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Acquisition of Intracranial Self-Stimulation in Medial Prefrontal Cortex of Rats Facilitated by Amphetamine

CHARLES H. K. WEST[1] AND RICHARD P. MICHAEL

Department of Psychiatry, Emory University School of Medicine, Atlanta, GA 30322 and Georgia Mental Health Institute, 1256 Briarcliff Road, NE, Room 504-N, Atlanta, GA 30306

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WEST, C. H. K. AND R. P. MICHAEL. *Acquisition of intracranial self-stimulation in medial prefrontal cortex of rats facilitated by amphetamine*. PHARMACOL BIOCHEM BEHAV 24(6) 1617–1622, 1986.—Two groups of rats were trained to lever press for intracranial self-stimulation (ICSS) in the medial prefrontal cortex (mPFC) using a uniform amount of stimulation for all animals. One group acquired the lever pressing task very gradually during saline pretreatment but dramatically improved its rate of acquisition during the third week of training when pretreated with *d*-amphetamine (0.5 mg/kg). Administration of amphetamine to the other group of rats before each of the first five training sessions greatly facilitated acquisition of the ICSS task, and a significant improvement in performance over the saline control group appeared on the third day of training. After ICSS performance had stabilized, testing the animals revealed a significant amphetamine-induced increase in rate over the dose range of 0.25 to 1.0 mg/kg. These effects of amphetamine suggest that ICSS in mPFC is sensitive to changes in catecholamine neurotransmission during both the acquisition and maintenance of this behavior.

Amphetamine Medial prefrontal cortex Self-stimulation Learning Reward Seizures

THE behavioral and neural processes underlying intracranial self-stimulation (ICSS) in the medial prefrontal cortex (mPFC) of the rat differ from those in the better studied hypothalamic sites. One difference is that the acquisition of the operant task proceeds considerably more slowly, taking a period of several (6–8) days, and this is unlike the more rapid acquisition (2–3 days) observed in hypothalamic or tegmental sites [4, 8, 17]. One view is that stimulation of mPFC somehow sensitizes this structure to subsequent stimulation in a process that is similar to the kindling of seizures in cortical and limbic structures. Presumably, stimuli which initially produce little or no positive reinforcement are able to do so upon repeated application over a period of days. Recent reports have indicated that certain manipulations can alter the speed of ICSS acquisition in mPFC. For example, prior application of non-contingent stimuli to mPFC or to sulcal prefrontal cortex increased the rate of acquisition [4,17]. On the other hand, non-contingent stimulation in the lateral hypothalamus prior to ICSS training was without such an effect on acquisition in mPFC [17]. Consistent with the kindling analogy, treatment with anticonvulsant drugs has been observed to attenuate the facilitatory effect of non-contingent electrical stimulation of mPFC prior to ICSS training [18]. Among the many neurotransmitters that might be affected by stimulation in the mPFC, it has been proposed that the mesocortical dopamine system, which terminates in prefrontal cortex, plays at least a modulatory role [10–12, 15]. These and other studies have suggested to us that examining the acquisition of ICSS in mPFC might provide additional information on the neural processes involved in learning, kindling and in the reinforcing effect of electrical stimulation in neocortex. We have, therefore, studied the effects of *d*-amphetamine, a dopamine agonistic drug, on the rate of acquisition of ICSS in the mPFC.

METHOD

Animals and Surgery

Male Sprague-Dawley rats weighing between 300–450 g at the time of surgery were implanted with 125 μm diameter bipolar platinum electrodes (Plastic Products Co., Roanoke, VA) under sodium pentobarbital (50 mg/kg, IP) anesthesia. With the incisor bar 5.0 mm above the interaural line, the coordinates were 10 mm anterior to the interaural zero, 0.8 mm left of the midline and 3 mm ventral to the dural surface

[1]Requests for reprints should be addressed to Charles H. K. West, Ph.D., Biological Psychiatry Research Laboratory, Georgia Mental Health Institute, 1256 Briarcliff Road, NE, Room 504-N, Atlanta, GA 30306.

