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Cocaine: Acute Effects on Reinforcement Thresholds for Self-Stimulation Behavior to the Medial Forebrain Bundle.[1]

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ESPOSITO, R. U., A. H. D. MOTOLA AND C. KORNETSKY. *Cocaine: acute effects on reinforcement thresholds for self-stimulation behavior to the medial forebrain bundle.* PHARMAC. BIOCHEM. BEHAV. 8(4) 437–439, 1978. – Reinforcing thresholds for self-stimulation behavior to the medial forebrain bundle were determined in rats by means of a rate-free psychophysical method. The acute administration of cocaine lowered the reinforcing thresholds independent of motor stimulatory effects. These results indicate that cocaine affects the sensitivity of the reward pathways in the brain, and further demonstrate the utility of rate-independent methods in the assessment of drug effects on self-stimultion behavior.

Cocaine Euphoria Self-stimulation Medial forebrain bundle Threshold determination

MORPHINE and amphetamine, both euphoria producing drugs of abuse with associated addiction liability [6], have been found to lower the reinforcing threshold for intracranial self-stimulation (ICSS) to reward areas of the brain in rats [2, 5, 9, 11]. In addition, it has been demonstrated that the acute administration of morphine to rats results in a lowering of the amplitude of the EEG recorded from the medial forebrain bundle–lateral hypothalamic area (MFB–LH) [10]. These findings support the contention that an important common mechanism of action of these drugs may be their ability to affect the sensitivity of the positive reinforcement pathways of the brain (i.e., those sites which support ICSS behavior). Thus it is reasonable to hypothesize that cocaine, also a euphoria producing agent in man with abuse liability [6], may exert similar effects on ICSS behavior.

Although the effects of cocaine on ICSS behavior in rats have previously been investigated [3,13], these studies (as similar recent investigations of the effects of amphetamine on ICSS behavior [1, 7, 8]) have utilized rate of lever pressing as their primary dependent measure. These investigations, although provocative, suffer from several logical and empirical problems associated with the use of rate of responding as the means to determine the reward value of ICSS [12], plus the general problem of non-specific effects (e.g., motor stimulation) that can easily confound the rate measure. Therefore the present study was designed to specifically investigate the acute effects of various doses of cocaine on a rate-free threshold determination for ICSS behavior to the MFB–LH in rats.

METHOD

Animals and Apparatus

Four male albino Fischer rats (Charles River Breeding Laboratories), weighing approximately 300 g, were stereotaxically implanted with bipolar stainless steel electrodes (0.0127 cm in dia. and insulated except at the tips). The electrodes were aimed at the MFB–LH. Prior to surgery all animals were anesthetized with Equi-Thesin (0.3 ml/100 g body weight). Coordinates for electrode placements were 4 mm posterior to bregma, ± 1.4 mm from the midline suture, and 8.5 mm ventral to the skull surface. After surgery all animals were injected intramuscularly with 60,000 units of penicillin (Bicillin), and then given at least 1 week for post-operative recovery before behavioral testing. Throughout the experiment the animals, when not being tested, were individually housed in standard steel cages and given ad lib access to food and water.

The animals were trained in an experimental chamber which consisted of a Plexiglas enclosure 20 cm × 20 cm. Mounted in an opening in one wall of the chamber was a

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wheel manipulandum, 15 cm long and 7.5 cm in dia. On one of the endplates of the wheel 4 equally spaced cams were positioned so that a microswitch was closed when the wheel was rotated, whereupon stimulation would be immediately delivered.

Biphasic square wave stimuli were delivered by a constant current stimulator (Nuclear-Chicago). Each stimulus consisted of a 500 msec train with a pulse width of 0.2 msec (and a delay of 0.2 msec between the positive and negative pulses) presented at 160 Hz. Pulse amplitude was varied according to the procedural requirements described below.

Procedure

Thresholds were determined by a procedure identical to one previously utilized to investigate the effects of morphine on ICSS behavior [5]. This procedure basically involves the use of discrete trials systematically presented over a range of stimulus intensities.

