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Amphetamine: Differential Effects on Defensive Flight and Motor Behavior in the Rat[1]

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MOLLENAUER, S., C. JACKSON AND T. POLLACK. *Amphetamine: Differential effects on defensive flight and motor behavior in the rat.* PHARMACOL BIOCHEM BEHAV **19**(1) 33–37, 1983.—As in previous research, hooded rats treated with an acute high dose of d-amphetamine sulfate (5 mg/kg free base) showed a dramatic defensive flight reaction to a novel stimulus (mechanical robot) that did not elicit flight from saline controls. Both the defense response and stereotypy behavior (repetitive movements and oral, licking chewing) were assessed at eight time periods after injection: 1, 15, 30, 45, 75, 105, 135, and 165 min. The defense response peaked early (15–30 min) after injection and showed a significant decline by 75 min, with no reemergence as stereotypy subsided. Stereotypy peaked later (45 min) and did not decline until 105 min. Tests in the absence of the robot provided a control for motor effects of the drug. Whereas stereotypy occurred in both Robot and No Robot conditions, the defense response occurred only in the Robot condition. These results were thought to provide further evidence that the effects of amphetamine on defensive flight could not be attributed to purely motor reactions. Thus, amphetamine-induced defensive flight may be an appropriate pharmacological model of affective psychosis. As such, it may be helpful in establishing differential pharmacological profiles for affective versus motor potencies of potential antipsychotic compounds.

Amphetamine Defensive flight Rat Affective behavior Psychosis Animal model

THE amphetamine psychosis that results from amphetamine abuse is thought to be an excellent model of paranoid schizophrenia [15], and, to some extent, a pharmacological parallel of undifferentiated schizophrenia [17]. Animal models of amphetamine psychosis have therefore been the subject of considerable interest and have played a large role in shaping current thought on the etiology of schizophrenia [10]. The most widely employed animal model of amphetamine psychosis has been the stereotypy model; with high doses or chronic treatment of amphetamine, animals exhibit restricted repetitive movements commonly called stereotypies [15]. The antipsychotic potency of neuroleptic drugs can generally be predicted from their capacity to antagonize these stimulant-induced stereotypies [4]. However, drugs selected with this model have invariably caused extrapyramidal side effects [1]. The propensity of the stereotypy model to select drugs having extrapyramidal side effects is not surprising in view of the fact that the nigrostriatal system plays an important role in the mediation of stereotypy behavior [5]. If antipsychotic and extrapyramidal actions can be dissociated pharmacologically, an animal model less closely tied to extrapyramidal function might play a valuable role in selecting clinically effective drugs.

Several laboratories have developed models of amphetamine psychosis that involve observations of animals' reactions to conspecifics (fight, flight) as they interact in naturalistic settings [8,11]. These approaches would seem to be highly promising in terms of ethological validity and their focus on affective rather than motor behaviors. At the same time, they are complicated by the difficulties of analyzing the behavior of interacting animals. In our laboratory we have recently developed a model that is derived from an ethological approach, but retains some of the advantages of traditional laboratory procedures. When observed in field or laboratory situations rats can be seen to exhibit species-typical defensive reactions such as freezing and flight [3]. These behaviors are natural, unlearned reactions that are normally elicited by any threatening stimulus. In our work with this paradigm, we have found that amphetamine treatment causes rats to exhibit the defense response of flight in response to a novel stimulus (mechanical robot, live white rabbit) that is normally nonthreatening, i.e., does not elicit the defense response from saline control animals [14]. In a more recent modification of this procedure the rat is placed on a slow-moving treadmill that carries it toward the stimulus. In order to retreat or flee from the stimulus the rat must move away from it continuously throughout the trial period. When saline control rats are tested in this apparatus, they permit themselves to be carried toward the stimulus (mechanical robot) and exhibit what appear to be investigatory behaviors; they orient toward the stimulus, poke their noses into its chamber and sniff. In contrast, rats treated

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with amphetamine show a dramatic defense response that would be comparable to flight in a field situation. This behavior does not resemble the "backward walking" that is elicited by extremely high doses of amphetamine (25mg/kg) [7]. Animals almost invariably move in a forward direction. Throughout the trial they typically orient toward the stimulus briefly and then turn and move rapidly to the back of the apparatus, spending most of the trial time at the extreme rear of the apparatus [13]. Trials conducted in the absence of the stimulus provide a control for the possibility that the defense response is attributable to motor effects. In the present research we have systematically explored the time course of the amphetamine-induced defense response and motor stereotypy (repetitive movements, licking, chewing). This work bears on the larger questions of whether the defense-response paradigm is capable of dissociating affective and motor behaviors and whether it has potential as a model of amphetamine psychosis.

