# COMBINED ACTION OF DIAZEPAM AND d-AMPHETAMINE ON FIXED-INTERVAL PERFORMANCE IN CATS[1]

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Cats trained under a fixed-interval 5-min schedule of milk presentation were injected with diazepam, amphetamine, and combinations of amphetamine and diazepam. Diazepam increased overall response rate as a function of the dose and disrupted the temporal pattern of responding. Low doses of amphetamine (0.5 mg/kg) usually increased the response rate; higher doses (1 to 2 mg/kg) either decreased the response rate or had little effect. Amphetamine always disrupted the temporal pattern of responding, even though it did not affect the overall rate. When doses of amphetamine that increased the response rate or left it unchanged were combined with diazepam, a potentiated increase in response rate occurred. When doses of amphetamine that decreased the response rate were combined with diazepam, the amphetamine-induced rate decreases were reversed at least partially. Less clear potentiation of disruption of the temporal pattern of responding was observed when amphetamine and diazepam were combined.

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The present research sought to describe the effects of diazepam (DZP) and *d*-amphetamine (AMPH) on positively reinforced behavior in cats, and to analyze the behavioral effects obtained when these two compounds, belonging to different pharmacological classes, were combined.

Frequently, pharmacologists have attempted to determine the mode of action of one drug by combining it with a second drug of known action. A similar approach at the behavioral level might provide important evidence about the mechanisms by which drugs affect behavior. Drug-combination studies in behavioral pharmacology are especially useful in testing the relevance of behavioral hypotheses in interpreting the effects of psychotropic compounds.

## METHOD

### Subjects

Six adult cats were used, Nr 20 (male) and 21 (male) in Exp. 1, Nr 22 (female), 23 (female), 24 (male), and 25 (male) in Exp. 2. All had an experimental history, ranging from 1 to 2 yr, on fixed-interval (FI) schedules, but had never been given drugs. They lived in groups of two to three animals in large home cages, were fed a standard ration (50 g) of wet food for cats, and received milk as the reinforcer in the experimental cage. When no experi-

ment took place, on weekends, an equivalent amount of milk was given in the home cage.

### Apparatus

The cats were studied in a cubic experimental cage (edge: 45 cm) equipped with a response lever 7.5 cm above the floor and protruding 5 cm from the wall. An electrically operated tap was used to dispense milk automatically. Each reinforcement consisted of 0.2 ml of milk, delivered in a tray to the left of the response lever. A 15-w bulb provided light during the experiment. The experimental cage was enclosed in an isolating compartment, providing partial sound isolation. The controlling units, composed of standard relay circuits, counters, and cumulative recorder, were located in another room.

### Procedure

Subjects were put in the experimental cage for 90 min daily, at approximately the same hour each day, Monday to Friday. Stable behavior under FI 5-min was obtained after four

to eight weeks. The results averaged from the last 10 sessions were used as control values.

The drug study was then started, according to a schedule slightly different in the successive Exp. 1 and 2.

Experiment 1 sought to obtain the dose-effect relationship for five doses of DZP and three doses of AMPH, and for the 15 possible combinations of the two drugs. Doses in mg per kg of body weight were as follows:

DZP  : 0.03, 0.0625, 0.125, 0.25, 0.50
AMPH : 0.5, 1, 2

Experiment 2 attempted to verify in four other cats the potentiated rate increase observed in Exp. 1 when DZP and AMPH were combined. Two doses of each drug were selected and administered alone and in the four possible dose combinations. The doses were in both cases the smallest and the largest doses used in Exp. 1, *i.e.*, 0.03 and 0.5 DZP and 0.5 and 2 AMPH. In addition, the effects of drug combination were determined during one session under an extinction schedule.

The drugs were administered i.p. 15 to 20 min before the session. AMPH was dissolved in natrium chloride solution. DZP was administered in the injectable solvent supplied for clinical use.[2] When the effects of two compounds were studied, two injections were made. Drug sessions took place every other day, provided that the subject's behavior had returned to the control baseline the day before. Drugs were never administered on a day following a weekend without experimental session.

*Data Recording and Analysis*

Responses and reinforcements were recorded on a cumulative recorder and digital counters. In addition, responses were counted on a set of eight counters. Each of these counters recorded the responses emitted during one of the successive fractions of 37.5 sec that divided the 5-min interval.

The analysis of results is based on the overall response rate and on the pattern of responding during the fixed interval. The rate under drug was compared to the control value

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by computing the output ratio, obtained by dividing the number of responses under drug by the number of responses averaged from 10 control sessions preceding the pharmacological tests (Smith, 1964). As a measure of the pattern of responding during the fixed interval, the index of curvature proposed by Fry, Kelleher, and Cook (1960) was computed.

The numbers of responses recorded in the eight 37.5-sec subdivisions of the 5-min interval were divided by the number of intervals in the session. These averaged values were used as a basis to compute the curvature index. As for overall rate, pre-drug controls were derived from 10 sessions.

The index of curvature is given by the formula

$$I = \frac{(n-1) \; R_n - 2 \sum_{i=1}^{n-1} R_i}{n R_n}$$

where n is the number of subdivisions in the time interval; Rn the cumulated number of responses obtained by summing up the responses emitted in the last subdivision plus all the responses emitted in the $(n-1)$ preceding subdivisions. The maximal value of the index depends on the number of subdivisions used. With eight subdivisions the maximum is 0.875. As the positively accelerated rate typical of FI schedule was preserved throughout the pharmacological tests, the index retained its positive value.

