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# Effects of Interactions Between Amphetamine and Food Deprivation on Covariation of Muricide, Consummatory Behaviour and Activity

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RUSSELL, J. W., G. SINGER AND G. BOWMAN. *Effects of interactions between amphetamine and food deprivation on covariation of muricide, consummatory behaviour and activity.* PHARMACOL BIOCHEM BEHAV **18**(6) 917–926, 1983.—Four experiments were conducted to study covariation of muricide, consummatory behaviour and general activity under conditions involving interactions between food deprivation and anorexia induced by low doses of d-amphetamine. Two basic experimental designs were used: (a) dosage of amphetamine was varied, with duration of deprivation constant at 22 hr; and (b) deprivation was varied, with a constant amphetamine dose of 1.0 mg/kg body weight. In general, effects of both types of manipulation on eating, drinking, general activity, and muricidal behaviour were consistent with earlier reports of effects when either deprivation or amphetamine-induced anorexia was varied separately. Rank order correlations supported the conclusions that relations between muricide, eating behaviour and general activity were both dose and time dependent. However, there also was evidence that such covariations existed for some, but not all, parameters of these behaviours, eg. differences in the median effective doses of amphetamine; differences in threshold doses; and differences in durations of deprivation required to counteract effects of amphetamine. A dissociation of muricide and eating behaviour was strikingly evident when all satiated animals injected with normal saline engaged in muricidal behaviour and when increasing deprivation had no significant influence on carcass consumption or muricidal behaviour of saline treated subjects. The present results are interpreted as essentially inconsistent with concepts in which covariation of muricide and consummatory behaviour is considered to depend: (a) upon a common set of physiological conditions, or (b) upon one being the antecedent of the other. However, results are consistent with a model in which each behaviour has its own basic physiological condition(s) which may be activated concomitantly.

Muricide Eating Drinking General activity Stereotyped behaviour Amphetamine Food deprivation

THERE has been considerable debate over the past fifteen years about possible relations between consummatory behaviour and muricide. The most simplified hypothesis which has been proposed is that muricide is a form of food-getting behaviour [26]. Because eating and drinking are innate genetically determined behaviours essential for maintenance of nutritional levels and fluid balance within the body for survival, it would be expected that, was this hypothesis valid, muricide would appear universally in all members of the species. Yet in early experiments [18] it was reported that, while the propensity to attack could be increased by changes in tissue conditions accompanying food deprivation, not all animals would kill. In fact, of 21 rats which failed to kill in this experiment, 15 starved to death in the presence of mice. Clearly the relation is more complex than is encompassed by such a relatively simple hypothesis.

In an earlier report [31] we have presented evidence showing significant covariation of muricide and eating behaviours. In these experiments deprivation occurred "naturally" as a function of the subjects' own feeding rhythms during a 12/12 hr light/dark circadian cycle. Food intake was found to covary significantly with muricide, while no such relationship was found between muricide and water intake. Covariation of muricide and eating does not, however, establish a causal relation between the two. A relationship between increased food deprivation and muricidal behaviour has been previously reported [5, 27, 28]. In other more complex studies the research designs have involved joint manipulation of food deprivation and some other potentially significant independent variable such as water deprivation, time of testing, etc. The general hypothesis in such studies being that muricidal behaviour may be a function of such interac-

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tions rather than of either variable alone. This research approach is illustrated by studies of effects of prior food competition on subsequent behaviour to mice [16, 24, 37]. The results suggest that competition for food prior to weaning probably does not influence muricidal behaviour.

In the four experiments presented here, we have also used an interactive approach, but one of a different nature. Advantage has been taken of the anorexic effect of d-amphetamine to observe muricidal behaviour under two conditions: (a) when food deprivation was held constant and dose of amphetamine varied systematically, and (b) when dose of amphetamine was held constant and period of food deprivation varied.

This interactive approach provided an opportunity to examine the relationship between hunger and muricide. It has been well established that d-amphetamine is a powerfully acting anorectic drug [6, 8, 9]. It is presumed that the drug is exerting its action on catecholamine and indolamine systems in the central nervous system [3, 14, 21]. If various doses of amphetamine reverse the effects of food deprivation on muricide this would add support to the interpretation that muricide, at least during the initial exposure to mice, is dependent on hunger induced by food deprivation.

Amphetamine, however, affects a variety of other behaviours in addition to inducing anorexia. It exerts central stimulating actions producing arousal and hyperactivity. Within limits it facilitates goal-directed, operant responding [8]. It has been shown to suppress muricidal behaviour in a dose-dependent manner [11,20] and produce abnormal behavioural patterns at high doses [34]. The molar effectiveness, of different behaviours relative to doses of d-amphetamine has been shown to vary almost 100-fold, however, its molar effectivensss on eating and drinking lies within a close range [22]. These behavioural effects of amphetamine have a bearing on the present experiments in which eating, drinking and activity, as well as muricide, were the dependent variables studied. In the first experiment, it was hypothesised that if muricide is "driven" by a state of hunger, then amphetamine induced anorexia should suppress muricide in a dose dependent manner in food deprived rats.

## EXPERIMENT 1: DOSE-DEPENDENT EFFECTS OF d-AMPHETAMINE ON MURICIDAL BEHAVIOUR OF FOOD-DEPRIVED RATS

Four dose levels of amphetamine were used in this experiment with food deprivation held constant at 22 hr.

### METHOD

*Animals*

Five groups of twenty naive male Wistar derived rats from the La Trobe University stock, ranging in age from 90–100 days at the time of testing were used. All were housed in individual wire mesh cages, measuring 40×20 cm, in a room maintained at 22±1°C. The room was illuminated on a 12 hr *ON* and 12 hr *OFF* light/dark cycle. They were given ad lib water, with food available only as required by the experimental design.

Naive adult Swiss mice (100 mice), weighing approximately 30 g, were used as prey.

