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Conditioned Drug Effects and Absence of Tolerance to *d*-Amphetamine Induced Motor Activity[1]

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TILSON, H. A. AND R. H. RECH. *Conditioned drug effects and absence of tolerance to d-amphetamine induced motor activity.* PHARMAC. BIOCHEM. BEHAV. 1(2) 149–153, 1973.–Tolerance development to *d*-amphetamine induced motor motor activity was studied under various experimental conditions. Following seven daily habituation sessions, female, albino rats were subjected to 7 daily sessions in which NaCl was injected IP 30 min before placement into activity cages (NaCl controls). In the next 9 days, the rats underwent 3 drug sessions, each separated by 2 NaCl controls, in which *d*-amphetamine (0.5, 10. or 1.7 mg/kg) was likewise injected before placement. A course of repeated drug administration followed for the next 14 days. One group of rats was injected with the drug 30 min before placement into the activity cage, a second group received the drug 30 min after each session as a control for conditioned activity effects, while a third group received NaCl. On the fifteenth day, all rats received *d*-amphetamine 30 min before placement as a test for tolerance development. This session was followed the next day by a test for conditioned motor effects in which NaCl was injected IP 30 min before the session. Dose related increases in motor activity were observed during the drug control sessions. The magnitude of the drug effect did not decrease following any of the conditions during the course of repeated drug administration. Animals repeatedly injected with the drug 30 min after or with NaCl 30 min before each session were affected by *d*-amphetamine approximately the same as they were before repeated injections. Rats administered *d*-amphetamine 30 min before sessions during the course of repeated injections showed an enhanced response to *d*-amphetamine during the test for tolerance. The magnitude of the change was related to the magnitude of the conditioned motor activity response. These experiments emphasize the importance of learned or conditioned variables that may result from repeated drug administration in conjunction with behavioral tests.

d-Amphetamine Tolerance to motor activity Conditioned drug effects

TOLERANCE to the effects of *d*-amphetamine on positively reinforced, schedule controlled behavior has been reported by several investigators [1, 2, 13, 14, 17, 21]. It was generally believed that tolerance to *d*-amphetamine induced increases in motor activity does not develop [8], but recent studies have indicated that tolerance may develop under certain experimental conditions. For example, Herman *et al.* [5] reported that stimulation of motor activity by amphetamine gradually declines when the drug is placed in the drinking water and testing continues for 3–6 months. Seegal and Isaac [15] have also reported a decrease in the effectiveness of *d*-amphetamine to alter motor activity when tests are conducted under low levels of visual stimulation.

The failure of some investigators to produce tolerance to *d*-amphetamine induced stimulation may have been due to uncontrolled variables related to conditioning. This suggestion is supported by the report of Pickens and Crowder [10], who showed that drug related increases in motor activity may be conditioned in 6 consecutive daily sessions. The present study sought to examine the role that conditioning variables may play in the attenuation of tolerance development to the stimulant effects of *d*-amphetamine.

METHOD

Animals

Forty-eight female, albino (Sprague-Dawley) rats, weighing approximately 120–150 g at the beginning of the experiment, were used. Animals were housed in groups of four in air-conditioned quarters maintained at approximately 72°F under a 12 hr light-dark cycle (day-night rhythm). Food and water were freely available in the home cages.

Apparatus

Locomotor activity was measured in doughnut shaped

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activity cages equipped with four hinged panels spaced equidistantly along the circumference of a wire mesh floor [18,19]. Depression of a panel activated a microswitch connected to a digital counter and primarily measured linear locomotor activity rather than vertical movements related to rearing or grooming. Experiments were performed in a sound and light attenuated compartment equipped with a ventilation fan. Sessions were run 7 days per week at approximately the same time each day.

