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# Chronic Amphetamine: Effects on Defensive Flight in the Rat

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MOLLENAUER, S., M. WHITE, R. PLOTNIK AND B. PIPKIN. *Chronic amphetamine: Effects on defensive flight in the rat.* PHARMAC. BIOCHEM. BEHAV. 17(3) 381–384, 1982.—Hooded rats were injected with physiological saline or d-amphetamine sulfate for 13 days on a schedule designed to mimic patterns of abuse: one injection on days 1–11, two injections on day 12, and three injections on day 13; amphetamine dosage for the first three injections was 3.5 mg/kg and for all subsequent injections was 5.0 mg/kg. Amphetamine-treated rats (Amphet) showed a dramatic flight reaction in response to a novel stimulus (mechanical robot) that did not elicit flight from saline control animals. Tested on a slow-moving treadmill that carried them toward the stimulus, Amphet rats accumulated only 15% of the trial time at the front of the apparatus nearest the stimulus and accumulated approximately 75% of the trial time at the extreme rear of the apparatus, farthest from the stimulus. Control tests of Amphet rats in the absence of the stimulus ruled out interpretations in terms of motor behavior. In fact, a major advantage of the present procedure is that animals are able to execute the relatively simple defense response despite the occurrence of motor stereotypy. These results suggest that the defense-response paradigm is suitable for the study of chronic amphetamine and may provide a useful adjunctive to existing models of amphetamine psychosis.

Chronic amphetamine  Defensive flight reaction  Rat  Affective behavior  Animal model  Amphetamine psychosis

BECAUSE chronic amphetamine treatment in humans results in the development of an "amphetamine psychosis" that is virtually indistinguishable from paranoid schizophrenia [1], there has been considerable interest in the development of animal models of this condition [9]. The effects of amphetamine on motor behavior have been studied most intensively; it is well-established that chronic or high doses of amphetamine cause an increasing restriction of behavior to stereotyped movements, such as sniffing and repetitive head movements [12]. Other well-documented effects of high dose or chronic amphetamine have included heightened arousal, hyperreactivity or increased startle reaction [7]. Although far less is known about the effects of chronic amphetamine on affective or emotional behaviors in animals, it is now clear that the drug has profound effects on these behaviors as well. When rats have been studied in naturalistic settings, amphetamine treatment has caused profound alterations in their reactions (e.g., fight or flight) to conspecifics [10]. The study of amphetamine effects in naturalistic settings clearly holds great promise in terms of ethological validity. At the same time, this approach is complicated by the difficulties of analyzing the behavior of interacting animals. A paradigm that permitted the study of affective behaviors of individual animals might provide an important complement to these more ethological approaches. In our laboratory we have recently developed such a paradigm based on the rat's natural defensive reaction of flight, i.e., the tendency to move away from a threatening stimulus. In the initial work with this paradigm, a wide range of acute amphetamine treatment (1.0–5.0 mg/kg d-amphetamine) caused rats to move away from a stimulus (mechanical robot or live white rabbit) that did not elicit flight from saline controls. The effect bore a strong linear relationship to dose, the higher the dose the more time accumulated at the extreme rear of the apparatus [11].

For the present research with chronic amphetamine, we have modified this paradigm to require a more continuously active flight response. In this procedure rats were tested on a slow-moving treadmill that carried them toward a novel stimulus (mechanical robot). In order to remain distant from the stimulus a rat would have to execute fairly continuous flight behavior. The properties of the stimulus robot (e.g., spin rate) were adjusted in pilot work so that control animals allowed themselves to be carried forward toward the stimulus robot and to accumulate a high percentage of trial time at the front of the apparatus. Thus, it was possible to study the effects of chronic amphetamine on the reactions to a stimulus that did not elicit defensive flight from control animals. The schedule of chronic amphetamine injections was designed to mimic one of the common patterns of abuse in which amphetamine is taken about once daily over a period of days or weeks, with massive doses taken on the final day [1].

## METHOD

### *Animals*

The animals were 48 male, Long Evans hooded rats weighing 250 to 300 g at the beginning of the experiment. The animals were individually housed for one week prior to the start of the experiment. They were given unlimited access to water and were fed a fixed amount of food, 15 g lab chow, at

approximately the same time each day, 20 min after the time when injections were administered. Testing was conducted toward the end of the light phase of their 12-hour light-dark cycle.

