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Amphetamine: Effects on Defensive Flight or Avoidance in the Rat

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MOLLENAUER, S., M. WHITE, R. PLOTNIK AND P. B. TIFFANY. *Amphetamine: Effects on Defensive Flight or Avoidance in the Rat.* PHARMAC. BIOCHEM. BEHAV. 11(3) 325–329, 1979.—Treatment with a moderately high dose of amphetamine caused rats to retreat from a stimulus they would normally approach and explore (mechanical robot or live white rabbit). While saline-treated rats spent approximately equal amounts of time in the area of the apparatus near the stimulus, amphetamine-treated rats spent a high percentage of trial time in the area of the apparatus farthest from the stimulus. The drug effects were dose related (range: 1.75, 3.5 and 7.0 mg/kg) with higher avoidance time at higher doses, and significant linear trends accounting for much of the variance. The highest dose of amphetamine elicited response stereotypy. However, control conditions ruled out the possibility that the present results could be explained by competing motor responses of stereotypy or increased activity. Thus, apart from its actions on motor behavior, amphetamine treatment resulted in rats avoiding or retreating from an otherwise neutral stimulus.

Amphetamine Defense responses Defensive flight Fear Avoidance Psychosis Rat Stereotypy

TREATMENT with amphetamine can have profound effects on emotional responsiveness in humans and animals. In humans, clinical and laboratory studies have shown that chronic heavy usage can result in amphetamine psychosis, which includes extreme paranoia as one of its symptoms [12,17]. Effects on emotional behavior have also been observed in rats after acute or chronic treatment. Amphetamine-treated rats show an increased escape or flight response to conspecifics [2, 7, 9, 13]. In addition, studies with rats suggest that amphetamine either induces a physiological condition that is, itself, aversive, or a condition in which the environment is perceived as aversive [14,18]. If amphetamine causes the environment to be perceived as aversive, one might expect amphetamine-treated rats to show defensive flight or avoidance in the presence of a neutral stimulus. Pilot work was used to establish stimulus properties of a mechanical robot (speed of movement and noise level) such that untreated rats did not show avoidance. Experiment 1 explored the effects of a wide dose range of d-amphetamine on rats' approach and avoidance of this stimulus. Experiment 2 studied the effects of d-amphetamine on responses to a different stimulus, a live white rabbit. Since it has been shown that amphetamine can augment acoustic startle [3, 4, 11], the purpose of Experiment 2 was to assess the effects of amphetamine on responses to a neutral stimulus that was essentially noiseless and did not provide abrupt changes in stimulation.

METHOD

Animals

The animals for Experiment 1 were 93 male, Long Evans hooded rats, weighing 250–350 g at the start of the experiment, purchased from Simonson Laboratories. Three rats were discarded before testing as a result of injection difficulties. Rats were housed individually two days prior to testing; they were given unlimited access to food and water on the first day but were food deprived on the second. Rats were tested toward the end of the light phase of their light-dark cycle; the cycle was 13 hr light and 11 hr dark.

The animals for Experiment 2 were 30 male, hooded Long Evans rats, weighing 250–350 g at the start of the experiment, purchased from Simonson Laboratories. Rats were housed, fed and tested under the same maintenance conditions as in Experiment 1.

Apparatus

The apparatus was a rectangular chamber constructed of wood and divided by a Plexiglas wall into two compartments; one housed the stimulus robot and the other was used to test the rat. The test compartment was 38 × 43 cm with walls 69 cm high. The compartment was open at the top, and the high walls prevented the rats from jumping out. The floor of the test chamber was made of 1/4 in. white plastic mesh and was marked off into six equal areas by bisecting the width and trisecting the length. The test chamber was lighted by a 75 W red electric light suspended 1.5 m above the floor of the apparatus.