Procedure

Upon receiving the animals, they were randomly divided into 4 groups (I, II, III, IV) of 12 rats per group according to the sequencing of drug and test exposure. Each group was further divided into 3 subgroups (4 rats per subgroup; A, B, C) according to the dose of *d*-amphetamine to be administered: A to receive 0.5 mg/kg: B to receive 1.0 mg/kg; and, C to receive 1.8 mg/kg. Four to five days later, the rats were given seven consecutive daily habituation sessions in which they were placed in motor activity cages for a period of 30 min. Following habituation, the rats were given seven daily sessions in which 1 ml/kg of isotonic saline (NaCl) was injected intraperitoneally (IP) 30 min before placement into the cage (NaCl control). On the following day, sessions to determine the effect of each dose of *d*-amphetamine on motor activity were initiated (drug controls). Rats in Groups I, II, and III received IP injections of *d*-amphetamine sulfate (K and K Labs, Plainview, New York) dissolved in NaCl. Injections were performed 30 min before placement of the animal into the activity cages. Three drug injections at each dose level were given in a period of 9 days and each drug session was separated by 2 daily NaCl control sessions. Rats in Group IV, however, always received NaCl (1 ml/kg).

Over the next 14 days, the effects of repeated injections of *d*-amphetamine on motor activity were investigated. Rats in Groups I A, B and C received *d*-amphetamine 30 min before placement into the activity cage, while rats in Groups II A, B and C were injected with the drug 30 min following the termination of each session. Animals in Groups III A, B and C and IV A, B and C received 1 ml/kg of NaCl 30 min before each behavioral session. On the fifteenth day of injections, the animals were tested for drug tolerance. Rats in the first three groups were injected IP with *d*-amphetamine and those in Group IV with NaCl 30 min before placement into the motor activity cage. In the next daily session, all 48 rats received NaCl 30 min before the session as a test for conditioned motor activity.

Analysis of Data

The data are activity counts obtained during 30 min behavioral sessions. A mean control level of activity (100%) based on 7 days of initial NaCl controls, in addition to the NaCl control sessions separating each of the drug controls (total of 13), was determined for each rat. Subsequent motor activity counts were converted to a percent of each animal's own mean control value, and group means were determined.

Significant differences between the means of two groups were examined by means of a one-tailed, Student's *t*-test. Differences between means obtained from the same group of animals were tested by a matched pair *t*-test. The accepted level of significance was set at $p<0.05$.

RESULTS

Habituation

All animals showed a progressive decline in motor activity in the first week of habituation sessions. Subsequent 30 min sessions in which NaCl was injected 30 min before placement into the activity cage provided a baseline of activity for each animal. The stability of the baseline is indicated by the performance of rats receiving NaCl during the entire course of the experiment (Group IV; Table 1).

Drug Control Sessions

The IP injection of *d*-amphetamine 30 min before placement into the activity cage produced dose related increases in motor activity (Table 1). The activity of rats receiving 0.5 mg/kg (subgroups A) of *d*-amphetamine ranged from 198–230% of control (mean of 218%), while the activity of rats injected with 1.0 (Subgroups B) and 1.8 mg/kg (Subgroups C) ranged from 304–341% (mean of 320%) and from 512–612% (mean of 552%), respectively. All drug induced increases in motor activity were statistically different from corresponding mean values of rats receiving NaCl (Group IV, all subgroups). There were no differences in the group means of rats receiving the same dose of *d*-amphetamine.

Tolerance Development

There were no indications of tolerance to the motor activity effects of *d*-amphetamine under any of the conditions of these experiments. In fact, rats in Group I (*d*-amphetamine 30 min before the session) showed an enhanced response to *d*-amphetamine after several consecutive days of injections. For example, the motor activity of rats in Group I A, receiving the initial dose of 0.5 mg/kg of *d*-amphetamine was 226% of control, while on the fourteenth day of injections, the same dose produced motor activity that was 302% of control. Following 15 daily injections of 0.5 mg/kg, 14–15 days of 1.0 mg/kg, and 7–15 days of 1.8 mg/kg, the mean activity values of the rats in Groups I A, I B and I C were statistically greater than the respective group means under drug control conditions. A two-way analysis of variance of motor activity revealed a significant time ($F=15.92$, $p<0.01$) and dose effect ($F=11.21$, $p<0.01$), as well as a significant interaction ($F=3.54$, $p<0.025$).

