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Enhanced Self-Stimulation Responding from the Substantia Nigra after Chronic Amphetamine Treatment: A Role for Conditioning Factors

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KOKKINIDIS, L. AND R. M. ZACHARKO. *Enhanced self-stimulation responding from the substantia nigra after chronic amphetamine treatment: A role for conditioning factors. PHARMAC. BIOCHEM. BEHAV. 12*(4) 543–547, 1980.—Rats treated with 2 mg/kg of d-amphetamine and tested for self-stimulation responding supported from the substantia nigra (pre-trial group), showed a progressive augmentation in rates of self-stimulation responding relative to control animals following repeated drug/test pairings for 10 days. A similar behavioral profile was not observed among animals that received behavioral testing followed by drug administration (post-trial group) during the chronic phase. On test day (Day 11), rats that received repeated drug/test pairings during the chronic phase exhibited a facilitated self-stimulation response to a low test dosage of d-amphetamine (0.5 mg/kg) which otherwise had no behavioral effect, whereas rats exposed to chronic test/drug pairings during the chronic phase did not show enhanced self-stimulation rates to the test dosage of d-amphetamine. Animals chronically treated with pre-trial injections of amphetamine also showed facilitated self-stimulation responding when tested with saline, relative to animals that were chronically treated with post-trial injections of amphetamine and tested with saline. These findings were not parallelled by drug-induced changes in locomotor activity. Response sensitization after chronic amphetamine treatment does not appear to involve the accumulation of the drug in adipose tissue. A role for conditioning factors in the development of the response sensitization is discussed.

Self-stimulation Substantia nigra Chronic d-amphetamine administration Response sensitization Conditioning factors Adipose tissue

THE consequences of long-term amphetamine treatment are dependent upon the physiological and behavioral measures under investigation. Whereas some of the physiological and behavioral effects of amphetamine are observed to diminish following repeated exposure to the drug, e.g., perseveration, anorexia [3, 5, 6], other behaviors do not appear to undergo tolerance, e.g., stereotypy, locomotor activity [2, 3, 4]. Indeed, the intensity of these drug-induced behaviors is enhanced rather than mitigated following long-term exposure to the drug [9, 10, 11]. For example, stereotypic behaviors ordinarily observed following amphetamine administration are larger in magnitude and have an earlier onset following drug injection when the organism has had prior exposure to the drug [6, 9, 11]. Similarly, the locomotor effects of amphetamine do not undergo tolerance [5,6], but rather are enhanced if animals have received repeated administration of the drug [8,13].

Recently it has been demonstrated that chronic amphetamine treatment has pronounced effects on intracranial self-stimulation [7]. In particular, following chronic exposure to the drug, a low dosage of d-amphetamine which ordinarily had no effect on behavior, facilitated self-stimulation responding supported from the substantia nigra [7]. Moreover, although chronic exposure to a high dosage of the drug for 5 days was sufficient to produce an enhancement in response rates, the behavioral sensitization was greatest following 25 days of long-term amphetamine treatment [7].

The mechanisms which subserve the behavioral sensitization are not well understood, however, several possibilities exist which may account for these effects. Since chronic amphetamine administration results in decreased synthesis of dopamine [3,9], produces a depletion of dopamine [3], and has neurotoxic effects on dopamine receptors [1], it might well be the case that the enhanced behavioral response to amphetamine following chronic exposure to the drug involves supersensitive dopamine receptors [3,4]. A

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more behaviorally based approach attributes the response sensitization observed after long-term amphetamine administration at least in part to conditioning factors [8]. That is, some of the behavioral effects of amphetamine are dependent upon stimulus factors [6,8], and the pairing of these stimuli to the behavioral effects of the drug may modify subsequent performance. Specifically, following long-term amphetamine treatment, the drug-induced behavioral effects may be associated with the neutral stimulus complex present during the injection/test procedure, resulting in an increment in response strength. Thus, animals chronically treated and tested with amphetamine show an increased locomotor response to the drug relative to non-amphetamine animals when drug treatment is substituted with saline [8,13]. Although conditioning factors do not account for all the variance [9], the present investigation was designed to replicate earlier findings involving the chronic effects of amphetamine on self-stimulation and to determine whether conditioning factors play a role in the behavioral sensitization.