RESULTS

*Experiment 1*

*Pre-drug control.* The stabilized pattern of responding under FI 5-min shows the "scalloping" seen in many different species. A typical cumulative record is shown in Fig. 1A. The mean number of responses emitted per minute was 4.3 for Cat 20 and 2.9 for Cat 21. The index of curvature was 0.64 and 0.67 respectively.

*Effects of DZP.* In both Cats 20 and 21, DZP produced an increase in the rate of responding, striking at doses of 0.125, 0.25, and 0.50 mg/kg for Cat 20, and at doses of 0.25 and 0.50 mg/kg for Cat 21. The number of responses was multiplied by a factor of 2 to 4, as can be seen from Fig. 2.

Cumulative curves for Cat 20 are shown in Fig. 1, B to F.

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[2]The formula of the solvent for 5-mg DZP is: benzylic alcohol, 15.7 mg, aethylic alcohol, 80.6 mg, N,N-dimethylacetamid, 94 mg, tetrahydrofurfuryl alcohol polyaethylen glycol, 434 mg, aqua q.s. ad. 1 ml (Hoffmann-Laroche, Valium[B]).
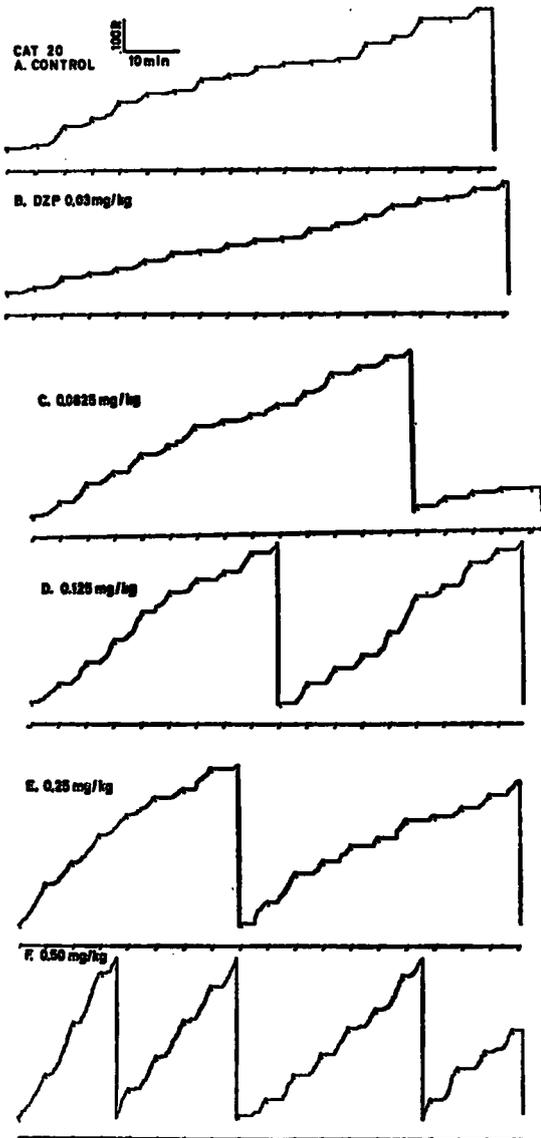
Fig. 1. Cumulative response records of Cat 20 on FI 5-min, showing the dose-effect relationship for DZP. A: Control; B to F: increasing doses of DZP. The pen tracing the horizontal line at the bottom of each graph was deflected when the 5 min elapsed; it came back to the initial position when the reinforced response was emitted.

The temporal pattern of responding obtained after stabilization of FI 5-min was altered by the drug. This is apparent from cumulative curves in Fig. 1, B to F. Figure 3 shows the decrease of the index of curvature as a function of the dose.

*Effect of AMPH.* The effects of AMPH administered alone are indicated in Fig. 2 and 3 by horizontal lines across the graphs.

In Cat 20, AMPH produced no increase in response rate at the doses selected. No significant effect was obtained at the dose of 0.5 and 1 mg/kg. Responding was almost completely suppressed after 2 mg/kg.

In Cat 21, AMPH at the dose of 0.5 mg/kg doubled the number of responses. A less significant increase was observed for the two higher doses (see Fig. 2).

A disruption of the temporal pattern of responding, as expressed by the index of curvature, was observed; this disruption was not correlated with increases in overall response rate. In Cat 20, the index dropped sharply with 1 mg/kg, though the number of responses did not deviate significantly from the control value. In Cat 21, the temporal pattern was disrupted increasingly with dosage, though the output ratio was at a maximum with the smallest dose (see Fig. 3).

Table 1 shows the effect of the three doses of AMPH on the rate in each of the successive subdivisions of the interval for Cat 21. Results are expressed in responses per minute. Larger doses tended to increase the low rate in the first parts of the interval and to decrease the high rate typical in the last subdivisions.

Table 1

Number of responses/min in each of the eight subdivisions of FI 5-min under AMPH for Cat 21.

|  | *Successive Subdivisions of FI* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
|  | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
| control | 0 | 0 | 0.2 | 0.3 | 1.3 | 3.0 | 6.6 | 12.2 |
| 0.5 mg/kg AMPH | 0 | 0 | 0 | 3.2 | 5.6 | 8.5 | 13.4 | 17.6 |
| 1 mg/kg AMPH | 0 | 0.5 | 0.8 | 2.7 | 4.9 | 8.6 | 7.0 | 10.4 |
| 2 mg/kg AMPH | 0 | 0.8 | 2.6 | 6.2 | 5.8 | 6.7 | 7.7 | 8.8 |

*Effect of DZP + AMPH combinations.* The most frequent effect, when the two drugs were combined, was a potentiation of the rate-increasing effect of DZP (see Fig. 2).

