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Postcocaine Depression and Sensitization of Brain-Stimulation Reward: Analysis of Reinforcement and Performance Effects

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KOKKINIDIS, L. AND B. D. McCARTER. *Postcocaine depression and sensitization of brain-stimulation reward: Analysis of reinforcement and performance effects.* PHARMACOL BIOCHEM BEHAV **36**(3) 463–471, 1990.—The effects of acute and chronic cocaine administration on intracranial self-stimulation (ICSS) were evaluated in a two-hole nose-poke discrimination paradigm. Analysis of ICSS rates as a function of current intensity revealed that cocaine increased rates of responding in a dose-dependent manner (5.0–20.0 mg/kg), resulted in a shift to the left of the rate-intensity function, and decreased thresholds for half-maximal responding. Brain-stimulation reward was modified by chronic exposure to cocaine, however, the direction of change was dependent on the schedule of drug administration. Repeated daily administration of cocaine (40.0 mg/kg) and ICSS testing 24 hr postinjection decreased rates and increased reward thresholds. A response depression was also observed when time-dependent variations in ICSS performance were evaluated after repeated cocaine administration. Using a different chronic cocaine/test schedule (30.0 mg/kg, twice daily), a sensitization of ICSS and decreased reward thresholds developed when rate-intensity functions were determined after 5-day drug intervals. These findings were discussed in terms of the role of dopamine in modulating central reward processes. It was suggested that depressed reward-system functioning might reflect reduced dopamine synthesis following cocaine withdrawal, and the ICSS sensitization was related to long-term compensatory changes in dopamine neurotransmission possibly involving presynaptic mechanisms.

Brain-stimulation reward Ventral tegmental area (VTA) Cocaine Dopamine (DA) Depression Sensitization

IT is well accepted that the euphoric consequences of cocaine administration play a significant role in the abuse potential of this drug (48). Considerable animal research has been conducted in order to delineate the neurochemical and neuroanatomical substrates modulating cocaine-induced reward. Like other stimulant drugs, cocaine is readily self-administered in animals (42), and increases ICSS responding with a corresponding decrease in reward thresholds (10,14). While cocaine induces a variety of neurochemical effects, there is substantial evidence implicating the involvement of mesolimbic and mesocortical DA activity in the maintenance of self-administration behavior (18, 19, 43). Mesolimbic DA activity also modulates brain-stimulation reward (52), with both cocaine administration and rewarding electrical stimulation of lateral hypothalamic and ventral tegmental brain regions, resulting in increased extracellular DA levels in the nucleus accumbens (21,38).

In addition to its reward-enhancing effects, cocaine intake alters mood, invoking a dysphoric state in humans after withdrawal from repeated use (16,17), and the depressive symptoms associated with cocaine withdrawal can act as a negative reinforcer perpetuating the continued use of the drug (9). Research with animals involving amphetamine has revealed that chronic administration of this stimulant elicits a pronounced and sustained depression in rates of ICSS responding after drug withdrawal (27–29), and a corresponding increase in reward threshold (7,31). The development of a postamphetamine depression of ICSS suggests that reward-system hypoactivity may be responsible for the dysphoric symptoms associated with drug abuse in humans, resulting from a decrease in DA synthesis and subsequent DA depletion after chronic amphetamine treatment (26).

The long-term effects of stimulant administration on ICSS are not limited to the development of withdrawal depression. Sensitization of brain-stimulation reward is also observed after repeated drug exposure and this typically can be elicited by drug challenge (27), exposure to a stressor (2), or brain stimulation (44). In an ICSS paradigm, for example, animals will show depressed brain-stimulation reward when tested during amphetamine withdrawal. However, increased responding and decreased reward thresholds developed after challenge with a low dose of amphetamine (26). Mechanisms subserving the sensitization effect appear to involve long-term changes in DA neurotransmission (46), with recent research demonstrating an amphetamine-elicited enhancement of DA neuronal release in nucleus accumbens and striatum after repeated drug exposure (45,47). Consistent with these neurochemical observations, amphetamine-induced ICSS sensitization has been observed from both the nigrostriatal and mesolimbic pathways (27, 28, 39).

The aim of the present series of experiments was two-fold. First, to elaborate on the dose-response effects of acute cocaine administration on ICSS, and second to assess changes in ICSS

rates and reward thresholds after exposure to several schedules of chronic cocaine administration. This was accomplished by determining the effects of cocaine on ICSS rate-intensity functions after acute and repeated drug injections, and by evaluating time-dependent changes in ICSS responding following cocaine administration.

EXPERIMENT 1

The purpose of Experiment 1 was to assess the effects of several doses of cocaine on brain-stimulation reward using a two-hole nose-poke discrimination ICSS paradigm. In this ICSS task a previously neutral stimulus (light) is associated with brain stimulation. By alternating the light onset between holes at predetermined intervals, animals learn to adjust their responding accordingly, and receive rewarding brain stimulation only when responding is directed to the signalled hole. In this way, correct (reinforced) and incorrect (nonreinforced) responses can be measured within a session at a particular current intensity.

This procedure was based on the premise that the error measure may provide a sensitive index of changes in performance. The extent to which animals continue to respond into the unsignalled hole is, to some degree, a reflection of the behavioral arousal associated with brain-stimulation reward. While error responding may reflect a variety of factors, e.g., response perseveration, or even associative changes (extinction), it allows for the determination of reinforcement and performance effects within the same ICSS test session.