Observations of muricidal behaviour were made on each rat in its home cage.

*Drug*

Four doses of dextroamphetamine sulphate (Smith Kline and French) were chosen on the basis of reports [9,20] from earlier experiments of levels which produced anorexia without significant incapacitation: 0.5, 1.0, 2.0 and 3.0 mg/kg body weight. Normal saline was used as the control substance and for amphetamine solutions. Volumes injected were calculated on the basis of 1 ml/kg body weight.

*Procedure*

All animals were habituated to the conditions prevailing in the experimental room for at least 1 week prior to testing. Rats were then randomly assigned to one of five groups with the restriction that there was no more than 20 rats in each group.

Twenty-two hours before testing, food was removed from all cages while water was maintained ad lib. Ten minutes prior to testing, rats were weighed and injected intraperitoneally with one of the doses of amphetamine or saline. All animals were tested for killing during a 1 hr trial interpolated in the middle of the 12 hr light period. Testing consisted of placing a previously weighed female mouse in the front of a cage and recording the amount of time it took the rat to kill. If a kill did not occur within the hour, the mouse was removed and the rat classified as a non-killer. When the animal killed a mouse, the carcass was removed at the end of the testing session and the carcass weighed. The amount of carcass consumed was determined by subtracting the carcass weight from the initial mouse weight.

### RESULTS

*Muricide*

The percent of rats exhibiting muricidal behaviour at the various doses of d-amphetamine are summarized in Fig. 1. There is a clear trend for the frequency of muricide to decrease with increasing dose levels. That this negative dose dependency is statistically significant is established by trend analysis using the Spearman Rank statistic: $r_s = -0.93$, $p<0.05$.

*Response Latency*

A Kruskal-Wallis one-way ANOVA confirmed that there were no significant differences among dosage groups in latency of the muricidal behaviour ($H(4)=3.96$, $p<0.05$), the median latency for all groups being approximately 64 sec.

*Carcass Consumed*

Figure 2 summarizes the data on amount of carcass consumed by animals for four doses of drug and saline. Again there appears to be a negative dose dependency with little or no consummatory behaviour in animals at the high dose levels. The significance of this trend is shown by the Spearman Rank statistic: $r_s=0.90$, $p=0.05$. That there were significant differences among the groups was shown by a Kruskal-Wallis ANOVA ($H(4)=11.5$, $p<0.05$) and by Mann-Whitney analysis in which all amphetamine treated groups differed significantly from the control group ($p<0.05$).

The behaviour of animals at the 2.0 and 3.0 mg/kg dose levels was bizarre. Without exception, the few animals which killed, spent the time remaining in the test period in biting the carcass over its entire body surface. This resulted

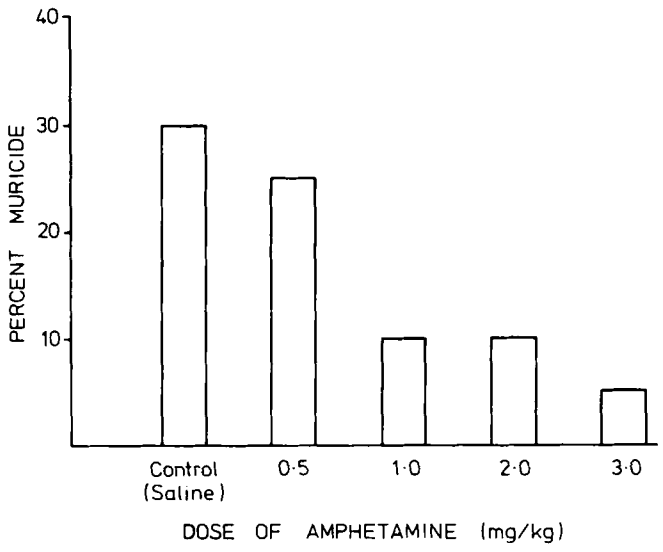


FIG. 1. Dose dependent relations between dose levels of d-amphetamine and frequency of muricide (deprivation constant at 22 hr).

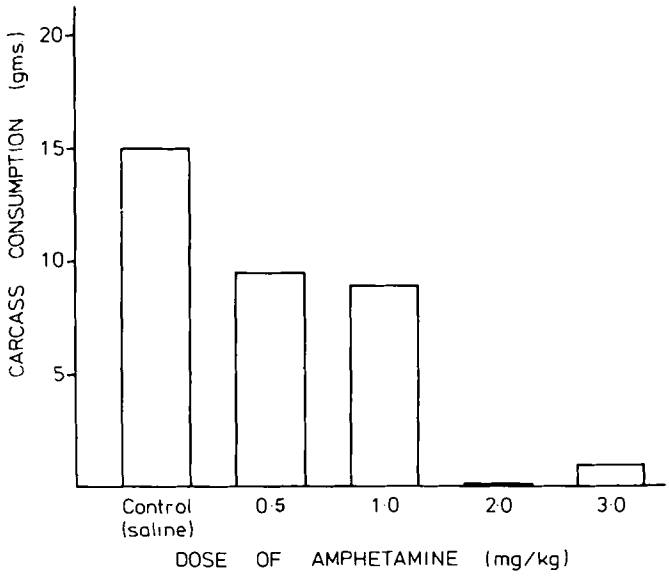


FIG. 2. Dose dependent relations between dose levels of d-amphetamine and amount (g) of carcass consumed (deprivation constant at 22 hr).

in multiple wounds to the carcass after the lethal bite, which inevitably occurred in the neck region. By comparison, control animals began eating the carcass at the position of the lethal bite and usually proceeded to eat towards and including the brain. Animals in the higher dosage groups would persevere at biting the carcass rather than commencing ingestion, the final consummatory act.