In Cat 21, where AMPH alone increased response rate slightly, and where the rate increase due to DZP was negligible below the dose of 0.25 mg/kg, a sharp increase was observed when the two drugs were combined, even at the smallest doses. The control rate was multiplied by a factor of 6 to 9 when the highest dose of DZP or of AMPH was included in the combination. The potentiation was
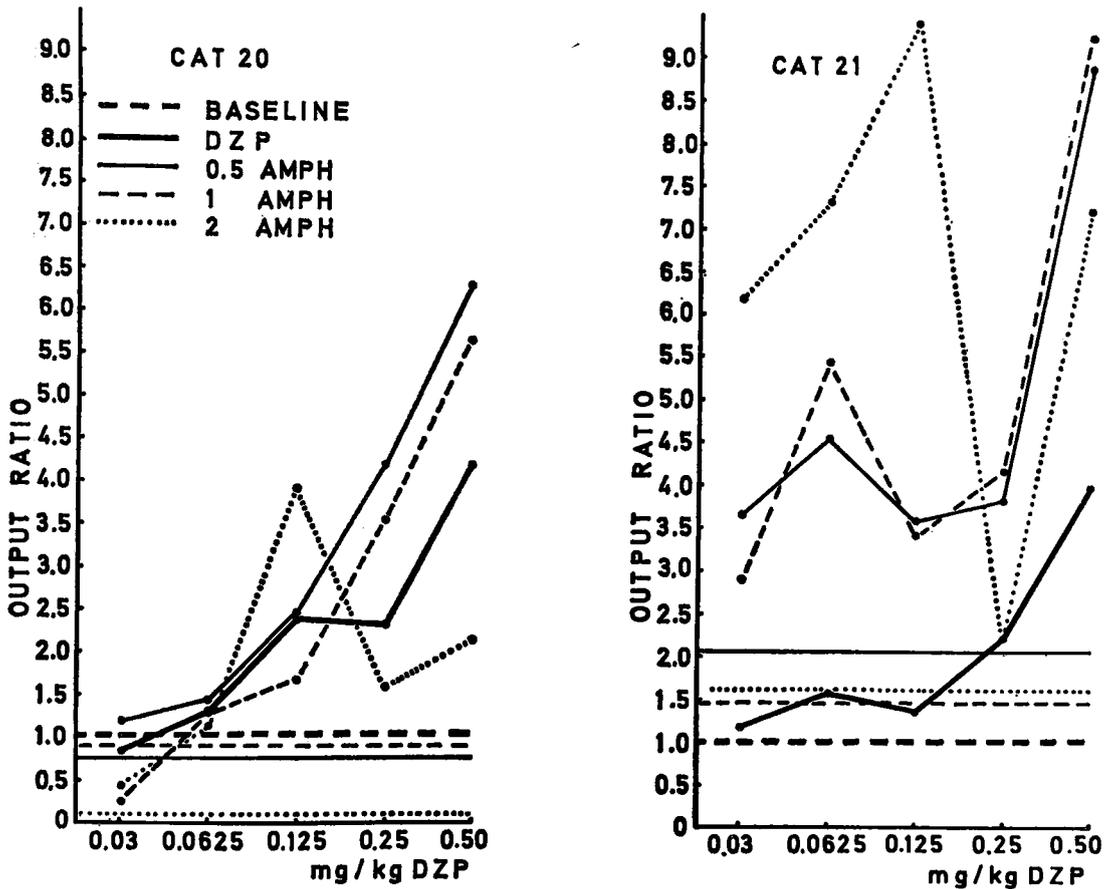
Fig. 2. Experiment I. Change in rate under DZP and AMPH, alone and in combination. Ordinate: the output ratio, computed by dividing the total output under drug by the control output. Abscissa: doses of DZP. The heavy solid curve is for DZP alone. The other curves are for doses of AMPH + DZP, as indicated in the upper-left corner. Results obtained with AMPH alone are given by the horizontal lines.

observed with 14 of the 15 possible combinations (one atypical result was obtained with 0.25 mg/kg DZP + 2 mg/kg AMPH). The phenomenon is illustrated by sample cumulative curves in Fig. 4.

In Cat 20, where AMPH either decreased response rate or had little effect, DZP usually partially reversed the AMPH rate decrease, or DZP rate increases were potentiated by those AMPH doses that had little effect when given alone. These relationships were particularly true when the dose of DZP was high.

The effect of the drug combination on the temporal pattern of responding cannot be summarized in a single relationship. The changes in the index of curvature shown in Fig. 3 do not strictly correlate with the variations in rate as seen in Fig. 2. At the higher end of the DZP dose-response curve, AMPH

combined with DZP did nothing that DZP alone did not do: the index was close to the value obtained with DZP alone. At lower doses of DZP combined with 0.5 mg/kg AMPH, AMPH potentiated the changes seen after DZP alone. The effects on rate of the same dose combinations were negligible in Cat 20. In Cat 21, the potentiated increase in rate was far less pronounced than with higher doses, while the index of curvature reached the lowest value obtained in this experiment. Finally, the deteriorating effect of high doses of AMPH was at least partially antagonized by low and medium doses of DZP.

