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The Influence of Environmental Variables on Amphetamine-Induced Activity in the Preweanling Rat[1]

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RASKIN, L. A. *The influence of environmental variables on amphetamine-induced activity in the preweanling rat.* PHARMACOL BIOCHEM BEHAV **19**(2) 187–191, 1983.—The present experiments investigated how environmental stimuli influence a behavioral response to amphetamine treatment in preweanling rats. In the first experiment, 15-day-old rats received either 1 mg/kg d-amphetamine or saline and were observed in the presence of their home shavings or in a novel cage. Results showed that although familiar cues from the home bedding decreased activity of the saline treated pups, these cues did not influence the activity-enhancing effect of amphetamine. In Experiment 2, 15-day-old pups were placed in a cage with an anesthetized adult conspecific and non-directed activity, around the cage, as well as directed activity on top of the adult were recorded. Following amphetamine treatment, animals tested with a conspecific showed no increase in non-directed activity, a finding which replicates previous observations. In the present experiment, however, a significant increase was seen in the activity directed towards or on top of the anesthetized adult. These experiments reveal that familiar cues per se are not sufficient to influence amphetamine-induced activity in young rats. Activity is influenced, specifically if familiar cues are associated with a discrete object. In the presence of a familiar stimulus, non-directed activity is decreased and directed activity is enhanced. These data are discussed in terms of clinical findings which show that amphetamine acts to focus, or direct activity of the hyperactive child, and further underlines the importance of environmental variables in influencing the amphetamine response.

Amphetamine Preweanling rat Familiar stimuli Hyperactivity

SEVERAL years ago, Campbell and Randall [5] reported that amphetamine-induced locomotor activity in the 15-day-old rat is greatly influenced by environmental stimuli. A 15-day-old rat tested alone in a novel cage showed a dramatic increase in motor activity following amphetamine treatment. If, however, an anesthetized adult conspecific was placed in the cage with the amphetamine treated rat pup, drug-induced activity was virtually eliminated. Strikingly, this particular environmental manipulation has no effect on amphetamine-induced activity in older rats. Thus, this appeared to be a paradoxical drug effect which occurred during early development and was strongly determined by external factors. Such a finding was of particular interest because of its parallel to the paradoxical calming effect observed when hyperactive children are given amphetamine under specific environmental conditions [1,2]. In spite of the link with clinical investigations a question still remains from that research: Why does the presence of an anesthetized adult alter the behavioral response a preweanling rat exhibits following amphetamine treatment? The present experiments were designed to address that question.

One possible explanation for the phenomenon reported by Campbell and Randall [5] relates to the sensitivity of the young rat to familiar olfactory and tactile cues. In the non-drugged state the hyperactivity normally observed at 15-days [4,9] is completely suppressed when the pup is tested in the presence of any one of several familiar cues. Placing either an anesthetized conspecific [10], a tube heated to a rat's body temperature [7], or even shavings from the home nest [6] in a test cage with a 15-day-old, dramatically decreases the young animal's activity. Given that familiar cues suppress activity in the normal young rat, this environmental variable may also be capable of suppressing the hyperactivity which is usually induced by amphetamine.

Another explanation for this phenomenon must also be considered. The presence of an anesthetized conspecific may appear to inhibit drug-related activity in the young rat, not simply because it is a familiar cue but, more specifically, because it is a discrete familiar stimulus towards which the animal can direct its activity. It has been suggested that following amphetamine administration in rats, as well as humans, there is a "qualitative shift" in activity from being non-directed to being oriented towards a specific goal [17]. In the young rat, for example, amphetamine increases the tendency for the animal to seek out and orient towards its home nest area [20].

This notion has also gained support from the clinical literature which shows that treatment of hyperactive children with amphetamine results in a decrease in irrelevant motor behavior and an increase in performance on cognitive tasks

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[11, 12, 13]. Thus, perhaps in the presence of a salient object, such as an anesthetized adult, a young rat treated with amphetamine would direct its activity towards that object. If this is the case, one would see the young animal exhibit some kind of motor activity on or around the anesthetized conspecific. Perhaps such a familiar object would enhance directed stereotyped responses in the pup, given that an increase in stereotypy has been observed when adult guinea pigs are tested in a familiar cage [16]. It is also possible that the drug-treated pup would exhibit locomotor activity, and that this activity would be exclusively directed towards the conspecific.