The test compartment was separated from the stimulus compartment by an opaque guillotine door which could be raised during testing and a fixed Plexiglas wall drilled with 1/4 in. holes spaced approximately 4 cm apart. The robot chamber was 38 × 41 × 69 cm, enclosed at the top but left open in the back. The chamber was illuminated by a 75 W white light; the light bulb was suspended directly above the robot, but was inset into the top of the chamber so that the bulb itself was not visible from the rat chamber.

A large mirror was placed over the test chamber so the rats could be observed with minimal distraction. White noise was also used to mask extraneous auditory cues.

For Experiment 2 the apparatus was modified as follows. The Plexiglas barrier separating the test and robot chambers was replaced with 1 × 2 in steel mesh in order to permit greater exposure of the stimulus rabbit. Pilot work had indicated that this wire mesh (as opposed to Plexiglas) tended to elicit exploration even in the absence of a stimulus; therefore, the rear wall of the apparatus was also replaced with wire mesh, backed by an opaque wall and shielded in front by an opaque guillotine door identical to the one at the front of the apparatus. The doors at the front and back of the test chamber were rigged to raise simultaneously.

Stimulus Robot

The stimulus robot was constructed from sheet metal and was electrically operated. The robot was suspended from a tripod, such that it hung approximately 1 cm above the wire mesh floor.

The robot alternated between walking and turning. During the walk period, the legs moved two strokes per sec, approximately 2 cm per stroke for 2.5 sec. During the spin period, the lower section of the robot turned at a rate of three revolutions per sec for approximately 4.5 sec.

The robot was activated in its spin period at the beginning of the test trial and remained on throughout the trial. While the robot was activated, it made a loud grinding noise; during the walk period, the noise level was 83 dB, rising to 94 dB during the rotation period.

Stimulus Rabbit

A two-kg male albino rabbit was used as the stimulus in Experiment 2. The rabbit was habituated to the enclosure for 30 min prior to testing. The animal was never observed to move during the drug tests.

Design and Drug Treatment

Experiment 1. The rats were randomly assigned to one of two test conditions (Robot-No Robot) and one of four drug treatments (Saline; 1.75, 3.5, 7.0 mg/kg d-amphetamine sulfate) in a 2 × 4 factorial design. Amphetamine was dissolved in physiological saline; all injections were administered intraperitoneally in a volume 1 ml/kg 30 min prior to testing. The n's for groups were unequal: for the Robot condition the n's were 9, 10, 10, and 11 in order of increasing dose; for the No Robot condition the n's were 16, 12, 12, and 10.

Experiment 2. Rats were randomly assigned to one of two drug treatments, either Saline or 3.5 mg/kg d-amphetamine sulfate, and to one of two stimulus conditions, either Rabbit or No Stimulus, in a 2 × 2 factorial design with n = 7 or 8 per cell. Injection procedures were the same as for Experiment 1.

Procedure

Rats were tested individually by an experimenter who was blind to drug treatment. A rat was first placed in the test chamber for a five-min habituation period; it was placed in the center third of the test chamber facing the robot chamber. During the habituation period the opaque door shielding the robot was down, all lights were off except the red light illuminating the test chamber and the rat was free to move around the chamber.

Robot test trials were conducted as follows: After the five-min habituation period, the rat was not removed from the test chamber; it was guided gently with gloved hand to the front third of the apparatus, the area nearest the robot chamber, and confined there by a Plexiglas guillotine door for a period of 10 sec. At the end of 10 sec, the rat was exposed to the robot before being released from the front of the apparatus. The sequence was as follows: The light in the robot chamber was turned on, the robot was activated and the opaque door was raised exposing the robot. The Plexiglas door confining the rat near the robot was raised immediately (approximately 1 sec) after the robot was exposed, and the rat was permitted to move freely about the test chamber for the one-min test. The test in the No Robot condition was identical to that of the Robot condition except that the robot was not activated or exposed during the one-min period.

The testing procedure used for Experiment 2 was essentially the same as for Experiment 1 except that the rabbit stimulus was employed and the habituation period was reduced from 5 min to one.