This complex picture clearly indicates that the effects of the combined drugs on the temporal pattern of responding are not merely a reflection of the effects on overall rate. It must be noted that the potentiated increase in over-
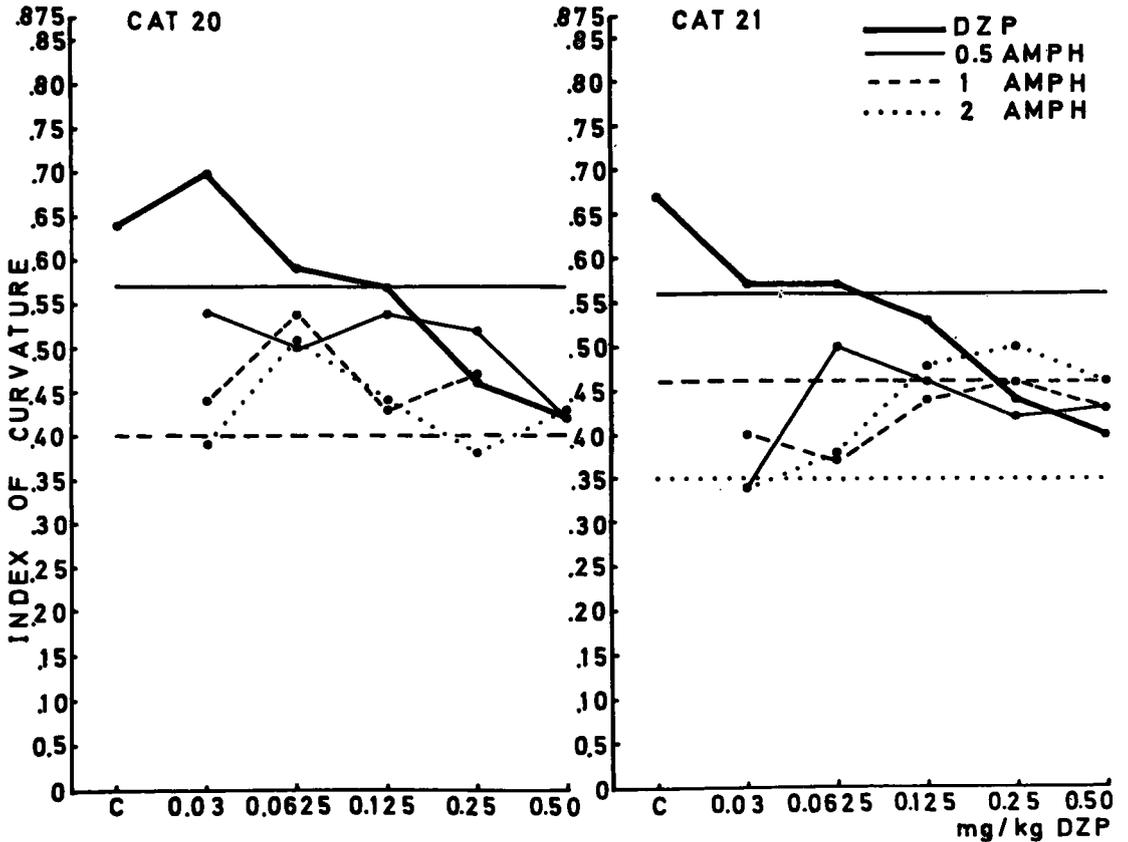
Fig. 3. Experiment I. Change in curvature index as a function of DZP and AMPH, administered alone and in combination. Ordinate: index of curvature computed from Fry, Kelleher, and Cook's formula. Abscissa: C = pre-drug control value, followed by the doses of DZP. The heavy solid curve is for DZP alone. The other curves are for doses of AMPH + DZP, as indicated in the upper right corner. Results obtained with AMPH alone are given by the horizontal lines; the index of curvature was not computed for Cat 20 under 2 mg/kg AMPH because responding was almost completely suppressed at that dose.

all rate never concealed a sharp increase of output during the first part of the interval (the classical "pausing period" of the FI) that would counterbalance an unchanged or reduced rate during the final part. An analysis of the proportions of responses emitted in each of the eight successive subdivisions of the 5-min interval, the details of which are not reported here, did not show any differential effect on "low" and "high" control rates that were typical of the first and the last part of the interval respectively.

Expressed in absolute figures, the increase was far more marked during the last 37.5-sec subdivision, despite the higher initial value, than during any of the first four periods, in which the initial value was close to zero.

Table 2 shows a typical example, drawn

from the results of Cat 21, for the combination 0.5 mg/kg DZP + 0.5 mg/kg AMPH, corresponding to cumulative curve F in Fig. 4.

Table 2

Number of R/min in each of the eight subdivisions of the FI under one DZP + AMPH combination in Cat 21.

