Behavioral History as a Determinant of the Effects of *d*-Amphetamine on Punished Behavior

Abstract. *Food-maintained responding by four squirrel monkeys (*Saimiri sciureus*) was suppressed by the presentation of electric shock (punishment). Two of these monkeys were experimentally naive and two had a history of responding maintained by both shock-postponement and shock-presentation schedules. In accord with earlier studies,* d-*amphetamine did not increase punished responding by naive monkeys. However, it did increase punished responding in the others. Subsequently,* d-*amphetamine also increased punished responding by the initially naive monkeys after they were trained under a shock-postponement schedule. Even though not evident in current behavior, an organism's prior experience can influence the behavioral effects of drugs.*

The behavioral effects of drugs are known to depend to a large extent on characteristics of behavior prevailing at the time of administration. One of the most important and well documented of these features is the rate or frequency with which a given behavior occurs under control or nondrug conditions (*1*, *2*). Another factor that has recently been shown to be of significance is the environmental context in which behavior occurs. For example, although amphetamine does not usually increase behavior suppressed (that is, punished) by electric shock, it will do so when responding is also maintained under different stimulus conditions by a shock-postponement schedule (*3*). This finding suggests that the behavioral effects of a drug can depend on factors other than momentary aspects of ongoing behavior.

Although ongoing behavior is a product of both prior experience and prevailing environmental conditions, known determinants of drug effects such as the response rate in the absence of drugs and the environmental context are usually apparent at the time a drug is administered. The experiment reported here shows that an organism's prior behavioral history can profoundly modify the effects of a drug even when the influence of such experience is not evident in current behavior.

The effects of *d*-amphetamine were examined in four male squirrel monkeys (*Saimiri sciureus*) for which responding maintained by food was also punished by shock. Two of these monkeys had previously been trained under a shock-postponement (avoidance) schedule; responding was subsequently maintained by the presentation of shock (*4*). The other two monkeys had no previous exposure to shock. Despite the apparently similar performances of all monkeys, *d*-amphetamine increased punished responding only in those monkeys having prior experience with shock.

The monkeys were seated in a Plexiglas restraining chair (*5*) for the 2-hour session. Throughout the experiment the monkeys were maintained at 80 percent of their free-feeding weights and were housed individually in cages with water freely available. The chair was equipped with stimulus lamps, a response lever (BRS/LVE 1352; Lehigh Valley Electronics), a pellet dispenser (model D-1; Gerbrands) and brass electrodes that rested on a shaved portion of the monkey's tail. The tail was held motionless by a stock. Food pellets (300 mg; Noyes banana-flavored) could be delivered into a recessed receptacle located on the front wall. Shock (5 ma, 200 msec, 650 volts a-c) was delivered through series resistance to the tail.

Two monkeys (MS-7 and MS-12) were initially trained under a shock-postponement schedule for which a response postponed for 25 seconds shocks that were otherwise scheduled to occur every 5 seconds (*6*). This schedule alternated with a second condition, associated with stimulus lamps of a different color, under which the first response occurring after 5 minutes produced a food pellet (5-minute fixed-interval schedule). After approximately 1 month, the shock-postponement schedule was deleted, and responding was maintained by the presentation of shock; as with food delivery, shock was produced under a 5-minute fixed-interval schedule. During this phase of the experiment, similar patterns of responding were maintained by the presentation of shock (associated with red lights) and the presentation of food (associated with white lights). This multiple schedule remained in effect for approximately 1 year (*7*). At the end of this time, the shock-presentation schedule was eliminated, and responding was maintained under the fixed-interval schedule of food alone. When responding was stable (after about 1 month), a punishment condition was put into effect; the first response after the 5-minute period still produced food, but each 30th response produced an electric shock, which, over a 2-week period, reduced responding to a point below that maintained by food alone. Reduction by the presentation of shock indicates that punishment had occurred.

Table 1. Overall response rates (responses per second) of unpunished and punished responding. Data for unpunished responding represent the mean of the last three sessions before the introduction of the 30-response shock schedule. Data on punished response rates are from control (nondrug) sessions (Thursdays) or from a day on which saline was administered; they represent the mean of eight sessions. Monkeys MS-7 and MS-12 had prior training under schedules of shock postponement and shock presentation. Monkeys MS-18 and MS-21 were experimentally naive.