## DISCUSSION

### *Dose Dependencies*

In Experiment 1 it was shown that, under conditions of constant food deprivation, there was a significant dependency between muricide and dose level of d-amphetamine. Similar dependencies have been reported by other investigators [17, 19, 20]. All previous designs have differed from the conditions of Experiment 1, which emphasised the interaction between a state (food-hunger) that covaries with muricide [31] and effects, for example, anorexia, of a pharmacological manipulation that counteracts that state. It is not surprising that the dose levels at which muricide is suppressed differ between these reports and the present experiment.

The absence of an effect on response latencies between dose levels was a surprising finding. It could be assumed, particularly at the higher doses, that amphetamine might influence the fluency of attack. Our data, however, must be interpreted with some caution as the number of animals exhibiting muricide at the three highest doses were very small with only one animal present in the 3 mg/kg group. More subjects need to be assessed before the effects of d-amphetamine on response latencies can be elucidated.

The decrease in carcass consumption with increasing doses of d-amphetamine was consistent with the majority of other reports that one of the behavioural effects of low doses of amphetamine is to induce anorexia. The Spearman Rank correlation between frequency of muricide and carcass consumption was $r_s=0.82$ which is 0.08 less than the accepted level of confidence, $p<0.05$, yet is of the same order of magnitude as the corresponding correlation reported in our earlier experiments ($r_s=0.89$, $p<0.05$), when laboratory chow rather than animal carcass was ingested [31].

### *Bizarre Behaviour*

One of the most interesting findings in Experiment 1 was the very bizarre behaviour induced by the two highest doses of d-amphetamine. Under conditions of the experiment consummatory behaviour may be viewed as involving a series of related responses. Food becomes available through the muricidal act. This is followed by "dissection" of the carcass and, finally, by chewing and swallowing. The effect of amphetamine at the two highest doses was to interrupt this seriatum responding immediately after muricide and preceding the "dissection" stage. The failure to begin the "dissection" appeared to be due to the animal perseverating on the muricidal act, i.e., repeatedly biting the mouse over its entire body surface. This quality of the drug induced response might be characteristic of perseverative behaviour reported for effects of amphetamine on other behaviours, e.g., repetitive [4] and stereotyped responding [30,34].

One possible explanation for the reduced carcass consumption for the high dose groups might be that the animals are "prevented" from consuming the animal because of perseverative biting rather than to attenuation of hunger per se. This interpretation is consistent with that of Cole [9].

## EXPERIMENT 2: DOSE-DEPENDENT EFFECTS OF d-AMPHETAMINE ON CONSUMMATORY RESPONSES OF FOOD-DEPRIVED RATS

Having obtained information about dose-dependent effects of d-amphetamine on muricide and carcass consumption under conditions of food deprivation, this experiment was designed to study effects of the same treatments on consummatory responses measured by food (laboratory

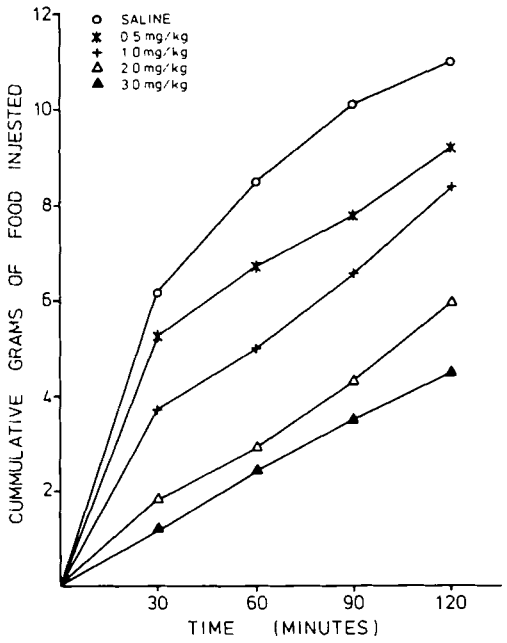


FIG. 3. Dose and time dependent relations between dose levels of d-amphetamine and food intake (deprivation constant at 22 hr).

chow) and water intake. This was necessary in order to establish amphetamine anorectic effects under the same deprivation condition and dose levels used in Experiment 1. In addition, this would allow for later comparisons between the amounts of carcass consumption and food consumption.

## METHOD

### *Animals*

Five groups of ten 90–120 day old naive male rats from the same Wistar derived stock as those in Experiment 1 were used. All were housed in individual cages and under the same conditions of ambient temperature and diurnal illumination as in the previous experiment. They were given at least one week to habituate to the laboratory environment prior to the start of the experiment proper. During this period food and water were available ad lib.

### *Procedure*

The procedure for this experiment was identical to that used in Experiment 1 except the sample size per drug group was ten and food and water intakes were the dependent measures.

All testing was carried out in home cages. Food intake was determined by weighing the food dishes prior to testing and reweighing at 30 min intervals to determine the amount, in grams, consumed. Spillage was added to all weighings. Water intake during each of the four 30 min periods was recorded in ml.

## RESULTS

### *Food Intake*

Trends in the consummatory response as measured by food intake (g) are shown in Fig. 3. There appear to be both dose and time dependencies as shown in the rank orders of food intake at each time of measurement and at each dose level over all measurements.

TABLE 1

TUKEY POST-HOC TESTS FOR EFFECTS OF VARIOUS DOSES OF d-AMPHETAMINE ON FOOD INTAKE

| Dose (mg/kg) | 0.5 | 1.0 | 2.0 | 3.0 |
|---|---|---|---|---|
| 0.0 | 1.18* | 2.62* | 4.49* | 6.49* |
| 0.5 | — | 0.81 | 3.18* | 4.68* |
| 1.0 | — | — | 2.37* | 3.87* |
| 2.0 | — | — | — | 1.50* |

Expressed as mean differences between groups.
*$p<0.05$, Tukey H.S.D. = 1.23.