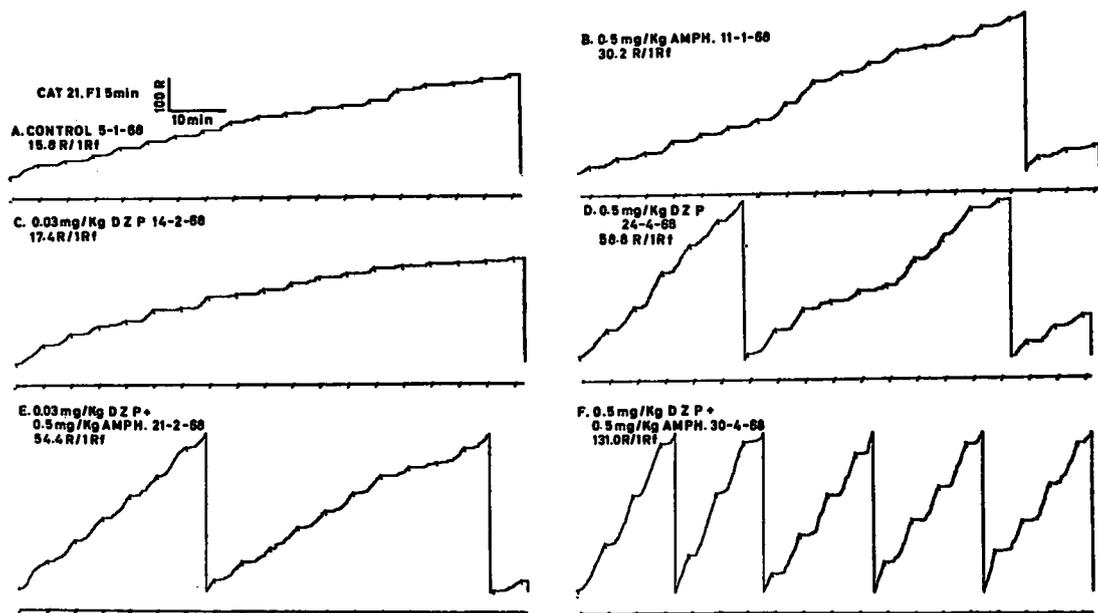| | Successive Subdivisions of FI | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| [1]control | 0 | 0 | 0.2 | 0.3 | 1.3 | 3.0 | 6.6 | 12.2 |
| 0.5 mg/kg [2]DZP + 0.5 mg/kg AMPH | 0 | 1.9 | 8 | 20.8 | 35.5 | 41.8 | 46.9 | 54.6 |
| increase (2 minus 1) | 0 | 1.9 | 7.8 | 20.5 | 34.2 | 38.8 | 40.3 | 42.4 |

Fig. 4. Cat 21. Cumulative response records illustrating the potentiation between DZP and AMPH. The average number of responses per interval is given with each curve (n R/1Rf).

*Experiment 2*

*Pre-drug control.* The mean numbers of responses per minute, giving the reference value for computing the output ratio, are as follows:

| Cat Nr | responses/min |
|--------|---------------|
| 22 | 5.4 |
| 23 | 5.9 |
| 24 | 5.7 |
| 25 | 8.6 |

The general pattern of responding was the same as in Exp. 1, but the total output was higher than in Cat 20 and 21. Except for Cat 24, the temporal pattern of responding was somewhat different from that of the subjects in Exp. 1. Control values of the index of curvature are shown on the first point of solid curves in Fig. 6.

*Effect of DZP.* The effects of DZP on the rate of responding are shown in Fig. 5. An increase in the number of responses was observed in all subjects after administration of 0.5 mg/kg. It was most pronounced in Cat 22 and 24, the output under drug corresponding to 250 and 256% of the control, respectively. The dose of 0.03 mg/kg produced either an increase (Cat 22 and 24) or a slight decrease (in Cat 23 and 25, practically negligible in the former animal). These results are in line with the results of Exp. 1.

The temporal pattern of responding was altered after administration of the higher dose in all subjects except Cat 25. The smaller dose either: failed to affect the index of curvature (Cat 22), reduced it less markedly than the higher dose (Cat 24), or increased it (Cats 23 and 25) (see Fig. 6).

*Effect of AMPH.* The response rate was clearly increased after 0.5 mg/kg for all animals except Cat 25. Under 2 mg/kg, the number of responses was close to the control value except for Cat 24, which showed a decrease. The index of curvature was altered to approximately the same extent for the two doses.

*Effect of DZP + AMPH combinations.* As can be seen from Fig. 5, a potentiation of the rate increase was observed in Cat 22 for all four combinations; in Cat 23 when DZP was combined with 0.5 mg/kg AMPH, and in Cat 24 and 25 for the combination 0.5 mg/kg DZP + 0.5 mg/kg AMPH. An antagonizing effect of the amphetamine-induced depression in rate was observed in Cat 24 with the combination of 0.5 mg/kg DZP + 2 mg/kg AMPH.