METHOD

Animals

The animals were 160 male, Long Evans hooded rats purchased from Simonsen Laboratories and 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 on the day of the experiment. Testing was conducted toward the end of the light phase of their 12-hour light-dark cycle.

Apparatus

The apparatus was similar to that used in previous research [13]. It was a rectangular wooden chamber painted dark gray. The floor of the chamber consisted of a treadmill that moved (2.0 mm/sec) toward the front of the test chamber, where the stimulus robot was positioned behind a perforated Plexiglas 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 treadmill chamber was 38×43 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 Plexiglas barrier in which numerous 1.25 cm holes had been drilled. The robot was positioned less than 2.54 cm from this Plexiglas barrier and was illuminated from above by a 75-watt white light.

Stimulus Robot

The stimulus robot was the same as used in previous research and is described in detail in that work [13]. Briefly, the robot was constructed from sheet metal and was electrically operated. It was suspended from a tripod, such that it hung approximately 1 cm above the apparatus floor. When activated the robot alternated between walk periods in which the body of the robot remained stationary and the legs moved, and spin periods in which the entire robot, body and legs, turned at a rate of three revolutions per sec.

Design and Drug Treatment

In Experiment 1 the time course of the defense response and stereotypy were explored systematically following an acute high dose of d-amphetamine (7.0 mg/kg, approximately 5.0 mg/kg free base, IP, in a volume of 1 ml/kg). The drug was supplied by Smith, Kline and French. In this experiment all rats were injected with amphetamine and tested with the robot at one of eight time periods after injection (1, 15, 30, 45, 75, 105, 135, or 165 min). In Experiment 1 and in Experiment 2, rats were randomly assigned to treatments, 10 per group, with restrictions on time of test noted below.

In Experiment 2 selected time points were studied with appropriate Saline and No Robot controls. The design of this experiment was a 2×2×2 factorial. Rats were injected with 7.0 mg/kg d-amphetamine sulfate (5 mg/kg free base) or with physiological saline, IP in a volume of 1 ml/kg, and were tested at one of two time points, 30 or 105 min after injection. Half the rats in each group were tested with the Robot and half with No Robot. Both experiments were run in replications with time of day counterbalanced across injection time and test time. All injections were administered between 1 and 3 p.m.

Procedure

Stereotypy observations. At the designated time after injection an animal was observed in its home cage for one minute by a trained observer blind to drug treatment and time of injection. The animal was also observed for stereotypy during the defense response test. Stereotypy was rated on a scale based on that developed by Segal [16]. Behavior was rated on duration (% time) and intensity (1–3) of repetitive movements and oral stereotypy, with the final score=(duration × intensity). For repetitive movements, a score of one was characterized by rhythmic, predictable, but relatively smooth movements and a score of three was characterized by highly restricted, rhythmic, jerky movements. For oral stereotypy a score of one was characterized by slight chewing movements and a score of 3, by vigorous chewing, licking or biting.

Defense response test. Immediately after the home-cage observations the animal was placed individually in the treadmill apparatus for the defense-response test. With the treadmill activated the rat was placed on the treadmill at the front of the apparatus and given a one-minute habituation period 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 Plexiglas barrier. For Robot trials the robot was activated when the door was raised; for No Robot trials the robot was not activated, but the lighted chamber was exposed. Since the rat was placed at the front of the apparatus, retreat from the robot required an active move to the back of the apparatus. Back time was recorded when the rat occupied the back third of the apparatus. In order to accumulate high Back time, indicating the defense response of flight, the rat had to move continuously to the back of the apparatus.