[13]. Animals were housed in groups of three or four and provided with food and water ad lib throughout the experiment. Between tests, they were maintained in a colony rat room with lights on between 7:00 a.m. and 7:00 p.m.

Apparatus and Testing

Following a recovery period of two weeks, rats were assigned randomly to one of two drug treatment groups and were tested in 30-minute sessions five days per week between 12:00 and 6:00 p.m. At the start of a session, animals were connected to a stimulus source and were placed inside a sound-attenuating, lighted chamber (inside dimensions: 31×30×29 cm). Depressing a lever on one wall of the chamber 10 cm above the grid floor activated a stimulator. Stimuli were generated by two Model S44 stimulators (Grass Instrument Co., Quincy, MA), each connected to a stimulus isolator and a constant current unit. Stimulus parameters were the same for all animals throughout training and drug testing. Each 200 msec stimulus train consisted of monophasic, square wave pulses 1 msec in duration at a current intensity of 150 μA and at a frequency of 100 Hz. The polarity of successive stimuli was reversed, so that every other stimulus was of the same polarity. Animals were trained with a maximum of 500 experimenter-administered stimuli during each session. Once the combined number of experimenter- and self-administered stimuli reached 500, no further experimenter-administered stimuli were given for that session. However, animals were free to obtain more than 500 stimuli per session by lever pressing spontaneously. The effect of this procedure was to equalize the amount of daily stimulation each animal received during the critical process of acquisition before the lever pressing task was fully learned.

Drugs and Protocol

d-Amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% saline with concentrations calculated as free base. All injections were given 15 minutes before the start of the session in a volume of 1 ml/kg. Before each of the first five training sessions, Group 1 animals (n=6) were administered *d*-amphetamine (0.5 mg/kg, SC) and Group 2 animals (n=6) were administered saline (SC). Both groups received daily saline injections during the second week of testing (days 8–12) and *d*-amphetamine (0.5 mg/kg) injections during the third week (days 15–19). One additional animal initially in Group 2 was omitted from data analysis because it failed to show any lever pressing behavior after more than three weeks of training. All other animals displayed ICSS behavior, although some required more priming by the experimenter than others.

The effect on the rate of ICSS of two additional doses (0.25 and 1.0 mg/kg, SC) of *d*-amphetamine was subsequently tested in 11 animals (one was eliminated due to severe decrement in performance). Before each fifteen minute test, rats received five consecutive daily injections of saline followed by five consecutive daily injections of amphetamine followed by a repetition of the saline treatment, and this regimen was repeated for each dose of amphetamine in a counterbalanced design. The mean rate of lever pressing per session during a week of amphetamine treatment was compared with the rates during the preceding and succeeding weeks of saline. The effect on ICSS of 0.5 mg/kg *d*-amphetamine was obtained by comparing the values from the second week of testing (saline) with the values from the third week of testing (amphetamine) for Group 1. The rates obtained for 30-minute sessions were divided by two for comparison with the rates for 15-minute sessions.

Data Analysis and Histology

Throughout these experiments, the stimuli that each animal received were initiated either by the experimenter during priming or shaping (experimenter-administered stimuli) or by the animal itself pressing a lever (self-administered stimuli). Thus, one measure of ICSS performance was the percentage of stimulations by the animal, which consisted of the number of self-administered stimuli divided by the total number of stimuli per session (experimenter-administered + self-administered stimuli) expressed as a percentage. Results for the acquisition experiment are given in two forms: (1) total number of lever presses per session and (2) the percentage of stimulations by the animal. Results from tests on the rate of ICSS as affected by amphetamine are expressed as the number of lever presses per 15 minutes.