Two atypical results were obtained. In Cats 23 and 24 with the combination of 0.03 mg/kg DZP + 2 mg/kg AMPH, the response rate was markedly reduced. In Cat 24, it resembled the effect of AMPH alone, and the results obtained with the larger dose of DZP indicate that the
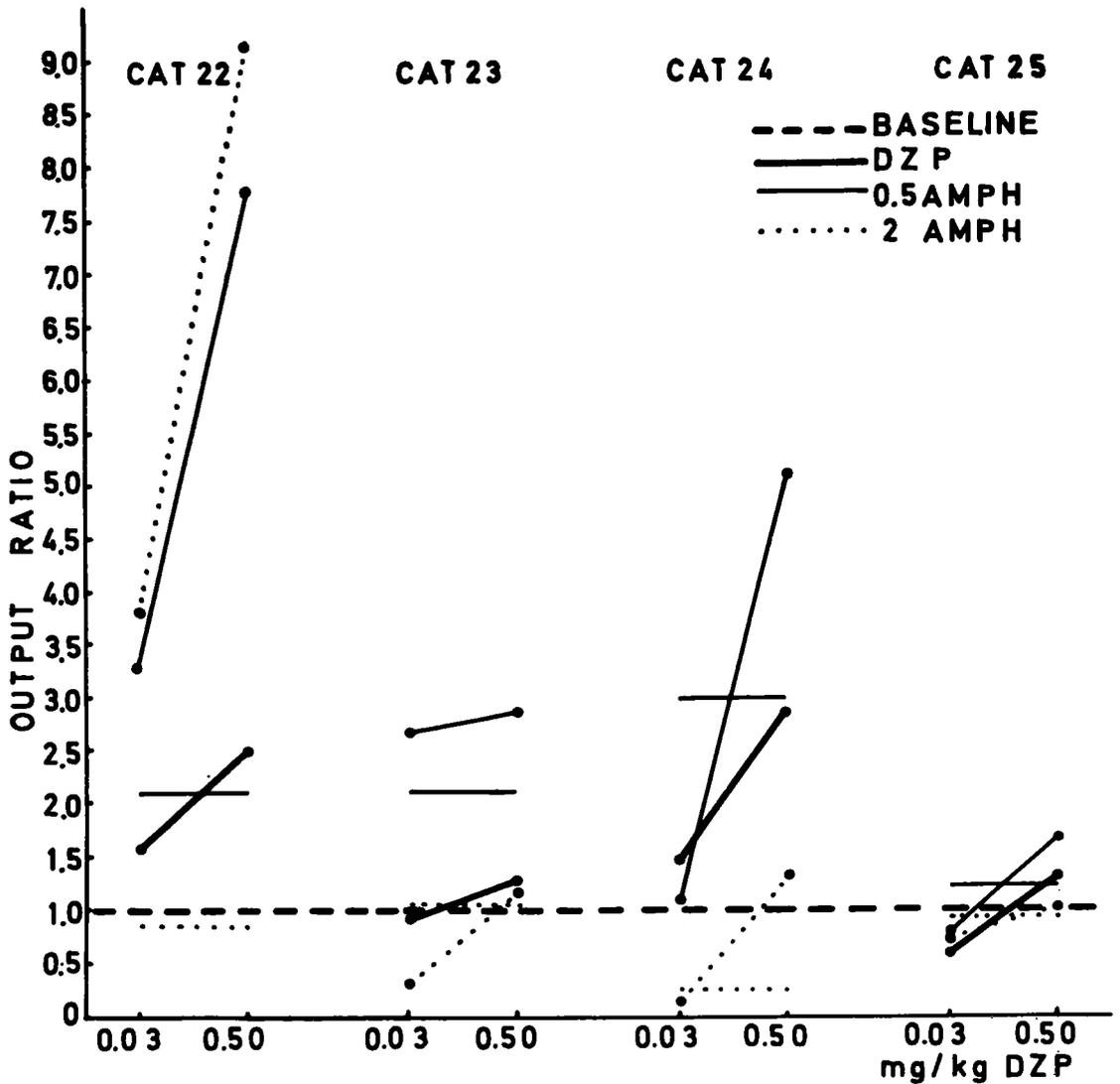
Fig. 5. Experiment II. Change in rate under DZP and AMPH, alone and in combination. Ordinate: output ratio; abscissa: doses of DZP. See Fig. 2 for key to reading.

smaller dose was insufficient to antagonize the effect of AMPH. In Cat 23, both drugs injected alone left the number of responses unaltered. A similar result was noted once in Exp. 1, in Cat 20 (DZP 0.03 mg/kg + AMPH 1 mg).

The index of curvature reflected in some cases a potentiation of the decreasing effect of both drugs and in some cases an antagonizing action, the decreasing effect of AMPH being partially attenuated by DZP (see Fig. 6).

*Experimental extinction.* When no milk was placed in the automatic dispenser and no drug was injected, experimental extinction showed the usual pattern: the rate of respond-

ing was high right after the first operation of the empty dispenser. Subjects were always placed in the experimental cage when a fixed-interval cycle was completed, so that their first response was reinforced. The high rates, typical of a beginning extinction session, occurred during the first and second interval. By the third interval, responding was almost completely eliminated. Cumulative curves for the four subjects are shown in Fig. 7A.