Subject	Responses	
	Unpunished	Punished
	Experienced	
MS-7	0.123	0.039
MS-12	0.344	0.208
	Naive	
MS-18	0.116	0.023
MS-21	0.194	0.086

This punishment condition was used with two other monkeys (MS-18 and MS-21) previously trained only under a food-presentation schedule. With these monkeys, the first response after 5 minutes produced food. After responding stabilized, the punishment schedule was imposed and responding was suppressed. As with MS-7 and MS-12, white lamps illuminated the chamber during this condition. For all monkeys, a 60-second time-out followed each food pellet, during which responding had no scheduled consequences. Daily sessions consisted of 20 cycles of the 5-minute fixed-interval schedule (plus time-out). Responding was stabilized for at least 2 weeks before the first drug administration. Table 1 shows rates of food-maintained responding for all four monkeys before and after responding was punished.

The effects of *d*-amphetamine sulfate were examined in all four monkeys as they responded under identical punishment conditions. The drug was dissolved in saline and injected intramuscularly immediately before the session, usually on Tuesday and Friday, given that performance on the preceding day was stable in comparison with performances prior to the beginning of the drug sequence. Each dose was administered at least twice in a mixed order.

After the effects of *d*-amphetamine on punished responding were determined with the experimentally naive monkeys, the concurrent schedule of food delivery and punishment was removed. A shock-

postponement schedule identical to that described above was then established (correlated with red lights), and responding was maintained under this condition for approximately 2 weeks. At the end of that time the shock-postponement schedule was removed and responding was again maintained by food and punished by shock. When responding stabilized at rates comparable to those obtained during the previous drugging regimen (approximately 3 weeks), the effects of *d*-amphetamine were redetermined. The procedures and dose ranges were identical to the earlier ones.

As has been reported (*2, 3, 8*), increases in punished responding as a function of *d*-amphetamine did not occur in those monkeys not having a history of responding maintained by shock postponement and shock presentation (Figs. 1 and 2).

The failure of *d*-amphetamine to increase punished responding of subjects without this history is consistent with other findings; despite the general tendency of amphetamine to increase responding occurring at relatively low rates, low rates of punished responding are usually either unaffected or decreased further by amphetamine. However, *d*-amphetamine did produce substantial increases in punished responding by those monkeys with a history of responding maintained by shock postponement and the presentation of shock (Figs. 1 and 2). Responding decreased for all monkeys at the highest doses of amphetamine.

Exposure to a shock-postponement schedule alone was sufficient to reverse the effects of *d*-amphetamine on punished behavior. When the two formerly naive monkeys were returned to the punishment condition after being exposed to the shock-postponement schedule, their response rates were nearly identical to those obtained during the initial determination of the *d*-amphetamine dose-effect curve (0.030 and 0.082 responses per second for MS-18 and MS-21, respectively; compare with Table 1). The effects of amphetamine, however, differed dramatically. Whereas punished responding had not increased before, increases with amphetamine did occur after the animals had responded under the shock-postponement schedule. A dose of 0.1 mg per kilogram of body weight produced maximum increases of 170 and 313 percent of control responses for MS-18 and MS-21, respectively (compare with Fig. 1).

