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Diminished Reaction to a Novel Stimulus During Amphetamine Withdrawal in Rats[1]

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SCHREIBER, H., R. BELL, L. CONELY, M. KUFNER, J. PALET AND L. WRIGHT. *Diminished reaction to a novel stimulus during amphetamine withdrawal in rats.* Pharmac. Biochem. Behav. 5(6) 687–690, 1976. – This experiment determined whether reaction to a novel stimulus was diminished in a dose-dependent fashion following 8 consecutive days of d-amphetamine administration. Thirty-two male rats were injected with saline, 0.5, 2.5, or 5.0 mg/kg of d-amphetamine (each N = 8). On the ninth day, all animals received saline injections and were tested (a) in the presence of a novel stimulus, or (b) in the absence of a novel stimulus. Reaction to the novel stimulus varied inversely with the dose of d-amphetamine which had been received during the drug administration period. This reduction in reaction to the novel stimulus did not seem to depend on (a) the level of amphetamine-induced stereotypy at the end of the drug administration period (b) general reduction of activity, or (c) interference by drug-conditioned responses.

d-Amphetamine Withdrawal Reaction to novelty Stereotypy Locomotor activity
Chronic drug administration Carry-over effects

SEVERAL studies have shown that, when rats are injected with relatively high doses of amphetamine, they show decreased attention to stimuli in the environment – a deficit which occurs independently of the stimulation of other behaviors [1, 3, 6, 10]. Conceivably, responsiveness to external stimuli might be reduced by a process which persists into drug withdrawal. Thus, the present study determined (a) whether reduced reactions to novel stimuli would be exhibited during withdrawal from d-amphetamine, and (b) whether this diminished reaction to novelty depended upon any of three other possible explanations, the level of amphetamine-induced stereotypy exhibited before withdrawal of the drug, general lethargy, or interference from drug-conditioned responses.

METHOD

Animals

Thirty-two male Sprague-Dawley rats were individually housed in standard laboratory cages (18 cm wide × 25 cm long × 17.5 cm high) in a windowless colony room with controlled temperature. Because of space and use considerations in this colony room, continuous lighting was maintained. Purina rat chow and water were continuously available.

Apparatus

The animals were tested in standard LeHigh Valley operant chambers with Plexiglas walls and grid floor (20 cm wide × 23 cm long × 19.5 cm high). Operant chambers, which were nonfunctional at the time of testing, were housed in 4 similar, separate testing rooms.

Procedure

The rack of cages containing the rats was wheeled into a staging area adjoining the testing rooms 15 min before injections began. Animals received 0.0, 0.5, 2.5, or 5.0 mg/kg of d-amphetamine sulfate calculated as the weight of the salt, 30 ± 5 min before testing began (N of each treatment group = 8). Comparable volumes of saline vehicle were injected SC in all animals. Four observers noted and agreed upon several behaviors at a pretest session during which all animals were injected and each tested for 6 min in the test chambers. Following an 8 day interval, the injection period (1 daily injection for 8 consecutive days) began. The animals were given the same dose of d-amphetamine which they had received in the pretest session. The 4 observers checked one of the several behaviors on a checklist at 5 sec intervals during the 2 min testing. Each of the observers saw the same 2 animals per treatment group daily. The order of injection was randomized daily per observer. A stereotypy score was formed by summing the number of 5 sec intervals when (a) repetitive sniffing with the nose thrust through the grid, (b) repetitive licking and biting of the grid, or (c) repetitive bobbing head movement was exhibited. A locomotor

[1] A preliminary report of this data was presented to the Southwestern Psychological Association Meeting, Albuquerque, N.M., 1976.

activity score was formed by summing the number of 5 sec intervals when (a) rearing, (b) general body movement, or (c) ambulation was exhibited. Other behaviors (grooming, defecations, passive and other) formed separate categories of response. All animals received saline on withdrawal day (the day following the injection period). Sixteen animals (4 from each treatment group, 1 per observer) were tested as before. Sixteen animals (4 from each treatment group, 1 per observer) were tested with a rat-sized, felt doll in the chamber. A reaction to novel stimulus score was formed by summing the number of 5 sec intervals in which the rat touched, tugged or bit the felt doll or oriented its head toward the doll with its nose within approximately 10 cm of it. The animals were weighed 1 day before the pretest session, 1 day before the injection period and on the seventh day of the injection period; injections were revised accordingly.