Contrasting with this pattern, the curves obtained under drug (0.5 mg/kg DZP + 0.5 mg/kg AMPH), indicate a surprising resistance to extinction. The rate of responding
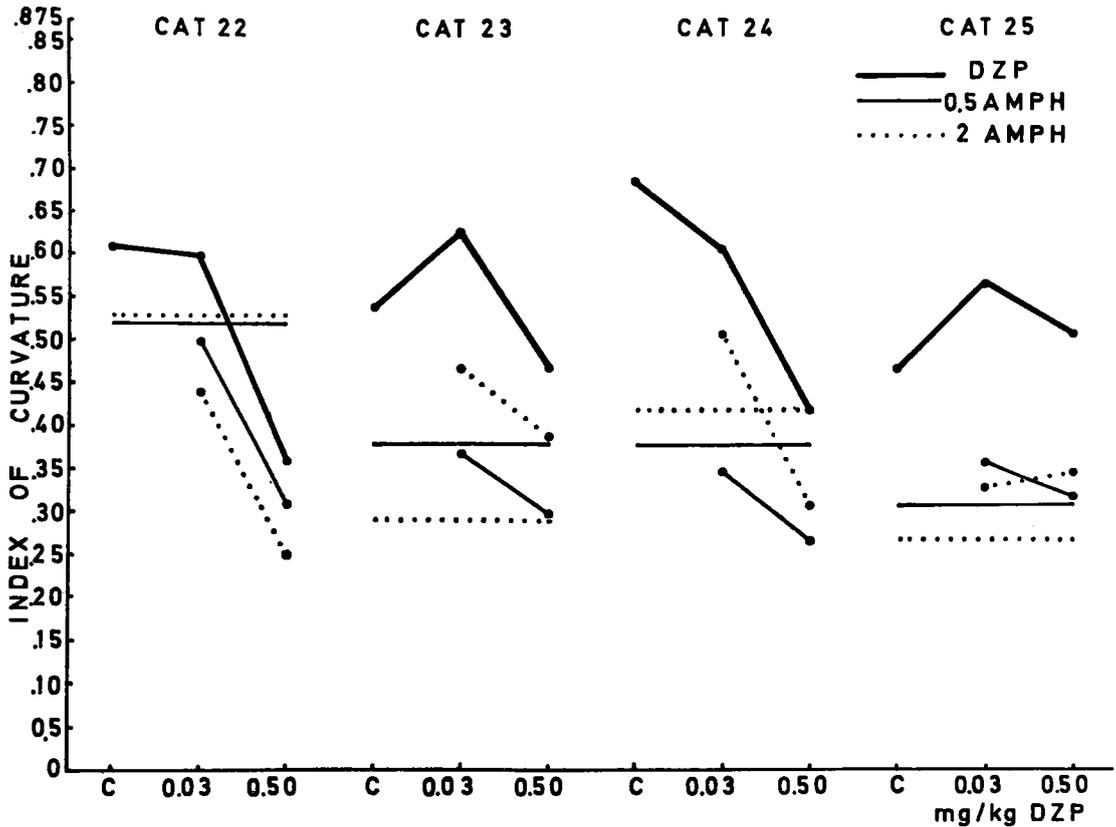
Fig. 6. Experiment II. Change in index of curvature under DZP and AMPH, alone and in combination. Ordinate: index of curvature. Abscissa: C = pre-drug control value, followed by doses of DZP. See Fig. 3 for key to reading.

was sustained throughout the session, reaching a level close to the one observed under identical pharmacological conditions in a normal fixed-interval session (see Fig. 7B).

*Increased food deprivation test.* It is highly improbable that an increase in hunger would explain the increased rate of responding under DZP, AMPH, or their combination. A similar hypothesis was discarded in a study on chlordiazepoxide (Richelle, 1962) in which food deprivation was extended over a 72-hr period, in addition to the permanent deprivation maintained throughout the experiment. Moreover, AMPH is known as an anorexic drug and, supposing that a change in hunger might explain the effect of DZP, it would not explain the potentiation of AMPH, nor the cases of antagonism.

For four subjects deprived of food in their home cage for 48 hr, the rate of responding was not changed.

DISCUSSION

The rate-increasing effect of benzodiazepines has been confirmed repeatedly for chlordiazepoxide (Richelle, 1962; Richelle and Djahanguiri, 1964; Richelle, Xhenseval, Fontaine, and Thone, 1962; Cook and Kelleher, 1963). DZP has been reported to increase the response rate in rats in intracranial self-stimulation experiments (Olds, 1966). Our results show that this action on operant behavior is not restricted to one species and is not specific to hypothalamic electric reinforcement. Olds' interpretation of her results, resorting to "specific facilitatory effects on hypothalamic reward systems" seems too simple to account for the rather widespread rate increasing action of diazepines on operant behavior. We cannot *a priori* rule out the possibility that this action is related to an effect on hypothalamic reward systems, or, in other words, to some basic motivational mech-
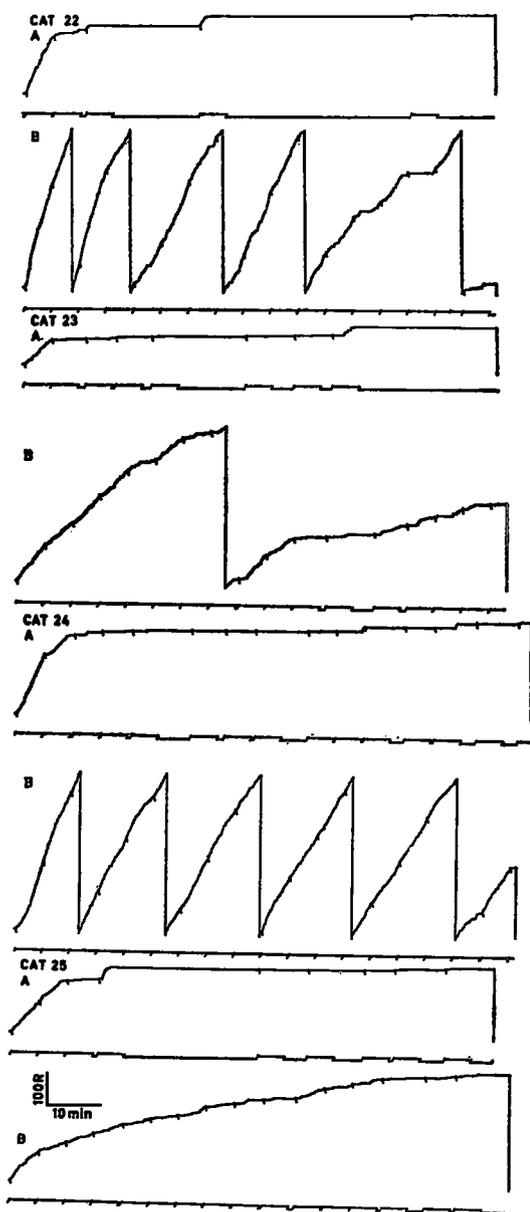
Fig. 7. Experiment II. Experimental extinction without drug (curves marked A) and under 0.5 mg/kg DZP + 0.5 mg/kg AMPH (curves B) in four individual subjects.

