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Environment-Specific Conditioning and Sensitization With (+)-Amphetamine

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MAZURSKI, E. J. AND R. J. BENINGER. *Environment-specific conditioning and sensitization with (+)-amphetamine.* PHARMACOL BIOCHEM BEHAV **27**(1) 61–65, 1987.—Learning variables have an important role in determining the behavioral effects of some pharmacological treatments. Environmental control of sensitization and conditioning of the stimulant effects of (+)-amphetamine (AMPH) were studied in two experiments. Rats were given 6 1-hr habituation sessions in automated activity chambers conducted every second day. Two days later the 12 rats in the paired group in each study received AMPH (2.0 mg/kg) followed immediately by placement in the chambers for 1 hr whereas rats in the unpaired groups received saline. All rats were injected the following day and left in their home cages afterwards. At this time the paired groups received saline and the unpaired groups received AMPH. Three days later a second pairing and subsequent home cage injection was administered, using the same procedure. Immediately prior to the test session (4 days after the last pairing session) all rats in the sensitization experiment received AMPH and those in the conditioning study received saline. During pairing sessions AMPH treated rats exhibited more vertical activity than controls. On the saline test session in the conditioning study there was still a significant group difference demonstrating environment-specific conditioning. There was no evidence of sensitization on vertical activity; however, a significant difference in horizontal activity was seen on the AMPH test session. Results suggest that these two phenomena can be dissociated behaviorally and may not follow the same time-course.

(+)-Amphetamine Environment-specific conditioning Sensitization Rats Horizontal activity Vertical activity

PSYCHOMOTOR stimulants, such as (+)-amphetamine (AMPH), demonstrate alterations in effect with repeated administration. For example, the anorexia and disruption of operant responding produced by AMPH typically exhibit tolerance [6, 8, 25]. Other effects, such as increased locomotion and production of stereotyped behaviors, more often exhibit sensitization, or an enhanced response over the course of treatment [5, 9–12, 14, 18, 19, 21, 23, 27]. However, sensitization has not been found to be universal. It appears that presentation of the drug on an intermittent basis produces sensitization, whereas continuous exposure results in tolerance [14, 15, 17]. Furthermore, more widely spaced trials appear to produce stronger effects than daily drug treatment [1, 11, 21].

Attempts to account for sensitization include physiological changes such as alterations in receptor numbers or sensitivity, pharmacokinetic changes, and learning factors [1,7]. Several lines of research support the notion that learning is involved. Animals given repeated injections of AMPH or cocaine showed stronger sensitization to the drug if it was later given in the same room where the drug was previously administered rather than in an environment unassociated with the drug [10, 18, 27]. Although some studies have indicated the presence of sensitization without an associative component [20,24], the bulk of evidence suggests a stronger effect with discrete cues. In similar studies, conditioned effects have been observed by administering saline to animals in an environment previously associated with a stimulant drug. These animals were found to be more active there than control groups, with the same drug history, but the drug and environment unassociated. The difference between the groups ruled out the possibility that the enhanced activity was solely a physiological response to drug treatment irrespective of associative factors [3, 4, 9, 16, 18, 22, 23, 26, 27]. One study demonstrated that as few as 6 pairing sessions were needed to produce environment-specific conditioned locomotion with AMPH [16]. Typically however, a larger number of pairing sessions is administered in an attempt to produce a robust conditioned effect.

The present study sought to examine environment-specific conditioning and sensitization. It was hypothesized that if conditioning principles contribute to each of these phenomena to a similar degree they should coexist and should exhibit similar time courses. By utilizing identical procedures to attempt to produce environment-specific sensitization and conditioning, these possibilities were examined.

METHOD

Subjects

Forty-eight male Wistar rats, initially weighing 250 (±25) g, were individually housed in a climatically controlled (21±1°C) environment kept on a 12 hr light (0600–1800 hr)/dark cycle. Purina Rat Chow and water were freely available in the home cage.

Apparatus

All behavioral testing was conducted in 6 automated activity monitoring chambers. Details of the apparatus have been previously described [2]. Briefly, each Plexiglas chamber (41×50×37 cm) was equipped with sets of infrared emitters and detectors at 5 and 15 cm above the wire rod floor, which provided independent indices of horizontal and vertical activity. Each chamber was enclosed in a plywood box insulated with Styrofoam painted flat black. A 2.5 W light was mounted on the ceiling and a small fan provided ventilation and background noise for each box.

