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BRIEF COMMUNICATION

Environment-Dependent Sensitization to Amphetamine-Induced Circling Behavior

KELLY L. DREW AND STANLEY D. GLICK

Department of Pharmacology and Toxicology, Albany Medical College
47 New Scotland Avenue, Albany, NY 12208

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DREW, K. L. AND S. D. GLICK. *Environment-dependent sensitization to amphetamine-induced circling behavior.* PHARMACOL BIOCHEM BEHAV **31**(3) 705–708, 1988.—Sensitization to amphetamine-induced circling behavior in nonlesioned, female rats was studied. Experiments were designed to determine the effects of time spent in the test environment prior to and following the administration of amphetamine and of the time between injections of amphetamine on the environment-dependent nature of the sensitization process. One group of rats was allowed to habituate to the test apparatus prior to injection of the drug. In this group, the drug was administered in the apparatus and the rats remained there for the duration of drug action. Another group of rats was placed in the apparatus only during the time of peak drug action. These rats were administered amphetamine in their home cages and were not allowed time to habituate to the test apparatus. Amphetamine was administered 2 times and injections were separated by either 1 or 7 days. To determine if the sensitization was dependent on the environment in which the drug was previously experienced, one-half of each of these groups of rats were kept in their home cages following the first injection of amphetamine and experienced the effects of the second injection of amphetamine in the test apparatus. The other half experienced the effects of both injections of amphetamine in the test apparatus. Sensitization was found to occur only in rats that experienced the effects of the first drug injection in the test environment.

Amphetamine Sensitization Reverse-tolerance Circling Conditioning Striatal-asymmetry

WE have reported previously that amphetamine-induced circling behavior in nonlesioned rats increases from the first to the second injection (2–4). Other laboratories have reported sensitization to amphetamine-induced circling behavior in nonlesioned rats (10) as well as to amphetamine-induced locomotor activity (12), stereotypy (as evidenced by an initial decrease in activity followed by an increase in poststereotypic activity) (1,8) and circling behavior in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway (9).

Some investigators have shown that sensitization to amphetamine occurs only when rats receive the drug in the environment where they are subsequently tested; i.e., that sensitization can be significantly influenced by, and thus dependent on, the test environment (12). Operationally, this type of sensitization will be referred to as environment-dependent sensitization. Others have shown that a significant degree of sensitization can occur via a process that is not significantly influenced by the test environment (1,9). Sensitization of this latter type will be referred to as environment-independent sensitization. Furthermore, Robinson *et al.* (10) have shown that a single injection of amphetamine in vivo produces an enhancement of amphetamine-stimulated dopamine release from striatal tissue in vitro. These in virto findings suggest that changes in dopamine release contribute to the behavioral sensitization produced by stimulant drugs and that an association between the test apparatus and the drug is not necessary for the expression of these changes.

Procedures designed to maximize Pavlovian conditioning by limiting exposure of the animal to the test apparatus to the time of peak drug effects (2, 3, 12) have been suggested to contribute to an environment-dependent component of sensitization (1). On the other hand, time between injections may allow for adaptations to occur which contribute to sensitization that is influenced less by environmental factors (9).

Circling behavior observed in normal rats is enhanced by amphetamine and other dopamine agonists and is thought to result from an endogenous asymmetry of the nigrostriatal pathway (7,4). Females have been shown to circle in response to amphetamine and to sensitize to the amphetamine-induced response more than males (10). Some rats fail to circle in response to amphetamine; these "nonlateralized" rats (about 30% of the rats tested in this study) make very few net rotations, may switch direction from one test to the next and have very low preferences for one direction over another on any given test (10). The following experiment was, therefore, designed to determine if the procedure employed or the time interval between injections would influence the environment-dependent nature of the sensitiza-

tion to amphetamine-induced circling behavior in nonlesioned female rats. Only lateralized rats were included in the analysis.

METHOD

Circling behavior was measured with an automated rotometer apparatus [cf. (2)] consisting of a clear Plexiglas cylinder (30.5 cm diameter × 30.5 cm height) set on a flat, wire mesh floor above a box of wood shavings. The sensing device distinguished full turns (four consecutive 90° turns) from quarter turns. The number of net turns (NET) defined as the number of full turns made in the dominant direction minus the number of full turns made in the nondominant direction was used as the dependent measure.

Subjects

Female Sprague-Dawley rats weighing between 200 and 300 g were purchased from Zivic Miller Laboratories (Pittsburgh, PA). Rats were housed in groups of 4/cage, maintained on a 12 hr light/12 hr dark cycle and were provided food and water ad lib.

