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BRIEF COMMUNICATION

Prior Drug Experience and Effects of Amphetamine on Schedule Controlled Behavior[1]

H. A. TILSON AND R. H. RECH

Department of Pharmacology, Michigan State University, East Lansing, Michigan 48823

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TILSON, H. A. AND R. H. RECH. *Prior drug experience and effects of amphetamine on schedule controlled behavior.* PHARMAC. BIOCHEM. BEHAV. 1(1) 129–132, 1973.–Food deprived rats were trained to lever press for food pellets on a fixed ratio 40 schedule of reinforcement. Following 18 days of training, rats received *d*-amphetamine IP (1.0 mg/kg) as a drug control. Three days later, half of the animals were given *d*-amphetamine and half were given NaCl for six days. Tolerance to the disruptive effects of *d*-amphetamine on FR responding was not noted in the drugged group. Both groups received 14 more daily sessions with NaCl followed by 12 additional days of drug. Rats with previous drug experience exhibited tolerance in 6 days, while the other group required 12 days. In a second study, rats were trained to respond on an unsignalled continuous avoidance schedule for 8–10 weeks. Two groups of rats were given 7 daily drug sessions in which *d*-amphetamine (1.0 mg/kg) was administered IP. Each drug session was followed by 2 daily NaCl control sessions. In the first 3 drug sessions of one group, *d*-amphetamine was injected IP 30 min after the end of the session. All other injections were given immediately before placement into the operant chamber. During the first session in which the drug was injected before placement into the chamber, response increases were significantly higher in rats with drug experience outside the behavioral situation than in drug naive subjects. These studies emphasize the importance of prior drug exposure when investigating behavioral effects of drugs.

Prior exposure to effects of drugs Schedule controlled behavior
Drug-environment interactions

THE FREQUENCY of drug administration is an important variable in the behavioral effects produced by a given drug dose. For example, in rats responding on a food reinforced, fixed ratio (FR) schedule of reinforcement, repeated daily administration of *d*-amphetamine results in tolerance to the disruptive effects of the drug [1, 2, 6]. Increases in continuous avoidance responding by CNS stimulants are reportedly less in rats having little or no experience with drug administration than in the same rats after three or four injections of the same drug [4].

In the present communication, we report on the influence of the experimental history of the subject and the subsequent development of tolerance to the behavioral effects of *d*-amphetamine, as well as the enhancement of continuous avoidance responding in rats.

METHOD

Animals. Sprague-Dawley rats (Spartan Research Animals, Haslett, Mich.) weighing 125–175 g at the beginning of the experiments were used. The rats were housed in groups of 2–4 in air-conditioned, temperature controlled quarters (approximately 72°F), which were maintained on a day-night rhythm (6:30 a.m. to 6:30 p.m.).

Apparatus and procedure - FR responding. In the first experiment, 8 male rats were used to investigate the effects of experience with the drug and the behavioral environment on the development of tolerance to *d*-amphetamine. The animals were deprived of food and maintained on a deprivation schedule at 70–75% of their free feeding body weight for the duration of the experiment. Each animal was trained to press a lever for food reinforcement in an operant chamber (BRS-Foringer Electronics Co., Model RC-002) contained within a ventilated, sound and light attenuated outer chamber. After 8–10 days of training, all rats were responding on a FR40 schedule of reinforcement during 40 min behavioral sessions. Ten consecutive daily sessions followed and in the last 5 sessions, IP injections of isotonic saline (NaCl controls; 1.0 ml/kg) were given immediately before placement of the animal into the

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operant chamber. On the next day, 1.0 mg/kg of *d*-amphetamine sulfate (K and K Labs, Plainview, New York) dissolved in NaCl was injected in order to determine the disruptive effect of the drug on FR responding. Following two additional daily NaCl control sessions, the rats were randomly divided into 2 equal groups. Rats in Group A were given 6 consecutive daily injections of *d*-amphetamine (1.0 mg/kg) and those in Group B received NaCl. The effect of *d*-amphetamine on FR responding was determined by dividing the number of reinforcers obtained during the drug sessions by the mean number of reinforcers received during the preceding 5 NaCl control sessions (% of control reinforcers) [6].

