*MOLECULAR ANALYSES OF THE EFFECTS OF
d-AMPHETAMINE ON FIXED-INTERVAL SCHEDULE
PERFORMANCES OF RATS*

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A series of doses (0.5 to 2.0 mg/kg) of *d*-amphetamine was administered to rats whose lever pressing was maintained by fixed-interval 30-s, 60-s, or 120-s schedules of reinforcement by sucrose delivery. Under both saline and *d*-amphetamine conditions, molecular features of responding were reliably described in terms of the distribution of postreinforcement pauses and local response rate following the onset of responding. Postreinforcement pause always varied from interval to interval but, on average, shortened under the drug. Local response rate (response rate exclusive of pause time) tended to decrease under the drug, and where acceleration occurred within runs of responses, it was reduced by the drug. All of these effects were dose-related. These findings suggest that fixed-interval behavior can be analyzed effectively at a molecular level, and that the effects of *d*-amphetamine are best described as disruption of temporal discrimination.

Key words: fixed-interval schedule, postreinforcement pause, local response rate, *d*-amphetamine, lever press, rats

A fixed-interval (FI) schedule provides for reinforcement of the first operant response that occurs once a specified interval has elapsed. The interval is usually timed from the previous reinforcement. Early investigators (e.g., Skinner, 1938) described the pattern of behavior generated by the schedule as a "scallop" in which a postreinforcement pause (PRP) is followed by a gradual increase in response rate, with a maximum being reached towards the end of the interval. However, Cumming and Schoenfeld (1958) reported that with prolonged exposure to a very long interval value (FI 30 min), pigeons developed a "break-and-run" pattern in which an initial pause was followed by an abrupt transition to a constant high rate. Sherman (1959) found evidence for similar effects in rats exposed to FI schedules of 10 s and 240 s. A crucial difference between the two accounts is whether or not acceleration occurs within runs of responses. This is difficult to determine from visual inspection of cumulative records, which provided the primary data in early studies. More recent studies have examined individual response runs and obtained evidence of acceleration early in the run—in rats (Lowe &

Harzem, 1977), in pigeons (Shull & Brownstein, 1970), and over longer periods, in monkeys (Dews, 1978).

Fixed-interval schedules have been widely used in the investigation of drug effects upon operant behavior, particularly in tests of Dews' (1958) hypothesis of rate dependency. Rate dependency can be proposed (a) in a weak form, which states that with other variables held constant, the rate of the operant response in the undrugged subject may influence the drug effect; (b) in an intermediate form, which specifies the nature of the relationship between the predrug response rate and the drug effect; or (c) in a strong form, which states that the drug effect depends entirely on the predrug rate, and that other variables have their effects through their effects on response rate (Dews & Wenger, 1977).

There is general support for at least the weak and intermediate forms of rate dependency from studies comparing different conditions of an experiment or comparing different components within a multiple schedule (Robbins, 1981). In addition, several investigators have examined the series of increasing response rates that occur within the FI scallop to see whether *d*-amphetamine exerts rate-dependent effects at this molecular level. Results have apparently been positive: Low rates early in the interval were elevated and high rates later in the interval were depressed. This result has been obtained with rats (Heffner,

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Drawbaugh, & Zigmond, 1974), pigeons (McMillan, 1968), and squirrel monkeys (McKearney & Barrett, 1978). However, the drug was not necessarily affecting a range of response rates differentially, but may have been affecting mainly the durations of PRPs and the subsequent periods of responding.

This question can be resolved only by examining data from individual intervals. Such an analysis was carried out by Branch and Gollub (1974) with pigeons under FI 40-s, 100-s, and 300-s schedules of reinforcement with food. They showed that the intermediate rates previously reported in the middle of an interval were artifacts of averaging occasions when that time was within the PRP with occasions when responding occurred at a relatively high rate. Moreover, they found that most of the drug effect was accounted for by changes in the PRP distribution rather than by changes in local rate per se. There was evidence for acceleration within runs of responses, but this was not systematically affected by *d*-amphetamine under FI 100 s and 300 s. There was less within-response-run acceleration at FI 40 s, and it was more disrupted by drug administration.