Work from this laboratory has shown that both correct and error responses are facilitated by increasing current levels, indicating that the incorrect response measure is sensitive to the behavioral arousal associated with the rewarding effects of higher current intensities. Moreover, drug treatments may have differential effects on these measures. For example, chronic exposure to desipramine increased the number of correct responses and shifted the rate-intensity function to the left without appreciably modifying the number of errors (32). These results were interpreted to suggest that the reward-enhancing effects of chronic exposure to this tricyclic antidepressant [see also (13)], were not related to nonspecific changes in performance (e.g., motor hyperactivity) typically seen after withdrawal from desipramine.

METHOD

Subjects

Sixteen naive male Wistar rats procured from the Canadian Breeding Farms and Laboratories (Quebec) served as subjects. Rats weighed 250–300 g at the initiation of the experiment and were permitted free access to food and water throughout the duration of the experiment. Rats were housed individually in a regular 12-hr light/dark cycle and testing was carried out during the light portion of the cycle.

Apparatus

ICSS testing was conducted in four identical black Plexiglas boxes (60 cm in length × 50 cm in width × 35 cm in height). Two holes, 4 cm in diameter and 10 cm apart, were located in the centre of the floor of each box. A ring of lights embedded in the black Plexiglas floor with an opaque cover (2 cm in width) surrounded each hole. Three infrared photobeam units were mounted in the Plexiglas of each hole 0.5 cm from the top, and disruption of the photobeams by a nose-poke response resulted in electrical brain stimulation. A constant current stimulator (Schnabel Electronics, Saskatoon) delivered brain stimulation which consisted of a monophasic square wave with a pulse duration of 0.1 msec and a pulse frequency of 100 Hz. Once initiated by a nose-poke response the stimulation had a duration of 0.5 sec. All boxes were interfaced to a Commodore 64 computer whose software controlled the presentation of electrical stimulation, the discrimination procedure which involved alternating the onset of lights around each hole at predetermined intervals, as well as recording the number of nose-poke responses in each hole during ICSS testing.

Procedure

Surgery. Subjects were anesthetized with sodium pentobarbital (Somnotol, 60 mg/kg) and were stereotaxically implanted with bipolar electrodes (MS-303/1, Plastic Products Co.) in the medial forebrain bundle at the level of the lateral hypothalamus (AP −1.5 mm from bregma, L ±1.5 mm from midline suture an V −8.5 mm from the skull surface) (36). Electrodes had 0.5 mm of the tips scraped and the tips were separated by 0.5 mm and were implanted perpendicular to the horizontal plane. The incisor bar was adjusted for each animal such that the horizontal plane was level for both posterior and anterior portions of the skull.

ICSS training. Seven days after recovery from surgery animals were trained for ICSS. During the training session the light surrounding one of the holes was always on and a nose-poke into this hole resulted in electrical brain stimulation, whereas responding into the unsignalled hole was not reinforced. After stable response rates were established at a current level which was adjusted for each individual subject discrimination training was initiated. Light onset was alternated between holes every 30 sec for a 5-min ICSS session. Animals received electrical brain stimulation only when a nose-poke response was made in the hole signalled by the light. When animals performed correctly on at least 90% of the total responses made during the session the alternation time was reduced to 20 sec and the ICSS test duration to 4 min. This procedure was continued until the alternation time was 10 sec with an ICSS trial duration of 2 min.

Current-response baseline and drug treatment. Once animals mastered the discrimination paradigm baseline rates of responding were established as a function of descending and ascending current presentation. This involved placing animals into the apparatus and allowing them a 5-min ICSS session at their individual optimal current intensity (ranged from 36–50 μA RMS). The current was then set at 44 μA (midpoint of optimal range) and was decreased by increments of 4 μA in a stepwise fashion. Correct and incorrect response rates were recorded for 2 min at the following current levels: 44, 40, 36, 32, 28, 24, 20 and 16 μA. After completion of the descending phase of the test session current was increased by 4 μA for 8 steps and the number of correct and incorrect responses was recorded for 2 min at each level of the ascending mode of current presentation.

After stable baseline rates were established each subject was tested for ICSS in the descending and ascending current modes immediately following an intraperitoneal (IP) injection of saline, 5.0, 10.0, and 20.0 mg/kg of cocaine hydrochloride. Since a repeated drug administration procedure was used in this experiment, the order of drug presentation was randomized for each animal, and 48 hr between drug treatments was allowed to minimize carry-over effects. During this drug-free period subjects received daily ICSS testing and stable baseline response rates were maintained.