Animals receiving repeated injections of the drug 30 min after each session (Group III, Table 1) continued to show increases in motor activity during the test for tolerance of approximately the same magnitude as that obtained during the drug control sessions. Likewise, rats receiving *d*-amphetamine only during drug control sessions and on the test day for drug tolerance (Group III) showed similar increases in activity in both portions of the experiment (230–512% as compared to 239–492% of control, respectively).

Conditioned Motor Activity

Only those rats receiving *d*-amphetamine 30 min before each session during the course of repeated injections (Group I) showed conditioned increases in motor activity (Table 1). In the test for conditioned effects on Day 16, the IP injection of NaCl 30 min before placement into the chamber produced increases in the motor activity of animals in Group I that were statistically greater than the

TABLE 1

THE EFFECTS OF REPEATED ADMINISTRATION OF d-AMPHETAMINE ON MOTOR ACTIVITY IN THE RAT. MEAN PERCENT OF NACL CONTROL MOTOR ACTIVITY ± S.D.*

Group† and Dose (mg/kg)	NaCl Control	Amphetamine Control	Daily Injections			Amphetamine (Tolerance Test)	NaCl (Conditioning Test)
			1	7	14	Day 15	Day 16
Group I							
A-0.5	100 ± 23	226 ± 40	201 ± 41	240 ± 36	302 ± 99	315 ± 63‡	167 ± 40§
B-1.0	± 25	304 ± 93	285 ± 88	301 ± 94	415 ± 78‡	449 ± 115‡	184 ± 39§
C-1.8	± 32	612 ± 90	763 ± 170	1087 ± 268‡	1093 ± 271‡	1141 ± 247‡	367 ± 89§
Group II							
A-0.5	100 ± 20	198 ± 20	98 ± 17	109 ± 12	90 ± 14	201 ± 13	96 ± 8
B-1.0	± 26	341 ± 27	106 ± 26	98 ± 12	98 ± 18	336 ± 104	109 ± 9
C-1.8	± 23	533 ± 155	96 ± 18	105 ± 15	102 ± 8	554 ± 144	105 ± 8
Group III							
A-0.5	100 ± 26	230 ± 44	102 ± 9	102 ± 4	108 ± 23	239 ± 52	99 ± 13
B-1.0	± 31	316 ± 57	106 ± 10	120 ± 10	101 ± 18	335 ± 95	101 ± 18
C-1.8	± 32	512 ± 41	102 ± 16	106 ± 20	107 ± 22	492 ± 51	106 ± 13
Group IV							
A-(0.5)[a]	100 ± 23	96 ± 7	97 ± 16	106 ± 16	99 ± 17	95 ± 11	94 ± 8
B-(1.0)[a]	± 26	98 ± 7	97 ± 12	95 ± 13	102 ± 18	93 ± 10	99 ± 21
C-(1.8)[a]	± 19	104 ± 13	97 ± 18	95 ± 9	99 ± 11	94 ± 8	108 ± 11

*See text for description of treatment for each group

†Four different rats were used in each subgroup

‡Statistically different from corresponding amphetamine control, matched pair t-test, $p<0.05$

§Statistically different from corresponding mean value of rats in Group IV, t-test, $p<0.05$

[a]Doses are presented in parenthesis to emphasize the fact that these subjects were not treated with drug, but received saline injections according to the same schedules as utilized in comparable subgroups A, B and C under Groups I, II and III.

respective means of the other groups. However, the differences between the means of the subgroups in Group I on the test day for conditioning were not significant (t-test). The activity of rats in Groups II and III did not vary significantly from the respective group mean of rats always receiving NaCl (Group IV).