In the second portion of this study, all animals were given 14 additional daily sessions. The last 5 sessions were NaCl controls and the mean number of reinforcers received served as control values (100%) for this part of the investigation. In the next 12 daily sessions, both groups of rats received *d*-amphetamine (1.0 mg/kg) to test for the rate of tolerance development (i.e., responding within 5% of each animal's mean control responding).

Avoidance responding. Eight female rats were trained to lever press on an unsignalled continuous avoidance schedule. Automated programming equipment controlled the presentation of shock every 5 sec (approximately 2mA and 0.2 sec duration; Lehigh Valley shocker-scrambler; Model 1531). Onset of shock was delayed by 30 sec following a response on the designated lever. The length of the sessions was 60 min. Each animal received 8--10 weeks of training and baseline experience before the study began, and sessions were usually run seven days per week. Following 5 days of NaCl control sessions (1 ml/kg, administered IP immediately before placement into the operant chamber), the rats were divided into two groups. Subsequently, rats in both groups received IP injections of *d*-amphetamine (1.0 mg/kg) on three separate drug sessions. Rats in Group C (N=3) were injected with the drug 30 min after the end of these sessions, while those in Group D (N=5) were injected immediately before placement into the operant chamber. These 3 drug sessions were followed by four additional sessions in which *d*-amphetamine (1.0 mg/kg) was injected IP before placing rats of both groups into the chamber. All drug sessions were separated by two daily NaCl controls (72 hr). The number of responses emitted by each rat during drug sessions was expressed as a percent responding of the NaCl control session on the preceding day.

In both experiments, statistical differences between group means were analyzed by a *t*-test, and differences between mean values obtained from the same group of rats were analyzed by a matched pair *t*-test. The accepted level of significance was set at $p<0.05$.

RESULTS

Fixed ratio responding. Following 13 days of training, the responding of the rats on the FR schedule of reinforcement was characteristic of that described elsewhere [3]. The number of reinforcers delivered in the next 5 daily sessions (NaCl controls) was not more than 10% of the mean NaCl control value of each animal. The average number of reinforcers received by the rats in Group A during the NaCl controls was 98.1± 7.9, (mean±1 S.D.) while rats in Group B averaged 108.3±8.1 reinforcers per session. The IP injection of 1.0 mg/kg of *d*-amphetamine produced decreases in responding which were 40 and 45% of control for Groups A and B, respectively (Fig. 1a). These values were significantly lower than the average NaCl control responding of the respective groups, but were not significantly different from each other. Seventy-two hr later, *d*-amphetamine (1.0 mg/kg) produced a decrease in the responding of Group A (50% of control) and following 5 successive drug sessions, responding was 48% of control. Rats in Group B received six NaCl injections during this part of the experiment and responded near 100% of control. The mean percent of responding for Group A (drug), relative to its control, was significantly lower than the corresponding values of Group B (NaCl) on each of the six days.