RESULTS AND DISCUSSION

At the termination of the experiment rats were overdosed with sodium pentobarbital and perfused intracardially with 0.9% saline followed by 10% Formalin. The brains were removed and frozen

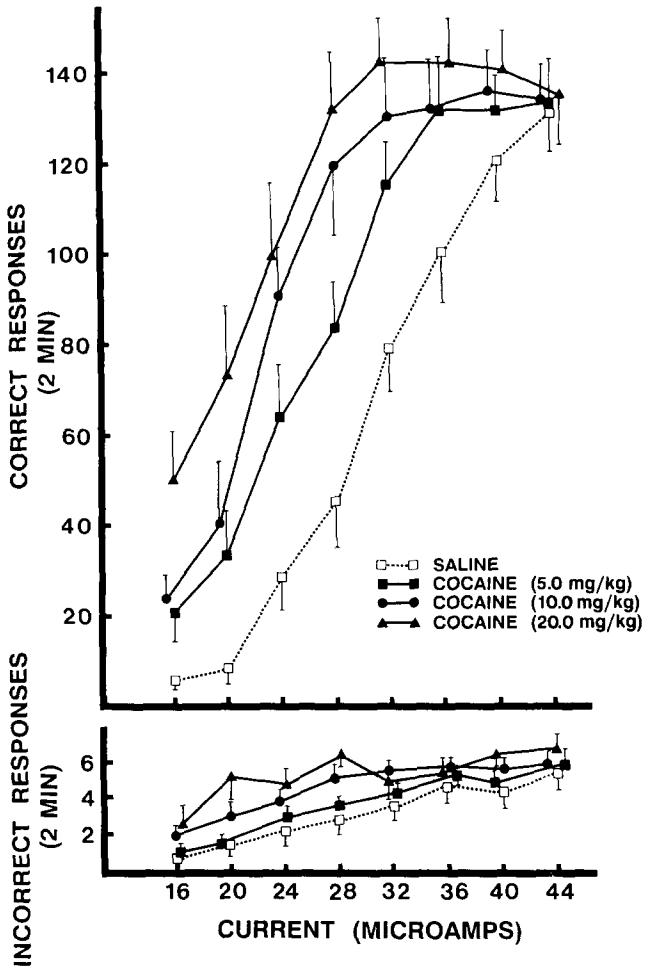

FIG. 1. Mean ICSS rates (correct responses) and incorrect responses (±S.E.M.) as a function current intensity and acute drug injection (saline, 5.0, 10.0 and 20.0 mg/kg of cocaine).

coronal sections were cut at 40 μm and stained with thionine for verification of electrode tracts. In those animals that completed the experiment histological examination of electrode sites confirmed that placements were in the region of the lateral hypothalamus (AP range −1.0 to −1.6 mm from bregma) (36). Three animals were discontinued from ICSS testing due to low levels of responding resulting from inaccurate electrode placements and one animal did not complete the experiment because of head cap loss.

ICSS response scores were averaged over ascending and descending current presentation modes and the mean number of correct and incorrect responses as a function of current intensity and drug treatment is depicted in Fig. 1. A two-way analysis of variance with repeated measures on both factors yielded significant main effects for Drug Treatment, $F(3,33)=16.22$, $p<0.001$, and Current Intensity, $F(7,77)=72.29$, $p<0.001$, as well as a Drug Treatment × Current Intensity interaction, $F(21,231)=3.89$, $p<0.001$. As shown in Fig. 1, ICSS rates of saline-treated animals increased as a function of current presentation, and cocaine injection further enhanced responding in a dose-dependent fashion resulting in a shift to the left of the rate-intensity functions. Newman-Keuls multiple comparisons of the simple main effects involved in this interaction ($\alpha=0.05$) showed that all doses of the drug increased ICSS responding at the 16–36 μA intensities relative to saline.

Miliaressis *et al.* (34) indicated that frequency threshold measures involving zero- and half-maximal responding are a good indication of the reinforcing efficacy of electrical brain stimulation. In this experiment we varied current (30), and not frequency, and since our lowest current intensity (16 μA) maintained ICSS behavior, it was not possible to analyze current thresholds for zero-responding performance. However, thresholds for half-maximal responding were determined for each animal, and one-way analysis of variance of these data revealed that cocaine modified reward thresholds, $F(3,33)=20.20$, $p<0.001$. Newman-Keuls multiple comparisons ($\alpha=0.05$) indicated that the current necessary to maintain half-maximal responding was significantly decreased by all three doses of cocaine (see Table 1).

Error scores were increased as a function of current level, $F(7,77)=12.08$, $p<0.001$, and cocaine treatment further enhanced the number of incorrect responses made during ICSS testing, $F(3,33)=8.22$, $p<0.001$. As can be seen in Fig. 1, the 10.0 and 20.0 mg/kg doses of the drug increased error responding, while injection of 5.0 mg/kg of cocaine did not modify the incorrect response measure. These results suggest that the discrimination procedure used in the assessment of ICSS is sensitive to both the reinforcing and the behavioral-activating effects of electrical brain stimulation and that cocaine can influence both of these measures (see the General Discussion section).

EXPERIMENT 2

In Experiment 2, changes in brain-stimulation reward were

TABLE 1

MEAN (±S.E.M.) REWARD THRESHOLDS (μA, RMS) FOR HALF-MAXIMAL ICSS RESPONDING AFTER ACUTE AND REPEATED COCAINE ADMINISTRATION IN EXPERIMENTS 1 AND 2