EXPERIMENT 1

In order to investigate whether conditioning factors are involved in the increased responsivity to amphetamine in a self-stimulation paradigm after chronic drug treatment, rats received repeated injections of amphetamine such that the systemic effects of the drug were either congruent with behavioral testing (pre-trial injection), or occurred following behavioral testing (post-trial injection). If conditioning factors play a role in the behavioral sensitization induced by chronic amphetamine treatment, then animals in the pre-trial group should show a larger response to a test dosage of amphetamine than animals in the post-trial group which received an equal amount of drug during the chronic phase.

Such a paradigm is also useful in determining whether the enhanced self-stimulation responding evidenced after long-term amphetamine treatment involves the accumulation of amphetamine in adipose tissue [12]. It has recently been suggested that amphetamine is stored in fat mobilizable pools, and following chronic amphetamine treatment release of stored amphetamine may be responsible for the enhanced behavioral response to the drug [12]. If this is the case, then in contrast to the first prediction animals in both the pre- and post-trial groups should show enhanced self-stimulation rates following a test dosage of amphetamine, since both groups received a comparable amount of amphetamine during the chronic phase.

METHOD

Subjects

Sixty naive male rats (Charles River CD, outbred albino) from the Canadian Breeding Farms and Laboratories served as subjects. Rats weighed 300–350 g at the initiation of the experiment and were housed individually and permitted free access to food and water throughout the duration of the experiment. Rats were housed in a regular 12 hr light/dark cycle and testing was carried out during the light portion of the cycle.

Apparatus

The apparatus was similar to that described by Zacharko and Wishart [15] and consisted of a Plexiglas box 60 cm in length, 20 cm in width and 30 cm in height. Two photobeam units were mounted 2.5 cm above the grid floor and 6.0 cm from each end of the box. When the photobeams were interrupted by either head or body movements into and out of the beam, electrical brain stimulation was initiated. Response rates and the duration of brain stimulation were recorded on LeHigh Valley timers and counters during 10-min test sessions. Brain stimulation was delivered from an Ortec Dual Channel Stimulator with a standard current intensity of 30 μA (biphasic square wave) and a pulse frequency of 100 Hz.

Procedure

Subjects were anesthetized with sodium pentobarbital (45 mg/kg) and were stereotaxically implanted in the substantia nigra with an insulated bipolar nichrome electrode (Plastic Product) which had 0.5 mm of the tip separated and scraped. The coordinates for electrode placement were anterior-posterior −4.5 from bregma, lateral +2.5 from the midline suture, vertical −8.5 from a flat skull surface. Following a 7 day postoperative period rats were tested for self-stimulation for a 10 min test session daily for 5 consecutive days. Following baseline testing rats were assigned to one of three groups (n=20/cell). Animals assigned to the three experimental groups were selected to equalize average baseline responses among the three groups. During the 10 days following baseline testing subjects in the first two groups received daily IP injections of either d-amphetamine sulfate (2 mg/kg) or saline and were subsequently tested for self-stimulation for a 10 min session 30 min following injection (pre-trial). Rats in the third group received a daily IP injection of 2 mg/kg of d-amphetamine immediately following testing (post-trial). On test day (Day 11), rats were subdivided such that half the animals in each group (n=10/cell) received an IP injection of either saline or 0.5 mg/kg of d-amphetamine. Thirty minutes after injection rats were tested for self-stimulation. At the termination of the experiment rats were sacrificed under chloroform anesthesia, perfused with 0.9% physiological saline followed by 10% Formalin and the brains were removed for histological verification of electrode placement. Frozen coronal sections were cut at 40 μm, and stained with thionin.

RESULTS

Histological verification of electrode placements revealed that in all cases placements were in the region of the substantia nigra.

The mean rate of self-stimulation responding during the chronic and test day phase of Experiment 1 is depicted in Fig. 1. Analysis of variance of the self-stimulation rates during the chronic phase yielded a significant Group×Days interaction, $F(20,570)=11.31$, $p<0.001$. Subsequent Newman Keuls multiple comparisons ($\alpha=0.05$) of the simple main effects involved in this interaction revealed that baseline self-stimulation rates were comparable among the three experimental groups and performance of control animals remained consistent over the 10 test sessions during the chronic phase. Injection of d-amphetamine (2 mg/kg) 30 min prior to testing increased self-stimulation rates relative to baseline performance, and to performance of control animals during the entire chronic phase. Moreover, with repeated drug/test pairings the facilitative effects of amphetamine were increased and response rates on the last 7 days of testing were significantly higher than response rates on the first day of drug treatment. In contrast to these findings, post-trial injections of amphetamine had no effect on self-stimulation responding as compared to baseline rates. Relative to the performance of

