.89†	−0.03	−0.27
C-F	−0.24	−0.36	+0.03	+0.60*

*Significant at 0.05 level (df=11).
†Significant at 0.01 level (df=11).

DISCUSSION

The results of the present study indicate that food deprivation differentially influences the general effects of amphetamine on food consumption, activity, and food-dish contact time. While differences in the amount of food deprivation did not significantly alter the dose-ordered increase in activity produced by the drug, differences in the amount of food deprivation did significantly affect the dose-ordered decrease in feeding produced by the drug as well as the drug's effect on food-dish contact time. Such a conclusion is clearly supported by the significant Deprivation effect ($p<0.01$) and significant Drug Dose × Deprivation Interaction ($p<0.05$) on food consumption and by the significant Drug Dose × Deprivation Interaction ($p<0.01$) on food-dish contact time. While the significant Deprivation effect on food consumption appeared to be due, in large measure, to differences in the effectiveness of 0 and 12 hr deprivation versus 24 and 36 hr deprivation on the drug's depression of feeding, the significant Drug Dose × Deprivation Interaction was, in part, the results of a similar depression of feeding produced by the 1.0 mg/kg dose of the drug under all deprivation conditions (see Fig. 1). Although a total cessation of feeding was not produced by the 1.0 mg/kg dose of the drug under any of the food deprivation conditions, all of the deprivation groups consumed an average of 10 pellets or less, thereby approaching a "floor" effect with this dose. Since any such "floor" effect has the potential for producing an artifactual interaction between drug dose and food deprivation, future feeding experiments should be designed to skirt this pitfall and to provide a more definitive test of this interactive assumption. In the case of food-dish contact time, the significant Drug Dose × Deprivation Interaction was due, in large measure, to the marked increase in contact time produced quite selectively by the 1.0 mg/kg dose under 0 and 12 hr food deprivation (see Fig. 3). It is important to note, however, that the interactive effects of drug dose and food deprivation on food-dish contact time involved no evidence of a similar "floor" effect, suggesting that, at least in this case, the interactive assumption can be made free of any such artifactual bias. In general, these findings suggest that, under the conditions of the present study, the importance of food deprivation to the measurement of amphetamine's effects appears to depend upon the specific behavior (food consumption or food-dish contact time but not activity) that is identified.

The drug-dose correlation data (see Table 1) also suggest that food deprivation differentially influences the interrelationship of amphetamine's effects on feeding, activity, and food-dish contact time. While the drug-dose correlation of feeding with activity demonstrated a significant correlation under all food deprivation conditions except 0 hr deprivation (where it nearly approached borderline significance), correlations of activity with food-dish contact time and of food-dish contact time with feeding demonstrated greater varia-

bility and, in general, were much more dependent upon differences in the amount of food deprivation when significant. Thus, as was the case with general measures of amphetamine effects, the importance of food deprivation to the interrelationship of the drug's effects appears to depend upon the specific interrelationship (activity with food-dish contact time and food-dish contact time with feeding but not so much feeding with activity) that is identified.

The present findings of a significant negative correlation of amphetamine's effect on feeding and activity with differences in amount of food deprivation are consistent with previous findings of a significant negative correlation of the drug's effect on feeding and activity (rearing) with differences in test arena size [5] and suggest that such an interrelationship of the drug's effect is a rather robust phenomenon. Furthermore, these findings have considerable relevance to the proposed view that amphetamine's depression of feeding is due to the drug's hypermotility action which produces behavior that competes with feeding [2,10]. If one assumes that significant negative correlations reflect the potential for such incompatible effects, such findings suggest that, under the conditions of the present study, the potential for the drug's effect on feeding being due to its competing hypermotility action is high. Such an assumption of competing effects is also consistent with the evidence that the central neurochemical mechanisms mediating amphetamine's action on feeding and activity demonstrate a considerable amount of overlap and are probably activated simultaneously by specific doses of the drug [6].

The present findings of a significant positive correlation of amphetamine's effect on activity and food-dish contact time under 0 and 12 hr food deprivation and a significant positive correlation of the drug's effect on food-dish contact time and feeding under 36 hr food deprivation were unexpected and deserving of comment. Apparently, the effect of amphetamine on food-dish contact time under 0 and 12 hr deprivation parallels the effect of the drug on activity and may reflect the psychomotor-stimulating action of the drug. In contrast, the effect of amphetamine on food-dish contact time under 36 hr deprivation parallels the effect of the drug on food consumption and may possibly reflect a feeding motivational deficit. In any event, the effects of amphetamine on food-dish contact time were quite unstable, with such instability probably representing certain aspects of the drug's hypermotility action or anorexic action, depending upon differences in the amount of food deprivation.

Finally, the results of the present study suggest that there is particular merit in a concurrent assessment of amphetamine's effects on various behaviors rather than an independent assessment of the drug's effect on isolated behaviors. As has been previously pointed out [6,12], it is only under such experimental conditions that one can examine the interrelationship of amphetamine's effects on different behaviors and arrive at meaningful conclusions about the complex action of such a drug.

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