Procedure

Both studies utilized similar procedures with the exception of treatment on the test session. Initially all rats were given 6 1-hr habituation sessions in the chambers, each occurring every second day. Each rat was always placed in the same chamber, and tested at the same time of day. When not in an activity monitoring sessions, all rats remained in their home cages in the colony room.

Two days after habituation had ended, all rats received a 1-hr drug-environment pairing session. Twelve rats in each study comprising the 'paired groups' received an intraperitoneal injection of 2.0 mg/kg of AMPH (Smith Kline & French) dissolved in distilled water immediately prior to the session. The other 12 rats, the 'unpaired group,' were given identical treatment but they received saline (0.9%) instead of AMPH. The next day all rats were given an injection of the compound they had not received in conjunction with the session. Following this injection they were left in their home cages for the duration of that day and the following two days. The following day (4 days after the first drug-environment pairing) all rats were given a second drug-environment pairing session and subsequent home cage injection following the same procedure as for the first.

The test session was conducted 4 days following the second drug-environment pairing. In the sensitization study all 24 rats received AMPH (2.0 mg/kg) immediately prior to the 1-hr session. In the conditioning study all rats received saline immediately prior to the test session.

Statistics

All data analyses were conducted utilizing analyses of variance (ANOVA). Horizontal and vertical activity were analysed separately. The accepted level of significance for all tests was set at $p<0.05$.

RESULTS

Sensitization

Figure 1 shows the mean (±SEM) horizontal and vertical activity levels (counts per 10 min) of the rats during habituation, each pairing session, and the test session. The 6 habituation sessions were analysed using a three-way ANOVA with time, session, and group as the factors (note that the data points in the figure are averaged over the six sessions). There were no significant effects with horizontal activity suggesting a lack of intra- or inter-session changes, or any group differences prior to drug treatment. There were however significant time, $F(5,110)=92.77$, $p<0.001$, and session, $F(5,110)=23.98$, $p<0.001$, effects during habituation with vertical activity. The session effect indicated a decline in activity across sessions whereas the time effect indicated a decrease in frequency of vertical activity within the sessions.

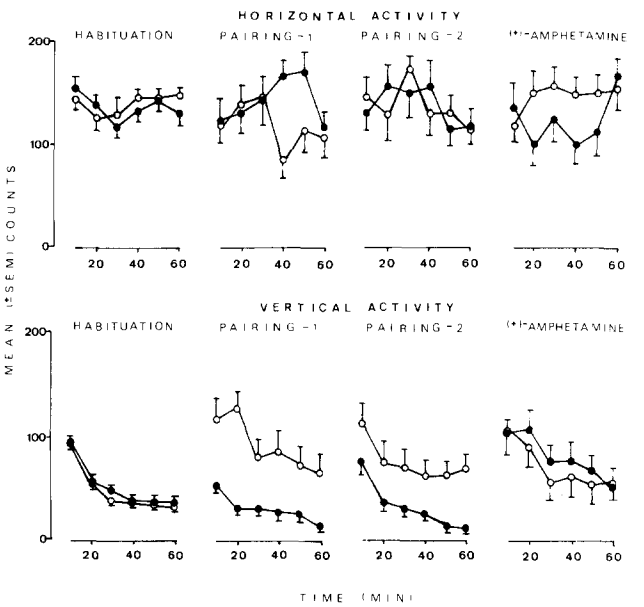

FIG. 1. Mean (±SEM) activity counts per 10 min on horizontal and vertical measures during the six habituation sessions combined, each pairing session, and the AMPH challenge session for rats given AMPH (○) or saline (●) prior to each pairing session. N=12 in each group.

The two pairing sessions were each examined separately. With horizontal activity there was only one significant effect; in the first session the group pretreated with AMPH had fewer counts than saline treated rats $F(1,22)=4.71$, $p<0.05$. The stimulant effects of the drug were apparent with vertical activity. In both sessions there was a significant group effect, rats receiving the drug being more active than controls (session 1, $F(1,22)=11.02$, $p<0.01$; session 2, $F(1,22)=4.87$, $p<0.05$). In both cases the time effect was also significant (session 1, $F(5,110)=7.72$, $p<0.001$; session 2, $F(5,110)=30.41$, $p<0.001$), suggesting that vertical activity decreased across the session regardless of drug treatment.

The test session revealed a slightly different pattern of activity. Although all rats received AMPH prior to this session, those who had previously had AMPH-environment pairings were significantly more active on the horizontal measure, $F(1,22)=5.35$, $p<0.05$. The group effect was not significant with vertical activity but, as in habituation and pairing, there was a significant time effect, $F(5,110)=4.60$, $p<0.01$.