The magnitude of the conditioned motor activity in Group I appeared to be related to the dose of *d*-amphetamine administered during the course of repeated injections. If the change in activity from NaCl controls (100%) obtained after the injection of NaCl on Day 16 is subtracted from the activity produced by *d*-amphetamine after 15 days of repeated injections, the resulting values fall in a line that approximates the dose response values obtained under drug control conditions (Fig. 1).

Effects of Drug Injections on Body Weight

d-Amphetamine has well-known anorexic effects [8,22] and the motor activity of the animals receiving repeated drug administration may have been affected by changes in the level of food deprivation. However, artifactual changes in motor activity should also have been expressed in the group of rats receiving the drug 30 min after the session (Group II), as well as in those receiving the drug 30 min before placement (Group I). The rats repeatedly injected with the drug showed rapid development of tolerance to any anorexic effects. Table 2 shows that the percent change in body weight following repeated administration of 1.8 mg/kg of *d*-amphetamine was approximately the same for all groups (17–19% increases). Similar results were obtained in the experiments with the lower doses of *d*-amphetamine.

DISCUSSION

In the context of the classical conditioning paradigm, the pharmacological responses of the drug may be viewed as an unconditioned stimulus, while neutral stimuli attending the drug administration serve as conditioned stimuli [11]. As with previous reports [7, 10, 11, 12], this study indicated that repeated administration of a central nervous system stimulant such as *d*-amphetamine results in conditioned motor activity, assuming optimal conditions for learning are present. More importantly, we have demonstrated that the magnitude of the conditioned activity is related to the dose of *d*-amphetamine used during the course of repeated administration, a finding that is in accord with established laws of classical conditioning. For example, the intensity of the unconditioned stimulus is related, within certain limits, to the magnitude of the unconditioned response, and the latter generally correlates with the magnitude of the conditioned response [6]. Other investigators have reported different degrees of conditioned decreases in motor activity associated with low to moderate doses of chlorpromazine [7]. However, no conditioned effects were observed after higher doses. It is possible that neurolepsis after larger doses of the drug may have affected

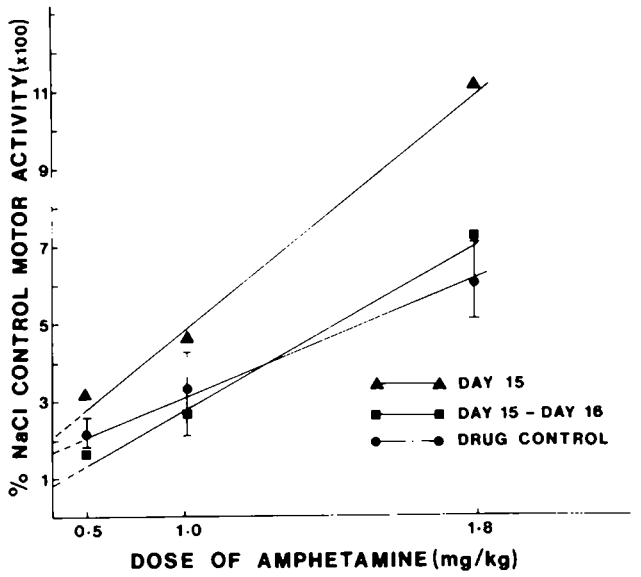

FIG. 1. Dose related stimulation of motor activity by *d*-amphetamine before and after the course of repeated injections. These animals were always injected 30 min before the session (Group I). The drug control values (circles) are group means ± SD of four rats per dose. Each rat was administered three drug sessions each separated by two daily NaCl control sessions (72 hr). Injections of *d*-amphetamine followed for 15 consecutive days, producing an enhanced response to the drug (15th day activity marked by triangles). The next day, NaCl was injected 30 min before the session revealing a conditioned motor effect. Subtracting the conditioned activity from the potentiated drug response on day 15 produced a dose response curve (squares) similar to that obtained during the drug controls.

the ability of the rats to be conditioned.