Two-way ANOVA for repeated measures provided main effects that support this observation. The dose-dependent effect, $F(4,135)=17.95$, $p<0.01$, when further analysed by Tukey post-hoc tests showed significant differences between all groups ($p<0.05$) except the 0.5 and 1.0 mg/kg group comparison (Table 1).

Analysis of the temporal pattern of food intake for the combined groups showed the greatest intake occurred during the first 30 min with relatively little change during subsequent intervals. The overall time effect was significant, $F(3,135)=68.59$, $p<0.01$, and is attributable to the set of comparisons between intake during the first 30 min and the remaining three time intervals (Tukey H.S.D. = 0.49, d's = −2.14, −2.29 and −2.32, respectively; all $p$ values <0.05). There were no significant differences among the later intervals.

It is also important, especially for comparison between food intake and other behavioural variables, that dose effects were due entirely to differences in consummatory responses during the first 30 min of ad lib access to food. This appears in Fig. 3 as the different levels of food ingested during that period. The rates of eating were very different among the five dosage groups: all began at zero, the intercept point for all curves in Fig. 3, and achieved levels at the end of 30 min which varies systematically from highest for the saline to lowest for the 3.0 mg/kg group. In Fig. 3 the essentially parallel lines beyond 30 min indicate that rates of consumption for the various dosage groups were similar during the last 90 min of the test period. Statistical verification of the significance of this observation comes from the Drug × Time interaction effect, $F(12,135)=12.21$, $p<0.01$, in the ANOVA of the overall data. This was further supported by Tukey H.S.D. tests which showed that saline treated animals consumed significantly more food during the first 30 min than did the 1.0, 2.0 and 3.0 mg/kg groups (Tukey H.S.D. 1.53; d's = −2.47, −4.2 and −4.92 respectively; and $p$ values <0.05). Similar tests indicated that the above groups also differed from the 0.5 mg/kg subjects (Tukey H.S.D. = 1.53; d's = −1.82 and −2.45 respectively; $p$ values <0.05) and that the comparison between animals at the two highest points was not significant ($d=-0.63$, $p>0.05$).

### *Water Intake*

ANOVA for data on water intake also showed significant

TABLE 2

TUKEY POST-HOC TESTS FOR EFFECTS OF VARIOUS DOSES OF d-AMPHETAMINE ON WATER INTAKE

| Dose (mg/kg) | 0.0 | 0.5 | 1.0 | 2.0 | 3.0 |
|---|---|---|---|---|---|
| 0.0 | — | −2.44* | 1.44 | 2.97* | 3.60* |
| 0.5 | — | — | 3.88* | 5.41* | 6.04* |
| 1.0 | — | — | — | 1.53 | 2.16* |
| 2.0 | — | — | — | — | 0.63* |

Expressed as mean differences between groups.
*$p<0.05$, Tukey H.S.D.=1.76.

dose, $F(4,45)=7.74$, $p<0.01$, and time, $F(3,136)=5.03$, $p<0.01$, dependencies. Results of Tukey post-hoc tests, summarised in Table 2, establish that the main effect at most dose levels was to suppress water intake.

The exception occurred with the 0.5 mg/kg dose, at which water intake consistently exceeded that in all other groups. Similar analyses for specific differences in time dependencies showed that the only significant difference was between the 30 min and the 120 min measures (Tukey H.S.D.=0.96; d=1.41; $p<0.05$). Because all animals had been maintained on ad lib access prior to the start of the amphetamine treatment, the absolute levels of total water intake were relatively low (saline, 7.0 ml; 0.5 mg/kg, 9.4 ml; 1.0 mg/kg, 5.3 ml; 2.0 mg/kg, 3.9 ml; 3.0 mg/kg, 3.7 ml) when compared, for example, with 23 hr water-deprived rats of like age, whose consumption is in the range of 20–30 ml [31]. Amount consumed increased systematically as measured at each time interval during the 2 hr test period.

## DISCUSSION

### *Dose Dependencies*

Data from Experiment 2 shows that following 22 hr of food deprivation, the anorectic effect of d-amphetamine are significantly dose dependent. The regularity with which food consumption decreased with increasing dose level is shown by trend analysis using Spearman Rank correlation which provided an $r_s=1.00$, $p=0.01$. Clearly the higher the dose of d-amphetamine, the greater was its influence in counteracting the effects on consummatory behaviour of food deprivation. If food intake under saline conditions is 100%, the suppressing effects of the various doses of amphetamine were: 0.5, 17%; 1.0, 26%; 2.0, 46%; and 3.0, 60%. The median effective dose ($ED_{50}$) of amphetamine for suppressing activation of the food-hunger drive under conditions of 22 hr food deprivation is calculated to be 2.29 mg/kg.

Results of the present experiment also showed that the effects of d-amphetamine on another consummatory system, water-thirst, were dose-dependent although the system was not activated by experimental deprivation. Animals maintained on ad lib access to water during the period of 22 hr food deprivation drank relatively little water under the test conditions, but the total amount they did drink were dose dependent, as by a Spearman Rank Correlation of $r_s=0.90$, $p=0.05$. Using water intake under saline conditions as the 100% baseline, effects of the various doses of amphetamine on drinking were: 0.5, 36% increase; 1.0, 22% suppression; 2.0, 45% suppression; and 3.0, 52% suppression. The increase found at 0.5 mg/kg was an unexpected result. While catecholamine agonists can increase water consumption in hypothalamic sites, increases due to peripheral administration have not to the authors' knowledge been reported. The $ED_{50}$ for suppressing the increase in total water intake during the test session is estimated as 2.86 mg/kg.

### *Time Dependencies*

Similar analyses can be carried out for studying effects of d-amphetamine on food and water intakes at various times following administration of the various doses of the drug for determining the nature of time dependencies under the conditions of food deprivation held constant at 22 hr. A Spearman Rank Correlation Coefficient of $r_s=-0.80$ ($p>0.05$) between 30 minute periods and amount consumed reflects the fact that most eating occurred during the first 30 min of the test period. The regular increase in water intake during the test session is reflected by a correlation of $r_s=+1.00$ ($p=0.05$).