Thus, the purpose of the present experiments was twofold: First, we sought to determine whether familiar environmental cues are sufficient to suppress amphetamine-induced activity, or if a discrete familiar object is necessary for such an effect. Second, the behavior of amphetamine-injected 15-day-old rats was closely observed in the presence of a discrete familiar object in order to assess any "qualitative shifts" in activity which may have occurred.

EXPERIMENT 1

In Experiment 1, pups were tested either in a cage containing their home nest shaving or in a novel cage following saline or amphetamine administration. The first experiment determined if familiar cues per se influence amphetamine-induced activity.

Subjects

Subjects were thirty-six preweanling male and female Sprague-Dawley rats bred and raised in the Amherst College colony. At 3 days of age all litters were reduced to eight animals and left untouched until they were tested at 15 days of age. Bedding in the maternity cage was changed once weekly. A split litter design was employed so that 2 animals from each of 4 litters were placed in each experimental condition. All litters were housed on a reverse light cycle (14 hr light/10 hr dark) and maintained on ad lib food and water.

Apparatus

Locomotor activity was measured in individual polypropylene maternity cages (47.4×26×15 cm) with mesh tops. The maternity cages contained either familiar shavings from the pups' home bedding which had been unchanged for seven days, or clean pine shavings. The cages were housed in a test room which was lit by a 50 watt red light bulb.

Drugs and Dosage

Experimental animals received either 1 mg/kg d-amphetamine sulfate dissolved in 0.9% saline or the saline vehicle. This dose was chosen because the dose response curve data of Campbell and Randall [5] have shown this to be an optimal dose for producing hyperactivity in a 15-day-old tested in isolation. In addition, this is a dosage which reportedly produces locomotion but not stereotypy in 15-day-old pups [8]. All injections were given IP with a microsyringe.

Procedure

At the beginning of the dark portion of the diurnal cycle, pups were removed from their litter and injected with either the drug or vehicle solution. Pups were then randomly assigned to one of the previously mentioned test cages and given a 30-min habituation period. Following habituation, activity levels were observed for a 1-hr session by an experimenter who was unaware of the animals' drug treatment. Activity was measured as previously reported [6,14] whenever the animal moved up and down along the axis of the cage. In this case a line was drawn across the shorter axis of the cage top, dividing the cage in two equal parts. An activity count was scored each time the animal moved across the center line. An activity count was recorded every 20 minutes during the hour.

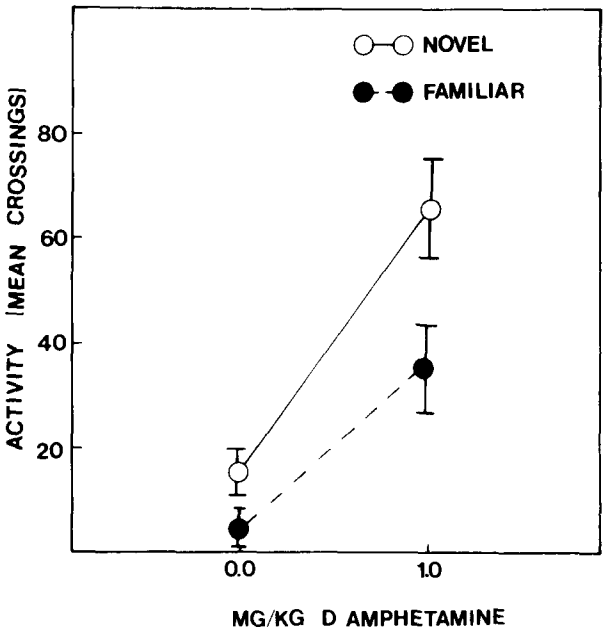

FIG. 1. Effects of saline and amphetamine on activity of 15-day-old rats tested in a novel (clean bedding) or familiar (home bedding) cage.

Data Analysis

The mean number of activity counts for all subjects in a group were calculated during 20 minute periods. All data were analyzed using Analysis of Variance for factorial designs.