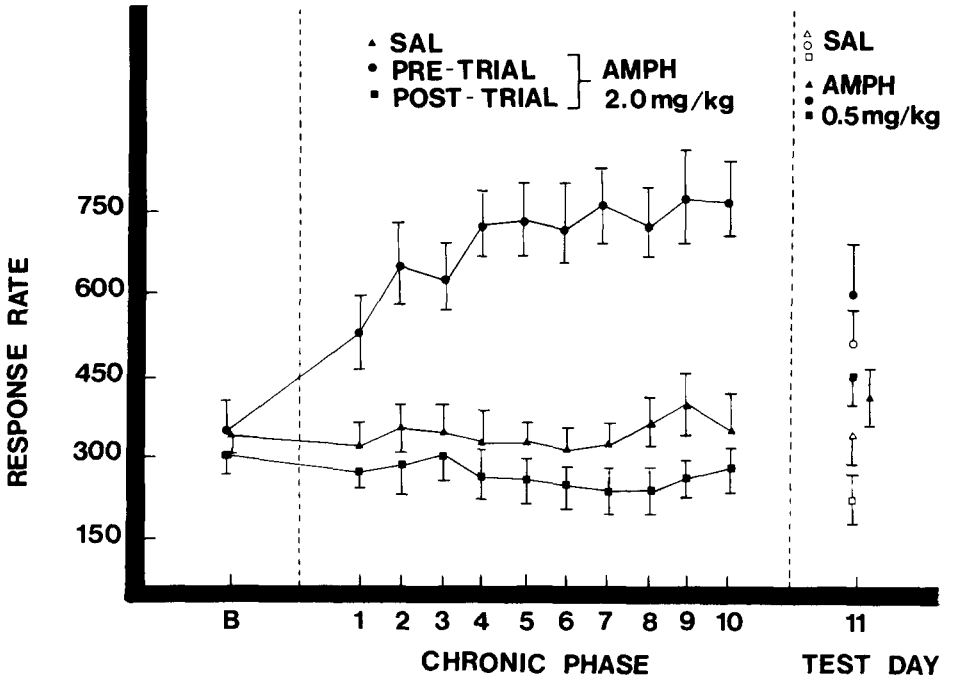

FIG. 1. Mean self-stimulation rates (±SEM) as a function of chronic drug treatment (saline, pre-trial amphetamine or post-trial amphetamine) over ten days, and test day (Day 11) drug treatment (saline or 0.5 mg/kg of d-amphetamine). The dosage of amphetamine used during the chronic phase was 2 mg/kg. B on the abscissa, represents the mean baseline self-stimulation rates of each of the three groups.

control animals, however, response rates after post-trial injections of the drug were significantly lower on Days 7, 8 and 9. These findings are consistent with previous work involving the effects of chronic amphetamine treatment on self-stimulation behavior [7].

Analysis of variance of self-stimulation rates during test day yielded significant main effects for Chronic Drug Treatment, $F(2,54)=7.15$, $p<0.01$ and Test Day Drug Treatment, $F(1,54)=9.72$, $p<0.01$. Although the interaction did not reach statistical significance Newman Keuls multiple comparisons ($\alpha=0.05$) of the simple main effects were carried out since an a priori prediction concerning the interaction was made [14]. Performance of animals chronically treated and tested with saline was not significantly different from that observed among animals chronically treated with saline and tested with d-amphetamine (0.5 mg/kg). Chronic post-trial injections of amphetamine had no effect on the self-stimulation response to the test dosage of the drug and performance was comparable to that observed among saline-amphetamine animals. In contrast, animals receiving pre-trial drug/test pairings during the chronic phase showed significantly higher self-stimulation rates after 0.5 mg/kg of the drug than did saline-amphetamine animals. Therefore, it appears that conditioning factors play a role in the development of the response sensitization. That is, although both the pre-trial and post-trial groups received an equivalent amount of amphetamine during the chronic phase, only the pre-trial groups showed enhanced response rates on test day. The finding that conditioning factors may play a role in the response sensitization is also evident when performance of the three groups on test day is considered after saline treatment. Rats that received chronic pre-trial injections of amphetamine and tested with saline showed significantly higher self-stimulation rates relative to animals exposed to chronic post-trial injections of the drug during the chronic phase and saline on test day. Self-stimulation rates among animals in the amphetamine (pre-trial)—saline groups were also higher than that observed among saline-saline animals ($p<0.01$).