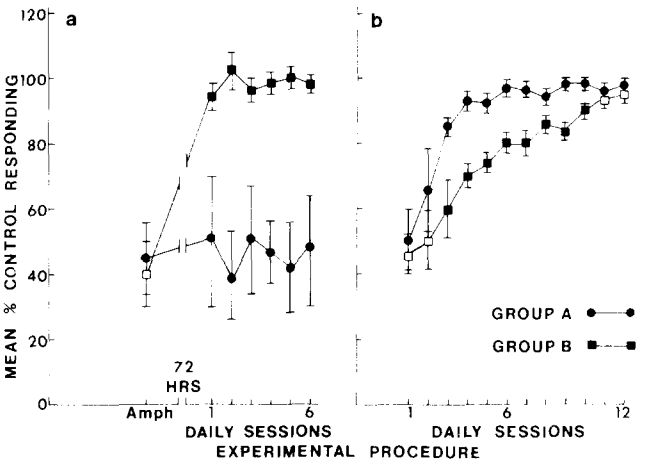

FIG. 1. The effect of drug experience on the development of tolerance to the disruptive effects of 1.0 mg/kg of *d*-amphetamine on FR 40 responding. The data are mean percent of NaCl control responding ± 1 S. D. of 4 rats in each group. The number of reinforcers delivered in 40 min during drug sessions was divided by the mean number of reinforcers delivered during 5 NaCl controls preceding each of the two portions of the experiment (Figs. 1a and 1b). Mean NaCl control values (100%) for the first half of the experiment were 98.1 and 108.3 for rats in Groups A and B, respectively (Fig. 1a). Corresponding NaCl control means for the same two groups of rats during the second portion of the study (Fig. 1b) were 122.6 and 136.5 reinforcers per sessions, respectively. After 18 days of training and baseline FR 40 responding, 1.0 mg/kg of *d*-amphetamine disrupted behavior of both groups to about 40% of control (Fig. 1a). Seventy-two hours later, rats in Group A (circles) received *d*-amphetamine (1.0 mg/kg) for six consecutive daily sessions. Rats in Group B (squares) were likewise given NaCl. Fourteen days of baseline responding (including 5 NaCl controls) followed and, as seen in Fig. 1b, 1.0 mg/kg of *d*-amphetamine was given for 12 consecutive days to rats of both groups. Filled squares denote statistical differences between the mean of Group B and the corresponding mean of Group A ($p<0.05$, *t*-test). Unfilled squares denote the lack of a statistical difference between corresponding means.

The baseline responding of rats in either Group A or B tended to increase during the next 9 sessions of no injections, but responding in the following 5 NaCl control sessions indicated that the baseline had stabilized. Rats in Group A received an average of 122.6±9.9 reinforcers per session while rats in Group B received 136.5±13.1 reinforcers per session. On the day following the last NaCl control, *d*-amphetamine (1.0 mg/kg) produced decreases in the responding of Group A (50% of control) and Group B

(46% of control), which were significantly lower than the mean NaCl control responding of each group (Fig. 1b). In spite of the increases in baseline responding from original control levels (approximately 25%), drug-induced disruption of FR 40 responding was about the same as that obtained during the initial exposure to *d*-amphetamine (40--50% of control responding). After three successive days of *d*-amphetamine injections, the responding of the rats in Group A (85.5% of control) was significantly higher than that of Group B (50% of control). The differences in responding between the two groups remained significant until the eleventh day. Rats in Group A reached the criterion of tolerance (95% of NaCl control) by the sixth day, while the rats in Group B did not become tolerant until the twelfth day of injections.

Avoidance responding. Responding on the unsignalled avoidance schedule was stable after 8--10 weeks of performance. Daily variation in the number of lever presses was not more than 7--8% during the NaCl control sessions preceding each drug session. The average number of responses during the NaCl control sessions was 385±81 (mean ± 1 S.D.) and 409±93 responses per 60 min session for rats in Groups C and D, respectively. Responding by rats in Group C during drug sessions one, two and three (drug 30 min after termination of the session) was similar to NaCl control performances on the preceding day. When *d*-amphetamine (1.0 mg/kg) was given to rats in Group C immediately before the session for the first time (fourth drug session), responding during this and all subsequent drug sessions was significantly greater than the sessions when the drug was given 30 min afterwards (Sessions 1--3). The injection of *d*-amphetamine (1.0 mg/kg) to animals in Group D immediately before placement into the operant chamber (Session No. 1) produced a slight increase in responding (112% of control; Table 1). However, this value was not significantly different that the mean value of the animals injected with *d*-amphetamine 30 min after the session (Group C, 105.9% of control). Subsequent administration of *d*-amphetamine produced increases in the responding of Group D which were significantly higher than the mean value of the initial drug response for this group. The initial response increase seen in rats of Group C following administration of *d*-amphetamine before placement into the operant chamber (Session No. 4, 177.1% of control) was significantly greater than the corresponding drug effect observed in Group D (Session No. 1; 112% of control).