### *Covariation*

As would be expected from the fact that the dose dependent trends for both food and water intakes were significant and both negative in sign, the Spearman Rank Correlation Coefficient for covariation of the two were substantial and significant: $r_s=+0.90$, $p=0.05$. By comparison covariation of trends for time dependent effects, although substantial, was not significant ($r_s=-0.80$, $p>0.05$).

## EXPERIMENT 3: DOSE-DEPENDENT EFFECTS OF d-AMPHETAMINE ON ACTIVITY OF FOOD-DEPRIVED RATS

Because d-amphetamine's central stimulating actions include arousal and hyperactivity, it was desirable to observe effects on locomotor activity of the same treatments used in Experiments 1 and 2. In addition, determining a dose response curve under these experimental conditions was necessary in order to choose an appropriate dose for Experiment 4.

## METHOD

### *Animals*

An experimental design analogous to that used in the previous experiment required 50 naive male rats from the same La Trobe University stock. Each was housed individually and under the same conditions of ambient temperature and illumination as in Experiments 1 and 2. Habituation to the laboratory environment occurred during 7 days preceding the experiment. The animals were assigned randomly to the five groups, 10 per group.

### *Apparatus*

For measurement of motor activity an Aminex field detector was used. Aminex uses a tuned oscillator circuit to supply high-frequency current to an input coil which creates a field around the test chamber. Movement within the chamber produces momentary changes in voltage induced in the output coil. This signal is amplified and an output pulse counted. Such devices have been used extensively to evaluate effects of drugs on motor activity.

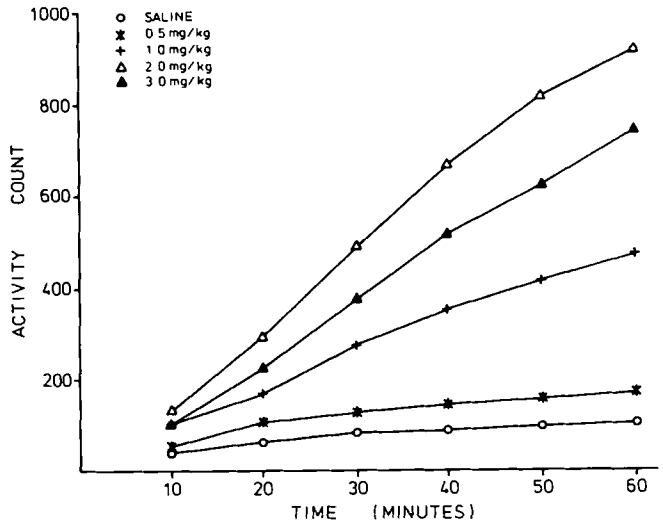


FIG. 4. Dose and time dependent relations between dose levels of d-amphetamine and activity (deprivation constant at 22 hr).

*Procedure*

Two days before testing, subjects were removed from their home cages and placed in plastic boxes of approximately the same size as the home cages. They were maintained on ad lib food and water until 22 hr prior to testing when only the food was removed. All testing took place during the middle of the 12 hr light period, i.e., 4–8 hr after lights went on. Ten minutes before testing each animal was injected IP with saline or one of the following doses of d-amphetamine sulphate: 0.5, 1.0, 2.0 or 3.0 mg/kg in a volume of 1 ml/kg body weight. There were ten subjects per group. Ten minutes later the boxes were placed on the Aminex activity meter, the equipment turned on and the subject given a 20 min warm-up prior to the start of the test session proper. Testing continued for 1 hr during which activity counts were recorded every 10 min.

## RESULTS

*Dose and Time Dependencies*

The cumulative activity counts for each of the different dosage groups is summarised in Fig. 4.

Examination of the total counts at 60 min shows that the relation between dose level and activity was curvilinear. Activity increased regularly with increasing doses to reach a maximum at 2.0 mg/kg. Thereafter a decrease in activity began. The overall effect was significantly dose dependent, $F(4,45)=7.90$, $p<0.01$. Further analysis using Tukey's post-hoc test provided the comparisons summarised in Table 3. All differences in activity between doses were significant except at the lowest and highest dose levels.

There was also a significant time dependency, $F(5,225)=7.38$, $p<0.01$. Tukey tests (Table 4) established that, with two exceptions which are difficult to interpret, comparisons of all other differences between activity levels at different times after injection of d-amphetamine were significant.

There was no significant Drug × Time interaction, $F(20,225)=1.40$, $p>0.05$.

TABLE 3

DIFFERENCES BETWEEN EFFECTS OF VARIOUS DOSES OF d-AMPHETAMINE ON LOCOMOTOR ACTIVITY (n=10)

| Dose (mg/kg) | Saline | 0.5 | 1.0 | 2.0 | 3.0 |
|---|---|---|---|---|---|
| 0.0 | — | 60.7 | 361.0* | 812.3* | 621.3* |
| 0.5 | — | — | 300.3* | 751.6* | 560.6* |
| 1.0 | — | — | — | 451.3* | 260.3* |
| 2.0 | — | — | — | — | 191.0 |
| 3.0 | — | — | — | — | — |

*$p<0.05$, Tukey H.S.D.=231.69.