With respect to the total duration of electrical brain stimulation received by animals in the three groups during the chronic phase, analysis of variance revealed a significant Group×Days interaction, $F(20,570)=2.57$, $p<0.01$. Pre-trial injections of the drug that enhanced response rates also produced an increased duration of brain stimulation relative to the remaining groups. Therefore, the duration per response was comparable between the three groups in the chronic phase of Experiment 1 and ranged from 0.30 to 0.55 sec of brain stimulation per response. The same was true for the test day results. Groups showing higher response rates after amphetamine treatment also showed higher total durations, [$F(1,54)=13.61$, $p<0.01$, for Drug on Test day main effect], which resulted in comparable amounts of electrical brain stimulation per response between groups. For example, the mean (±SEM) duration per response of the six groups on test day was 0.44 ± 0.06 and 0.50 ± 0.13 for the saline-saline and saline-amphetamine groups; 0.33 ± 0.04 and 0.30 ± 0.05 for the pre-trial saline and amphetamine groups; 0.55 ± 0.12 and 0.42 ± 0.04 for the post-trial saline and amphetamine groups. This range of electrical brain stimulation per response is consistent with that typically used in other self-stimulation paradigms.

EXPERIMENT 2

The results of Experiment 1 showed that repeated drug/test pairings modified rates of self-stimulation respond-

TABLE 1

MEAN NUMBER OF PHOTOBEAM CROSSINGS (± SEM) AS A FUNCTION OF CHRONIC DRUG TREATMENT AND TEST DAY DRUG TREATMENT

	Chronic phase										Test day	
	1	2	3	4	5	6	7	8	9	10	Saline	d-Amph (0.5 mg/kg)
Saline	50.5 ±5.8	48.5 ±4.9	54.1 ±4.9	55.9 ±5.5	57.4 ±5.4	51.8 ±5.8	62.5 ±7.2	60.6 ±4.5	58.6 ±4.5	65.7 ±3.8	71.9 ±5.8	77.1 ±15.8
Pre-trial (d-amph 2.0 mg/kg)	76.5 ±5.7	67.6 ±6.6	70.7 ±7.0	76.7 ±8.4	71.8 ±8.7	81.3 ±8.0	71.2 ±8.0	82.2 ±10.2	72.0 ±9.3	89.4 ±10.2	79.0 ±7.7	85.9 ±9.7
Post-trial (d-amph 2.0 mg/kg)	55.7 ±4.9	44.8 ±6.5	28.6 ±5.6	40.1 ±6.1	33.6 ±5.6	41.2 ±5.9	26.0 ±4.8	42.0 ±6.0	35.9 ±5.8	39.6 ±5.7	55.2 ±6.5	72.9 ±10.8

ing supported from the substantia nigra. Since a photobeam task was used to elicit self-stimulation behavior in Experiment 1, the purpose of Experiment 2 was to determine whether the increased self-stimulation rates after repeated drug/test pairings were not reflective of more photobeam interruptions due to increased levels of motor activity resulting from long-term amphetamine treatment.

METHOD

Sixty naive male rats served as subjects in Experiment 2. Apparatus specifications were identical to those described in Experiment 1, with the exception that interruption of the photobeams was used as an index of locomotor activity. Naive, unoperated rats were randomly assigned to one of three groups (n=20/cell). As in Experiment 1, two groups of rats received daily injections of either saline or 2 mg/kg of d-amphetamine and were tested for locomotor activity during a 10 min session 30 min after injection. The third group received 2 mg/kg of d-amphetamine after behavioral testing. These patterns of drug administration and testing were carried out for 10 consecutive days. On test day (Day 11), rats were subdivided such that half of the animals in each group (n=10/cell) received an injection of either saline or 0.5 mg/kg of d-amphetamine. Thirty minutes following injection subjects were tested for locomotor activity for a 10 min session.

