Pharmacology Biochemistry & Behavior, Vol. 12, pp. 723–727. Printed in the U.S.A.

Deprivation-Dependent Effects of Amphetamine on Concurrent Measures of Feeding and Activity

SHERWOOD O. COLE

Department of Psychology, Rutgers University, Camden, NJ 08102

Received 18 October 1979

COLE, S. O. *Deprivation-dependent effects of amphetamine on concurrent measures of feeding and activity*. PHARMAC. BIOCHEM. BEHAV. 12(5)723–727, 1980.—The effects of d-amphetamine (0.0, 0.5, 1.0, 2.0 mg/kg) on feeding, ambulatory activity, and rearing of male Holtzman rats were investigated in an open-field arena under 4 different conditions of food deprivation (0, 24, 48, 72 hr). Differences in the amount of food deprivation significantly influenced the drug's effect on feeding and rearing, but not on ambulatory activity. Also, differences in the amount of food deprivation appeared to more significantly and reliably influence in interrelationship (correlation) of feeding with ambulatory activity than the interrelationship of feeding with rearing or rearing with ambulatory activity. These findings suggest that the amount of food deprivation differentially influences difference concurrent measures of amphetamine effects as well as differentially affecting the interrelationship of the drug's effects. The importance of the correlation data to the potential incompatibility of amphetamine's effects as well as to an assessment of the different activity measures is briefly considered.

d-Amphetamine Food deprivation Anorexia Ambulatory activity Rearing Interrelationship of drug effects

WHILE it has long been recognized that food deprivation may influence the experimental effects of amphetamine [2], the more specific importance of such deprivation to an assessment of particular measures of the drug's effect remains unclear. For example, in the case of feeding measures, the effects of amphetamine appear to interact with food deprivation [3,6], although such a conclusion may depend upon how one determines drug dosage in feeding studies; i.e., as absolute dosage or mg/kg dosage [7,10]. Regarding other behavioral measures that have been examined, the data are sparse and equally inconclusive. While the effects of amphetamine on food-dish contact time differ with differences in the amount of food deprivation, the effects of the drug on ambulatory activity appear to be relatively insensitive to differences in the amount of deprivation [5]. Furthermore, until rather recently [5], there seems to have been little or no concern for determining the importance of food deprivation to different measures of amphetamine effects under conditions where such measures are assessed concurrently (within same observation period) rather than independently (in different observation periods). The present study addresses itself to this particular issue.

While some data on the importance of food deprivation to the concurrent assessment of amphetamine's effects on feeding and activity have been previously reported [5], the present study expands the investigation in the following specific ways: (a) it increases the range of both the drug doses and food deprivation conditions used; (b) it examines activity in an open-field arena where the subject has an increased opportunity for ambulatory movement; and (c) it assesses two measures of activity (ambulatory movement and rearing) rather than only ambulation. In addition to determining the importance of food deprivation to the general effects of amphetamine on concurrent measures of feeding and activity, the present study also examines the importance of food deprivation to the interrelationship (correlation) of the drug's effects on these behaviors. The second of these objectives permits one to determine whether amphetamine's depression of feeding might be due to the drug's incompatible (competing) hypermotility action. A competing response hypothesis of amphetamine effects has been proposed by several authors [1, 8, 9] and has important implications concerning the practical use and theoretical interpretation of the drug's action.

METHOD

Subjects

Forty adult, male Holtzman rats (300–450 g) were subjects. They were housed individually under standard laboratory conditions and had ad lib access to water in the home cage. They were also permitted ad lib access to Purina laboratory chow in the home cage, except when otherwise specified in the food deprivation procedure.

Apparatus

A 80×80 cm open-field arena, which had been used successfully in previous drug assessment studies, served as the test apparatus. The floor of the arena was divided into sixteen 20 cm squares with the use of a marking pen to provide a standardized basis for scoring ambulatory movement. The walls of the arena were 30 cm high, and a plastic food cup, firmly attached to one wall of the arena at floor level, permitted free access to food. However, water was not available in the test arena. Fluorescent lighting directly above the testing area provided uniform illumination of the arena.

Procedure

Initially, the 40 subjects were assigned randomly to one of 4 food deprivation groups (0, 24, 48, 72 hr). While under their appropriate food deprivation condition, all subjects were administered two 30-min adaptation sessions, separated by approximately 1 week. During adaptation sessions, subjects were permitted to explore the open-field and to eat freely 45 mg precision food pellets placed in the food cup.