Experiment 1 (Acute)							
Saline	5.0 mg/kg	10.0 mg/kg	20.0 mg/kg				
30.2±1.1	24.1±1.6	22.7±1.3	19.6±1.0				
Experiment 2 (Chronic)							
Drug Schedule 1							
Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
27.8±1.0	32.2±1.6	29.2±1.1	32.5±2.1	31.6±2.2	32.6±1.6	31.8±1.8	34.6±2.0
Drug Schedule 2							
Baseline	Day 6	Day 12	Day 18	Withdrawal			
29.0±1.2	24.6±1.4	19.8±0.69	23.5±1.9	24.8±1.7			

evaluated after repeated exposure to cocaine. Previous research has shown that long-term exposure to cocaine does not modify ICSS reward thresholds (14). More recently, however, it was demonstrated that rats allowed to self-administer cocaine showed increased reward thresholds for brain stimulation after drug withdrawal, the magnitude of which was dependent on the amount of cocaine consumed during the self-administration procedure (33). It was of interest to determine whether similar changes in reward would be observed after chronic exposure to cocaine that was experimenter-administered. Thus, in Experiment 2, two schedules of high-dose cocaine treatment were used and shifts in ICSS rate-intensity functions were assessed. In addition, we wished to elaborate on the findings of the self-adminstration study which involved medial forebrain ICSS by evaluating brain-stimulation reward from the VTA. Recent lesion studies and in vivo analysis of extracellular DA has provided strong support for the involvement of this neurotransmitter in VTA-ICSS (12,38), as well as modulating the rewarding effects of acute cocaine administration (21,38).

METHOD

Drug Schedule 1

Twelve naive male Wistar rats served as subjects. All particulars concerning subjects, surgical procedure, and apparatus specifications were similar to those described in Experiment 1, with the exception that electrodes were implanted in the VTA (AP −2.8 mm from bregma, L ± 1.4 mm from midline suture, and V −8.6 mm from the skull surface) (36). After recovery from surgery, animals were trained for ICSS using the discrimination procedure described in Experiment 1, and baseline rates of responding were determined after descending and ascending current presentation. Once stable rate-intensity functions were achieved, the baseline of each animal was determined by using the mean rate of responding at each current level for the last 3 days of ICSS testing. Animals were then tested daily for ICSS after 7 steps (4 μA) of descending current presentation starting at 40 μA followed by 7 ascending current steps. The range of optimal current intensities used during training ranged from 32–48 μA, and the 40 μA intensity represented the midpoint of this range. Immediately after the ICSS session animals were injected IP with 40.0 mg/kg of cocaine hydrochloride. This test/drug procedure was continued for 7 consecutive days.

Drug Schedule 2

Twelve naive male Wistar rats were implanted in the VTA and trained in the two-hole discrimination ICSS task. After stable rate-intensity functions were achieved the chronic cocaine schedule commenced. This involved two daily injections of 30.0 mg/kg of cocaine 4 hr apart. ICSS testing was conducted after every 5th day of consecutive drug treatment 20 hr after the second cocaine injection on Days 6, 12, and 18. Animals were then withdrawn from the drug and tested for ICSS 5 days later (Day 23).

RESULTS AND DISCUSSION

Histological analysis of electrode sites revealed that in those animals that completed the experiment electrodes were situated in the VTA (AP range −2.6 to −3.2 mm from bregma) (36). The results from three animals were discarded from the experiment (Drug Schedule 1) due to the development of motor seizures, also the data from two rats exposed to Drug Schedule 2 were not used as a result of inaccurate electrode placements.

Drug Schedule 1

ICSS rates as a function of descending and ascending current presentation were averaged and analysis of variance of this data showed significant main effects for Test Day, $F(7,56)=2.41$, $p<0.05$, and Current Intensity, $F(6,48)=46.99$, $p<0.001$, as well as a significant Test Day × Current Intensity interaction, $F(42,336)=1.56$, $p<0.02$. Rate-intensity functions from Baseline, Days 1, 3, 5, and 7 are depicted in Fig. 2. As can be seen in this figure, lower ICSS rates and a shift to the right of the current-response curve were evident after just one injection of 40 mg/kg of the drug when ICSS testing was conducted 24 hr postinjection. While there was some recovery at Day 3, rates of responding remained depressed on Days 5 and 7. Newman-Keuls multiple comparisons ($\alpha=0.05$) showed that rates were significantly lower at the 24–40 μA current levels on Day 7 and at the 24–32 μA levels on Day 5.

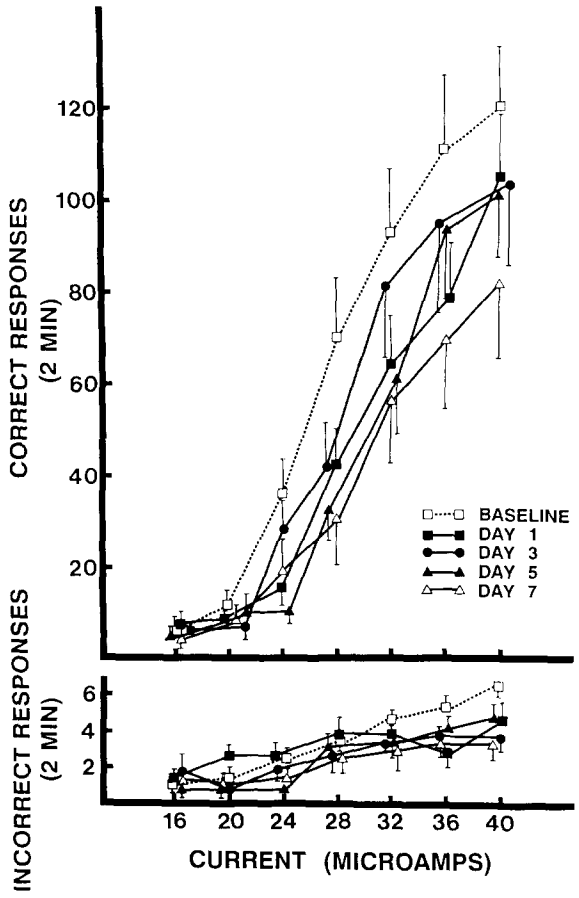

FIG. 2. Mean ICCS rates (correct responses) and incorrect responses (±S.E.M.) as a function of current intensity on ICSS Test Days 1, 3, 5, and 7 (Drug Schedule 1). Rate-intensity functions were determined 24 hr after each daily injection of 40.0 mg/kg of cocaine.