*Apparatus*

The apparatus was a rectangular chamber in which the floor was replaced by a treadmill that moved (3.5 mm/sec) toward the front of the test chamber, where the stimulus robot was positioned behind a wire mesh barrier. The apparatus was located in a dark room and was illuminated by a 60-watt red light suspended 90 cm above the apparatus. The test compartment was 38 cm × 43 cm and had walls 69 cm high; it was open at the top and animals were observed by way of a mirror suspended above the apparatus. The test compartment was separated from the robot compartment by an opaque guillotine door, which could be raised during testing, and by a fixed 2.54 cm × 5.08 cm steel mesh wall. The robot was positioned less than 2.54 cm from this wall and was illuminated from above by a 75-watt white light. The details of this arrangement have been described previously [11].

*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 body of the robot was a cube 15 cm × 18 cm, and 10 cm long; the legs were rectangular in shape, 10 cm × 5 cm and 14 cm long. The body of the robot was painted white and the legs were painted in black and white vertical stripes 2 cm in width.

The robot alternated between walking for 2.5 sec and spinning for 4.5 sec. During the walk period, the body of the robot remained stationary and the legs moved two strokes per sec, approximately 2 cm per stroke. During the spin period, the entire robot, body and legs, turned at a rate of three revolutions per sec. The robot was engineered to achieve maximum spin rate within several seconds. Pilot work had suggested that rate of spin was the critical factor in determining whether the robot would elicit a defense response.

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.

*Design and Drug Treatment*

Rats were randomly assigned, 24 to each of two drug conditions, chronic amphetamine injections (Amphet) or chronic saline injections (Sal). All animals received one injection per day for 11 days at the same time each day, late afternoon; on day 12, the day prior to test, they received two injections, one at the usual injection time and one at midnight; on the day of the test they received three injections, one early morning, one at midday and one at the usual injection time. The Amphet rats were given 3.5 mg/kg d-amphetamine sulfate for the first three injections and 5.0 mg/kg d-amphetamine sulfate for all subsequent injections. The Saline rats received physiological saline for all injections. All injections were administered IP in a volume of 1 ml/kg. The last injection was administered 30 min before testing.

Within each drug condition, half the animals, randomly selected, were tested with the robot (Robot) and half were tested without the robot (No Robot). For all animals data was taken during a one-minute habituation period with no stimulus and during the one-minute test period.

*Procedure*

Rats were tested individually for defensive flight as follows. With the treadmill activated the rat was placed on the treadmill at the front of the apparatus and given a one-minute habituation trial without the robot stimulus. At the end of this habituation period the rat was moved again to the front of the apparatus and the opaque door was raised exposing the robot behind the wire mesh barrier. For Robot trials the robot was activated when the door was raised; for No Robot trials the robot was not activated but the door was raised exposing the robot and the lighted chamber. During both Habituation and Test periods, two measures were recorded: Front time, the cumulative amount of time that the rat spent with its head and shoulders in the front third of the apparatus, nearest the robot compartment, and Back time, the cumulative time the rat spent with head and shoulders in the back third of the apparatus, farthest from the robot compartment. In order to accumulate low front time and high back time the rat was forced to move continuously against the action of the treadmill.

## RESULTS

The Front and Back time data for both Habituation and Test periods are summarized in Fig. 1. These data were analyzed using mixed design ANOVAs in which Test period (Habituation vs Test) was a within-subject factor, and both Drug condition (Amphet vs Saline) and Stimulus condition (Robot vs No Robot) were between-subject factors. Prior to analysis, the Front time data were transformed using common log of (x + 1) in order to correct for heterogeneity of variance. This trasnformation is recommended for time scores in which there are zero scores [13]. From inspection of Fig. 1, it is clear that the robot did not elicit flight or avoidance from saline-treated rats (open bars). In the Robot test, Saline rats spent 50% of the trial time at the front of the apparatus nearest the robot (M=30 sec) and spent only 30% of the trial time at the back of the apparatus farthest from the robot (M=18 sec). These scores did not differ significantly from those of saline-treated rats tested in the No Robot condition. Irrespective of stimulus condition, amphetamine-treated rats (hatched bars) responded to the treadmill apparatus with decreased Front time and increased Back time; this response was very stable across Habituation and No Robot test periods, and resulted in significant main effects for Drug in the analyses of both Front time and Back time, $F(1,44)=21.0$, $p<0.01$, and $F(1,44)=37.9$, $p<0.01$, respectively. Introduction of the Robot, however, caused a dramatic potentiation of the Amphet rats' tendency to move to the back of the apparatus. In the Robot test, Amphet rats spent less than 15% of the trial time at the front of the apparatus near the robot (M=8 sec). In the ANOVA of Front time there was a significant 3-way interaction showing that the suppression of Front time for Amphet rats was significantly greater in the presence of the robot, $F(1,45)=4.5$, $p<0.05$. The interaction effect was confirmed by Newman Keuls