Statistical analysis. All statistical analyses were performed according to Kirk [5]. The reaction to novel stimulus scores were analyzed with a completely randomized analysis of variance design. The stereotypy scores and the locomotor activity scores were transformed with a square root transformation ($\sqrt{X+1}$) in order to meet the homogeneity of variance assumption of analyses of variance. Stereotypy and locomotor activity scores were independently analyzed with split-plot factorial (4 × 8) analyses of variance for the injection period (A=dose; B=day of injection period). Stereotypy and locomotor activity scores for withdrawal day were independently analyzed with completely randomized factorial (4 × 2) analyses of variance (A=dose received during injection period; B=presence of doll). Except as otherwise noted, comparisons between means were performed with Tukey's test. Except as otherwise noted, the significance level for all results reported here was set a $p < 0.05$.

RESULTS

Injection period. The animals receiving saline showed a low level of stereotypy and a high level of locomotor activity relative to the other groups. The elevated level of locomotor activity shown by the saline group may have been due to the brevity of the test period. Although the animals receiving saline showed a significant days of injection effect in locomotor activity, $F(7,196) = 2.2149$, this locomotor activity pattern oscilated over days of injection. As may be observed in Fig. 1, there was no significant increase or decrease in locomotion from Day 1 to Day 8. Neither the stereotypy, $q(4,28) = 2.9958$, nor the locomotor activity, $q(4,28) = 2.1284$, of the 0.5 group was significantly greater than that of the saline animals. The animals receiving 2.5 mg/kg of d-amphetamine showed increasing stereotypy over days of injection, $F(7,196) = 8.2323$, $p < 0.01$. Whereas the animals receiving 2.5 mg/kg of d-amphetamine did not significantly differ in stereotypy from the saline animals of Day 1, they did differ significantly from them by Day 8, $q(4,224) = 11.2843$. Along with the increasing stereotypy, animals receiving 2.5 mg/kg d-amphetamine showed decreasing locomotor activity over days of injection, $F(7,196) = 12.4881$, $p<0.01$. Whereas the 2.5 group showed essentially the same amount of locomotor activity as the saline group on Day 1, they showed significantly less locomotor activity by Day 8, $q(4,224) = 8.4063$. The animals receiving 5.0 mg/kg of d-amphetamine showed virtually the maximum amount of stereotypy and virtually no locomotor activity. The 5.0 mg/kg of d-amphetamine animals showed significantly more stereotypy, $q(4,28) = 14.9495$, $p<0.01$ and significantly less locomotor activity, $q(4,28) = 10.15$, $p<0.01$, than the saline animals throughout the injection period. These results, as shown in Fig. 1, produced a significant dose by days of injection interaction for stereotypy, $F(21,196) = 3.144$, $p<0.01$, and for locomotor activity, $F(21,196) = 3.699$, $p<0.01$.