During these studies, several animals sporadically displayed signs of seizure activity. The first convulsions appeared on the second day of testing, and the number of animals having convulsions progressively increased during the first two weeks. Typically, an animal would recuperate within a minute or so, and lever pressing would be resumed. When appropriate, the session length was adjusted to compensate for time lost due to the seizure. There were no significant differences between Groups 1 and 2 for the total number of seizures recorded (Student's *t*-test). To assess the significance of differences between groups during acquisition, analyses of variance using a two-factor mixed design for repeated measures on one factor, were performed [2]. F-ratios were further evaluated using Tukey's test (two-tailed) to compare daily means. A completely randomized analysis of variance was used to compare rates of ICSS at the different doses of amphetamine. *t*-Test for related measures was used where animals served as their own controls.

When testing was completed, rats were heavily anesthetized with sodium pentobarbital and were perfused transcardially with 0.9% saline followed by 10% formalin. Brains were removed, and frozen sections were cut at 50 μm. Sections were stained with cresyl violet in order to localize accurately the sites of the electrode tips.

RESULTS

Effects of d-*Amphetamine on the Acquisition of Lever Pressing*

In Group 1 animals, the administration of 0.5 mg/kg *d*-amphetamine before each of the first five training days greatly facilitated the acquisition of lever pressing for ICSS in mPFC. This was confirmed by the analysis of variance which showed a highly significant interaction between days 1–5 (week 1) of acquisition and drug effect for both the number of lever presses made by animals, $F(4,40)=7.23$, $p<0.001$, and the percentages of stimulations made by animals, $F(4,40)=9.32$, $p<0.001$. The facilitatory action of amphetamine was evident by the second training day, and both measures of ICSS performance were significantly increased ($p<0.01$) for Group 1 animals by day 3, as shown in Fig. 1. By day 5, the numbers of lever presses per session were 722±182 (mean±SEM) for Group 1 and 62±32 for Group 2, while the percentages of stimulations made by animals were 76±14% for Group 1 and 13±7% for Group 2.

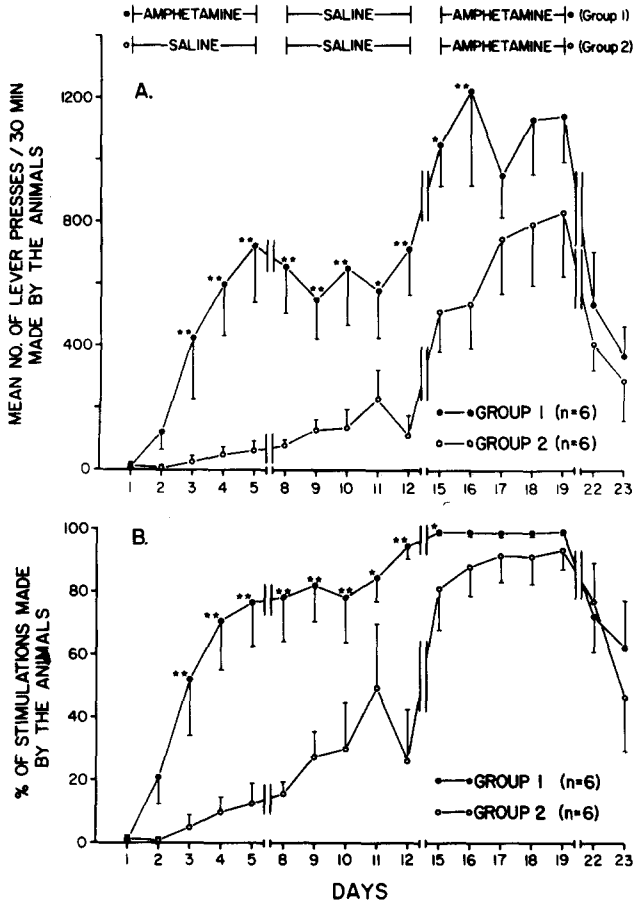

FIG. 1. Acquisition curves for intracranial self-stimulation in rats represented (A) by mean numbers of lever presses made by the animals, and (B) by the percentages of stimulations made by the animals during the 30-min sessions. Regimen of daily pretest drug treatment with either saline or 0.5 mg/kg *d*-amphetamine is indicated above graphs for Group 1 (●) and Group 2 (○). Number of days from first training session (Day 1) given on abscissa. Vertical bars give standard errors of means. n=Number of animals per group. Significant differences between daily means for the two groups (analysis of variance, Tukey's test) indicated by stars (★$p<0.05$, ★★$p<0.01$).