During the one-min test the amount of time the rat spent in each third of the apparatus was recorded; Front time was the total time the rat spent with its head and shoulders in the third of the test chamber closest to the stimulus; Back time was the total time spent with head and shoulders in the third of the chamber farthest away from the stimulus. Low Front time coupled with high Back time would indicate that the rat was avoiding the stimulus. The number of lines crossed or re-crossed by the rat during the one min trial was also recorded as a measure of activity.

In Experiment 1, rats were randomly selected (n=64) from each drug treatment and robot condition and observed for stereotypy approximately 25 min after injection. Rats were rated by an observer blind to the drug treatment on a three point scale: 0, for rats showing no stereotypy, 1, for rats showing intermittent stereotyped head movements, sniffing and gnawing, and 2 for rats showing almost constant stereotyped activity.

RESULTS

The data from the robot test are summarized in Fig. 1. From the Front and Back time scores of saline-treated rats it appears the robot was not an aversive stimulus for undrugged animals. Saline-treated rats tested with the robot spent about equal amounts of time at the front (nearest the robot) and at the back (farthest from the robot). Also, the amount of time spent at the front by Saline rats tested with the robot was virtually identical to that of Saline rats tested with no robot. The pattern of results for Amphetamine rats appears quite different, with the drug causing a dose-related retreat from the stimulus robot, i.e., decreased Front time and increased Back time. In the ANOVA of these data there was a significant interaction between drug treatment and test condition for both Front and Back time, $F(3,82)=3.43$, $p<0.05$ and $F(3,82)=4.95$, $p<0.01$, respectively. The main effect of Robot was also significant for Front and Back time, $F(1,82)=26.66$, $p<0.001$ and $F(1,82)=46.9$, $p<0.001$, respectively. The main effect for drug was not significant in either case. Individual means were compared using the Newman Keuls test with an 0.05 level of significance. The depression of Front time in the Robot condition was significant for both the 3.5 and 7.0 mg/kg doses as compared to Saline and also as compared to the same-dose treatments in the No Robot condition. The increases in Back time were

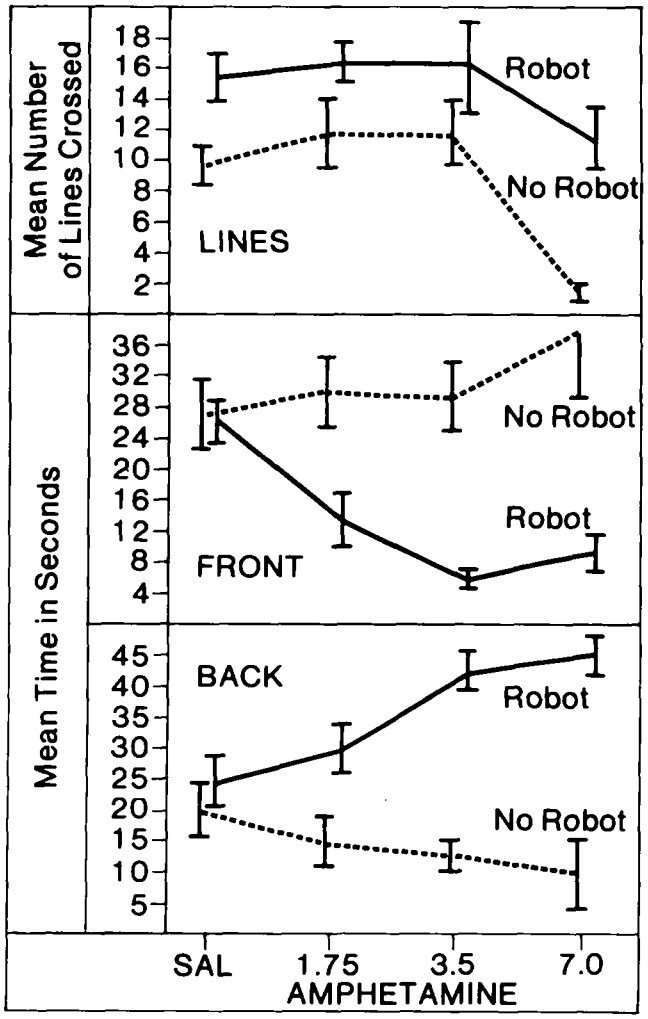

FIG. 1. Mean Back time and Front time in seconds (± SE_m) and mean number of Lines crossed (± SE_m) as a function of drug treatment during Robot and No Robot tests.