Given the importance of the rate-dependency hypothesis in behavioral pharmacology (e.g., see Barrett, 1984) and the widespread use of *d*-amphetamine in generating relevant data, Branch and Gollub's results are most important. If analysis at a more molecular level suggests that the drug primarily affects pausing within schedule performance, we would be unwise to couch our larger-scale or molar-level descriptions solely in terms of response rates. The present study sought to replicate Branch and Gollub's findings with rats, a species widely used in the investigation of rate dependency. In addition, a number of molecular techniques of analysis were used to identify those that might provide the most adequate and parsimonious account of behavior under FI schedules, and of its modification by drug administration.

METHOD

Subjects

Twelve experimentally naive male hooded rats, 90 days old and weighing from 420 to 470 g at the start of the study, were maintained at 80% of their free-feeding weights and were caged singly with water always available.

Apparatus

In two standard Campden Instruments test chambers (Model 410) fitted with dipper feeders, only the left levers were operative, and each required a force of 0.1 N to register a lever press. Each chamber was illuminated by a 2.8-W houselight. The reinforcer was 5-s access to 5% sucrose solution (by weight), timed from the operation of a switch attached to the clear plastic flap covering the food tray. The tray was lit by a 2.8-W light during availability of sucrose. Each chamber was situated in a sound-attenuating housing that masked extraneous noise. The experiment was controlled and the data were collected by a Nova® 2/10 minicomputer.

Procedure

Following shaping of lever pressing by reinforcing successive approximations and brief exposure to fixed-ratio 1 reinforcement, Rats F1 to F4 were exposed to an FI 30-s schedule of reinforcement, F5 to F8 were exposed to FI 60 s, and F9 to F12 were exposed to FI 120 s. Sessions were conducted 6 days per week. Each session lasted until 100 reinforcers had been delivered.

Day-to-day overall response rates were considered stable after 30 sessions, whereupon daily injections began. Following four administrations of isotonic saline (0.9%), an acute regime of administration of *d*-amphetamine sulfate (Sigma Chemical Company) began. Drug dosages were 0.5, 1.0, 1.5, and 2.0 mg/kg of body weight, dissolved in saline to give an injected volume of 1 mL/kg of body weight, and were injected intraperitoneally approximately 15 min before the start of a session. Dosages were administered in a semirandom order, the order being different for each rat. Three days were allowed to elapse between successive drug administrations. Saline was injected on all nondrug days in a volume of 1 mL/kg of body weight. Each rat received each dose on three occasions, and the saline days immediately preceding each drug day were used as control days for analyzing the drug effects.

RESULTS

Average Response Patterns

Figure 1 shows average response rates for successive 5-s segments of the interreinforcer

Fig. 1. Average response rates in successive 5-s segments of the interreinforcer interval for rats on FI 30 s under saline (dashed lines) and during four doses of *d*-amphetamine sulfate.

interval for the 4 rats trained under an FI 30-s schedule of reinforcement. The control curve is based upon the saline days preceding drug administration days ($N = 12$), and the curve for each drug dosage is the average over the three days during which that dose was administered. Presented in this fashion, all rats showed a scalloped control curve, but the response to the drug varied. There was a modest tendency for the curve to be flattened out at the highest dose, 2.0 mg/kg. Otherwise, in 2 rats (F2 and F4) low response rates early in the interval increased and high rates later in the interval decreased. With the other 2 rats (F1 and F3), response rates increased generally.

Figure 2 shows response-rate changes un-

der an FI 60-s schedule when *d*-amphetamine was administered. These effects were more consistent across subjects, but Rat F5 showed an increase in all rates at the end of the interval, except with the highest dose. Otherwise, there were small increases in the early and middle parts of the interval, and decreases at the end. The scalloped control curves were flattened out by the drug, particularly at the highest dose.

The clearest effects on average response rates were obtained with the FI 120-s schedule, as shown in Figure 3. All rats showed increases in rate in the early and middle parts of the interval. Drug-correlated decreases in the last part of the interval occurred in 15 of 16 curves. (The exception, with F11, was at

Fig. 2. Average response rates in successive 5-s segments of the interreinforcer interval for rats on FI 60 s under saline (dashed lines) and during four doses of *d*-amphetamine sulfate.

the lowest drug dosage.) With the higher doses (1.5 and 2.0 mg/kg), drug curves were much flatter than the control curves.