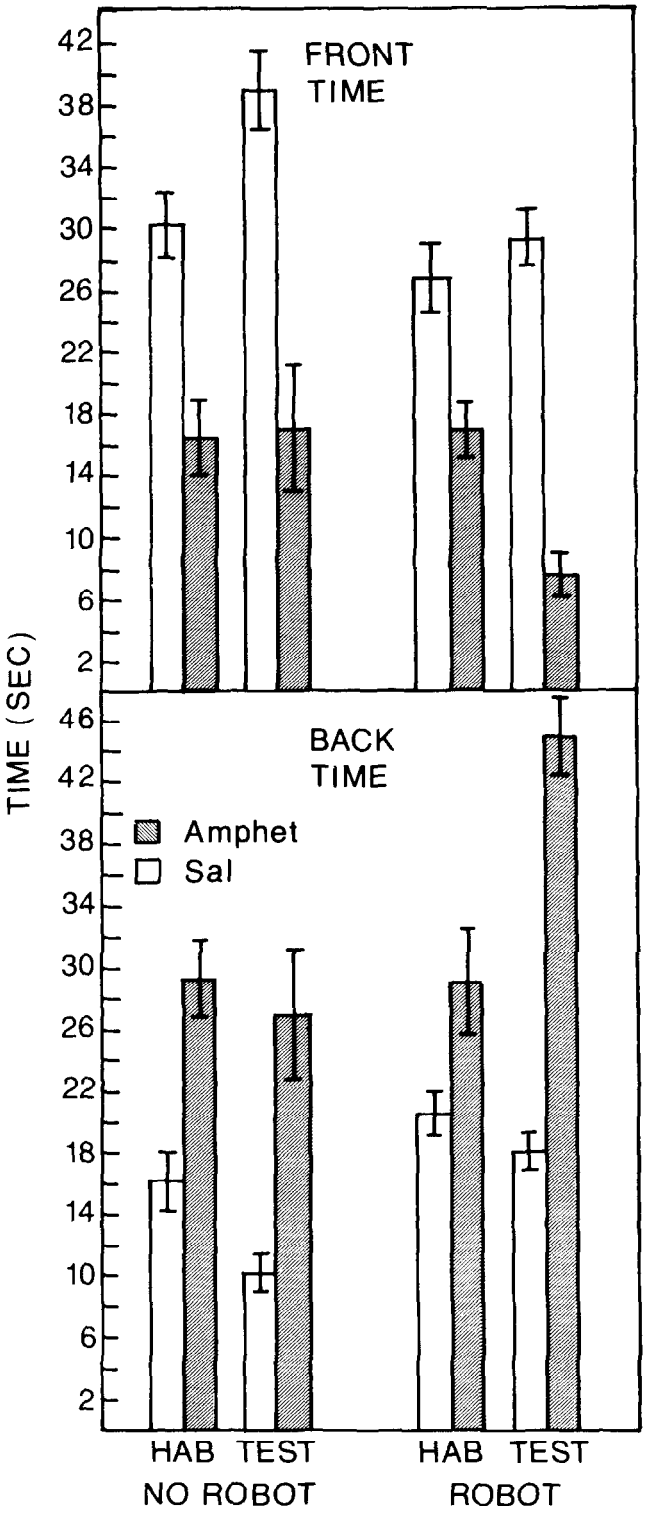


FIG. 1. Mean Front and Back time in seconds (±SEM) during habituation and test periods for rats treated with chronic amphetamine or saline. For rats treated with amphetamine, introduction of the robot caused a defensive flight reaction, reflected in a marked suppression of Front time and a marked increase in Back time.

comparisons of individual means at the 0.05 level of significance. Specifically, the Front time of Amphet rats tested with the robot was significantly less than that of both Saline rats tested with the robot and Amphet controls tested with No Robot; it was also significantly suppressed relative to the Amphet-Robot animals' own habituation data.