also significant for both the 3.5 and 7.0 mg/kg doses as compared to Saline, and for all three doses as compared to the same-dose treatments in the No Robot condition. None of the other individual comparisons were significant. The dose-response relationships were clarified using trend analyses corrected for unequal n's and unequal intervals [8]. As inspection of Fig. 1 suggests, the retreat behavior in the Robot condition increased as a function of dose. For Back time 90.7% of the variance was accounted for by a signficant linear trend ($p<0.01$). For Front time, 53% for the variance was accounted for by a linear component and 44% by a quadratic component, and both trends were significant. The quadratic component in Front time reflected the ceiling effect, i.e., the fact that Front time was maximally suppressed at 3.5 mg/kg amphetamine and showed no further suppression at 7.0 mg/kg. There were no signficiant trends in the No Robot data. The lack of trend in the No Robot data is an important feature of these results. It means that the effect of amphetamine was specific to the Robot condition and reflects retreat from the stimulus rather than spatial preference or responses to procedure.

The data on activity (number of lines crossed) are also shown in Fig. 1. This measure does not include some types of activity, such as rearing, that are known to be influenced by amphetamine [15]. Rather, it is a measure of the rat's movements about the apparatus. It was included to determine whether differences in retreat behavior could be attributed to changes in locomotor activity. A Fig. 1 shows, the pattern of results for Lines was quite different than the pattern for retreat. In the ANOVA of Lines data there was no significant interaction between test condition and drug treatment. There was a significant main effect of Robot, $F(1,82)= 20.98, p<0.001$, reflecting the fact that activity was generally elevated in the Robot condition. There was also a significant main effect of drug, $F(3,82)= 7.0, p<0.001$. The Newman Keuls test showed that the significant drug effect was attributable to the suppression of activity caused by the 7.0 mg/kg dose ($p<0.05$). None of the other doses differed significantly from Saline, and the interaction was not significant. In the absence of interaction and low-dose effects, trend analysis was not considered appropriate for these data.

The mean ratings for stereotypy for Saline, 1.75, 3.5 and 7.0 mg/kg amphetamine were 0.25, 0.56, 1.56, and 1.94, respectively. An ANOVA on these data showed a significant effect of drug, $F(3,60)= 34.33, p<0.001$, and t tests showed that the 7.0 and 3.5 mg/kg doses both differed significantly from Saline, $t(30) = 4.85, p<0.01$ and $t(30) = 8.45, p<0.01$, respectively. The 7.0 and 3.5 mg/kg doses also differed significantly from the 1.75 dose, $t(30) = 5.75, p<0.01$ and $t(30)=323, p<0.01$, respectively. The stereotypy ratings were made while the animals were still in their home cages, and quantitative ratings were not made while the rats were in the test apparatus. However, many of the rats, and virtually all of those in the 7.0 mg/kg group were observed to exhibit stereotypy in the test apparatus. This effect is reflected in the activity scores. In the No Robot condition, rats treated with 7.0 mg/kg amphetamine tended to remain where they were placed in the apparatus and engage in stereotypy. In the Robot condition, the 7.0 mg/kg rats moved to the back of the apparatus and alternated between periods of stereotypy and orientation to the stimulus.