Five out of six Group 1 animals were performing at the 95% self-administration level by day 11. In contrast, animals in Group 2, treated with saline for the initial training sessions, acquired the ICSS lever pressing task very slowly. Only two of these six animals were performing at the 95% self-administration level by day 11.

During the second week of testing when all animals received saline injections, the ICSS performance of both groups gradually improved (Fig. 1, days 8–12). Both measures of performance for Group 1 remained well above those for Group 2 throughout that week, having greatly improved the previous week during amphetamine administration. This indicated that the lever pressing performance of Group 1 animals stabilized at the higher level after amphetamine treatment was stopped. During the third week (Fig. 1, days 15–19), both groups received amphetamine injections before each session. From week 2 to week 3, Group 1 showed a further significant increase in the mean numbers of lever presses, although there was no significant increase in the mean percentage of stimulations made by the animals (Table 1). Group 2, which received amphetamine treatment for the first time during week 3, showed a highly significant increase ($p<0.001$) in both measures compared with the previous week (Fig. 1 and Table 1). In Group 2, 4 of the 6 rats were performing below the 20% self-administration level after 10 saline sessions, whereas three of these four rats reached the 95% level after three sessions with amphetamine pretreatment. No injections were given on the final two days (days 22 and 23) of this experiment, and both measures declined markedly. During subsequent testing, baseline ICSS performance remained around these lower levels.

Effects of d-*Amphetamine on ICSS Rates*

Comparison of data from Group 1 for weeks 2 and 3 indicate that 0.5 mg/kg *d*-amphetamine produced a significant increase of 74% in ICSS rates in these animals (Table 1). This effect was confirmed using two additional doses of *d*-amphetamine in the same experimental protocol. With 0.25 mg/kg, there was a 52% increase in rate and with 1.0 mg/kg, there was a 78% increase compared with saline (Fig. 2). The effect of amphetamine on ICSS rate was not dose-dependent, $F(2,25)=0.45$, NS, with the range of doses tested here.

To ascertain if repeated daily treatments with *d*-amphetamine without stimulation would materially affect ICSS, additional drug testing was performed in five rats. Animals were treated with 0.5 mg/kg *d*-amphetamine or saline for four consecutive days per week (Monday–Thursday) without ICSS, and were then given a single ICSS session on the Friday of each week, 24 hours after the last injection. Tests were run for three weeks in the order: first week amphetamine, second week saline, third week amphetamine. There were no significant differences between treatments (*t*-tests for related measures), and the data indicated that the administration of amphetamine without stimulation in mPFC did not produce any facilitation of ICSS.

Histological Findings

The locations of electrode tips for 10 of the 12 rats are shown in Fig. 3. Due to an error in the laboratory, the brains of 2 animals were lost. There was a tendency for rats with more posteriorly placed electrodes to show better overall ICSS performance. Since electrode sites for Group 1 tended by chance to be more posterior than Group 2, there was a possibility of an effect due to electrode placement. However, this was unlikely because Group 2 animals showed a brisk ICSS response with amphetamine treatment. The frequency of seizures was not correlated with ICSS performance. However, the two rats with most seizures had electrodes on the edge of the callosal fiber bundle, and animals with fewer convulsions had electrodes located more medially within gray matter.