diazepoxide in the same schedule when a locomotor response was used, and Cook and Catania (1964) under chlordiazepoxide in a fixed-interval shock escape schedule.

The present results with AMPH confirm, on the whole, the numerous reports on the behavioral action of this drug. The quantitative analysis of the change in rate in the different subdivisions of the fixed interval indicates the well-known tendency of AMPH to increase low rates and to decrease high rates at appropriate doses (Kelleher and Morse, 1968). However, at the dose of 0.5 mg/kg, an increase in rate was observed in the final part of the interval and is negligible in the first part. The resistance of the pause might be due to the long training of the present subjects, especially in the two cats used in Exp. 1.

The rate-increasing effects of diazepam were frequently potentiated by amphetamine; DZP also antagonized the rate-decreasing effect found with high doses of the latter drug. Potentiation by amphetamine of the rate-increasing effects of another diazepine, chlordiazepoxide, has been described by Rushton and Steinberg (1966). Similar phenomena were observed after combined administration of amphetamine and barbiturates (Rushton and Steinberg, 1963; Rutledge and Kelleher, 1965). The mechanism of this potentiation and antagonism are unknown.

Behavioral analysis shows that the potentiation of DZP effects by amphetamine is not a general effect: it is not observed for each dose combination in all individuals. The effects of amphetamine alone show great interindividual variability, but do not help to predict unequivocally the potentiation of diazepam effects. Another complication comes from the fact that the type of behavior being studied might be an important variable, the potentiation appearing in some cases and not in other cases, as was shown for amphetamine-barbiturate combinations. Further research should seek to verify the potentiation effect on behavior maintained by other schedules.

anism supposedly common to all positively reinforced behavior. However, a number of studies have shown that increases in response rate due to diazepines are not limited to positively reinforced behavior: Heise and Boff (1962) observed it in a Sidman avoidance schedule under nitrazepam and diazepam, Fontaine and Richelle (1969) under chlor-

## REFERENCES

Cook, L. and Catania, A. C. Effects of drugs on avoidance and escape behavior. *Federal Proceedings,* 1964, **23,** 818-835.

Cook, L. and Kelleher, R. T. Effects of drugs on behavior. *Annual Review of Pharmacology,* 1963, **3,** 205-222.

Fontaine, O. and Richelle, M.  Etude comparative de la chlorpromazine et du chlordiazepoxide sur une série de comportements avec renforcement positif et négatif chez le rat. *Psychologica Belgica*, 1969, **9**, 17-29.

Fry, W., Kelleher, R. T., and Cook, L.  A mathematical index of performance on fixed-interval schedules of reinforcement. *Journal of the Experimental Analysis of Behavior*, 1960, **3**, 193-199.

Heise, G. A. and Boff, E.  Continuous avoidance as a baseline for measuring behavioral effects of drugs. *Psychopharmacologia*, 1962, **3**, 264-282.

Kelleher, R. T. and Morse, W. H.  Determinants of the specificity of behavioral effects of drugs. *Ergebnisse der Physiologie*, Band 60, Springer-Verlag, Berlin 1968, 1-56.

Olds, M. E.  Facilitatory action of diazepam and chlordiazepoxide on hypothalamic reward behavior. *Journal of Comparative and Physiological Psychology*, 1966, **62**, 136-140.

Richelle, M.  Action du chlordiazepoxide sur les régulations temporelles dans un comportement conditionné chez le chat. *Archives internationales de Pharmacodynamie*, 1962, **140**, 434-449.

Richelle, M., and Djanhanguiri, B.  Effet d'un traitement prolongé au chlordiazepoxide sur un conditionnement temporel chez le rat. *Psychopharmacologia*, 1964, **5**, 106-114.

Richelle, M., Xhenseval, B., Fontaine, O., and Thone, L.  Action of chlordiazepoxide on two types of temporal conditioning in rats. *International Journal of Neuropharmacology.*, 1962, **1**, 381-391.

Rushton, R. and Steinberg, H.  Combined effects of chlordiazepoxide and dexamphetamine on activity of rats in an unfamiliar environment. *Nature*, 1966, **211**, 1312-1313.

Rushton, R. and Steinberg, H.  Dose-response relations of amphetamine-barbiturate mixtures. *Nature*, 1963, **197**, 1017-1018.

Rutledge, Ch. O. and Kelleher, R. T.  Interactions between the effects of methamphetamine and pentobarbital on operant behavior in the pigeon. *Psychopharmacologia*, 1965, **7**, 400-408.

Smith, C. B.  Effects of *d*-amphetamine upon operant behavior of pigeon: enhancement by reserpine. *Journal of Pharmacology and Experimental Therapeutics*, 1964, **146**, 167-174.