RESULTS

Experiment 1

The results from Experiment 1 are summarized in Fig. 1. It is clear from the figure that the time course for the defense response of flight (Back time) was quite different from that of

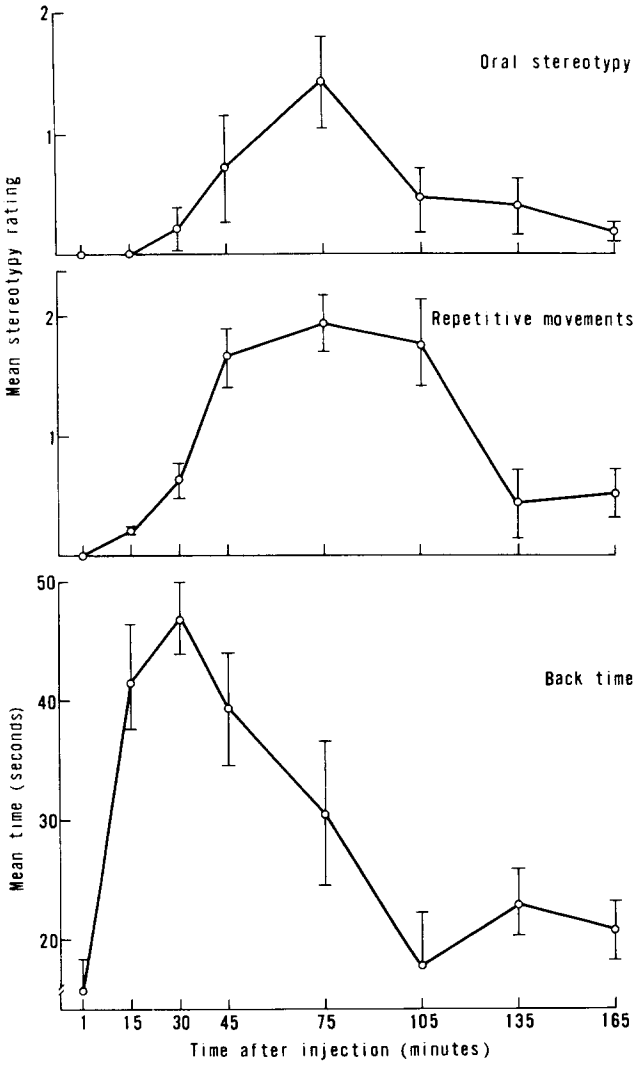

FIG. 1. Mean (±SEM) for stereotypy observations and defense response (Back time) for rats tested at different times after injection of 5 mg/kg d-amphetamine sulfate (n=10 per group). To accumulate high Back time, indicating defensive flight, the rat must move continuously to the back of the apparatus. The figure shows that the time course of the defense response differs from that of stereotypy.

stereotypy (repetitive movements and oral stereotypy). The data were analyzed by analyses of variance. For justification of parametric analysis of observational data, see [9]. For all three measures the ANOVAs indicated the expected significant effect of time, $F(7,72)=8.04$, $p<0.001$, $F(7,72)=10.78$, $p<0.001$, and $F(7,66)=4.44$, $p<0.001$ for Back time, repetitive movements and oral stereotypy, respectively. Follow-up comparisons were made using the Newman Keuls procedure ($p<0.01$). The defense response of flight, as indicated by Back time (lowest panel of the figure), peaked relatively early after injection. The Back time scores for rats tested at 15, 30 and 45 min all differed significantly from 1-min times, but not from each other. In contrast, the stereotypy response of repetitive movements (middle panel) peaked later after injection; there was a significant increase in repetitive movements from 30 to 45 min. The oral stereotypy response (upper panel) peaked still later, with a significant increase from 45 to 75 min. The duration of peak response also differed for the three behaviors. The defense response, Back time, showed a significant decrease at 75 min and had returned to baseline by 105 min. The back times for 105, 135 and 165 min did not differ from 1 min. In contrast, the peak response for repetitive movement stereotypy (middle panel) continued through 105 min, and did not decline until 135 min. The oral stereotypy response showed a very short period of peak response, with a significant decrease occurring between 75 and 105 min. In sum, the systematic exploration of time course in Experiment 1 suggested a very different pattern of results for the defense response and stereotypy behavior.