Self-administration studies have shown that infusion of *d*-amphetamine and other stimulants can reinforce and maintain other modes of responding [20]. An alternative explanation for the conditioned drug responses reported in this and other investigations is that the learned response is maintained by the reinforcing properties of the drug. In this regard, dose related increased in learned or conditioned activity may be associated with the magnitude of the reinforcing stimulus (i.e., intensity of the drug effect). The amount of reinforcement is a well-established variable influencing performance in the learning situation [3, 4, 9, 23].

Repeated injections of *d*-amphetamine under any of the conditions of these experiments did not result in tolerance development. Similar results have been reported by others [10, 13, 22, 24]. Rats injected with *d*-amphetamine 30 min before or 30 min after (to control for conditioned effects) activity sessions for fourteen consecutive days did not show tolerance on the test day when the drug was injected 30 min before placement into the activity cage (Day 15). Unlike results of Seegal and Isaac [15], our data indicate that rats receiving drug injections separated by 72 hr or 14 days also did not develop tolerance, even though testing was conducted under conditions of low visual stimulation. Since Seegal and Isaac did not publish the time intervening between each of their replications or how long each of their animals were used, it is not possible to compare directly their results with the data reported here.

TABLE 2

THE EFFECT OF REPEATED ADMINISTRATION OF 1.8 MG/KG d-AMPHETAMINE ON BODY WEIGHT

Treatment	Mean Body Weight* Group			
	IC	IIC	IIIC	IVC
NaCl control	221	212	219	209
Daily Injection				
1	223	216	223	215
2	222	217	228	222
4	229	225	238	225
7	238	233	246	236
10	247	241	251	238
14	255	244	259	247
Drug Tolerance Test	257	250	261	251
NaCl Test for Conditioning	259	250	259	248
% Change from NaCl Control	17	18	18	19

*Each value is the mean of four rats per subgroup. Initial body weights were obtained on the NaCl session preceding the course of repeated injections.

Only those rats injected with *d*-amphetamine 30 min before each daily session showed an enhanced response to the same dose following repeated injections. The potentiation observed in this and other studies [5, 11, 13] appears to be related to the conditioned motor activity response. In addition, the shift in the dose response curve of those animals receiving *d*-amphetamine 30 min before each session seemed to be related to the magnitude of the conditioned activity effect developed during the course of repeated administration. Although Tormey and Lasagna [22] indicated no enhancement of the drug response after repeated administration of amphetamine, 20 mg/kg of amphetamine for 26 days in their animals may have produced fatigue or interference due to toxicity. Herman *et al.* [5] reported an enhanced response to the drug during the first month of weekly tests. When the drug was administered via the drinking water, tolerance to the motor activity effects of *d*-amphetamine reportedly occurred in 3–6 months of monthly testing. The decreases in motor activity reported by Herman *et al.* may have been due to several factors, including the extinction of conditioned activity. For example, the effects of *d*-amphetamine administered in this fashion would be present to a greater extent in the home cage environment than in the testing cages. Furthermore, continual administration of the drug may have resulted in a decrease in the effectiveness of the drug to elicit motor activity. One of the dynamic laws of the reflex indicates that the reflexual (unconditioned) response diminishes in magnitude following repeated elicitation at high frequencies [16]. Finally, continuous administration of the drug in this fashion may have also resulted in the development of a behavioral or a neurophysiological toler-

ance whereby an accommodation occurred to the stimulant effects of the drug.

These experiments emphasize the importance of stimulus variables resulting from and attending the repeated administration of drugs. Results such as those presented above dictate particular caution in the interpretation of chronic drug studies, even with agents known to induce a metabolic tolerance, when behavioral measures are utilized to assess tolerance development.

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