Following adaptation, all subjects, while again under their appropriate food deprivation condition, were administered four 30-min drug test sessions (0.0, 0.5, 1.0, 2.0 mg/kg d-amphetamine SO_4 in 1 ml/kg 0.9% NaCl), with the order of the drug dose randomly assigned to animals over successive sessions. Approximately 1 week separated the last adaptation session from the first test session and each of the test sessions. Measures of feeding and activity were determined for each test session. Food consumption was measured by placing 300 precision pellets in the food cup 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. Activity was measured both in terms of the number of discrete rearing events (regardless of duration) and the number of squares entered by subjects. Hand counters were used to record activity data and, since a single observer recorded all data, no interjudge reliability measure was determined.

The testing procedure for any one subject was as follows. The animal, after having been food deprived for the specified number of hours (with the exception of the 0 hr deprivation group), was removed from the home cage, weighed, injected IP with the appropriate dose of d-amphetamine, and returned to the home cage. Thirty min later, the subject was placed in the open-field test arena, in front of and facing the food cup, to begin the 30-min test session. Upon completion of the session, the animal was returned immediately to the home cage to await the next session.

RESULTS

The effect of amphetamine doses on the food consumption of subjects in the different food deprivation groups is summarized in Fig. 1. Overall analysis of these data demonstrated a highly significant Drug Dose effect, $F(3,108) = 94.23$, $p<0.01$, a significant Deprivation effect, $F(3,36) = 27.04$, $p<0.01$, and a significant Drug Dose×Deprivation Interaction, $F(9,108)=7.86$, $p<0.01$. Further analysis (t-test for related samples following ANOVA; minimum 0.05 level of significance) of drug dose data indicated that, in the 0 hr deprivation group, none of the drug doses differed significantly from the vehicle (0.0 mg/kg) or from each other. However, in the case of both the 24 and 48 hr deprivation groups, all 3 drug doses (0.5, 1.0, 2.0 mg/kg) differed significantly from the vehicle and from each other, with the exception of the 1.0–2.0 mg/kg comparison. In the 72 hr deprivation group, all 3 drug doses differed significantly from the vehicle and from each other.

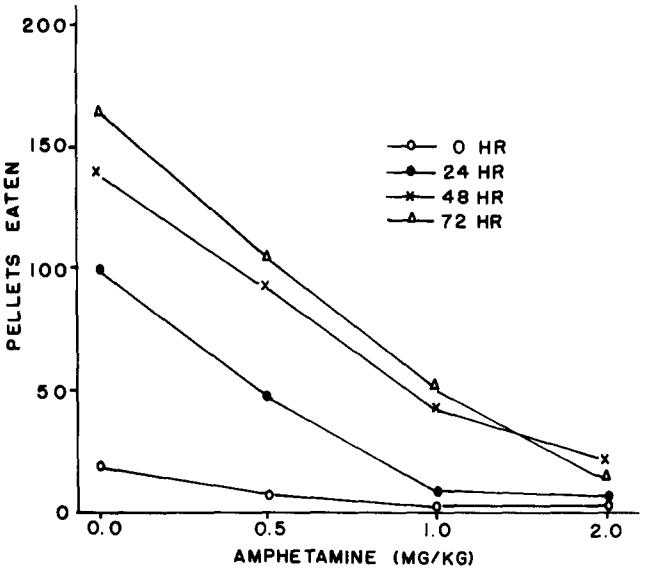

FIG. 1. Mean number of pellets eaten by subjects in 0. 24, 48, and 72 hr food deprivation groups under different d-amphetamine dose (0.0, 0.5, 1.0, 2.0 mg/kg) conditions. Drug doses were assigned randomly to subjects over four 30-min test sessions.

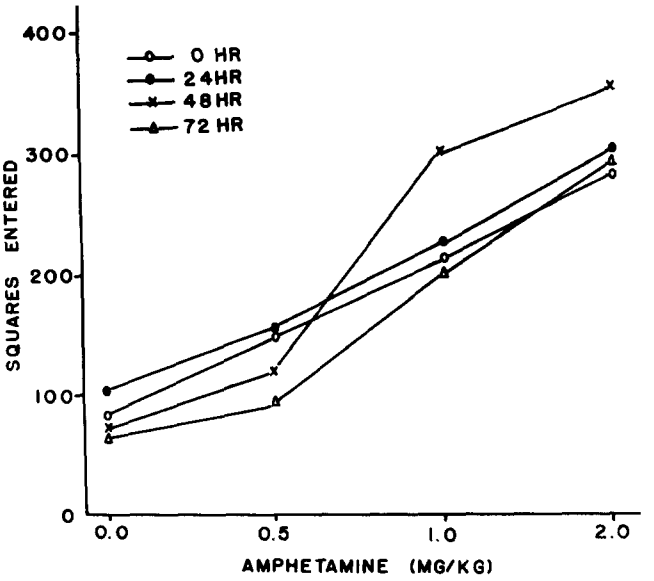

FIG. 2. Mean number of squares entered by subjects in 0, 24, 48, and 72 hr food deprivation groups under different d-amphetamine dose (0.0, 0.5, 1.0, 2.0 mg/kg) conditions. Drug doses were assigned randomly to subjects over four 30-min test sessions.