Procedure

In this experiment the circling response induced by amphetamine was measured on two occasions which were separated by either 1 or 7 days. Rats experienced the effects of the first injection of amphetamine in either their home cages or in the rotometer apparatuses, however, all rats experienced the effects of the second injection of amphetamine in the rotometer apparatuses. Two different procedures of drug administration were employed. These procedures differed with respect to the time subjects remained in the rotometer apparatuses before and after administration of amphetamine. They were adopted because they have been used previously when investigators have suggested that the environment dependent nature of sensitization to amphetamine is dependent on the procedure employed. (Reference to the organization of Table 1 may help to clarify the experimental design.)

Specifically, one group of rats was injected with amphetamine 15 min after placement in the rotometers and circling behavior was recorded for the next 60 min. (This procedure has been described previously by Glick *et al.* (5) and Robinson *et al.* (10) and will be referred to as the '60-min procedure.') Other rats were placed in the rotometers 30 min after injection of amphetamine and circling was recorded for the next 30 min. (This procedure was utilized previously by Tilson and Rech (12) and Drew and Glick (2,3) and will be referred to as the '30-min procedure.')

Experiments designed to study Pavlovian conditioning of drug-induced responses (2,3) often include a group of control rats that receive saline prior to placement in the test apparatus and amphetamine following removal from the apparatus. Rats which experience the drug in the context of the test apparatus are then given an injection of saline at a later time to control for the number of injections. To determine the effect of such saline injections on sensitization some rats treated according to the 30-min procedure were injected with saline 1 hr following removal from the apparatus (2 hr following the injection of amphetamine) as described by Drew and Glick (2,3), while others were not. On day 1 if rats were to remain in their home cages the second injection was administered 2 hr after the first injection.

Amphetamine was administered in a dose of 1.25 mg/kg (IP). This dose was found by Jerussi and Glick (7) to produce maximal rates of circling which was consistent in direction over the duration of action of the drug. *d*-Amphetamine sulfate purchased from Sigma Chemical Company, St. Louis MO, was dissolved in 0.9% sterile saline and injected in a volume of 1.0 ml/kg.

Statistical Analysis

Results from the 60-min and 30-min procedures were analyzed separately. Results from each procedure were analyzed in two ways by 3- or 4-way parametric analyses of variance (ANOVA). The purpose of the first approach was to compare the response following the second injection of *d*-A in rats that had received the first injection of *d*-A in their home cages to rats that had received the first injection in the rotometers. Factors included in this approach were group (i.e., rats that had received the first injection in the home cages versus rats that had received the first injection in the rotometers), intertrial interval (ITI) (i.e., time between injections), and time across the duration of drug action (i.e., 5 to 60 min following injection of *d*-A for the 60 min procedure and 30 to 60 min following injection of *d*-A for the 30-min procedure). Time over the duration of drug action was treated as a repeated measure. For subjects treated according to the 30-min procedure the occurrence or absence of an injection of saline 2 hr following the injection of *d*-A was also included as a separate factor in the analysis.

The purpose of the second approach was to compare the *d*-A-induced response on the first trial with the response on the second trial. Of course data obtained from rats that were not placed in the rotometers following the first injection could not be analyzed in this manner. Variables included as factors in this approach for the 60-min procedure were, trial (i.e., the first or second injection of *d*-A), intertrial interval and time across the duration of drug action. These same variables, as well as the occurrence or absence of an injection of saline 2 hr following the injection of *d*-A, were included in the analysis of the 30-min data. Time over the duration of drug action and trial were treated as repeated measures. If the direction of circling in any 5-min block was opposite to the direction of the cumulative response, for any individual rat, the number of net turns made by that rat during that block was assigned a negative value.

Because the circling response was not measured after the first injection of amphetamine in some rats, nonrotators were selected on the basis of the circling response following the second amphetamine injection. Nonrotators were defined as those rats which following the second injection of amphetamine, made fewer than 5 net turns or showed a preference for turning in a particular direction of less than 70% (calculated as the number of full turns made in the dominant direction divided by the total number of full turns, times 100).

RESULTS

Means of cumulative responses for each group are shown in Table 1. In Fig. 1 the circling response is shown as a function of time since injection, collapsed across intertrial interval for the 60-min procedure and across intertrial interval and occurrence of the saline injection for the 30-min procedure.