A trial began with the delivery of a noncontingent stimulus. A response (one-quarter wheel turn) within 7.5 sec of this stimulus resulted in a contingent stimulus, identical in all parameters to the noncontingent stimulus and terminated the trial. Failure to respond had no scheduled consequences and the trial terminated after 7.5 sec. Intervals between the trials varied around an average of 15 sec. Responses during the intertrial interval (error responses) resulted in a 15 sec delay before the start of the next trial. The initial non-contingent stimulation thus served both as a discriminative stimulus indicating availability of response-contingent stimulation and as a comparative stimulus in the sense that it was a predictor of the parameters of the contingent stimulus. Total responses and total intertrial responses were recorded, in order to assess nonspecific stimulatory effects of the drug and possible behaviorally disruptive effects, respectively.

Stimulus intensities were varied according to the classical method of limits [4] with slight modification. Stimuli were presented in alternating descending and ascending series with a step size of either 5 or 10 μA depending on the individual animal. Animals with low thresholds (i.e., below 35 μA) were trained on the 5 μA step size. Ten trials were given in succession at each step size or interval. A descending series was initiated at a previously determined intensity which invariably yielded a contingent response in at least 9 out of 10 trials, and then 10 more successive trials were conducted at the next lowest interval and so on. Five or more responses at a particular intensity were scored as a plus for the interval, while less than 5 responses were scored as a minus for the interval. Descending series were conducted until minus scores were achieved in 2 successive intervals. An ascending series was started at one step size below the lowest intensity in the descending series, and continued until a level was reached in which there were at least 9 responses out of 10 trials, whereupon a descending series would be initiated at least one interval above the last intensity used in the ascending series. Threshold was determined by calculating the arithmetic mean ($\bar{x}$) in microamperes of the midpoints between intervals in which the animal made greater than 5 responses (a plus score) and less than 5 responses (a minus score).

Each day the animals were given 4 test series before (preinjection session) and 4 test series after injection (postinjection session). The animals were injected intraperitoneally with either cocaine hydrochloride dissolved in an isotonic saline vehicle, or the saline vehicle alone (all injections were in volumes of 1 ml/kg of body weight), and then immediately afterward the postinjection session was begun. The time required to complete either the preinjection or postinjection session varied from 60 to 90 min. The major dependent measure was the percentage change in threshold from the pre- to the postinjection session. (The percentage change was calculated as the postinjection session threshold minus the preinjection session threshold $\times$ 100 divided by the preinjection session threshold.)

The animals were first tested for 4 days with saline control injections and then drug days were begun. Animals No. 862, 863, and 865 were tested with 5 doses (1.0, 2.5, 5.0, 7.5 and 10 mg/kg) given in random order. Animal No. 864, who proved resistant to any discernable effect on the threshold procedure at these doses was also given additional testing at doses of 20, 40 and 60 mg/kg. Saline injection control days were also alternated between drug days to serve as a check for any nonspecific effects of the experimental protocol such as the injection procedure, the passage of time or repeated stimulation on the absolute threshold levels. Thus for each animal there was a total of 8–10 saline control days.

Following the completion of the testing the animals were sacrificed with an overdose of anesthesia (Equi-Thesin), perfused intracardially with saline and then Formalin. The brains were subsequently removed from the skull, fixed, embedded, and sliced at 40 μ. Mounted sections were stained with cresyl violet and luxol blue and subsequently examined under a light microscope in order to determine electrode placements.

RESULTS

The effects on the major dependent measure are summarized in Fig. 1. The range of percentage change in threshold from preinjection to postinjection on saline days is illustrated to the left. These scores tended to average around zero or else show very slight increases as with animal 863. (Absolute threshold values ranged from 18–60 μA).

With all the animals the 1 mg/kg dose proved ineffective in terms of producing any threshold lowering effect. For animals 865, 863 and 862 there were clear threshold lowering effects at doses ranging from 2.5 mg/kg to 10 mg/kg, with the optimally effective dose varying from animal to animal. Animal 864, who showed no effect at these lower doses was consequently tested with 20, 40 and 60 mg/kg. As can be seen from Fig. 1, this animal also showed threshold lowering effects at these higher doses. The 40 mg/kg dose produced a threshold lowering effect just beyond the saline range. It is noteworthy that, in all animals, doses above the ineffective dose of 1 mg/kg never produced any threshold increases.