Results and Discussion

Preliminary analyses revealed no sex differences in any treatment groups and consequently, values for males and females were combined. Figure 1 shows activity plotted as a function of drug doses for animals placed in either novel (clean bedding) or familiar (home bedding) cages. As is clear from the graph, the saline treated animals (0.0 d-amphetamine) are slightly more active when placed in a novel cage than when tested in a cage which is familiar. More interesting, it can be seen that regardless of the test environment, amphetamine-treated pups become significantly more active than their saline counterparts. These results were confirmed by an Analysis of Variance which revealed an effect of drug, $F(1,32)=20.09$, $p<0.05$, and effect of environment, $F(1,32)=4.5$, $p<0.05$, but no drug × environment interaction, $F(1,32)=2.60$, $p>0.05$. Thus, although the

baseline activity levels of young rats is decreased in familiar surroundings, amphetamine produces proportionately equal levels of heightened activity in these animals. Amphetamine-treated animals in a novel environment are in fact more active than those in a familiar environment, but this finding has more to do with baseline activity levels than with an actual change in the activity-enhancing effect of the drug.

The present findings replicate the phenomenon previously reported that familiar sensory cues produce quiescence in young rats [6, 18, 19]. Although sex differences in the activity of preweanlings in novel and familiar bedding have been reported previously [3], they were not observed in the present experiment. This discrepancy may be accounted for by the difference between the actual test paradigm of this and a previous report [3].

The present finding suggests that familiarity per se does not account for the apparent decrease in activity observed when amphetamine-treated pups are tested with an anesthetized conspecific. The presence of familiar cues may not influence the action of amphetamine unless these familiar cues are associated with a specific object. Given this finding it remained to be determined how the presence of a familiar object, such as an anesthetized adult conspecific actually influences amphetamine-induced activity. Campbell and his associates [5,7] have suggested that the presence of a familiar object completely suppresses amphetamine-induced activity. It is possible, however, that the salience of a familiar object may elicit drug-induced activity and thereby cause the animal to appear quiescent. This finding would be in keeping with recent investigations which challenge the notion that the action of amphetamine on hyperactive children is at all paradoxical. For example, Rapoport *et al.* [14] report that amphetamine actually decreases motor activity of hyperactive children, normal children and normal adults under specified conditions. In particular, if the individual is required to perform a task involving vigilance or sustained attention, amphetamine will potentiate directed task performance and attenuate irrelevant motor activity [1, 11, 12, 13].

The following experiment was designed to clarify the change in activity which occurs when young rats, treated with amphetamine, are tested in the presence of a conspecific.

EXPERIMENT 2

In this experiment, 15-day-old rats were given either amphetamine or saline and tested in the presence of an anesthetized male adult as was previously reported [5], however, in the present experiment, activity was measured somewhat differently. Non-directed activity around the cage was measured, and, in addition, the activity exhibited around and on top of the anesthetized conspecific was recorded. Stereotyped responses such as head weaving, sniffing and rearing were also measured. In this way one can determine if the presence of a familiar object actually leads to a reduction in amphetamine-induced activity, or if amphetamine-induced activity does not diminish but rather becomes directed towards that object.

Subject and Apparatus

Subjects were 16 preweanling male and female Sprague-Dawley rats bred, raised and housed in the same manner as the subjects in Experiment 1. A split litter design was again employed so that no more than 2 animals from each litter were used in each experimental condition. As in Experiment 1, the locomotor activity of each subject was observed at 15 days of age in polypropylene maternity cages (47.5×26×15 cm) with mesh tops. In Experiment 2 the test cages contained clean pine shavings, and an anesthetized adult male rat that was placed along one of the 26 cm walls of the cage.

FIG. 2. Non-directed and directed activity of 15-day-old rats tested in the presence of an anesthetized adult conspecific.

Procedure

Subjects were randomly assigned to either the drug or saline group and placed in the test environment. Again a 30 min habituation period was given and was followed by a 1-hour session during which activity scores were measured. In this experiment, two activity measures were taken. As in Experiment 1, an activity count was scored every time the animal moved across the longer axis of the cage. An activity count was also scored each time the animal moved along the shorter wall of the cage with the anesthetized adult male rat. The activity was either directly in front of the conspecific or actually on the animal's ventrum. The activity which was displayed along the longitudinal axis of the cage is referred to as "non-directed" activity whereas activity on or in front of the anesthetized conspecific is referred to as "directed activity." In addition, stereotyped responses such as head weaving, rearing and sniffing were recorded.