Comparison of the responses following the second injection of *d*-A between rats that had experienced the effects of the first injection of *d*-A in the rotometers and rats that had not, showed that the response on the second trial was greater

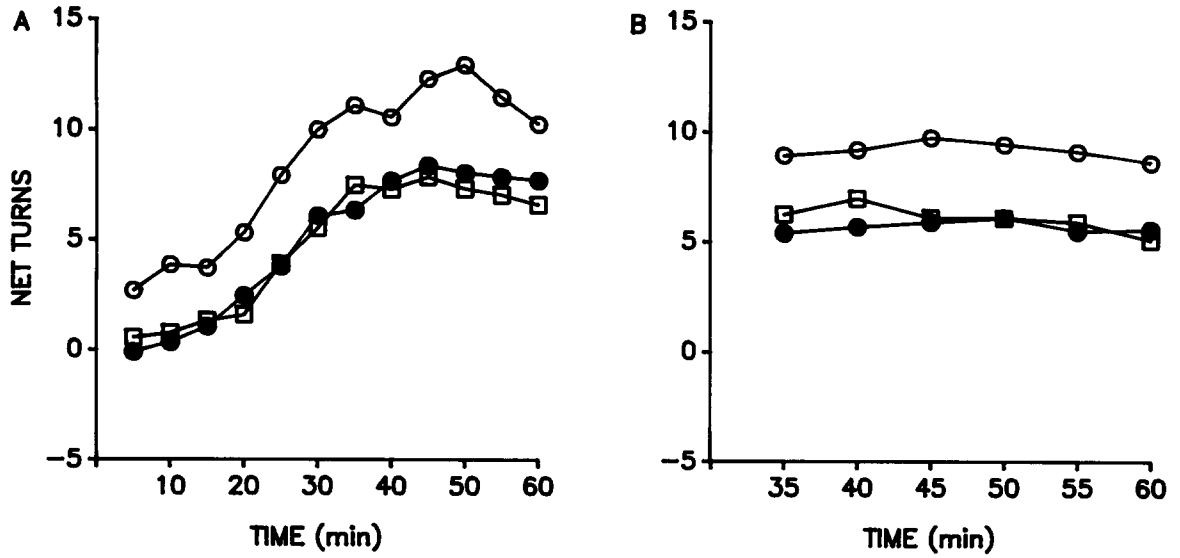

FIG. 1. The data from Table 1 are shown collapsed across intertrial interval and occurrence of saline injection and expressed as a function of time since injection for animals treated according to the 60-min procedure (A) or the 30-min procedure (B). Circles represent the response in the RR group following the first injection (closed) and the second injection (open). Squares represent the response in the HR group following the second injection.

in rats that had been in the rotometers following the first injection. A 3-way ANOVA performed on results obtained from the 60-min procedure following the second injection of *d*-A revealed significant main effects of group ($p=0.0026$) and of time ($p<0.0001$). No other main effects or interactions were significant.

Similarly, a 4-way ANOVA performed on the results obtained from the 30-min procedure, following the second injection of *d*-A (i.e., group × ITI × time × occurrence of saline injection 2 hr after the first injection of *d*-A), revealed a significant main effect of group ($p=0.0017$). No other main effects or interactions were significant. The main effects of group, for each procedure, demonstrate that the amphetamine-induced response, following the second injection of the drug, was greater in rats that had experienced the effects of the first injection in the rotometer environment than in rats that had experienced the effects of the first injection in their home cages.

Comparison of the circling response induced on the first and second trials showed that the response on the second trial was greater than the response on the first trial. A 3-way ANOVA (i.e., trial × ITI × time) performed on results obtained by the 60-min procedure, following the first and second injections of *d*-A, in rats that received both injections in the rotometers revealed significant main effects of trial ($p=0.0002$) and time ($p<0.0001$). Likewise, a 4-way ANOVA performed on results obtained by the 30-min procedure, following the first and second injections of *d*-A, in rats that received both injections in the rotometers (i.e., trial × ITI × time × occurrence of saline injection 2 hr after the first injection of *d*-A) revealed a significant main effect of trial ($p<0.0001$). No other main effects or interactions for either procedure were significant. The main effects of trial, in each analysis, demonstrate that the amphetamine-induced response was greater following the second injection of the drug than following the first injection.

DISCUSSION

These results indicate that the sensitization to amphetamine-induced circling behavior observed in this laboratory does not occur unless the effects of the first injection of the drug is experienced in the test apparatus. Brief habituation to the apparatus or a 7-day interval between injections of amphetamine did not alter the environment-dependent nature of the sensitization process. Pharmacokinetic factors cannot account for the sensitization observed in this study because rats that did not sensitize, i.e., those that received the drug in the home cage on day 1, received identical amounts of *d*-A, according to an identical dosage regimen, as did the rats that sensitized; i.e., those rats that received the drug in the rotometer apparatus on day 1.