RESULTS

The mean number of photobeam crossings as a function of drug treatment during the chronic phase and test day of Experiment 2 are depicted in Table 1. Analysis of variance of the activity data during the chronic phase yielded a significant Groups×Days interaction, $F(18,513)=2.23$, $p<0.01$. As can be seen in Table 1, locomotor activity of saline treated animals was consistent over the 10 day chronic phase. Relative to saline treated animals, d-amphetamine produced a significant increase in locomotor activity on all days during the chronic phase with the exception of Day 7. Although animals which received repeated drug/test pairings showed increased motor activity, the locomotor response to amphetamine was not observed to increase as a function of repeated testing, as was the case with self-stimulation. For example, locomotor activity during the first day of testing was not significantly different than that observed on the last day of testing. In contrast to pre-trial injections of amphetamine, post-trial injections of the drug significantly depressed motor activity during the chronic phase relative to the remaining groups. As compared to saline animals this depression was significant from Days 3–10 with the exception of Day 6. With respect to the test day results (Day 11), significant differences were not observed between the different experimental groups, regardless of the chronic drug regimen or test day drug treatment employed. Therefore, it appears that the findings of Experiment 1 were not paralleled by changes in locomotor activity after chronic exposure to amphetamine.

DISCUSSION

Consistent with previous reports [7], chronic amphetamine administration produced pronounced effects on self-stimulation responding supported from the substantia nigra. Repeated injections of 2 mg/kg of the drug induced a progressive augmentation of self-stimulation responding. Moreover, it appears that conditioning or learning variables play a role in the observed behavioral sensitization. That is, animals that received repeated injections of d-amphetamine such that the systemic effects of the drug were congruent with behavioral testing, showed facilitated self-stimulation rates to a test dosage of the drug which otherwise had little or no behavioral effect on performance. In contrast, animals that received drug treatment following behavioral testing during the chronic phase, did not exhibit increased self-stimulation rates to amphetamine treatment on test day relative to saline-amphetamine animals. Since the behavioral sensitization was observed only among animals that received chronic pre-trial injections of amphetamine, but not among rats that were treated with chronic post-trial drug injections, it is likely that conditioning factors play a role in the development of the response sensitization. That conditioning variables are involved in the increased self-stimulation rates after chronic exposures to amphetamine is also evident when performance on test day is considered after saline treatment. Animals that received chronic drug/test pairings and tested with saline showed increased self-stimulation rates relative to that observed among animals exposed to chronic post-trial injections of amphetamine and saline on test day.

Several possibilities exist which may account for the be-

havioral effect of chronic amphetamine treatment on self-stimulation responding. As discussed previously, amphetamine may be stored in adipose tissue and the accumulation of the drug in mobilization pools may be responsible for the sensitized response to amphetamine after chronic exposure to the drug [12]. In the present study, however, this possibility is unlikely given that the response sensitization observed on test day was only evident among animals that received chronic pre-trial injections of the drug, and not among rats that were exposed to chronic post-trial injections, despite the fact that both groups received comparable amounts of amphetamine during the chronic phase. A more likely possibility involves the effects of chronic amphetamine treatment on dopamine receptors. Specifically, chronic amphetamine treatment may result in dopamine receptor supersensitivity [4]. Thus, with respect to amphetamine-induced stereotypic behaviors, it has been demonstrated that the enhanced efficacy of dopaminergic neuronal transmission may be responsible for the occurrence of augmented stereotypic behaviors following chronic amphetamine treatment [2,3]. As was observed with stereotypic behaviors, chronic administration of high doses of d-amphetamine in the absence of drug-test pairings produced enhanced self-stimulation responding from the substantia nigra after a low test dosage of the drug which ordinarily had no behavioral effect, suggesting a role of dopamine receptor supersensitivity [7]. In the present study, however, the development of hypersensitive dopamine receptors following chronic amphetamine treatment cannot account for all the variance. If the enhanced response rates observed on test day among animals repeatedly exposed to drug/test pairings and tested with amphetamine was the result of dopamine receptor supersensitivity, then a similar behavioral response should have been observed among rats that received chronic post-trial injections of the drug. Further to this point, a model involving the development of dopamine supersensitivity cannot adequately account for the augmented rates of responding after saline injection on test day among the chronic pre-trial amphetamine animals.

Although modified receptor sensitivity following long-term amphetamine treatment may be involved in the sensitized behavioral response to amphetamine following chronic administration of high dosages of the drug [7], the findings of this investigation suggest that with respect to chronic administration of the drug in moderate dosages, the contiguity between the systemic effects of the drug and the behavioral test situation play a role in the development of the response sensitization.

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