DISCUSSION

The results of the present study have shown that repeated daily administration of *d*-amphetamine facilitated the acquisition of the lever pressing task for ICSS in mPFC. This facilitation, which was observed as early as the second day of ICSS training, continued throughout the five days of drug administration. After ICSS performance had stabilized, *d*-amphetamine in the dose range of 0.25–1.0 mg/kg signifi-

TABLE 1

ICSS PERFORMANCE DURING TREATMENT WITH SALINE (WEEK 2) OR WITH 0.5 mg/kg AMPHETAMINE (WEEK 3)

	Group 1		Group 2	
	Mean No. of Lever Presses*/Session	Mean % Stimulations by Animals	Mean No. of Lever Presses*/Session	Mean % Stimulations by Animals
Week 2 (Saline)	630 ± 140	83 ± 10	138 ± 45	30 ± 11
Week 3 (Amphetamine)	1099 ± 143	99 ± 1	684 ± 138	89 ± 9
	$p<0.02$	NS	$p<0.001$	$p<0.001$

*By animals.

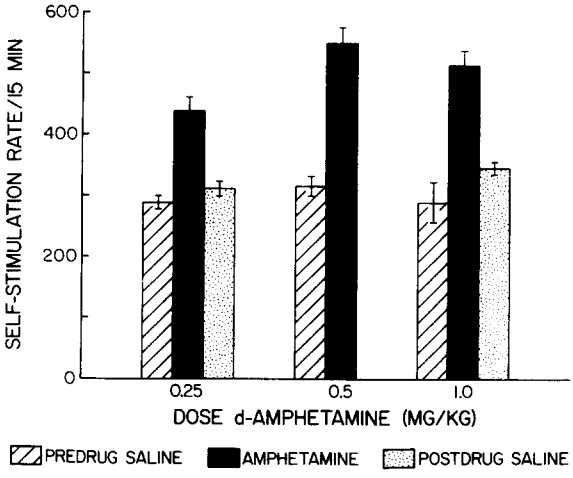

FIG. 2. Effects of different doses of amphetamine on the rate of intracranial self-stimulation in the medial prefrontal cortex of rats (15-min sessions). Amphetamine (solid bars) administered daily for five days significantly increased ($p<0.001$) rates relative to the preceding (hatched bars) and following (stippled bars) five day periods of saline treatment.

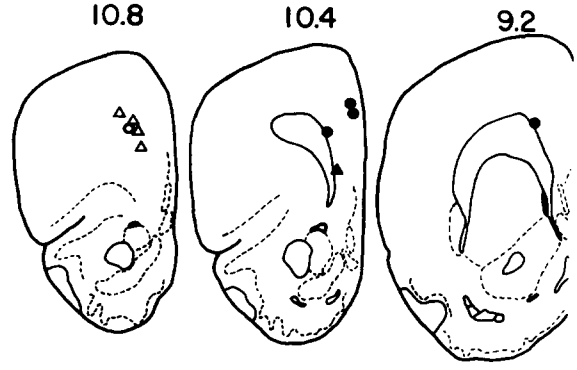

FIG. 3. Locations of electrode tips for Group 1 (circles) and Group 2 (triangles) shown on coronal sections through frontal cortex (from atlas of Pellegrino, Pellegrino and Cushman [13]). Numbers above sections give planes anterior to interaural zero. Symbols indicate good (solid) or poor (open) self-stimulation performance.

cantly increased lever pressing rates. However, with the doses used here the effect was not dose-dependent unlike results typically obtained from hypothalamic sites [8,19].