Study of the time course of the response revealed that following the second injection of *d*-A, sensitization was evident at all time points. Circling behavior was not interrupted by periods of stereotypy in the sensitized animals. A similar increase in response, independent of time, was reported by Groves and Segal (6) for activity and by Robinson and Becker (10) for circling behavior in unlesioned rats following administration of comparably low doses of amphetamine.

It remains unclear why environment-dependent sensitization was observed here and by others (12) while sensitization largely independent of the environment has been observed elsewhere (1,9). Differences in sensitization to amphetamine-induced responses have been observed between rats of different strains and between Sprague-Dawley rats obtained from different suppliers (5,8). The proportion of environment-dependent or independent mechanisms contributing to the enhanced responsiveness may vary between different populations of rats as well.

Sex differences in sensitization to amphetamine have also been clearly established (10). Environment-dependent sensitization has, including the present study, been demonstrated only in female rats (12). Sensitization to am-

TABLE 1

FAILURE FOR PROCEDURE OR INTERTRIAL INTERVAL (ITI) TO AFFECT ENVIRONMENT-DEPENDENT SENSITIZATION TO AMPHETAMINE-INDUCED CIRCLING RESPONSE (NET)

Procedure	ITI (days)		HR	RR	
			d-A2	*d*-A1	*d*-A2
60 min	1	mean	68.6	69.5	101.1
		sem	10.7	11.3	18.1
			(n=17)	(n=13)	
	7	mean	44.7	50.1	103.1
		sem	10.8	8.2	18.3
			(n=16)	(n=14)	
30 min	1 saline	mean	31.2	30.9	54.1
		sem	5.2	5.4	7.0
			(n=12)	(n=22)	
	no saline	mean	34.2	31.1	50.8
		sem	3.4	5.2	7.3
			(n=13)	(n=21)	
	7 saline	mean	37.4	40.5	61.6
		sem	6.6	9.6	11.9
			(n=10)	(n=14)	
	no saline	mean	43.8	38.6	56.2
		sem	9.9	9.7	10.2
			(n=10)	(n=9)	

Following a 15-min habituation period circling behavior (NET) was recorded from 0–60 min after an injection of 1.25 mg/kg *d*-amphetamine (60-min procedure); or, rats were placed in the rotometer apparatus 30 min after the injection and circling behavior was recorded for the next 30 min (30-min procedure). Animals in the HR group received the first injection of amphetamine (*d*-A1) in the home cage (H) and a second injection of amphetamine (*d*-A2) in the rotometers (R). The RR group received both injections in the rotometers. Injections were separated by 1 or 7 days. Some rats treated according to the 30-min procedure received an injection of saline 2 hr following the injection of amphetamine (saline); others did not (no saline). While there were significant main effects of group on *d*-A2 and trial in the RR groups for both procedures (see Fig. 1) no other main effects (i.e., ITI or occurrence of saline injection) or interaction effects were significant.

phetamine, independent of the environment, has been shown to occur in male rats (1) and ovariectomized female rats (9). While no differences in sensitization to amphetamine-induced circling behavior were found to exist between intact and ovariectomized female rats or castrated male rats (10), no study has addressed the contribution of environmental variables to sensitization to amphetamine in male and female rats. Another possible explanation, not addressed in this study, is that sensitization to amphetamine is enhanced when the drug is experienced for the first time in a novel environment.

Perhaps two processes contribute to the enhanced responsiveness to amphetamine following repeated administration. One of these processes may occur in the absence of environmental factors while the other does not. Pavlovian conditioning has been shown to influence drug-induced responses [Siegel (11)]. It is easy to suggest that the environment-dependent sensitization observed here results from a Pavlovian conditioning process. However, failure for habituation to the test apparatus to reduce the degree of sensitization argues against the role of conditioning in this environment-dependent process. Additional work in this laboratory also suggests that sensitization to amphetamine does not behave like a simple Pavlovian-conditioned response (3).

In summary, this study revealed that the circling response following a second injection of *d*-A was greater in rats that experienced the effects of both the first and second injection in the rotometer environment than in rats that experienced the effects of the first injection in their home cages. In fact, there was no evidence of sensitization in the latter group. Sensitization to amphetamine was dependent on the environment when the drug was administered in the rotometer following a 15-min habituation period and when the drug was administered in the home cage and rats were placed in the rotometers only during the time of peak drug action. It is clear, therefore, that the two procedures employed did not influence the environment-dependent nature of the sensitization process. Likewise, a delay of 7 days between the first and second injection of *d*-A failed to affect the environment dependency of the sensitized response.

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