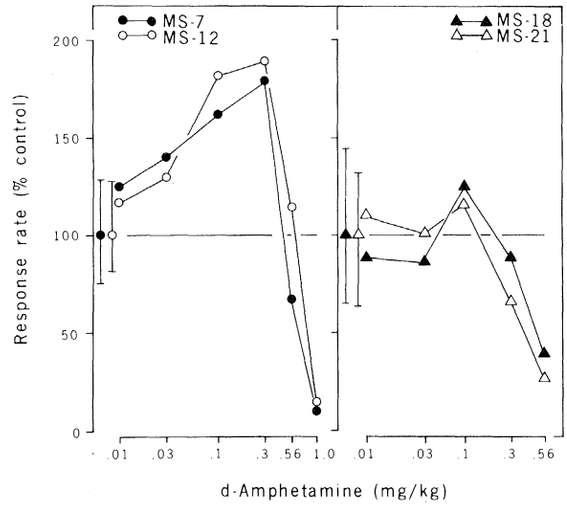

Fig. 1. Effects of *d*-amphetamine on punished responding in squirrel monkeys with (MS-7 and MS-12) and without (MS-18 and MS-21) prior experience under shock-postponement and shock-presentation schedules. When the drug was administered, responding by all four monkeys was maintained under a 5-minute fixed-interval schedule of food delivery and was suppressed by the delivery of a 5-ma shock after each 30th response. Vertical bars represent the ranges of response rates on control days (usually Thursdays) when drugs were not given or when saline was administered.

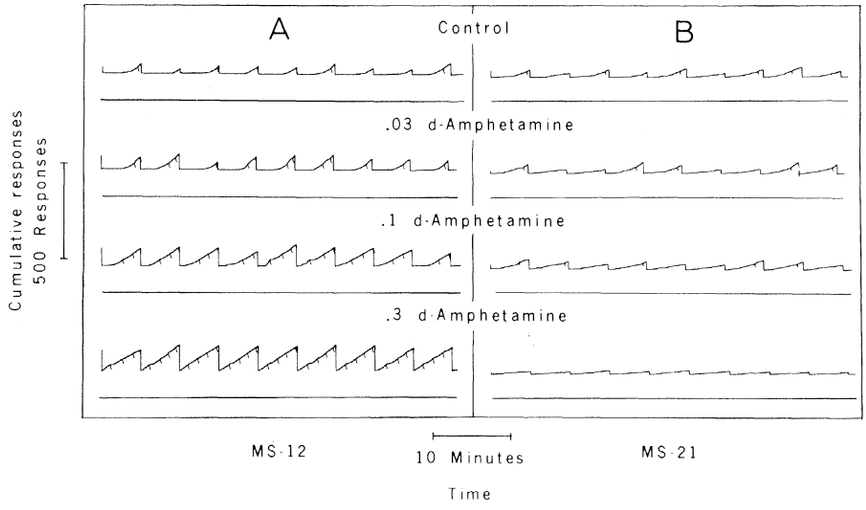

Fig. 2. Cumulative response records depicting (A) increases in punished responding with amphetamine after a history of responding under shock-postponement and shock-presentation schedules and (B) the absence of increases in punished responding when no previous exposure was given to schedules of shock. Shock delivery is indicated by a diagonal mark. The recording pens returned to the original position when food was delivered.

These results are similar to those of earlier research that demonstrated increases with amphetamine in responding that is both maintained by food and is also punished when responding is being maintained under other conditions by shock postponement or by the presentation of shock (*3, 9*). Amphetamine has also been shown to increase punished responding maintained by the termination of a stock-associated stimulus (*10*). Together, these findings suggest that the total environmental context, the event that maintains responding, and the organism's behavioral history are significant factors in determining behavioral changes produced by drugs.

Current behavior depends critically on what has occurred in the past. For example, the manner in which an event affects behavior may depend more on the organism's behavioral history than on the nature of the event (*4, 11*). The development and maintenance of schedule-controlled operant behavior exemplifies the significance of the dynamic and continuing interaction between former and current behavioral consequences. The ongoing rate and pattern of responding reflect the combined effects of a previous history of reinforcement and control by existing schedule contingencies. These factors are also important determinants of the effects of drugs on behavior. In this experiment, however, the contribution of the organism's previous history to the ongoing response rate and pattern was not apparent in the behavior until after the drug was administered.

The results of this study suggest that the effects of drugs on punished behavior, and probably on other behaviors as well, are complexly determined. There has been a tendency to regard the process of punishment as a unitary behavioral phenomenon and to describe the effects of drugs on "punished behavior" in

categorical terms. The results of studies in behavioral pharmacology have forced a reevaluation of the concept of punishment and, at the same time, have yielded a greater understanding of variables contributing to the effects of drugs on behavior. Just as all behaviors controlled by reinforcement are not similarly affected by drugs, neither are all behaviors suppressed by punishment (*9, 12*). The effects of drugs do not depend simply on whether a behavior is reinforced or punished but depend on other more complex and multiple determinants of those behaviors. As demonstrated in this study, prior experience can leave residual effects that, although not manifest in current behavior, can nonetheless significantly influence the behavioral effects of drugs.