In summary, there was considerable inter-subject variability in the data presented in this fashion. Generally, however, the scalloped curve was flattened, or lost curvature, under the drug. This often meant that low rates early in the interval increased, whereas high rates at the ends decreased. Sometimes this effect was marked by a general increase in response rates, particularly at the shortest fixed-interval studied (30 s).

Molecular Analyses

To obtain a more complete account of behavior and the changes under the drug, the PRP and the numbers of responses in each succeeding 2-s interval up to reinforcer delivery were recorded separately for each interreinforcer interval. This enabled the following to be calculated: (1) the distribution of PRPs; (2) the rate and pattern of responding as a function of the duration of responding within

each interval; (3) the local rate of responding as a function of time through the interreinforcer interval; and (4) the local rate of responding as a function of time since responding began. Details regarding each measure and the results obtained from it will be described in turn.

Distribution of PRPs

In order to eliminate occasions where an isolated one or two responses occurred prior to the initiation of a run of responses, the termination of the PRP was defined as the third response occurring after a reinforcer delivery. Using this measure and excluding intervals with fewer than three responses, median PRP duration under saline was consistent across animals under a given schedule and averaged 25.5 s, 46.7 s, and 88.9 s under FI 30 s, FI 60 s, and FI 120 s, respectively. Median PRP tended to decline with drug dosage, particularly under FI 120 s. At the highest dose it averaged 17.6 s, 39.4 s, and 38.4 s for FI 30 s, FI 60 s, and FI 120 s, respectively.

Fig. 3. Average response rates in successive 5-s segments of the interreinforcer interval for rats on FI 120 s under saline (dashed lines) and during four doses of *d*-amphetamine sulfate.

Fig. 4. Histograms showing the distributions of postreinforcement pauses in 2-s intervals for one rat on an FI 30-s schedule, under four doses of *d*-amphetamine and during the saline (control) sessions preceding each set of drug sessions. Data from intervals with fewer than three responses were excluded.

Fig. 5. Histograms showing the distributions of postreinforcement pauses in 4-s intervals for one rat on an FI 60-s schedule, under four doses of *d*-amphetamine and during the saline (control) sessions preceding each set of drug sessions. Note that the left-most bar represents all postreinforcement pauses of less than 19.5 s. Data from intervals with fewer than three responses were excluded.

The effects of the drug on the distribution of PRP durations were much more striking. Figure 4 shows the distributions for Rat F3, in 2-s intervals, under saline and drug conditions. The distribution under saline was heavily skewed to the right, but pauses were quite variable from interval to interval. With increasing doses of *d*-amphetamine, the distribution shifted to the left—that is, the pauses became shorter but the within-animal variability remained. This pattern of changes in the PRP distribution was repeated in all rats under FI 30 s, including F2 and F4 that showed much less pronounced changes in the overall response pattern (see Figure 1).

Figures 5 and 6 present comparable data for Rat F7, under FI 60 s in 4-s intervals, and for Rat F9, under FI 120 s in 10-s intervals. Under saline, distributions again peaked late in the interreinforcer interval with pauses showing considerable variability. Shifts in the distributions with the drug were marked, with a large increase in relative number of shorter pauses at the higher doses. Note that in Figure 6 the mode of the distribution shifted from

over 80 s during saline sessions to under 20 s during sessions with the two highest drug doses.

Rate and Pattern as a Function of Duration of Responding

Because the data were collected separately within individual interreinforcer intervals, it was possible to assess whether the rate or pattern differed depending on the point at which responding was initiated. If responding began at 20 s in an FI 30 s, for example, would the rate be the same at 24 s as it would be if responding had started at 14 s? This was the most detailed analysis of responding attempted, and required that the interval-by-interval data be sorted according to the point at which responding started in each interval. As the PRP histograms in Figures 4 to 6 indicate, the distributions of these points were skewed and also changed considerably under the drug. Thus, there were varying numbers of runs of different lengths, and the most frequent lengths changed under the drug. In addition, only under the FI 30-s schedule were

Fig. 6. Histograms showing the distributions of postreinforcement pauses in 10-s intervals for one rat on an FI 120-s schedule, under four doses of *d*-amphetamine and during the saline (control) sessions preceding each set of drug sessions. Note that the left-most bar represents all postreinforcement pauses of less than 19.5 s. Data from intervals with fewer than three responses were excluded.

many runs of a given length recorded from one session (i.e., all the longer run lengths in one session of the FI 60-s or FI 120-s schedule might differ from each other).