The current necessary to maintain half-maximal responding was determined for each animal and one-way analysis of variance of this data indicated that thresholds were significantly increased, $F(7,56)=2.86$, $p<0.02$. Reward thresholds are shown in Table 1, and while current thresholds were elevated for all test days, Newman-Keuls post hoc comparisons ($\alpha=0.05$) indicated that only the increase seen on Day 7 was significantly higher from baseline.

It should be mentioned at this point, that one problem we encountered in this experiment was a susceptibility of some of the cocaine-treated animals to develop generalized motor seizures during ICSS testing. This effect probably resulted from the

interaction between repeated administration of a single high dose of cocaine and daily exposure to electrical brain stimulation. We did not see seizure development in animals exposed to Drug Schedule 2 where rats received daily intermittent injections of a lower dose of cocaine, and were tested for ICSS only 3 times during the 18-day drug/test schedule.

While the subjects that completed the experiment (N = 9) remained seizure-free during the 6 days of drug treatment, data from 3 animals that began the experiment were discarded, and several more animals developed seizures by the 8th and 9th day of the drug/test procedure. Continued testing of the nonseizure prone animals revealed that the postcocaine depression of ICSS was still evident after 9 days of drug withdrawal. This informal observation indicates that the reward-depressing effects of cocaine were fairly long-lasting.

As can be seen in Fig. 2, rates of responding at the highest current level (40 μA) were significantly lower than baseline on Test Day 7 suggesting a performance effect (30, 34, 52). Evaluation of ICSS on the other days, however, showed a shift to the right of the rate-intensity functions within the dynamic range of stimulation only. With respect to the incorrect scores, error responding was somewhat reduced at the 40 μA intensity on Day 7 indicating that withdrawal from repeated cocaine administration did induce a small change in behavioral arousal (see Fig. 2). However, analysis of variance revealed that while increasing current intensities elevated the number of incorrect responses made during ICSS testing, $F(6,48) = 19.26$, $p<0.001$, no significant effects were observed on the number of errors after repeated exposure to cocaine, $F(7,56) = 1.04$, $p>0.1$.

Drug Schedule 2

Changes in baseline rates of ICSS after chronic cocaine administration (2 daily injections of 30.0 mg/kg) are depicted in Fig. 3. A two-way analysis of variance with repeated measures on Current Intensity and Test Day (Baseline, Days 6, 12, 18, and withdrawal) yielded significant main effects for Current Intensity, $F(6,54) = 38.86$, $p<0.001$, and Test Day, $F(4,36) = 7.01$, $p<0.001$, as well as a Test Day × Current Intensity interaction, $F(24,216) = 3.68$, $p<0.008$. As shown in Fig. 3, and in marked contrast to the results from Drug Schedule 1, ICSS rates were increased relative to baseline responding and there was a shift to the left of the rate-intensity functions for all four test days. Thresholds for half-maximal responding were determined for each animal and one-way analysis of variance of these data showed a significant change in reward thresholds, $F(4,36) = 9.89$, $p<0.002$.

Subsequent Newman-Keuls multiple comparisons ($\alpha = 0.05$) indicated that repeated cocaine administration significantly decreased reward thresholds when ICSS testing occurred after 5-day drug intervals on Days 6, 12, and 18, and reward thresholds remained significantly lower after 5 days of drug withdrawal (see Table 1).

Analysis of the incorrect response scores revealed that while error responding was enhanced as a function of current presentation, $F(6,54) = 23.97$, $p<0.001$, repeated cocaine treatment did not modify error rates, $F(4,36) = 1.88$, $p>0.1$. Also the number of incorrect responses were not significantly altered when ICSS testing was evaluated after cocaine withdrawal (see Fig. 3).