JAMES E. BARRETT

Department of Psychology, University of Maryland, College Park 20742

References and Notes

1. P. B. Dews, *J. Pharmacol. Exp. Ther.* **122**, 137 (1958); D. J. Sanger and D. E. Blackman, *Pharmacol. Biochem. Behav.* **4**, 73 (1976); P. B. Dews and G. R. Wenger, in *Advances in Behavioral Pharmacology*, T. Thompson and P. B. Dews, Eds. (Academic Press, New York, 1977).
2. R. T. Kelleher and W. H. Morse, *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.* **60**, 1 (1968).
3. J. W. McKearney and J. E. Barrett, *Psychopharmacologia* **41**, 23 (1975).
4. The maintenance of responding by the response-produced *presentation* of electric shock was first documented by R. T. Kelleher and W. H. Morse [*J. Exp. Anal. Behav.* **11**, 819 (1968)] and has been further extended by J. W. McKearney [*Science* **160**, 1249 (1968)] and L. D. Byrd [*J. Exp. Anal. Behav.* **12**, 1 (1969)]. Factors responsible for the development and maintenance of responding are summarized by W. H. Morse and R. T. Kelleher [in *The Theory of Reinforcement Schedules*, W. N. Schoenfeld, Ed. (Appleton-Century-Crofts, New York, 1970); in *Handbook of Operant Behavior*, W. K. Honig and J. E. R. Staddon, Eds. (Prentice-Hall, Englewood Cliffs, N.J., 1977), pp. 174–200].
5. D. F. Hake and N. H. Azrin, *J. Exp. Anal. Behav.* **6**, 297 (1963).
6. M. Sidman, *Science* **118**, 157 (1953).
7. J. W. McKearney [*J. Pharmacol. Exp. Ther.* **190**, 141 (1974)] and J. E. Barrett [*ibid.* **196**, 605 (1976)] have used similar multiple schedules to examine the effects of various drugs on comparable performances maintained by the presentation of food or shock.
8. I. Geller and J. Seifter, *Psychopharmacologia* **1**, 482 (1960); H. M. Hanson, J. J. Witoslawski, E. A. Campbell, *J. Exp. Anal. Behav.* **10**, 565 (1967); J. E. Barrett and J. M. Witkin, *Pharmacol. Biochem. Behav.* **5**, 285 (1976).
9. J. W. McKearney and J. E. Barrett, in *Contemporary Research in Behavioral Pharmacology*, D. E. Blackman and D. J. Sanger, Eds. (Plenum, New York, in press).
10. J. W. McKearney, *J. Exp. Anal. Behav.* **26**, 281 (1976).
11. Several experiments [for example, M. Sidman, R. J. Herrnstein, D. G. Conrad, *J. Comp. Physiol. Psychol.* **50**, 553 (1957); R. J. Herrnstein and M. Sidman, *ibid.* **51**, 380 (1958)] have shown that the behavioral effects of a stimulus preceding response-independent electric shock could be modified after a history of responding under shock-postponement schedules.
12. D. E. McMillan, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1870 (1975).
13. Supported by grants AA-02104 and DA-01839 from the from the Public Health Service. I thank L. R. Gollub, J. L. Katz, J. W. McKearney, and W. H. Morse for helpful comments on the manuscript and J. A. Stanley and N. L. Gehman for technical and secretarial help.