The mechanism of enhanced ICSS acquisition with *d*-amphetamine is not known, but five possibilities will be mentioned. (1) Many studies have suggested that catecholamines are involved in learning (e.g., [1]). Therefore, animals might be capable of learning the operant task faster due to an amphetamine-induced increase in dopamine and/or norepinephrine neurotransmission. With this explanation, the strength of the stimulus is presumed to be near the reinforcement threshold in mPFC during the first week of training so that saline-treated animals acquire the ICSS task slowly whereas amphetamine-treated animals learn more rapidly. (2) The second explanation is closely related to the first. Perhaps amphetamine-treated rats are more motivated than saline-treated ones to perform the necessary work for stimuli that are barely above the reinforcement threshold. Thus, amphetamine-treated animals start to work sooner for stimuli of the same reinforcing value as those received by saline-treated animals. (3) The third explanation is that a generalized amphetamine-induced increase in the level of locomotor activity might facilitate the acquisition process. (4) The fourth explanation is that stimulation of mPFC might produce another reaction that interferes with the acquisition process, as has been proposed by Corbett and colleagues [5]. Amphetamine, then, might reduce this disruptive effect and allow more rapid acquisition. (5) Finally, it is possible that stimuli are more reinforcing to animals treated with amphetamine. If such an effect occurs, it is probably due to an increase in the rate of sensitization of mPFC to stimulation since both groups performed at an equal level on the first day of training. Any one or combination of these hypotheses might account for the facilitation we have observed in ICSS acquisition by amphetamine. Due to the wide spectrum of behavioral effects reported for amphetamine, it should not

be assumed that amphetamine increased reinforcement and/or learning during acquisition of the lever pressing task, although these remain viable possibilities. Since the aim of this paper is to simply describe the phenomenon, underlying mechanisms must be left for further investigation.

With the testing conditions used in this study, amphetamine enhanced both acquisition and subsequent rate of ICSS in mPFC, although several earlier studies described little or no increase in ICSS rates in mPFC implanted rats treated with *d*-amphetamine. Procedural differences may account for the difference. For example, in two studies, longer injection-ICSS test intervals (30–40 minutes) were used, and locomotor activity tests were performed between the amphetamine administration and the ICSS sessions [3,8]. In other studies, a random interval 10 second schedule was used to test the effects of amphetamine on rates [6, 7, 16], and animals were injected at the start of a 60 minute test session immediately following a 25 minute ICSS baseline period. In a paradigm involving a choice between two ICSS sites, amphetamine increased the preference for lateral hypothalamic over mPFC sites, but it also significantly increased the rate of ICSS in both sites [9]. However, Phillips and Fibiger [15], using a protocol rather similar to ours, reported that 1.0 mg/kg *d*-amphetamine (IP) produced an increase of 214% in mPFC ICSS rates in a group of rats prior to receiving lesions of ascending dopaminergic projections. Clearly, testing protocols are critical in determining the effects of this drug on ICSS. The protocol of 5 consecutive daily injections of amphetamine probably did not influence the observed effect on ICSS rates since there was no significant change in rate throughout each five-day drug test period and since a large rise in rate was observed on the first day of each drug treatment period.

It is increasingly apparent that the properties of ICSS in mPFC differ from those in other ICSS sites, including the lateral hypothalamus (e.g., [20,21]). Thresholds are higher and rates of acquisition are slower in mPFC. Also, we observed in this study that ICSS in mPFC was associated with overt signs of seizure activity. Although others have reported that seizures were rare, anticonvulsant drugs did attenuate mPFC sensitization in the same rats [18]. Strain differences and electrode placement may account for the differences. Whether or not seizure after-discharge activity is necessary to sustain ICSS in mPFC is not known. The development of seizures and the initial appearance of ICSS behavior appeared to be associated in time, although not all self-stimulating rats displayed signs of seizure and the phenomena were not closely correlated. The occurrence of convulsions probably did not affect the amphetamine induced facilitation of ICSS acquisition since there was little difference between groups either in total numbers of convulsions during the first week of training (Group 1=10; Group 2=12) or in the timing of the initial appearance of seizures. Since seizures can alter the behavioral responses to drugs [14], it is possible that they may have affected the sensitivity of animals to amphetamine. Furthermore, small seizures or after-discharge activity evoked by individual stimuli might limit the rate at which an animal can lever press for ICSS. This could explain why mPFC rats show little or no increase in ICSS rate with increased stimulus current intensity [8,16].

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