From Back time data it is clear that Amphet rats not only avoided the area nearest the robot; they continued to move to the extreme rear of the apparatus. The amphet rats tested with the robot spent approximately 75% of the trial time at the back (M=45 sec). The ANOVA of Back time also resulted in a significant 3-way interaction showing that the increased Back time of the Amphet rats was significantly greater in the Robot condition. Comparisons of individual means (Newman Keuls, 0.05 level) were consistent with the interaction. That is, the Back time of Amphet rats tested with the robot was significantly greater than the Back time of both Saline rats tested with the robot and Amphet controls tested in the No Robot condition. It was also significantly elevated relative to the animals' own Back time scores in the Habituation period. During robot trials, Amphet rats were frequently observed to make abortive escape attempts, jumping at the rear wall of the apparatus.

## DISCUSSION

Chronic amphetamine treatment caused rats to show a dramatic flight reaction in response to the stimulus robot. These animals accumulated a high proportion of the trial time at the extreme rear of the apparatus, despite the fact that this requires continuous flight behavior. An important feature of these data is the fact that the robot did not elicit a significant response from saline control animals. In fact, saline-treated rats tested with the robot spent approximately 50% of the trial time in the front third of the apparatus, nearest the robot. Thus, chronic amphetamine treatment caused rats to show a defensive flight reaction to a stimulus that was nonthreatening as defined by the behavior of control animals.

The present results show that the defense-response paradigm is suitable for study of chronic amphetamine effects. They also answer the major question raised by our previous work with a stationary floor: whether amphetamine-induced high back time was simply an indirect consequence of amphetamine-induced immobility. With the treadmill paradigm the rat could only accumulate high back time by moving repeatedly to the back of the apparatus. Moreover, the behavior of rats on the treadmill is to be distinguished from that of the "backward walking" elicited by extremely high doses of amphetamine (25 mg/kg) [4]. In both Robot and No Robot conditions animals almost invariably move in a forward direction. In robot tests, Amphet rats orient briefly toward the stimulus and then turn and move rapidly to the back of the apparatus. Thus, the behavior elicited in this situation more nearly approximates the active response of flight as seen in a field situation.

Amphetamine is known to have profound effects on motor behavior [9,12]. From the present results, however, it seems unlikely that the defensive flight reaction can be attributed to purely motor effects. Rather, defensive flight seems to be superimposed on the motor consequences of the drug. Most relevant to this issue is the No Robot control, specifically the fact that Amphet rats tested with the robot had significantly lower Front time and higher Back time than Amphet rats tested with No Robot. Amphet rats in both

conditions were observed to engage in stereotypy, such as repetitive movements, but if anything, stereotypy would be expected to interfere with the execution of the flight response. It is one of the major strengths of the present paradigm that rats can execute the simple response of flight despite the motor effects of amphetamine.

Another feature of these results was the fact that amphetamine-treated rats, regardless of stimulus condition, responded to the treadmill with a tendency to move toward the back. Observations of the animals suggested that contact with the wire mesh at the front of the apparatus elicited a startle response and acted as an aversive stimulus. This interpretation is consistent with some of our more recent work with a slower treadmill speed (11 mm/sec). In this condition, Amphet rats continue to show a slight suppression of Front time, but do not differ from Saline controls in Back time unless the stimulus robot is present (Mollenauer, unpublished research). It has been shown previously that amphetamine augments tactile startle response [7,8]. The present results suggest further that amphetamine may actually cause some forms of tactile stimulation to become sufficiently aversive to elicit an active avoidance response.

Behavioral effects of continued amphetamine treatment are of special interest as possible models of amphetamine psychosis. The animal model most widely used in the development of antipsychotic drugs has been that based on motor restriction or stereotypy [12]. The capacity of drugs to block stimulant-induced stereotypy has correlated well with antipsychotic potency [3]. However, drugs developed with the stereotypy model have invariably involved extrapyramidal side effects. It has been suggested that the use of the stereotypy model itself may have had the unfortunate consequence of early elimination of drugs that would lack extrapyramidal side effects [2]. Thus, it is clear that there is a need for animals models that focus on behaviors other than stereotypy [2,10]. Two laboratories have recently studied the effects of chronic amphetamine on social behaviors in rats [5,6]. Considering the differences in procedures and drug regimen, there was surprising concordance in the results from these laboratories. Although there were several points of difference relating especially to the social rank of the animal, the results from both investigations suggest that startleability and fight-flight are consistent features of continued amphetamine treatment. The present investigation has found comparable results, increased startle and defensive flight, using a single-animal approach. Whether the defensive-flight paradigm proves to be an effective model of psychosis will depend on whether it can successfully select antipsychotic drugs. We are presntly engaged in testing the relative potency of typical and atypical neuroleptics in attenuating amphetamine-induced defensive flight.

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