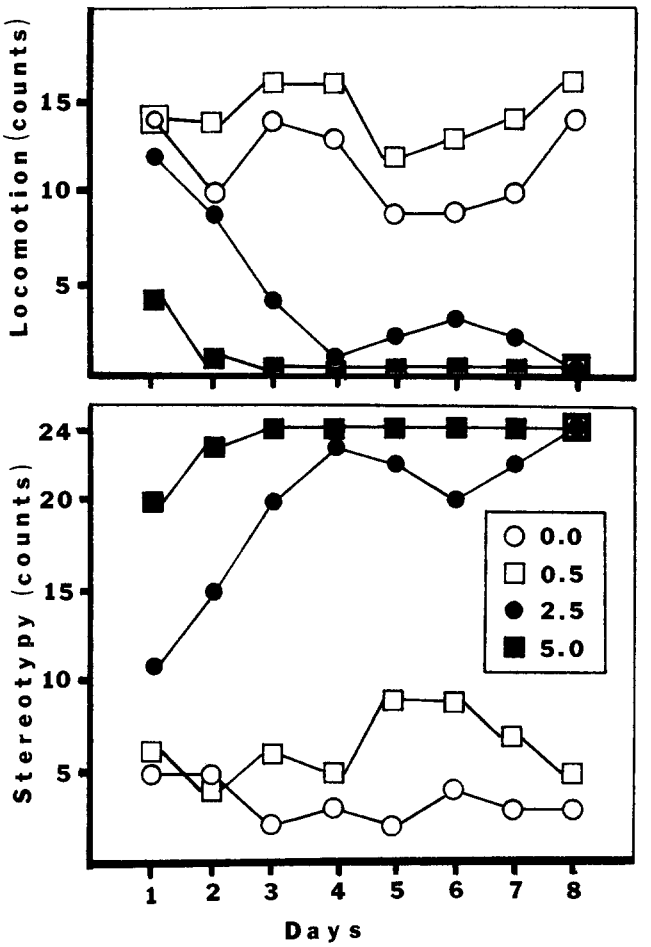

FIG. 1. Stereotypy and Locomotor Activity scores of animals receiving 0.0, 0.5, 2.5 and 5.0 mg/kg d-amphetamine during the injection period (all Ns = 8).

Withdrawal day. The results for withdrawal day, including reaction to novel stimulus, locomotor activity and stereotypy are presented in Table 1.

There was an inverse relationship between the dose of d-amphetamine which had been received during the injection period and the reaction to the felt doll on withdrawal day when all animals received saline, $F(3,12) = 3.5964$. The group which had received 5.0 mg/kg of d-amphetamine showed significantly less reaction to the felt doll than the saline group, $t(6) = 2.8997$. The group which had received 2.5 mg/kg of d-amphetamine failed to show significantly less reaction to the felt doll than the saline group, $t(6) = 1.9718$, $0.05<p<0.10$. However, a test for linearity of trend showed that there was a trend among the dose levels

TABLE 1

RESPONSES ON WITHDRAWAL DAY

Behavior	Dose History Groups 0.0 $\overline{X}$ (SD)	0.5 $\overline{X}$ (SD)	2.5 $\overline{X}$ (SD)	5.0 $\overline{X}$ (SD)
Reaction to Novel Stimulus	9.25(3.11)	8.25(3.89)	5.00(0.50)	3.00(1.41)*
Locomotor Activity				
With doll present	3.05(0.76)	3.27(1.33)	3.46(0.64)	3.62(0.46)
With doll absent	3.73(1.85)	3.53(1.04)	4.54(0.45)	3.57(1.02)
Stereotypy				
With doll present	1.93(1.42)	1.57(0.62)	2.81(0.89)	2.26(0.45)
With doll absent	1.39(0.30)	3.04(1.52)	1.49(0.60)	3.26(1.22)*

*$p<0.05$.

which was significantly linear, $F(1,12) = 10.426$, $p<0.01$. No test for nonlinear trends was significant.

Stereotypy scores on withdrawal day showed a significant dose effect only when the felt doll was absent, $F(3,24) = 4.1353$. The animals with a dose history of 5.0 mg/kg of d-amphetamine showed significantly more stereotypy than did the subjects which received saline, $t(24) = 3.9218$, $p<0.01$. However, neither the dose of d-amphetamine received during the injection period nor the presence of the felt doll had a significant effect on locomotor activity on withdrawal day.

Other behaviors observed on withdrawal day (grooming, defecation, passive and other) were so infrequent and so dispersed among the treatment groups that no statistical analysis was undertaken.