One final analysis was conducted on the group that received a total of 3 AMPH-environment pairings. A two-way ANOVA, with time and session as the factors, over the three sessions, yielded a significant session effect on horizontal activity, $F(2,22)=3.58$, $p<0.05$, the mean activity level increasing over sessions. A similar analysis on vertical activity showed only a significant time effect, $F(5,55)=7.50$, $p<0.001$.

Conditioning

The activity of rats in this experiment is illustrated in Fig. 2. These rats exhibited similar activity profiles to those in the sensitization study during the habituation and pairing sessions. During habituation there was a significant time effect, $F(5,110)=4.04$, $p<0.01$, suggesting a decline of horizontal

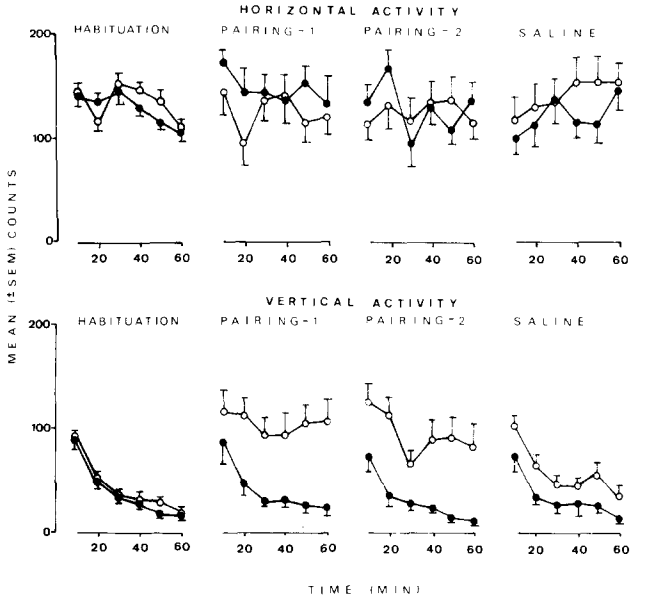

FIG. 2. Mean (±SEM) activity counts per 10 min on horizontal and vertical measures during the six habituation sessions combined, each pairing session, and the saline challenge session for rats given AMPH (○) or saline (●) prior to each pairing session. N=12 in each group.

activity across the session. Vertical activity during habituation exhibited significant time, $F(5,110)=101.59$, $p<0.001$, session, $F(5,110)=9.86$, $p<0.001$, and time by session effects, $F(25,550)=2.18$, $p<0.01$, again suggesting that there was a decline both within and across sessions, and no difference between the groups.

During the two pairing sessions there were no significant effects observed on horizontal activity. With the vertical measure there were significant time (session 1, $F(5,110)=4.55$, $p<0.01$; session 2, $F(5,110)=5.85$, $p<0.01$), and group effects (session 1, $F(1,22)=11.14$, $p<0.01$; session 2, $F(1,22)=12.82$, $p<0.01$), demonstrating a stimulant effect of the drug as well as a within session decline in activity.

In the saline test session, there were no significant effects observed on horizontal activity. However, there was a significant group effect with vertical activity $F(1,22)=6.01$, $p<0.05$, those rats previously having the drug associated with the chambers being more active. Again the time effect was also significant, $F(5,110)=26.57$, $p<0.001$.

DISCUSSION

The results indicate that measures of horizontal and vertical activity were somewhat independent as the two appeared not to covary. This finding suggests that each was assessing a different aspect of the rats' behavioral repetoire. Therefore, horizontal and vertical activity will be discussed separately.

Horizontal activity, which incorporates general ambulation and possibly some aspects of grooming, appeared to be little affected by the habituation process. Indeed, there was a significant within session decline only in the conditioning experiment and no between session decline in either experiment. Thus, it appeared that this measure of activity did not exhibit typical habituation within the parameters of the present study.