For all these reasons, the results are not presented in aggregate; however, the general findings were quite clear. Under saline with each schedule, most rats showed within-response-run acceleration of rate. When responding began earlier in the interval, it started at a comparatively low rate; at the end of the interval the rate reached a similar maximum, regardless of the starting point. Under the drug, within-response-run acceleration was reduced and terminal rate was diminished. Illustrative data from a rat under FI 30 s are shown in Figure 7. The longer curves in the figure were more variable because they were based on smaller numbers of observations. The results for the minority of animals that did not show within-response-run acceleration

were homogeneous, in that the relatively constant local response rate did not vary with the length of the run but did decline under the drug.

Local Rate of Responding as a Function of Time Through the Interreinforcer Interval

In Figure 7 the last point on each curve represents the final 2 s of the interreinforcer interval. Each set of curves could thus be collapsed into a single function that would give the average rate of responding, for those occasions when responding was occurring, as a function of time through the interval. The data are presented in this fashion in Figure 8 for all rats exposed to the FI 30-s schedule. Note that this differs from Figure 1 in that local response rates are averaged only over those occasions on which responding was occurring; therefore, the curves in Figure 8 do not extend

Fig. 7. Local response rates as a function of the duration of responding in an interval for one rat on an FI 30-s schedule, under four doses of *d*-amphetamine and during the corresponding saline (control) sessions. Separate curves are given for intervals in which responding occurred for different durations; curves from intervals with longer periods (up to 20 s) appear to the left of each panel and curves from intervals with shorter periods of responding (down to 2 s) appear to the right of each panel. The numbers on the *x* axis indicate the duration of responding in each curve and are centered below the curves they identify. Each mark on the *x* axis indicates a 2-s period.

over the whole 30-s interreinforcer interval. Under saline, the curves start around the middle of the interval. Most of the control curves show some within-response-run acceleration, whereas the others are flat, indicating a steady local response rate. Drug administration had three effects: (1) responding started earlier; (2) within-response-run acceleration was reduced; (3) maximum local rate was reduced. All these effects were dose-related.

Corresponding data obtained with the FI 60-s schedule are shown in Figure 9. The results are essentially the same as with FI 30 s. Under saline, 2 rats showed within-response-run acceleration and 2 did not. Drug administration had the same three dose-related effects. Finally, data obtained with the FI 120-s schedule are shown in Figure 10. Under saline, all 4 subjects showed within-response-run acceleration. Again, the drug had the same dose-related effects. The overall results with this method were consistent and also resemble the results of the more molecular method. We are therefore justified in collapsing the data

in this fashion, as no reliable molecular features are obscured.

DISCUSSION

Evidence is accumulating that alternative methods of describing operant behavior repay investigation. The presentation of sample cumulative records has declined in popularity (Church, 1979; Skinner, 1976) and has been replaced by data on overall rates under various conditions or by averaged response patterns. The present study suggests, however, that the behavior of rats maintained by FI schedules can be better understood if averaged response patterns are augmented by molecular analyses. Consistent and orderly data were obtained, both in the baseline conditions and following drug administration, when the PRP was recorded separately and local response rate was averaged across only those occasions when responding was occurring (i.e., when at least three responses had occurred in the current interreinforcer interval). It was then seen that

Fig. 8. Local response rates in 2-s intervals as a function of time since reinforcement for rats on an FI 30-s schedule, under four doses of *d*-amphetamine and during the corresponding saline (control) sessions. Local response rate at any point in the interreinforcer interval is averaged for only those intervals in which responding had begun by that time. Consequently, each curve covers only that part of the interreinforcer interval in which responding sometimes occurred. Points are plotted only when responding occurred at least three times in the sessions concerned.

Fig. 9. Local response rates in 2-s intervals as a function of time since reinforcement for rats on an FI 60-s schedule of reinforcement, under four doses of *d*-amphetamine and during the corresponding saline (control) sessions. Further details are as for Figure 8.