The effect of amphetamine doses on the ambulatory activity (squares entered) of subjects in the different food deprivation groups is summarized in Fig. 2. Overall analysis of these data demonstrated a significant Drug Dose effect, $F(3,108)=59.40$, $p<0.01$, but no additional significant

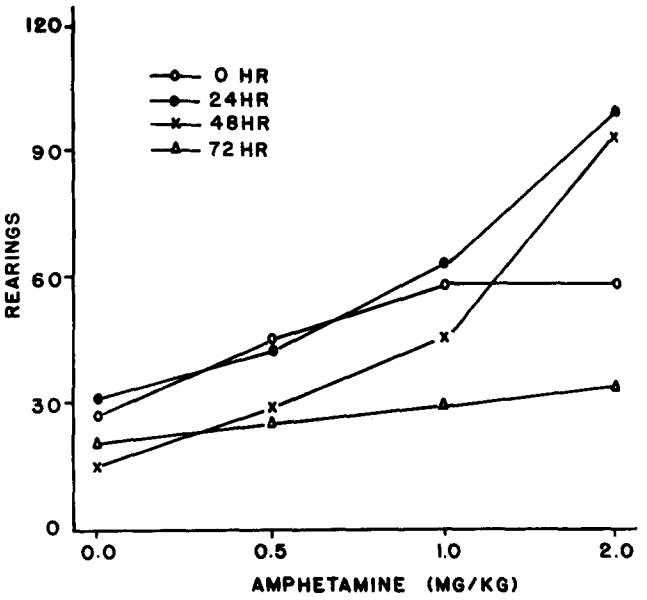

FIG. 3. Mean number of rearings by subjects in 0, 24, 48, and 72 hr food deprivation groups under different d-amphetamine dose (0.0, 0.5, 1.0, 2.0 mg/kg) conditions. Drug doses were assigned randomly to subjects over four 30-min test sessions.

sources of variance. Further analysis (t-test for related samples following ANOVA; minimum 0.05 level of significance) of drug dose data indicated that, in both the 0 and 24 hr deprivation groups, all 3 drug doses differed significantly from the vehicle and from each other. In the 48 hr deprivation group, the 1.0 and 2.0 mg/kg doses differed significantly from the 0.5 mg/kg dose and the vehicle, but doses within these pairs did not differ significantly from each other. In the 72 hr deprivation group, only the 1.0 and 2.0 mg/kg doses differed significantly from the vehicle, but all 3 drug doses differed significantly from each other.

The effect of amphetamine doses on the rearing of subjects in the different food deprivation groups is summarized in Fig. 3. Overall analysis of these data demonstrated a significant Drug Dose effect, $F(3,108)=18.75$, $p<0.01$, a significant Deprivation effect, $F(3,36)=3.15$, $p<0.05$, and a significant Drug Dose×Deprivation Interaction, $F(9,108)=2.36$, $p<0.05$. Further analysis (t-test for related samples following ANOVA; minimum 0.05 level of significance) of drug dose data indicated that, in the 0 hr deprivation group, only the 1.0 and 2.0 mg/kg doses differed significantly from the vehicle. In both the 24 and 48 hr deprivation groups, the 1.0 mg/kg, but not the 0.5 mg/kg, dose differed significantly from the vehicle, and the 2.0 mg/kg dose differed significantly from the vehicle and the two lower doses. In the 72 hr deprivation group, none of the drug doses differed significantly from the vehicle or from each other.