The absence of an unconditioned effect of AMPH on the horizontal activity is perhaps a surprising result, although it is consistent with previous data from our laboratory [2,4]. There is an abundance of literature demonstrating that the dose utilized here is a stimulant enhancing the frequency of locomotion [13]. However, there are several possible explanations for the present findings. The activity chambers used here (41×50×37 cm) were much larger than those typically used by others e.g., [18]. Perhaps the larger floor space was conducive to a high level of horizontal activity. Thus, it is possible that horizontal activity counts had shown a ceiling effect in the control group. This proposition is supported by the fact that during the habituation sessions there was little evidence of a decline in horizontal activity. Thus, any stimulant effect of the drug may have been masked by this high level of activity in the control group. The apparent decrease in horizontal activity seen in AMPH-treated rats on the first pairing day in the sensitization study might be accounted for by the possibility that some rats in the drugged groups were engaged in stereotyped behavior, although no attempt was made to systematically observe the rats during the sessions. As stereotypy is more focused and replaces locomotion it would interfere with and actually reduce horizontal activity [13]. If stereotypy provides an explanation for the decreased horizontal activity seen on pairing day 1, AMPH must have produced significantly less stereotypy on pairing day 2 or the sensitization test day where no decrease in horizontal activity was seen in the paired group. The observation that the unpaired group showed significantly less horizontal activity than the paired group on the sensitization test day provides a replication of the effect seen in the paired group during their first AMPH administration. Although the exact reason cannot be determined here, it appears that the first time AMPH is administered in these activity monitors there may be a decline in horizontal activity which wanes with subsequent treatment.

The AMPH and saline test sessions also yielded some interesting results on horizontal activity. In the drug-free test for environment-specific conditioning there was no significant difference between the paired and unpaired groups. Since there appeared to be no unconditioned stimulant effect, the absence of a conditioned stimulant effect is congruent with general learning principles. The results from the sensitization study suggest that horizontal activity did indeed undergo some sensitization. One criterion often used to identify sensitization is an increase in the recorded measure over repeated administration. Using this criterion sensitization was evidenced as the paired group showed more horizontal activity following each successive drug injection (a significant effect of sessions). A criterion used to determine if the sensitization effect was specific to the environment, i.e., under the influence of learning factors, is to compare the paired and unpaired groups on the AMPH challenge session. If the paired group shows a stronger effect again there is evidence of sensitization. This second criterion would also lead to the conclusion that sensitization occurred. Furthermore, since sensitization apparently occurred only in the drug-paired group, it follows that there was an environmental influence on sensitization. However, this influence did not extend to the test session for conditioning as no conditioned effect was observed. This is a surprising result in view of the notion that the same principles underlie each effect. It is possible that had there been more sessions a cumulative effect may have emerged.

In summary, activity recorded on the horizontal measure

appeared not to habituate. This finding, and the fact that AMPH may have initially depressed this measure, may explain the absence of an unconditioned stimulant effect, and subsequently the lack of a conditioned effect. However, examination of the group receiving the drug three times in the environment suggests that some sensitization was occurring.

Vertical activity, which incorporates jumping and rearing, exhibited a very different profile from that observed with horizontal activity. During the habituation sessions there was strong evidence of both within and between session declines, suggesting that this measure is highly susceptible to the habituation process.

The unconditioned effect of AMPH on vertical activity was very pronounced. The drug produced marked increases on all occasions that it was administered. These data are also consistent with earlier research [2,4].

During the test session in the conditioning experiment, the group previously having AMPH-environment pairings was significantly more active than the other group. Thus, there was evidence of environment specific conditioning. However, it should be noted that although the group exhibited a conditioned response, the conditioned effect was much weaker than the corresponding unconditioned effect. This finding is in good agreement with a number of previous studies [3, 4, 9, 16, 22, 26, 27]. An earlier study demonstrated that conditioning can be produced with as few as six drug-environment pairings [16]; the present report suggests that the effect can be produced with as few as two trials.

It is of interest that the unconditioned and conditioned effects with vertical activity seen here were apparently non-interactive. That is, the conditioned effect did not summate with the unconditioned effect to result in sensitization. The reason for the absence of the two aspects altering behavior simultaneously is not known. Perhaps the fact that the conditioned effect was not nearly as strong as the unconditioned effect may have been a factor. Thus, the relatively minor contribution of the conditioned effect could be masked by the much larger unconditioned effect.

It has been suggested previously that both sensitization and conditioning are related and may coexist. Although sensitization has been produced with minimizing the influence of discrete environmental cues [20,24], evidence suggests that a maximal effect occurs with the use of such cues [10, 18, 27]. As there is evidence suggesting that both sensitization and conditioning are strongly affected by environmental context it would follow that similar learning principles are involved (i.e., association of the drug and environment). The present study suggests that the horizontal and vertical activity seen in rats treated with AMPH were differentially affected. Conditioning without sensitization was observed with vertical activity whereas sensitization in absence of conditioning occurred with horizontal activity. Thus, the data suggest that the two phenomena can be dissociated.

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