Fig. 10. Local response rates in 2-s intervals as a function of time since reinforcement for rats on an FI 120-s schedule of reinforcement, under four doses of *d*-amphetamine and during the corresponding saline (control) sessions. Further details are as for Figure 8.

responding occurred at a relatively high rate from its initiation, rather than accelerating from a very low rate. However, within-response-run acceleration often did occur and appeared to do so much more frequently at longer FI values. When there was acceleration, local response rate was largely determined by the time since the last reinforcer delivery. These data are consistent with previous findings with rats (Lowe & Harzem, 1977) and pigeons (Branch & Gollub, 1974; Shull & Brownstein, 1970) at short and intermediate FI values.

The dose-related effects of acute intraperitoneal administration of *d*-amphetamine were threefold: There was a marked shortening of the PRP although it remained variable; the maximum local rate of responding declined; where within-response-run acceleration occurred under saline, it was diminished or abolished by the drug. These findings are consistent with previous studies of the within-interval effects of this drug on FI behavior in rats, although behavior at the molecular level presented here must be inferred from the data available in earlier reports. Most importantly, our findings with rats replicate those of Branch and Gollub (1974). They carried out a similarly detailed examination of the effects of *d*-amphetamine on behavior maintained by FI schedules, but used pigeons instead of rats and employed some different methods of analysis. Despite these differences, they came to remarkably similar conclusions. In particular, they concluded that averaging of response patterns tends to produce scallops artifactually because of long and variable PRPs, but that within-response-run acceleration does occur when that artifact is controlled for. They reported, as was true in our study, that the primary effect of the drug was to alter the distributions of PRPs. Unlike the present study, they did not find that within-response-run acceleration was abolished by the drug; however, their measure of acceleration differed from that used here.

When pausing was distinguished from periods of responding, two types of temporal control were seen: PRP duration was determined by the FI value, and within-response-run acceleration was more likely under longer FI values. Both these types of temporal control were systematically disrupted by the drug. This tended to produce rate constancy (Gon-

zales & Byrd, 1977; Heffner et al., 1974) or rate convergence (Ksir, 1981), but it may also be described as a direct effect on temporal control. Such a description may enable a parsimonious interpretation that integrates this phenomenon with others resulting from different procedures. Two recent studies used a temporal-discrimination procedure with rats in which a short (2-s) or long (8-s) stimulus was presented and then two retractable levers were inserted. A left response was then reinforced if a 2-s stimulus had occurred, and a right response was reinforced if an 8-s stimulus had occurred. Acute administration of 1 mg/kg (Maricq & Church, 1983) or chronic administration of 1.5 mg/kg methamphetamine (Meck, 1983) resulted in the "long" response being selected more often than in the absence of the drug in test sessions in which a range of stimulus durations was presented; that is, the rats "overestimated" the duration of the stimulus that had occurred. Perhaps early responding under FI schedules, and a consequent reduction in the PRP, may also be characterized as "overestimation" of elapsed time.

Rate dependency is generally supported by the effects of amphetamine on behavior maintained by FI schedules (see Dews & Wenger, 1977, for a review), and in the present study the effects on average response patterns were generally to increase low rates and to decrease high rates. However, our contention is that increases in low rates are best described as changes in temporal control—the probability of initiating responding changes, rather than the response rate. By this view, reductions in high rates in the interval are a separate effect of the drug. The best evidence for rate dependency unconfounded by other variables comes from studies such as that by McMillan (1969), who showed that food-reinforced key pecking by pigeons at low rates under either FI or fixed-ratio (FR) schedules could be increased by amphetamine, whereas high rates maintained by the same types of schedules could be decreased. Although these effects did not all occur at the same dosages, they seem to be good evidence for response rate per se indicating the drug effect. However, the important and unusual finding that low-rate FR responding could be increased by amphetamine occurred with FR 250. Cumulative records (McMillan, 1969, p. 31) show that

these ratios took as long as 20 min to complete. Thus the performance appeared to be under FI-like temporal control, and any disruption of that control would have produced increases in responding similar to those seen early in the interval under FI schedules in the present study.

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