Experiment 2

The defense response data (Back time) are summarized in the lower panel of Fig. 2. Considering first the Saline data (open bars), it is clear that the robot was not a particularly aversive stimulus for undrugged animals. The saline-treated rats showed no significant flight, as would be indicated by elevated Back time. In contrast, the amphetamine-treated rats tested at 30 min showed a dramatic elevation in Back time. The higher Back time of 30-min Amphet rats tested with the Robot was significantly greater than that of 30-min Saline rats tested with the Robot, $t(72)=5.25$, $p<0.01$. It was also significant as compared to 30-min Amphet rats tested with No Robot, $t(72)=4.9$, $p<0.01$. By 105 min the defense response was no longer significant. An important feature of these data is the fact that there were no significant differences between the Back time scores of Amphet and Saline rats tested in the No Robot condition.

The data for Repetitive movement stereotypy are summarized in the middle panel of Fig. 2, and the data for Oral stereotypy, in the upper panel. The Repetitive movement data are from the trial observations. The Oral stereotypy data are from the home cage; these observations could not be made during the trial due to lack of visibility. It is clear from the figure that the pattern of results for both forms of stereotypy was quite different from that for Back time. Saline-treated rats showed no stereotypy in any condition. As expected from Experiment 1, Amphet rats showed significantly higher levels of stereotypy at 105 min than at 30 min, regardless of the stimulus condition. For both sets of data this resulted in a significant interaction between drug and time of test, $F(1,72)=30.5$, $p<0.001$, and $F(1,72)=14.3$, $p<0.01$, for Repetitive movements and Oral stereotypy, respectively.

DISCUSSION

We have now amassed considerable data to show that d-amphetamine causes an emergence of defensive flight to an apparently benign stimulus ([13,14], and Experiments 1 and 2). Tests in the No Robot condition (Experiment 2) rule out the possibility that the emergence of defensive flight is an indirect consequence of amphetamine-induced motor behavior or procedural artifact. The present results show further that the time course of defensive flight differs from that of motor stereotypy (Experiment 1). An important feature of these results is the fact that the defense response did not show a significant reemergence as the amphetamine-induced stereotypy subsided at 135 and 165 min after injection. At still later time periods sampled in pilot research there was no suggestion of defensive flight (Mollenauer, unpublished research). The failure of defensive flight to reemerge is in contrast to the well-established relationship between