In order to determine the interrelationship of the effects of amphetamine on the concurrent measures of behavior, drug-dose correlations of feeding with ambulatory activity, feeding with rearings, and ambulatory activity with rearings were calculated for each subject in the different food deprivation groups. The individual correlations of the 10 subjects within each of the deprivation groups were then pooled to form a deprivation-group composite correlation, with these

TABLE 1

SUMMARY OF DRUG DOSE CORRELATIONS (PEARSON r) OF FEEDING WITH AMBULATORY ACTIVITY (F-A), FEEDING WITH REARING (F-R), AND AMBULATORY ACTIVITY WITH REARING (A-R) IN 0, 24, 48, AND 72 hr FOOD DEPRIVATION GROUPS

	Food deprivation groups			
Comparisons	0	24	48	72
F-A	−0.4158	−0.7713†	−0.8118†	−0.8690†
F-R	−0.3715	−0.6784*	−0.6683*	−0.4425
A-R	+0.7276*	+0.7383*	+0.7481*	+0.4713

*0.05 level of significance ($df=8$).
†0.01 level of significance ($df=8$).

results being summarized in Table 1. As is apparent, the drug-dose effects of d-amphetamine on feeding demonstrated a significant negative correlation with the effects of the drug on ambulatory activity (squares entered) in all food deprivation groups except the 0 hr group and with the effects of the drug on rearing in the 24 and 48 hr food deprivation groups. In contrast, the drug-dose effects of amphetamine on ambulatory activity and rearing demonstrated a significant positive correlation in all food deprivation groups except the 72 hr group.

DISCUSSION

With a more expanded range of drug dose and food deprivation conditions than has been used heretofore [5], the findings of the present study demonstrate that the importance of food deprivation to the general effects of amphetamine depends upon the particular measure of behavior one is identifying. While differences in food deprivation did not significantly alter the dose-ordered increase in ambulatory activity produced by the drug, differences in food deprivation did significantly affect the dose-ordered decrease in feeding produced by the drug as well as the drug's effect on rearing. Such a conclusion is clearly supported by the significant Deprivation effect ($p<0.01$) and Drug Dose×Deprivation Interaction ($p<0.01$) with feeding and by the significant Deprivation effect ($p<0.05$) and Drug Dose×Deprivation Interaction ($p<0.05$) with rearing. While the significant Deprivation effect on feeding appeared to be due, in large measure, to differences in the effectiveness of conditions within the 0–48 hr range, the significant Drug Dose×Deprivation Interaction was due, mainly, to the relatively flat dose-response function in the 0 hr deprivation group. Such an interpretation of the Interaction effect is confirmed by a re-analysis of feeding data with the 0 hr deprivation group deleted, which still yielded a significant Drug Dose effect ($p<0.01$) and a significant Deprivation effect ($p<0.01$), but did *not* yield a significant Drug Dose × Deprivation Interaction. Although the 0 hr deprivation group demonstrated a total cessation of feeding only under the 1.0 and 2.0 mg/kg dose conditions, the animals in this group consumed such a small number of pellets under the remaining conditions (0.0 and 0.5 mg/kg) that the dose-response function approached a "floor effect." While it is clearly recognized that such a "floor effect" has the potential for producing an artifactual interaction between amphetamine dose and food deprivation and should, therefore, probably be minimized or avoided whenever possible in drug studies, there may be occasions when such a "floor effect"

might serve as a useful yardstick for determining the minimum requirements for or limitations in the effectiveness of experimental variables. For example, such a "floor effect" observed in the present study demonstrates that some minimal amount of food deprivation is essential to the establishment of a reliable dose-response function. In the case of rearing, the significant Deprivation effect appeared to be due to differences in the effectiveness of conditions spread across the entire range of deprivation hrs investigated. It is important to note, however, that the significant Drug Dose×Deprivation Interaction with rearing involved no clear evidence of a similar "floor effect." While the dose-response function on rearing was relatively flat in the 72 hr deprivation group (which undoubtedly made a significant contribution to the Drug Dose×Deprivation Interaction), there was no indication that the flatness of the function was due to any tendency for rearing to be eliminated under these conditions. Thus, at least in the case of rearing, the assumption concerning the interaction of drug dose and food deprivation can be made free of any artifactual bias.

The drug-dose correlation data (see Table 1) also suggest that the importance of food deprivation to the interrelationship of amphetamine's effects on the different measures of behavior depends upon the particular interrelationship one is identifying. While the drug-dose correlations of feeding with ambulatory activity were relatively high ($p<0.01$) in all deprivation groups except 0 hr and showed a general progression with an increase in deprivation, drug-dose correlations of feeding with rearing and rearing with ambulatory activity were, in general, lower ($p<0.05$) and showed no consistent progression with an increase in deprivation. In fact, the drug-dose correlations of feeding with rearing and rearing with ambulatory activity demonstrated a sharp decline in the 72 hr group instead of a further increase as was observed in the drug-dose correlation of feeding with ambulatory activity in this group. Thus, differences in the amount of food deprivation (excluding 0 hr) appeared to more significantly and reliably influence the interrelationship of feeding with ambulatory activity than the interrelationship of feeding with rearing or rearing with ambulatory activity.