TABLE 4

TUKEY POST HOC TESTS FOR TIME DEPENDENT EFFECTS OF d-AMPHETAMINE ON ACTIVITY

| Time (min) | 10 | 20 | 30 | 40 | 50 | 60 |
|---|---|---|---|---|---|---|
| 10 | — | 39.1* | 51.2* | −19.8 | − 91.5* | −153.7* |
| 20 | — | — | −13.1 | −57.9 | −129.6* | −191.8* |
| 30 | — | — | — | −71.0* | −142.7 | −204.9* |
| 40 | — | — | — | — | − 71.7* | −133.9* |
| 50 | — | — | — | — | — | − 62.2* |
| 60 | — | — | — | — | — | — |

Expressed as mean differences between groups.
*$p<0.05$, Tukey H.S.D.=27.9

*Focused Stereotypy*

Observations of animals administered 3.0 mg/kg of d-amphetamine showed that the increase in activity occurring regularly at lower doses had begun to include qualitatively different responses. The animals spent a large part of the test session sniffing, performing circular head movements and a sequence of actions consisting of crouching, sitting up to sniff the top of the cage and returning to the crouched position.

## DISCUSSION

*Dose Dependency*

Results of Experiment 3 show the effects on general activity resulting from the interaction of varying dose levels of d-amphetamine and 22 hours of food deprivation. This general activity has been shown under other sets of conditions to be affected by the drug [6, 7, 15, 23]. In general, earlier reports are consistent with findings of Experiment 3. Certain features of those findings have particular relevance for the present studies. As is evident in Fig. 4, the saline animals under 22 hr food deprivation showed a relatively low level of activity throughout the test period. Taking activity of this group as the 100% baseline, the stimulating effects of the various drug doses were: 0.5, 185.7%; 1.0, 514.3%; 2.0, 1028.6%; and 3.9, 814.4%. The midpoint of activity was at the dose of 1.0 mg/kg which is consistent with data reported by Cole [9].

*Focused Stereotypy*

The threshold for induction of focused stereotypy in Experiment 3 occurred between the 2.0 and 3.0 mg/kg dose levels of d-amphetamine. The dosage threshold for such behaviour was considerably lower than in many earlier studies [10, 29, 30] and may well have resulted from synergism of the interactive effects of amphetamine and food deprivation. This is consistent with data reported by Gershon, Singer and Ho. Food deprivation has been shown to increase sensitivity to sympathomimetic drugs by a shift of the dose-response curve to the left [6,25]. The fact that a threshold for stereotypy was reached at dose levels below 3.0 mg/kg could account for the drop in activity observed at that level, that is, stereotypy could have competed with locomotor behaviour [34].

## EXPERIMENT 4: EFFECTS OF VARIATIONS IN FOOD DEPRIVATION ON MURICIDE IN RATS ADMINISTERED A CONSTANT DOSE OF d-AMPHETAMINE

In Experiment 4 independent variables were reversed; administration of d-amphetamine was limited to a constant dose level while the period of food deprivation was varied. The dose of 1.0 mg/kg of amphetamine was selected on the basis of Experiments 1, 2 and 3 in which that dose caused significant reduction in food consumption and muricide and an increase in locomotor activity without producing stereotyped or other bizarre behaviours. This experiment differed from the previous experiments in that all rats were selected on the basis of their spontaneous muricidal behaviour during pre-experimental tests.

### METHOD

*Animals*

Sixty-five male rats from the same stock and with the same characteristics as shown in the preceding experiments were used. They were housed under the conditions described above and were maintained on ad lib water, with food available only as required by the experimental design.

Sixty-five naive adult Swiss mice, each weighing approximately 30 g, were used as prey.

Observations on muricidal behaviour were made on each rat in its home cage.

*Procedure*

The rats were screened for spontaneous killing prior to the beginning of the experiment proper. Sixty-five rats that killed mice within a 1 hr test session on two successive days were included in the experiment; those that did not kill on both days were excluded.

The first phase of the experiment lasted 7 days during which the subjects were tested for mouse killing under conditions of ad lib food and water. All testing was conducted during the middle of the 12 hr light period. Mice were presented to the subjects from the front of the cage, the rats being given 1 hr to kill. After an animal killed, the carcass was removed immediately, preventing any opportunity for ingestion. Following the last test session on Day 7, animals were assigned randomly to one of six treatment groups. The experimental design required one group under each of three conditions of food deprivation (0.0, 11.0 and 22.0 hr) and two conditions of pharmacological treatment (normal saline and 1.0 mg/kg of d-amphetamine sulphate). The periods of food deprivation were chosen a priori on the basis of previous experiments performed in our laboratory.

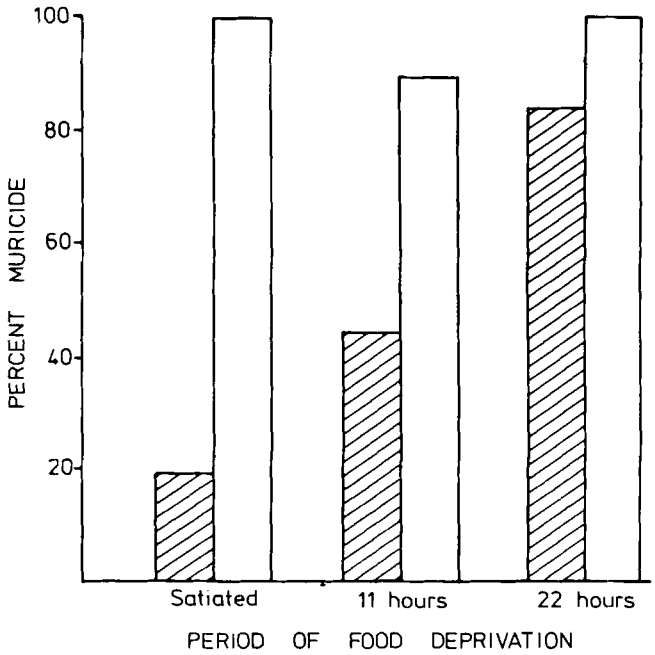


FIG. 5. Effects of various periods of food deprivation on frequency of muricide (amphetamine constant at 1.0 mg/kg). Hatched bans represent animals injected with amphetamine and clear bans represent saline treated animals.