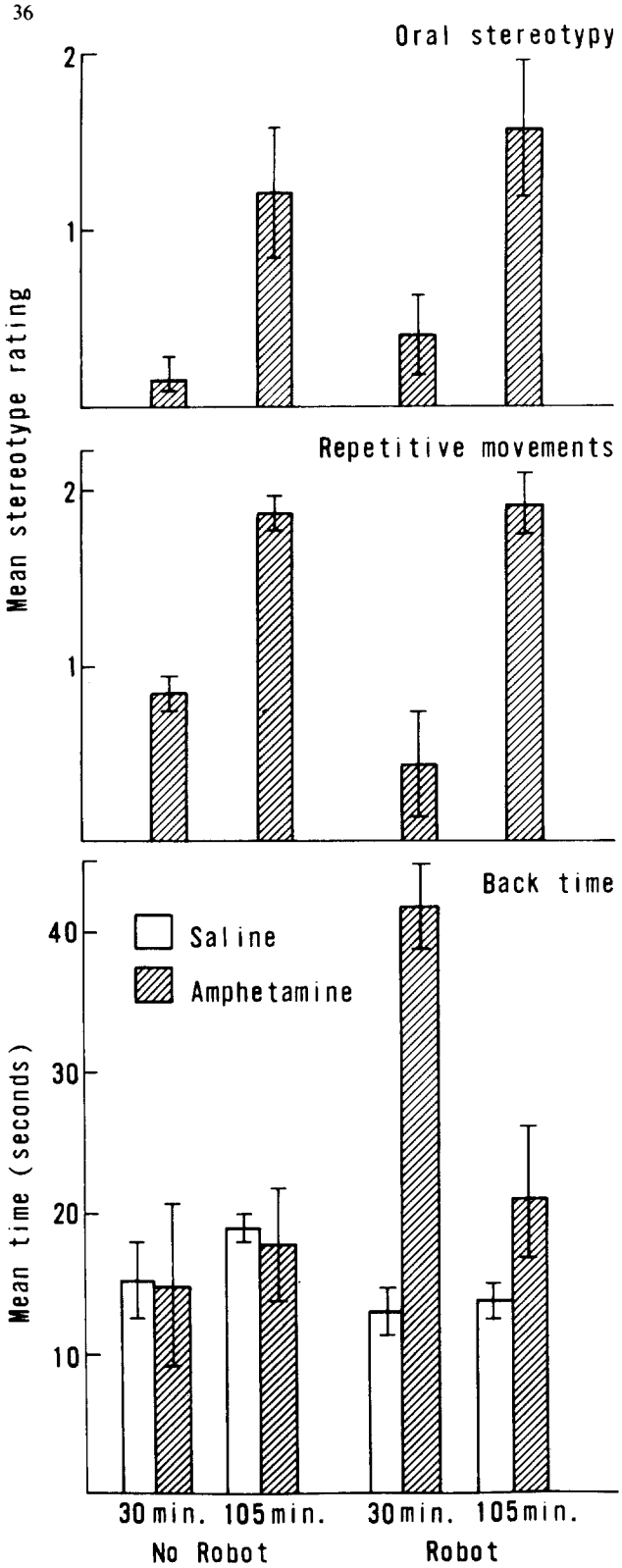

FIG. 2. Mean (±SEM) for stereotypy observations and defense response (Back time) for rats tested at 30 min or 105 min after injection of 5 mg/kg d-amphetamine sulfate or physiological saline (n=10 per group). Saline-treated rats did not show defensive flight in response to the Robot. When tested at 30 min after injection amphetamine-treated rats showed defensive flight in the Robot condition, but not the No Robot condition. Stereotypy was highest at 105 min and did not differ in Robot and No Robot conditions.

amphetamine-induced stereotypy and locomotor behavior. It has been shown consistently that moderatly high doses of amphetamine cause a biphasic effect on locomotor activity. Initial hyperactivity is followed by a period of suppressed locomotor activity while animals are engaged in stereotypy; hyperactivity later reemerges as the stereotypy subsides [15]. In the present research, the reemergence of locomotor activity, characterized by repeated rearings and agitated movements around the cage, was very apparent during home-cage observations at 135 and 165 min. The fact that defensive flight does not show this same pattern of reemergence is further evidence that the amphetamine-induced defense response is dissociable from the motor effects of the drug.

The data from Experiment 2 are particularly germane to the question of whether the defense response can be dissociated from motor behaviors. While stereotypy occurred in both stimulus (Robot) and no stimulus conditions, defensive flight occurred only in the stimulus condition. This feature of the results raises the possibility that the defense-response model may be capable of differentiating between antipsychotic and motor potencies of neuroleptic drugs. In this event, the motor actions of a drug would be manifest in both stimulus and no stimulus conditions whereas antipsychotic actions would be manifest in the stimulus condition only. The behavioral profile of the drug (antipsychotic vs. motor) would then be defined by the relative potency in these two conditions. We are presently engaged in testing the relative potencies of typical and atypical neuroleptics to alter the amphetamine-induced defense response and behavior in the no-stimulus control condition.

If the defense-response model does prove effective in differentiating between antipsychotic and motor actions it could be invaluable in the exploration of new pharmacological approaches to schizophrenia. Additionally, the model may be useful in elucidating neuropharmacological mechanisms of affective behavior. Since the neuroleptics are known to vary widely in their effects on receptor mechanisms, such as DA-sensitive adenylate cyclase, Ne-receptor coupled cyclic AMP and (^{3}H) haloperidol binding [6,18], their relative potencies in antagonizing the defense response may provide clues to the mechanisms mediating this affective behavior.

Finally, as a model for amphetamine psychosis, the defense response has several important advantages. The paradigm not only focuses on affective reactions as opposed to motor behavior, but allows for evaluation of purely motor effects in the No Robot condition. Previous research suggests that defensive reactions are mediated by limbic system structures [2, 12, 19]; thus, the defense response might prove an alternative to the current focus on the nigrostriatal system. Since the paradigm is based on a natural unlearned defense response, results are not complicated by questions of drug effects on learning/memory, pain sensitivity or appetitive motivation; at the same time, the advantages of traditional laboratory procedure, such as single animal observations, are still preserved. Finally, and perhaps most important for the study of chronic amphetamine effects, the time course of the defense response is such that defensive reactions can be studied prior to the onset of severe motor stereotypy.

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