The data from Experiment 2 are presented in Fig. 2. It seems clear from the time scores of Saline control rats that the rabbit was a neutral stimulus. Both Front and Back times of saline-treated rats tested with the rabbit were virtually the same as those of saline-treated rats tested with No Stimulus. The effects of amphetamine were the same as those obtained in Experiment 1. Amphetamine caused the rats to retreat from or avoid the stimulus rabbit as reflected in markedly depressed Front time and elevated Back time. In the ANOVA of both Front and Back times, the interaction between drug and stimulus condition was significant, $F(1,26)=5.25, p<0.05$ and $F(1,26)=9.4, p<0.005$, respectively. The main effect of stimulus was also significant in Back time, $F(1,26)=12.6, p<0.005$. In Newman Keuls comparisons (0.05 level of significance) the Saline rats tested with the rabbit did not differ from Saline rats tested with No Stimulus. However, the Amphetamine rats tested with the rabbit showed significantly more Back time and significantly less Front time than either the Saline rats tested with the rabbit or their Ampehtamine counterparts tested with No Stimulus.

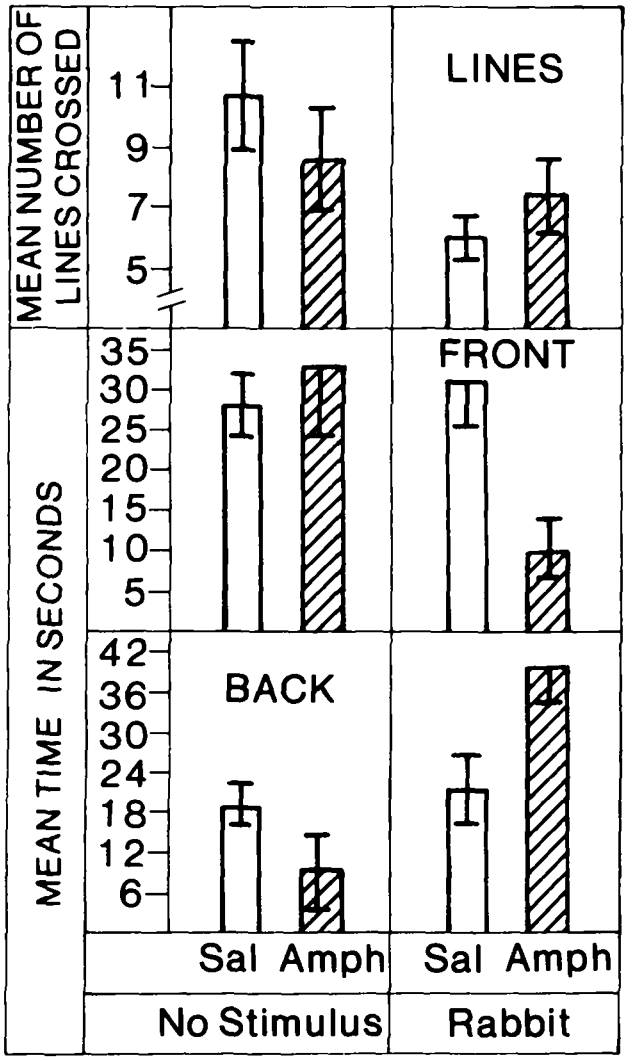

FIG. 2. Mean Back time and Front time in seconds (± SE_m) and mean number of Lines crossed (± SE_m) for Saline and 3.5 mg/kg Amphetamine in Rabbit and No Stimulus tests.

The data on activity are also shown in Fig. 2. As expected from Experiment 1, neither of the main effects nor the interaction was significant.

DISCUSSION

A wide dose range of d-amphetamine sulfate caused rats to avoid or retreat from a stimulus that was not avoided by saline-treated rats, and the effect was strongly dose-related. In tests with the robot, increasing dose caused increased avoidance, with a linear trend accounting for 90.7% of the variance in Back time (time farthest from the stimulus). A moderate dose of d-amphetamine sulfate (3.5 mg/kg) also caused rats to avoid a live white rabbit. This stimulus was essentially noiseless and motionless, and perhaps, most important, it was clearly neutral. Saline-treated rats spent over half of the trial time in the front third of the apparatus exploring the rabbit. In contrast, amphetamine-treated rats showed a marked suppression of Front time and a dramatic increase in Back time, spending almost 70% of the trial time in the third of the apparatus farthest from the rabbit. Thus, amphetamine caused an avoidance or retreat from a clearly neutral stimulus, and the avoidance response could not be attributed to acoustic startle or sudden movement cues.