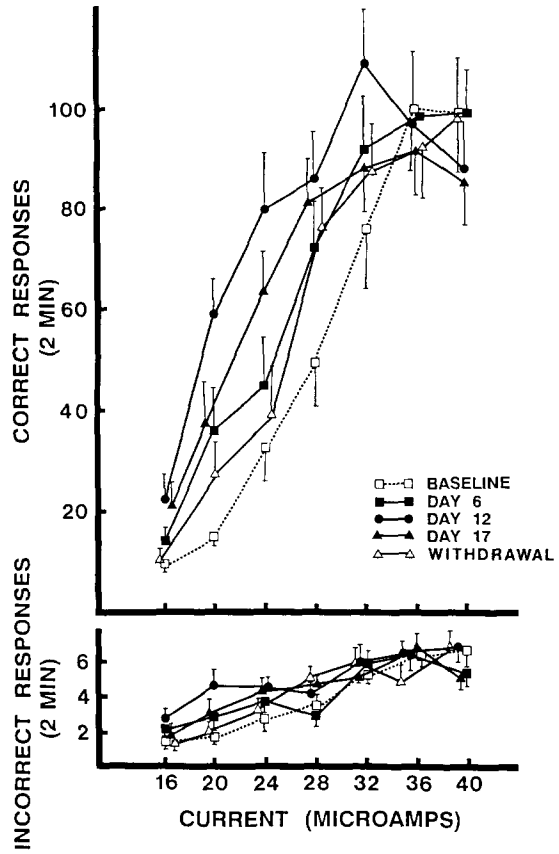

FIG. 3. Mean ICSS rates (correct responses) and incorrect response (±S.E.M.) as a function current intensity and repeated cocaine treatment (Drug Schedule 2). Rate-intensity functions were conducted after 5 consecutive daily injections of cocaine (30.0 mg/kg, twice a day) on Days 6, 12, and 18, and 5 days after drug withdrawal (Day 23).

EXPERIMENT 3

The results of Experiment 2 demonstrate that repeated exposure to cocaine can modify brain-stimulation reward, however, the direction of change is dependent on the drug/test schedule. The postcocaine depression of ICSS was evident only after animals were tested for ICSS daily, 24 hr after withdrawal from cocaine treatment. To elaborate more fully on the development of the postcocaine depression in terms of its temporal characteristics, we evaluated ICSS rates as a function of time after acute injection of the drug, and determined whether the temporal-response curve would be modified after repeated cocaine administration. A lower dose of the drug (20.0 mg/kg) was used in this experiment to minimize the possibility of generalized motor seizures developing after repeated daily drug exposure and electrical brain stimulation.

METHOD

Fifteen naive male Wistar rats served as subjects in this experiment. All other specifications concerning subjects with respect to age, housing, and feeding schedules are identical to those described in Experiment 1. Rats were implanted with bipolar stimulating electrodes in the VTA as previously described in Experiment 2. Subjects were trained for ICSS using the discrimination paradigm (see Experiment 1). Once stable rates of responding were established animals were placed into two groups based on equalized baseline ICSS rates. One group (N = 7) was injected with 20.0 mg/kg of cocaine and the other (N = 8) received an IP injection of saline. Immediately after drug injection animals were tested for ICSS for a 5-min session and the current intensity used for all animals in this experiment was 30 μA. This current level is

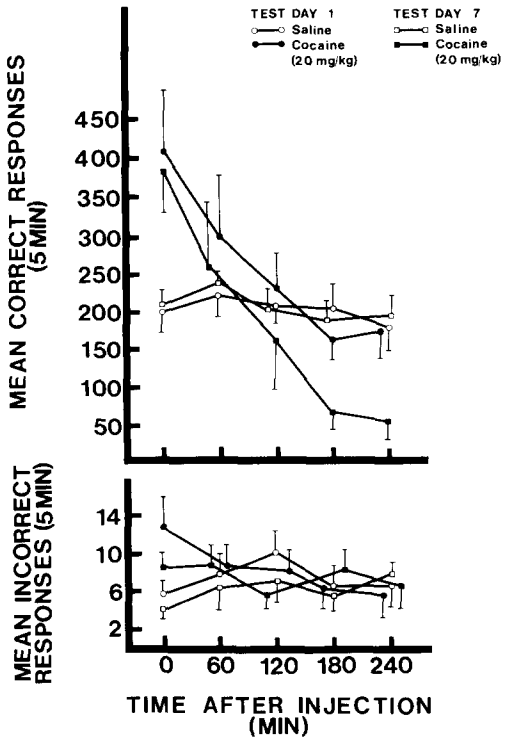

FIG. 4. Mean ICSS rates (correct responses) and incorrect responses (±S.E.M.) made during daily 5-min ICSS sessions at 0-, 60-, 120-, 180-, and 240-min intervals after injection of saline and 20.0 mg/kg of cocaine. The current used was 30 μA. Data after the 1st drug injection (Test Day 1) and the 7th drug injection (Test Day 7) are depicted.

within the dynamic range of reward stimulation, and was chosen since it does not induce asymptotic rates of responding thereby minimizing the possibility of performance effects influencing the data (30,52). Following this initial ICSS test, animals were retested for 5-min sessions at the 60-, 120-, 180-, and 240-min intervals after the initial drug injection. This procedure was continued daily for 7 consecutive days. Both correct and incorrect responses were recorded for each 5-min test period.

RESULTS AND DISCUSSION

All animals showed stable rates of ICSS responding and histological analysis revealed that electrode placements were situated in the VTA (AP range −2.6 to −3.0 mm from bregma) (36).

Analysis of variance of ICSS scores yielded a significant main effect for Time Interval, $F(4,52)=23.50$, $p<0.001$, and a Time Interval × Drug interaction, $F(4,52)=13.94$, $p<0.001$. No significant main or interaction effects for repeated drug testing (Test Day) were observed. Since the largest changes from baseline ICSS responding seen in Experiment 2 (Drug Schedule 1) occurred after 6 daily cocaine injections (Day 7), we reanalysed the data using ICSS scores from Test Day 1 and Test Day 7.