On day 8 animals were tested for muricidal behaviour under these conditions. The subjects were deprived of food in accordance with their assigned group (i.e., 0.0, 11.0 or 22.0 hr prior to testing) and injected intraperitoneally 10 min prior to the test session with either amphetamine sulphate or isotonic saline. Testing was carried out under the same conditions as on the previous 7 days with the exception that the mouse remained in the rat's cage for the full 1 hr test session irrespective of whether or not muricide occurred. Mouse carcass weights were recorded at the end of the test session and carcass consumption was determined by subtracting the weight of the mouse carcass from its initial body weight.

### RESULTS

*Muricide*

The percent of rats exhibiting muricidal behaviour after the various periods of food deprivation are summarised in Fig. 5.

Control animals were injected with normal saline prior to testing continued at the 100% level of muricide for which they had been selected. By comparison, a dose of 1.0 mg/kg amphetamine suppressed muricidal behaviour to an extent inversely proportional to the period of deprivation. Analysis by Fisher's Exact Probability test showed a significant difference ($p<0.005$) between satiated experimental and control subjects, but not between amphetamine and saline group at 11 hr and 22 hr deprivation. The deprivation time to produce 50% muricide under these conditions was calculated to be 12.7 hr.

*Response Latency*

Measures of response latencies during the 7 days of test-

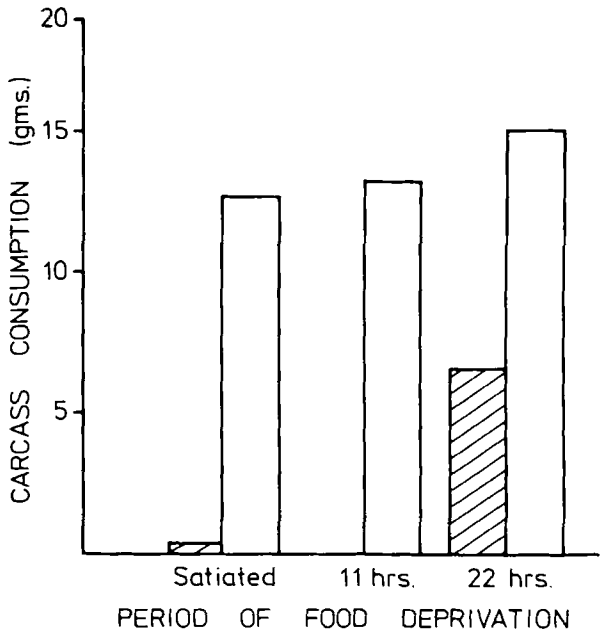


FIG. 6. Effects of various periods of food deprivation on amount (g) of carcass consumed (amphetamine constant at 1.0 mg/kg). Hatched bans represent animals injected with amphetamine and clear bans represent saline treated animals.

ing for muricidal behaviour which preceded the drug trials showed an initial decline from a median latency of 69 sec to 32 sec on the second day and leveling at a baseline of 23 sec thereafter. The magnitude of the latency on Day 1, 69 sec, was the same as that of the latency, 64 sec of rats in Experiment 1. Results of that experiment demonstrated that there were no significant differences in latency of the muricidal behaviour between amphetamine and control groups.

*Carcass Consumed*

Amounts of carcass consumed by both amphetamine and saline injected rats at the three levels of food deprivation are summarised in Figure 6.

There was a striking difference between the saline and amphetamine treated rats. The former consumed much greater amounts of carcass at all periods of deprivation. Although there was a trend towards increasing consumption with increasing deprivation among the saline groups, the tendency was very small and not significant.

The relationship was quite different among the amphetamine treated rats. Only one of the 0 hr (satiated) group and none of the 11 hr group consumed any portion of the dead prey, that is, amphetamine suppressed the consummatory behaviour exhibited by the saline treated rats. However, changes in tissue conditions associated with further deprivation began to counteract the suppression. At 22 hr deprivation consummatory behaviour of amphetamine treated animals was 45% of controls.

## DISCUSSION

*Deprivation Dependencies*

Data from Experiment 4 show that, under conditions of constant amphetamine treatment, muricidal behaviour is a function of extent of food deprivation. The dose of 1 mg/kg did not suppress killing completely even in satiated animals. Although the percentage of animals showing muricidal behaviour increased regularly with period of deprivation, deprivation did not begin to compensate for the effects of the dose of amphetamine until 22 hr. These results suggest that there are individual differences among rats in thresholds at which the hunger stimulating effects of deprivation counterbalance the anorectic effects of amphetamine. The median period of deprivation at which this occurred under conditions of Experiment 4 was approximately 12 hr.

The deprivation dependency for carcass consumption, under conditions of constant amphetamine treatment, had quite different characteristics. This consummatory behaviour was completely suppressed until between 11 and 22 hr deprivation. At 22 hr the amount of carcass consumed was 45% of the corresponding saline control rats, indicating that the median deprivation for counteracting the effects of amphetamine was somewhat above the levels tested. Further efforts should be made in order to describe this interaction.

*Carcass Consumption*

The fact that rodents are omnivorous animals is sometimes overlooked. Carcass consumption was not unexpected as the final act of muricidal behaviour. However, the extent to which it occurred under conditions of the present experiment was not anticipated. Earlier reports [25,37] have indicated that, once an animal has killed, it goes on killing irrespective of whether it is deprived or not. Results of Experiment 4 are consistent with this observation in that all saline-treated animals engaged in muricidal behaviour at all three levels of food deprivation. The unexpected observation is that these animals also engaged in consummatory behaviour in which amounts of intake were independent of the extent of food deprivation. This relation is inconsistent with that which characterises interactions between deprivation and normal food intake.

## GENERAL DISCUSSION

In the preceding discussion major features of the four experiments were considered individually. In this section an overview of them is provided.