The dose-response relationship found in the present research was of particular interest. Low versus high doses of amphetamine have often been shown to have differential effects which could be explained by different competing responses occurring at low versus high doses [10]. For low and moderate doses, amphetamine is known to increase locomotor activity and decrease exploration of novel stimuli [10,16]. In the present results, locomotor activity, measured by number of lines crossed, was very similar for saline and the two lowest doses of amphetamine in both Robot and No Robot conditions. Except for the highest dose, the dose-response curves for locomotion were essentially flat, while the curves for retreat or avoidance showed a linear increase with dose. Thus, locomotion was orthogonal to retreat and cannot account for the retreat response. Since the present measure of activity did not include measures of exploratory behavior (rearing, sniffing), the possibility remains that the lower doses of amphetamine caused a depression of exploratory behavior. Such a decrease in exploratory behavior might account for some modest decline in time spent near the stimulus. However, decreased exploratory behavior would not account for the marked suppression in Front time that was observed with both Robot and Rabbit stimuli. Nor could it account for the very pronounced elevation of Back time obtained with both stimuli. Apart from any decreased exploratory tendencies that may have occurred, the amphetamine-treated rats displayed a strong avoidance of both robot and rabbit as reflected in depressed Front time and elevated Back time.

High doses of d-amphetamine are known to produce stereotypy, which has been considered a model for amphetamine psychosis [5,15]. For this reason, and because stereotypy is known to interfere with other responses [10], an important question for the present research was whether the avoidance behavior might be an indirect consequence of stereotypy. In home-cage observations in the present research, severe stereotypy occurred at the highest dose and to lesser degrees at the lower doses. However, only the highest dose caused motor inhibition due to stereotypy in the test apparatus. This result was reflected in the activity data, in which there was a strong suppression at the high dose but no difference from saline at the lower doses. The pattern of results for avoidance behavior was entirely different in that effects were not confined to the highest dose. The middle and lower doses both caused a significant increase in Back time, and the middle dose also caused a suppression of Front time; in fact, the dose response curve showed a maximal suppression of Front time at the middle dose. Since the retreat or avoidance scores for the middle-dose amphetamine-treated rats were very similar to those of the high-dose rats, it seems unlikely that the stereotypy of the high-dose animals accounted for their avoidance. If anything, the stereotypy might be expected to interfere with the retreat or avoidance response. The fact that the retreat response could be executed despite stereotypy constitutes a major advantage of the present paradigm.

The present finding, that amphetamine caused rats to avoid stimuli not avoided by saline-treated rats, is consistent with amphetamine's known actions on emotional behavior. Acute and chronic treatment in rats causes increased flight and escape responses to other rats [2, 6, 7, 9, 13] as well as

aversion to associated tastes and spatial areas [14, 18]. Grilly [10] has suggested that amphetamine treatment, itself, creates a physiological state that essentially mimics that induced by noxious stimuli, such as shock. The present results suggest further that amphetamine can cause an apparently neutral stimulus to elicit flight or retreat. An analogous situation exists in instances of human amphetamine abuse, in which the drug elicits extreme fear or paranoia [12,17]. The paranoid reactions exhibited by amphetamine users typically emerge following chronic high-dose treatment. However, Segal and Janowsky [17] have speculated that paranoid tendencies may co-exist with the behavioral arousal observed at lower doses. In the present research, flight or retreat responses were observed at lower doses and became more pronounced at higher doses. In future research we plan to explore the effects of chronic amphetamine treatment on defensive flight or avoidance. Perhaps the neuropharmacological mediation of these flight responses is in some way analogous to the mediation of fear responses following amphetamine abuse.

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