DISCUSSION

The present results indicated that amphetamine-produced reductions in reaction to novelty were manifested during drug withdrawal. The animals which had received 5.0 mg/kg showed reduced reaction to novelty in comparison with the saline ones. Kirkby, Bell, and Preston [6] showed that, along with an increase in stereotypy, locomotion and startle response, 5.0 mg/kg of methamphetamine produced decrements in the orienting response and inhibited habituation of the startle response in rats. The decrement in the orienting response occurred at 5.0 mg/kg of methamphetamine, but not at 0.5 mg/kg of methamphetamine. Based on their study and others these authors suggested that this drug-produced deficit in the orienting response occurs at doses which elicit stereotypy, but not at lower doses, indicating the involvement of some process involving stereotypy, However, the present results suggested that the reduction in reaction to the novel stimulus during the withdrawal period was a general consequence of d-amphetamine administration, rather than being tied to the level of stereotypy exhibited at the end of the injection period. Animals receiving 0.5 mg/kg of d-amphetamine showed little stereotypy and a relatively high level of locomotor activity compared to the other d-amphetamine groups during the injection period. Animals receiving 2.5 mg/kg of d-amphetamine showed relatively little stereotypy at first, but, as the injection period progressed, stereotypy was increasingly exhibited while locomotor activity was progressively reduced. In fact, by Day 4 of the injection period, the 2.5 mg/kg group was exhibiting a reciprocal pattern of stereotypy and locomotor activity equivalent to that of the 5.0 mg/kg group, which showed virtually the maximum amount of stereotypy and the minimum amount of locomotor activity possible. If the reduction in reaction to novelty was tied to the level of stereotypy exhibited at the end of the injection period as suggested by Kirkby, Bell and Preston, then the 2.5 mg/kg group should have shown a reduction in reaction to novelty comparable to that shown by the 5.0 mg/kg group. However, the reduction in reaction to novelty in the 2.5 mg/kg group failed to reach the level of reduction shown by the 5.0 mg/kg group. Moreover, the reduction in reaction to novelty varied linearly with the amount of d-amphetamine which had been administered. Randrup, Munkvad, Fog and Ayhan [9] have suggested that stereotypy per se does not cause the functional impairment reflected in reduced exploration. Rather stereotypy and functional impairment may be features of the same underlying process. There were two other potential explanations for the reduction in reaction to novelty at amphetamine withdrawal shown in the present study. First, general lethargy could conceivable have reduced reaction to the novel stimulus. For example, withdrawal of dl-amphetamine (3 mg/kg) following prolonged administration in rats produced an inhibition of locomotor activity and decreased noradrenaline and 5-hydroxytryptamine levels in the cerebellum [7]. Although lethargy and depression are among the commonly reported aftereffects of chronic amphetamine administration in man, the aftereffects of chronic amphetamine administration in animals are reported to be relatively mild [4]. Moreover, reducing the reaction to the novel stimulus seemed to be a specific effect of d-amphetamine administration, not a consequence of lethargy, depression or some other general, activity-reducing aftereffect of chronic d-amphetamine administration, because withdrawal from d-amphetamine produced no observable effects on locomotor activity or on the other behaviors (grooming, defecations, passive and other). Second, drug-conditioned responses (in the present case, drug-conditioned stereotypy) conceivably could reduce responsiveness to a novel stimulus through response competition and interference. It seems well-established that the repeated pairing of drug-induced internal stimuli with the neutral stimuli surrounding injection and testing may produce the conditioning of certain responses (e.g., locomotion and stereotypy) in some cir-

cumstances of testing [2, 8, 12, 14]. Animals which had received 5.0 mg/kg of d-amphetamine seemed to show evidence of stereotypy when injected with saline on withdrawal day and tested in the absence of the felt doll. Thus, conditioned stereotypy may have interfered with the rats' investigation of the felt doll to produce the response decrement. This explanation, although feasible, was undermined by two considerations. First, the 2.5 mg/kg group, whose increase in stereotypy during the injection period would have fit the drug-conditioned response pardigm more suitably than the 5.0 mg/kg group's pattern of stereotypy, failed to show evidence of conditioned stereotypy when injected with saline. Second, there was no evidence of increased stereotypy among those subjects injected with saline on withdrawal day and tested with the felt doll present in the operant chamber. Thus, the interference, if it was present, occurred without an observable increase in stereotypy in the doll-present condition of testing. Moreover, the repetitive sniffing and head-bobbing which comprised the stereotypy score on withdrawal day may not have reflected true stereotypy. One identifying characteristic used to operationalize stereotypy has been the absence of forward locomotion [11]. The rats which had received 5.0 mg/kg of d-amphetamine showed no reduction in locomotor activity on withdrawal day. Since the reciprocity of locomotion and stereotypy which was seen during the injection period did not carry over into withdrawal day, stereotypy, as traditionally defined, had not been conditioned. Nonetheless, the conditioning of repetitive, stereotypy-like sniffing and head-bobbing, if not true stereotypy, may conceivably have interfered with the rats' reaction to the novel stimulus in the present study.

With the exception of the locomotor activity pattern of the 0.5 group, the pattern of stereotypy and locomotion found among the dose groups during the injection period in the present study paralleled the pattern of stereotypy and locomotion found among the equivalent dose groups of Segal and Mandell [13] for the corresponding time after injection. These authors showed that the effects of chronic d-amphetamine administration carried over into the withdrawal period to influence responsiveness to subsequent d-amphetamine injections. Moreover, the results obtained by Segal and Mandell suggested that the progressive change in stereotypy and locomotion and the carry-over effects of chronic amphetamine administration may have reflected a single process, changes in catecholamine biosynthetic capacity. Analogously, the reduced reaction to a novel stimulus shown by subjects which had received d-amphetamine in the present study may have reflected the same process.

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