This analysis of variance involved a 2 (Drug) × 2 (Test Day) × 5 (Time Interval) design with repeated measures on Test Day and Time Interval and yielded significant interactions for Drug × Test Day, $F(1,13)=7.67$, $p<0.02$, and a Drug × Time Interval, $F(4,52)=6.67$, $p<0.002$. Subsequent Newman-Keuls post hoc comparisons of the simple main effects ($\alpha=0.05$) involved in these interactions showed that responding during the first 5-min test session, which was conducted immediately after the first drug injection, was significantly increased relative to saline-treated animals (see Fig. 4). Response rates were then observed to decrease as a function of time after drug injection and were not significantly different from control subjects at the 60–240 time intervals. After 7 days of drug treatment, rates were still significantly higher after cocaine injection, however, responding rapidly decreased with repeated testing and was significantly depressed at the 180- and 240-min intervals compared to control subjects.

The depression of ICSS was not related to fatigue factors since a response decrement was not evident in saline-treated animals as a function of repeated ICSS testing (see Fig. 4). Also, error scores were not modified after repeated drug testing or drug administration indicating that the temporal depression was not due to nonspecific performance deficits involving fatigue. Analysis of variance of the incorrect response scores revealed no significant main effects for Drug Treatment, $F(1,13)=0.01$, $p>0.1$, Test Day, $F(1,13)=2.14$, $p>0.1$, or Time Interval, $F(4,52)=0.31$, $p>0.1$.

GENERAL DISCUSSION

Evaluation of ICSS responding as a function of current presentation yielded typical rate-intensity functions (30). Acute cocaine administration increased rates of responding in a dose-dependent manner and shifted the current-response curves to the left. Analysis of changes in thresholds for half-maximal responding indicated that exposure to cocaine decreased reward thresholds. Essentially, these findings replicate previous reports showing that cocaine modifies central reward processes by decreasing the threshold for electrical brain stimulation (14,21).

The use of the two-hole discrimination procedure in the assessment of ICSS revealed that in addition to elevating ICSS response rates, higher current intensities also increased the number of error responses made during the test intervals. This observation is consistent with earlier work from this laboratory and demonstrates that the discrimination task provides a sensitive measure of the behavioral-activating properties associated with brain-stimulation reward (32). The increase in nonreinforced behavior indicates that as the rewarding value of the stimulation increases, it becomes more difficult for animals to terminate responding. As mentioned earlier, the arousal effect may involve changes in associative (e.g., extinction) and nonassociative factors (e.g., response perseveration).

While cocaine increased error responding, this relatively small change in performance cannot account for the drug-induced shifts in the rate-intensity functions. In addition, it should be noted, that although cocaine (10.0 and 20.0 mg/kg) facilitated brain-stimulation reward and increased the number of incorrect responses, the shift to the left of the rate-intensity curve, and decrease in reward threshold seen after injection of 5.0 mg/kg of the drug was not accompanied by concomitant increases in error responding. Likewise, the sensitization of ICSS (Drug Schedule 2) and the observed postcocaine depression seen in Experiments 2 and 3, were not paralleled by significant alterations in the number of responses to the nonreinforced hole. Taken together, these findings indicate that the discrimination paradigm can be useful in analyzing, and in some instances, dissociating between reward and performance effects.

Repeated administration of cocaine resulted in significant changes in brain-stimulation reward that were dependent on the drug/test schedule. When animals were exposed to daily cocaine injections followed by ICSS 24 hr postinjection, an ICSS depression, shift to the right of the rate-intensity functions, and after 7 days of drug treatment, a significant increase in reward thresholds was evident. The depression of ICSS seen in this study is consistent with recent findings involving the effects of self-

administered cocaine on brain-stimulation reward (33), as well as with previous reports concerned with the long-term consequences of amphetamine withdrawal on ICSS (7, 26, 30).

Changes in ICSS also developed after exposure to twice daily injections of 30.0 mg/kg of cocaine. During this drug schedule, ICSS was conducted on 3 occasions after 5-day intervals of repeated drug treatment, and under these conditions, facilitated response rates, a shift to the left of the rate-intensity function, and decreased reward thresholds were evident. Thus, in marked contrast to the postcocaine depression seen during Drug Schedule 1, a sensitization of brain-stimulation reward developed when animals were exposed to Drug Schedule 2. A similar sensitization of brain-stimulation reward after long-term amphetamine administration has been reported (26). Typically, in these studies ICSS sensitization is seen after amphetamine challenge (27,28), however, baseline rates also have been reported to increase following repeated exposure to amphetamine (44).

The apparent contradictory results derived from the two chronic drug schedules can be better understood in terms of the role of DA in central reward processes, and the effects of acute and repeated cocaine on DA neuronal dynamics. There is growing consensus that DA plays an important modulatory role in ICSS, particularly in VTA-reward. For example, ICSS reward sites in the VTA appear to be closely related to the layer of DA cell bodies in this region (8). Consistent with this neuroanatomical relationship, Fibiger *et al.* (12) recently demonstrated that unilateral 6-OHDA lesions of ascending mesotelencephalic DA fibres disrupted VTA brain-stimulation reward from the same side of the lesion without affecting contralateral VTA-ICSS. Finally, from the neurochemical perspective, Phillips *et al.* (38) showed that DA in the nucleus accumbens is released after VTA-ICSS, and the amount of extracellular DA is positively related to current intensity. In agreement with this observation, lateral hypothalamic stimulation also has been shown to increase extracellular accumbens DA (21).