Results and Discussion

Figure 2 depicts the levels of activity exhibited when a 15-day-old rat is placed with an anesthetized conspecific. The left panel shows non-directed activity, that is, activity which results in the animal's movement along the longitudinal axis of the cage, plotted as a function of drug dose. It is clear from these results that regardless of the drug treatment, 15-day-old rats show very little non-directed activity when tested in the presence of an anesthetized conspecific. Student's t revealed no significant differences of drug treatment in non-directed activity, $t(14)=1.56$, $p>0.05$. The usual pattern of behavior for these young animals is to approach the conspecific and huddle beside it or on its ventrum. A striking

difference in activity between amphetamine and saline treated animals is seen, however, in the directed activity measure. The right panel of Fig. 2 depicts this difference and shows activity which is recorded every time the animal moves across or in front of the conspecific. Thus, pups with either amphetamine or saline treatment approach and remain in contact with the anesthetized conspecific, however, their behavior while in contact with the conspecific is dramatically different. Saline treated pups huddle with the adult and eventually fall asleep, while amphetamine treated animals run from side to side in front of or on top of a familiar object. Student's t test revealed a statistically significant drug effect for directed activity, $t(14)=2.28$, $p<0.05$. Although behaviors which are typically considered to reflect stereotypy were recorded, there were no consistent differences between saline and amphetamine treated pups at this drug dosage.

GENERAL DISCUSSION

Experiment 1 reveals that familiar tactile and olfactory cues from a pup's home bedding do not significantly influence the activity enhancing effect of amphetamine. In contrast, the second experiment shows that cues from an anesthetized adult do influence the behavior of young amphetamine-treated rats. The results of Experiment 2 are particularly intriguing in that they shed light on the findings reported by Campbell and Randall [5] which show that the presence of an anesthetized adult rat completely inhibits amphetamine-induced activity in the 15-day-old. Campbell and Randall [5] measured activity in the same manner as what, in the present experiment, is referred to as non-directed activity, and in this way the current findings replicate previous observations. However, additional information obtained in the present experiment shows that the 15-day-old amphetamine-treated animal does not become quiescent in the presence of a conspecific, but rather directs its activity toward that object. Perhaps the ability to observe activity along the ventrum of the anesthetized conspecific is facilitated in the present experiment by the use of a direct observation method rather than time lapse videography as used by Campbell and Randall [5].

The finding that amphetamine potentiates activity towards an anesthetized adult is consistent with the report showing that an amphetamine-treated, but not saline-treated 15-day-old, repeatedly followed an anesthetized adult which was pulled along the perimeter of an open field [10]. Administering amphetamine to young rats in the presence of certain stimuli does not, in fact, produce a paradoxical motor response, but rather produces a change in the nature of the activity which the animal displays. If familiar stimuli are scattered around the cage (as in the present first experiment), the animal's activity appears non-directed. If, however, familiar olfactory and tactile cues are associated with a discrete object, the animal's activity becomes oriented towards that object. Perhaps instead of suggesting that the action of amphetamine is paradoxical, one might suggest that in the young rat, amphetamine potentiates motor activity which is associated with normal adaptive species specific behavior. For example, in the very young pup, amphetamine increases activity which a pup directs towards its mother's nipple [15] and in the two-week-old rat, amphetamine increases activity which is oriented towards the home nest [20]. Further, in the present experiment it was shown that amphetamine increases the activity a 15-day pup exhibits along the ventrum of an anesthetized adult conspecific. Thus, the psychomotor stimulant is acting to increase motor behavior, but in the presence of certain relevant stimuli the nature of that motor response is changed.

This interpretation is in keeping with the rapidly growing view regarding the action of amphetamine on hyperactive children. Many researchers have questioned that this drug effect is at all paradoxical [11,17] and suggest that amphetamine acts to focus or direct activity in the hyperactive child. As mentioned earlier, such children may appear less active and fidgety after their medication because they are more able to focus their attention on a specific task, and thus exhibit less non-directed activity. The point of this author is not to equate directed activity in the rat with focused attention in a child, but rather to illustrate the similarity of the action of amphetamine on behavior in the young of these two species. The present paper further emphasizes the importance of environmental stimuli in the behavioral expression of a response to amphetamine treatment. That highly relevant, familiar stimuli are capable of eliciting amphetamine-induced activity in the young rat suggests that relevant or familiar stimuli may act to focus or elicit directed-activity in the drug-treated hyperactive child.

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