*Effects of Interaction on Consummatory Behaviour and General Activity*

The major feature of design in all experiments was the interaction between two independent variables: food deprivation, which activates eating and certain other behaviours; and administration of d-amphetamine, which suppresses eating (anorexia) and also affects other behaviours. Interactive effects were studied by holding one of these constant and varying the other. Experiments 2 and 3 were planned to provide information about the interactive effects as they appeared in measures of consummatory behaviour and general activity.

With food deprivation held constant at 22 hr, eating, drinking and general activity showed systematic changes related to dose levels of d-amphetamine. The dependencies were monotonic for eating and drinking, the consummatory behaviours being increasingly suppressed as dose increased. Dose related effects on general activity were curvilinear: activity increased with doses less than 2.0 mg/kg peaked at that level and then decreased. Responding during the test period

also showed significant time dependencies, responses increasing monotonically at different rates. These results are consistent with reports by other investigators (e.g. [9,12]). In fact, they are remarkably so when differences in experimental conditions are considered, as indicated, for example, by the very similar $ED_{50}$ for activity measures in the present experiments and that estimated from data provided by Cole [9].

*Muricide: Evidence for Covariation*

Systematic changes occurred in muricidal behaviour under both the main experimental conditions. There was a significant trend for the frequency of muricide to decrease with increasing doses of amphetamine (deprivation constant) and for an increase in muricide to accompany increases in period of deprivation (dose of amphetamine constant).

Not all parameters of muricidal behaviour were, however, affected similarly. Response latencies were almost identical for initial responding under the two conditions of Experiments 1 and 4, and there were no significant differences in latencies between groups in either experiment. This suggests that not only may different behaviours react differentially to interactions between food deprivation and d-amphetamine, but so may different parameters of the same response.

The sequence of acts constituting muricide would be expected to end with consummatory behaviour if the same biochemical mechanism(s) of action subserve(s) both. Comparison can be made of effects of varying doses of d-amphetamine (deprivation constant) when the incentive (food) was laboratory chow and when it was a freshly killed carcass. From Figs. 2 and 3 it is apparent that similar dose dependencies exist. A Spearman Rank Order analysis of the trends gives an $r_s=0.90$, $p=0.05$: suppression of eating increased significantly with increasing dose levels of amphetamine regardless of the nature of the incentive. Examination of the information summarised in Fig. 6 shows that suppressive effects of amphetamine on carcass consumption can be counteracted if tissue conditions underlying the food-hunger drive have been sufficiently altered by food deprivation.

*Muricide: Evidence for Dissociation*

Despite these conclusions about covariation certain results of the present series of experiments provide evidence for a dissociation between eating behaviour and muricide. Dissociation appears in two forms. One is parametric: although both behaviours are affected by the deprivation-amphetamine interaction, the thresholds and median effective doses for each are different. A second form of dissociation is all-or-none, that is, one of the behaviours is affected by the deprivation-amphetamine interaction, but the other is not.

Examination of the dose-effect data presented in Figs. 1, 2 and 3 provides a basis for parametric comparisons. As discussed earlier, the rank order trends in all cases indicate a significant decrease in muricide and in consumption of carcass and normal food with increasing doses of amphetamine. Also, in all cases, effects began to appear (the basal threshold) at the lowest dose of amphetamine administered. However, the ($ED_{50}$s) were quite different for different behaviours: muricide, 0.83 mg/kg; carcass consumption, 1.17 mg/kg and normal food, 1.46 mg/kg. Differences in a third parameter, the terminal threshold, are also apparent. Terminal thresholds for muricide and normal food consumption are not reached within the range of doses used in the present experiments. However, the terminal threshold for carcass consumption was reached at the dose 2.0 mg/kg. (As reported above, it was at this dose level that bizarre behaviours began to appear.) Relations between number of hours of deprivation and the anorexic effect of a constant dose of amphetamine (1.0 mg/kg) provide further evidence of disjunction between muricide and carcass consumption: the former persisted even in satiated animals, while the latter remained suppressed until counteracted by tissue conditions induced by more than 11 hr deprivation.

Undoubtedly the most striking evidence for a dissociation between eating behaviour and muricide is found in control groups in Experiment 4 which were injected with saline only. First, all control animals in the group that had normal food available ad lib engaged in muricidal behaviour. Second, there was no evidence that increasing hours of deprivation (which is associated with increased consumption of normal food and which at 22 hr was capable of counteracting amphetamine suppression of carcass consumption) had any significant influence on carcass consumption or muricidal behaviour of the saline-treated animals. In fact, those animals which were satiated ate an average of 13 g of carcass, which is far in excess of the amount of laboratory chow consumed after 22 hours deprivation in Experiment 2 during the first hour of testing.

It is important to remember that these statements apply to animals selected for their muricidal behaviour. Had the full population been sampled rather than the present subpopulation, the results would have been confounded by the presence of animals that were not muricidal. The objective of the present experiment was to observe deprivation-amphetamine interactions when such complications were minimised.

In evaluating the significance of the conclusions reached in the preceding two paragraphs more weight should be given to the assertions in the second than those in the first. Differences in various parameters of the effects of the deprivation-amphetamine interaction may reflect nothing more than the well-established fact that different behaviours have different thresholds of sensitivity to the same chemical agent. On the other hand, it would be irrational to conclude that muricide, which in selected killers is not affected, can be manifestations of a common set of physiological conditions.

*Some Implications*

In an earlier paper [31] we considered implications of covariation of two or more behavioural periodicities. Three possible models were considered, which are also applicable in the present circumstances. Such covariation may mean that all behavioural measures are manifestations of a common set of physiological conditions, that one is the antecedent of the others, or that each has its own basic condition(s) and that all of these are activated concomitantly. Our present results, when considered together with reports of other relevant studies (e.g. [19, 25, 35]), strongly suggest that the first two of these possibilities are unlikely. Other procedures will be necessary to test hypotheses arising from the remaining model.

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