Indirect evidence supporting a role for DA in reward comes from pharmacological studies assessing DA function with respect to incentive-motivational learning. Rewarding brain-stimulation consists of two components, a) an incentive-motivational effect, and b) a reinforcing effect that maintains the probability of response repetition (15). In the discrimination ICSS paradigm used in this study, a previous neutral stimulus (light) was associated with brain-stimulation reward, thus acquiring motivational properties similar to the reward. Much of the literature with respect to reward-related learning is consistent with the position that DA mediates the motivational properties of conditioned incentive stimuli [for recent reviews see (4,11)].

While cocaine affects several neurochemical systems, there is good reason to believe that increased brain-stimulation reward reflects drug-induced facilitation of DA neurotransmission. It appears that cocaine increases synaptic DA activity primarily by inhibiting reuptake processes (40,41), and, recently, [^{3}H]cocaine binding sites have been identified that are related to cocaine's affinity for DA reuptake inhibition (6, 24, 35). Using in vivo microdialysis, Bradberry and Roth (5) found cocaine to increase extracellular DA in the nucleus accumbens and VTA [see also (21)], and, as alluded to earlier, facilitated VTA-ICSS after cocaine administration was associated with drug-related increases in extracellular accumbens DA (38). The position that enhanced DA functioning modulates the cocaine-elicited increase of ICSS is compelling, as is the possibility that changes in DA neurotransmission might be involved in the reward depression seen after cocaine withdrawal. Specifically, following chronic exposure to cocaine tyrosine hydroxylase activity is decreased (20). Trulson *et al.* (49–51) found this effect to be pronounced and reported reduced tyrosine hydroxylase activity in both striatal and mesolimbic tissue for up to 60 days after drug withdrawal. It is interesting to note that these changes appear to be specific to this synthesizing enzyme and are not associated with depletions of DA neuronal stores. Chronic intermittent or continuous administration of high doses of cocaine (up to 100 mg/kg/day for either 10 or 20 days) did not produce DA depletions in cortical, hippocampal, hypothalamic, or striatal tissue (25). These findings also indicate that, unlike amphetamine, multiple injections of high doses of cocaine do not result in DA neuronal toxicity involving presynaptic terminal degeneration. While cocaine seems to be devoid of neurotoxic effects, the process by which repeated exposure to cocaine can modify synthesis without altering steady-state DA levels is not well understood (25).

In any event, it is clear that the reward system depression after cocaine withdrawal cannot be attributed to depletion of DA neuronal stores as previously speculated (9), and appears to involve more subtle changes in DA neurotransmission. Cocaine enhances DA receptor stimulation by inhibiting DA reuptake, and consequently increases negative feedback regulation of DA synthesis. Our behavioral observations involving the postcocaine depression (Drug Schedule 1) may be related to this neurochemical state, and the time-dependent changes in ICSS seen after repeated cocaine treatment (Experiment 3), might well reflect the decreased ability of DA synthesis in mesolimbic neurons to fully recover from the repeated drug-elicited increases in synaptic concentrations of DA.

With respect to the enhanced ICSS responding seen with Drug Schedule 2, there is reason to believe that repeated cocaine administration can produce long-term changes in DA presynaptic functioning which may modulate the development of behavioral sensitization. Converging information indicates that during cocaine withdrawal an increase in the release of DA is elicited in response to neuronal stimulation. Akimoto *et al.* (1) observed a significant elevation of striatal extracellular DA after cocaine challenge to animals with a prior history of cocaine exposure [see also (23)]. Repeated cocaine administration was shown to facilitate amphetamine-induced [^{3}H]DA release in striatal tissue at various stages of cocaine withdrawal (53). This increase in DA release seems to be dependent upon DA1 or DA2 receptor stimulation (37), and it is interesting to note that cocaine-induced sensitization of locomotor activity can be blocked by DA receptor antagonists (3). Finally, consistent with the facilitated release seen in DA terminal areas, the recent finding showing less potassium-induced release in the cell body regions of the VTA and substantia nigra, in animals pretreated with cocaine, implies reduced somatodendritic inhibition of impulse firing rates in response to neuronal stimulation (22).

Such changes in DA neurotransmission might account for the brain-stimulation reward sensitization seen when ICSS was conducted after 5-day intervals. With daily intermittent drug administration, and the passage of time between test sessions, these long-term alterations in DA neuronal dynamics become established, possibly to compensate for the cocaine-induced DA hypoactivity seen soon after withdrawal. Electrical brain stimulation, under these conditions, might result in enhanced DA release and increased ICSS responding. Moreover, like the postcocaine depression, these effects seem to be enduring since a significant sensitization of brain-stimulation reward was still evident 5 days after drug withdrawal.

Summarizing, ICSS rates are increased and reward thresholds decreased after acute cocaine injection. Repeated drug exposure results in reward depression when ICSS testing is conducted after the acute effects of cocaine injection dissipate. We speculate that such a reward-system hypoactivity might be involved in the dysphoria that develops after cocaine abstinence in humans, and in the compensatory increases in drug intake seen after repeated drug use. With the passage of time between ICSS test sessions a

reward-system sensitization was observed. This increase in brain-stimulation reward may be related to some aspects of the behavioral pathology associated with cocaine use. In human abusers, for example, DA hyperactivity might result in the excessive reinforcement and proliferation of inappropriate thoughts and behaviors that characterize cocaine-induced psychotic episodes.

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