10 January 1977; revised 18 April 1977

Neuronal Circadian Rhythm: Phase Shifting by a Protein Synthesis Inhibitor

Abstract. *A potent inhibitor of protein synthesis, anisomycin, was applied (10^{-6}M) in 6-hour pulses at specific phases in the circadian rhythm of endogenous compound action potential (CAP) activity recorded from the eye of* Aplysia *in vitro. The phase of the circadian rhythm was systematically advanced or delayed (up to 15 hours) depending on the specific phase at which the pulse was applied. The resultant phase response curve implicates protein synthesis on the eukaryotic ribosome as a fundamental part of the controlling processes that constitutes the circadian clock.*

Although the basic mechanism for circadian rhythms is thought to be endogenous to cellular biochemical processes, the exact nature of the regulatory system is unknown. Various models for the cellular clock have been proposed (*1*), including a sequential transcription model (*2*) and a membrane model (*3*) that takes into account the results of ionic studies (*4*). The involvement of protein synthesis in circadian rhythms has been tested on several occasions with the use of specific inhibitors. Early results on *Gonyaulax*, a dinoflagellate, were equivocal (*5*), but there was good evidence that cycloheximide increased the period of the rhythm in *Euglena* (*6*). Experiments with puromycin and cycloheximide on the rhythms in *Acetabularia* (*7*), an alga, and *Aplysia* (*8*), a gastropod, show that these inhibitors cause phase-dependent shifts in the rhythms. We now report that low concentrations ($10^{-6}M$) of anisomycin, a potent inhibitor of protein synthesis at the ribosomal level in eukaryotes (*9*), given in 6-hour pulses, either advanced or delayed the phase of the circadian rhythm in compound action potentials (CAP) from the eye of *Aplysia*, depending on the phase of the rhythm at which the inhibitor was applied. This result, together with the studies on other rhythms cited above, is strong evidence for the importance of ribosomal protein synthesis in the cellular regulatory mechanisms that constitute the circadian clock.

The interpretation of the effects of inhibitors on rhythms is dependent on the precision of the phase and the period of the rhythm, and on the degree to which the inhibitor is effective at low concentrations and free of side effects. The *Aplysia* eye rhythm and the inhibitor, anisomycin, meet these requirements exceptionally well. Anisomycin ($10^{-6}M$) applied to *Aplysia* central neurons (*10*) inhibited protein synthesis by 90 percent but did not interfere with RNA synthesis or the physiological function of the neurons. The eye rhythm (*11*) is regular in wave form, period and amplitude when continuously recorded in vitro for weeks (*12*), allowing the accurate determination of the rhythm before and after inhibitor treatment. A persistent change in period or a phase-dependent response to treatment is convincing evidence that the clock mechanism itself is affected (*13*).

Aplysia californica (100 to 300 g), obtained from Pacific Bio-Marine (Venice, Calif.) and kept in Albany in Instant Ocean aquariums in a light-dark period of 13 to 11 hours (LD 13:11) at 15°C were used. Eyes with attached optic nerves were dissected and placed in 125 ml of culture medium, maintained thereafter at 15°C in constant darkness (DD). The optic nerve was drawn into a tubing electrode (*12*) in the culture chamber allowing the CAP to be recorded continuously on a Grass polygraph. The culture medium was similar to one used previously (*12*). It contained 90 percent artificial seawater (ASW) and 10 percent nutrient mixture including MEM (minimum essential medium), essential and nonessential amino acids, vitamins, dextrose, penicillin, and streptomycin (Gibco) (*12*), but no *Aplysia* blood or glutamine. Anisomycin (Pfizer) was dissolved in ASW and added to the culture medium (*p*H 7.8) after the rhythm had been monitored for 1 or 2 days. It was administered in 6-hour pulses (30 eyes tested) or left in continuously for several days. After treatment, the inhibitor solution was removed, the preparation was washed with 250 ml of culture medium, and fresh medium was added under dim red light. Control experiments of changing the culture medium did not perturb the rhythm. Initial studies showed that the incorporation of tritiated leucine into trichloroacetic acid precipitable protein in the eye is inhibited by anisomycin up to 90 percent on days 1 to 3 and 40 percent on day 7 (*14*). Our finding agrees with that of others (*8, 10*), namely, that protein synthesis decreases with time after dissection in *Aplysia* neurons. Protein synthesis is preserved longer by the use of culture medium (*15*) as is the circadian rhythm (*12*) which does not exhibit the pronounced damping observed in seawater alone (*8*).

The CAP activity undergoes smooth changes in frequency in eyes maintained in DD and the persistent rhythm of fre-