TABLE 1

THE EFFECTS OF DRUG EXPERIENCE ON THE INITIAL INTERACTION BETWEEN *d*-AMPHETAMINE AND AVOIDANCE RESPONDING IN THE RAT

Drug Session No.†	Mean Percent of Control Responding* ± S.D. Group C (N = 3)	Group D (N = 5)
1	Drug 30 min after 105.9 ± 8.0	Drug 30 min before 112.2 ± 6.3
2	103.9 ± 5.9	193.8 ± 29.3‡
3	98.2 ± 9.0	207.0 ± 72.9
4	Drug 30 min before 177.1 ± 35.6‡	Drug 30 min before 194.5 ± 45.9
5	172.5 ± 21.7	182.2 ± 50.4
6	160.7 ± 17.6	191.0 ± 26.1
7	164.8 ± 21.2	178.7 ± 55.8

*Responding during drug sessions was calculated as a percent of the NaCl control session on the preceding day. Data reported are the mean of three and five animals for Groups C and D, respectively.

†Rats in Group C received 1.0 mg/kg of *d*-amphetamine IP 30 min after the end of sessions one through three. In all other sessions and animals *d*-amphetamine was injected immediately before placement of the rat into the operant chamber. All drug sessions were separated by 72 hr (2 NaCl control sessions intervening).

‡All subsequent means are statistically different than mean responding during Drug Session No. 1 of the same group (matched pair *t*-test).

DISCUSSION

These experiments emphasize the importance of the animal's past history when investigating the behavioral effects of drugs. The development of tolerance to the disruptive effects of *d*-amphetamine on FR responding has been reported to occur in 4--12 days using doses of 1.0--3.0 mg/kg [1, 2, 6]. In this study, rats receiving 18 days of baseline experience did not develop tolerance to the disruptive effects of 1.0 mg/kg of *d*-amphetamine during an initial course of 6 daily injections. After 14 additional days of baseline responding, these same animals showed tolerance to the effects of a second course of *d*-amphetamine within six days. The importance of experience with the schedule of reinforcement is also suggested by these studies. Animals receiving approximately one month of baseline performance, but only one drug injection, showed tolerance to the disruptive effects of *d*-amphetamine in the initial course of repeated injections. However, twelve consecutive days of drug injections were required to produce the criterion of tolerance in this latter group as compared to six days for the group having early drug experience.

In the continuous avoidance experiment, administration of 1.0 mg/kg of *d*-amphetamine to drug naive rats produced significant increases in responding in all sessions except the first. This data is in accord with those of Rech and Stolk [4] who reported gradual increases in avoidance responding by several CNS stimulants over a period of months. In the present study, it was evident that experience with the effects of the drug, even outside the behavioral situation, was capable of affecting the initial response to *d*-amphetamine. The initial increases in avoidance responding induced by *d*-amphetamine in rats having received prior drug treatments were near maximal. One possible explanation for this effect is that the 3 exposures to *d*-amphetamine before the drug and box pairing allowed for habituation of adaptation to disruptive novelty effects of the drug.

The results of these studies have obvious implications for the design of and subsequent interpretation of results from experiments on the behavioral effects of drugs. For example, if an unknown drug were substituted for *d*-amphetamine in this study, the results from the first part of the FR experiment might suggest that tolerance does not develop to the behavioral effects of the drug. Since there was a considerable length of time required for tolerance to develop in the drug naive animals in the latter part of the

same study, these data could have been taken as an indication of a metabolic factor, acting to prolong the duration of the drug effect. Furthermore, the small, nonsignificant effect on continuous avoidance responding with the initial injection might have suggested that this dose of the drug had little or no effect on this baseline of behavior. The past history of the organism is a well-known variable in the analysis of behavior [5] and investigators should stipulate in their reports any differences between drug naive and drug sophisticated animals when attempting to systematically replicate drug effects with a particular group of animals.

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