The significant negative correlation in amphetamine's effect on feeding and ambulatory activity across food deprivation conditions (excluding 0 hr) observed in the present study is consistent with previous findings based upon a more limited drug dose and food deprivation range [5] and suggests that such an interrelationship is a rather robust phenomenon. More importantly, these findings have particular 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 [1,9]. 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 depression of feeding being due to its competing excitatory action on ambulatory activity is high. While the excitatory effects of amphetamine on rearing may also compete with the requirements of feeding and thus contribute to the drug's depression of feeding, the magnitude and consistency of the negative correlations of feeding with rearing do *not* suggest that the effects on rearing comprise as important an incompatible principle as do the effects on ambulatory activity. It is also of interest to note that the present interpretation is consistent with the theory of Lyon and Robbins [9], which predicts that shorter behavioral "chains" (such as rearing) produced by amphetamine's arousal effects will be less disturbing to other behaviors (such as feeding) than will longer behavioral "chains" (such as ambulation).

The significant positive correlation in amphetamine's effects on ambulatory activity and rearing is not particularly surprising, although one might have expected such a correlation to be higher (since both measure arousal and no significant drug-induced stereotypy was present to interrupt either behavior) and to hold up better across deprivation conditions. While the correlation was remarkably stable across the 0–48 hr deprivation groups, the relatively flat dose-response function with rearing resulted in a substantial breakdown of the correlation in the 72 hr group.

Perhaps, the singly most important principle illustrated by these findings is the fact that, under conditions of the present study, ambulatory activity and rearing do not provide highly redundant measures of amphetamine'a general arousal effect. Accordingly, the value of multiple measures of general activity in drug-assessment studies should be obvious, particularly when one is interested in the degree to which arousal effects might influence other behaviors.

Finally, there would appear to be particular merit in a *concurrent* assessment of amphetamine's effects on different behaviors as was done in the present study instead of an *independent* assessment of the drug's effects on different behaviors. As has been previously pointed out [4, 5, 11, 12], such an experimental approach permits one to examine the interrelationships of the drug's effects on different behaviors and to derive more meaningful conclusions about the complex multiple action of the drug.

ACKNOWLEDGEMENTS

The d-amphetamine was generously supplied by Smith, Kline, and French Laboratories, Philadelphia, PA. Appreciation is expressed to Elizabeth Pokalo for assistance in data collection and Dorothy Cole for technical assistance in preparing the figures.

REFERENCES

1. Carlton, P. L. Cholinergic mechanisms in the control of behavior by the brain. *Psychol. Rev.* **70:** 19–39, 1963.
2. Cole, S. O. Experimental effects of amphetamine: A review. *Psychol. Bull.* **68:** 81–90, 1967.
3. Cole, S. O. On the combined effects of amphetamine and food deprivation: A reply to Gollub and Mann. *Psychopharmacologia* **16:** 426–429, 1970.
4. Cole, S. O. Brain mechanisms of amphetamine-induced anorexia, locomotion, and stereotypy: A review. *Neurosci. Biobehav. Rev.* **2:** 89–100, 1978.
5. Cole, S. O. Interaction of food deprivation with different measures of amphetamine effects. *Pharmac. Biochem. Behav.* **10:** 235–238, 1979.
6. Cole, S. O. and P. E. Gay. Interaction of amphetamine and food deprivation on a food-motivated operant. *Communs behav. Biol.* **6:** 345–347, 1971.
7. Gollub, L. R. and W. G. Mann, Jr. The interaction of amphetamine and body weight on a food-reinforced operant. *Psychopharmacologia* **15:** 64–72, 1969.

8. Grilly, D. M. Rate-dependent effects of amphetamine resulting from behavioral competition. *Biobehav. Rev.* **1**: 87–93, 1977.
9. Lyon, M. and T. Robbins. The action of central nervous system stimulant drugs: A general theory concerning amphetamine effects. In: *Current Developments in Psychopharmacology, Vol. 2,* edited by W. B. Essman and L. Valzelli. New York: Spectrum Publications (Distributed by John-Wiley and Sons), 1975, pp. 81–162.
10. MacPhail, R. C. and L. R. Gollub. Independence of the effects of d-amphetamine and food deprivation or body weight on the food consumption of rats. *Psychopharmacologia* **34**: 163–172, 1974.
11. Segal, D. S. and A. J. Mandell. Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. *Pharmac. Biochem. Behav.* **2**: 249–255, 1974.
12. van Rossum, J. M. and F. Simons. Locomotor activity and anorexigenic action. *Psychopharmacologia* **14**: 248–254, 1969.