The specificity of the threshold lowering effect was made evident by a number of factors. At all the doses tested cocaine failed to produce any increase in intertrial or error responding. The error response total for the preinjection periods was quite low, ranging from 4–8 responses/200 trials, and remained within this range after drug administration. This indicates that on this procedure the drug did not exert any behaviorally disruptive effects. Secondly, while the errors remained unchanged, the drug did produce (at some doses) an expected increase in

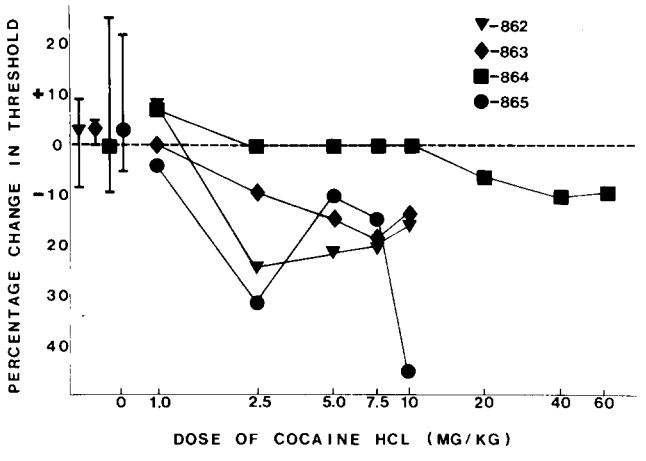

FIG. 1. Percentage change in threshold values from pre- to post-drug or saline treatment as a function of dose of cocaine for each of four animals. Means and range (vertical lines) of change in threshold after saline for each of the animals are indicated on the left.

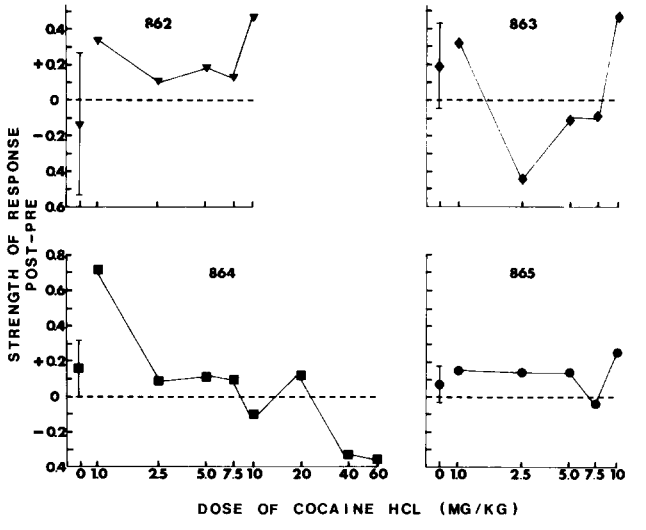

FIG. 2. Change in strength of responding from pre- to postinjection sessions for each animal. Response strength was measured as the number of microswitch closures per trial counting all trials in which the animal responded. Means and standard deviations for each animal on saline days are indicated to the left. A point above the horizontal line would indicate a postinjection increase in response strength.

strength of response (measured as microswitch closures/trial) (Fig. 2), although threshold reductions occurred independent of these effects. For instance animal 863, who showed threshold lowering effects at doses of 2.5 and 5 mg/kg, also showed decreases in response strength measures at these doses. Thus threshold reductions occurred concurrently with both response strength increases and decreases, while error responding always remained low. Histological analysis revealed all electrodes to be located in the MFB–LH. Animal 865 had the stimulating electrode within the MFB at the level of the fornix. Animal 863's placement was within the MFB, lateral and superior to the fornix and inferior to the mammillothalamic tract. Animal 862's placement was within the MFB, lateral and inferior to the posterior nucleus of the hypothalamus, and lateral and inferior to the mammillothalamic tract.

DISCUSSION

These results demonstrate that the acute administration of cocaine lowers the threshold for self-stimulation to the MFB–LH in rats, although the effective dose range may vary greatly between animals. Of particular interest is the specificity of this effect, as evidenced by the clear dissociation between response strength, error responses and threshold reduction. Threshold reductions occurred concurrently with both response strength increases and decreases, while error responding remained unchanged.

These findings with cocaine and our previous work with morphine [5, 9, 10] render support to the hypothesis that euphoria producing agents affect the sensitivity of areas of the brain which support ICSS. The method described herein appears to have utility for the testing of drugs with abuse potential.

REFERENCES

1. Carey, R. J., E. B. Goodall and G. F. Procopio. Differential effects of d-amphetamine on fixed ratio 30 performance maintained by food versus brain stimulation reinforcement. *Pharmac. Biochem. Behav.* **2**: 193–198, 1974.
2. Cassens, G. P. and A. W. Mills. Lithium and amphetamine: opposite effects on threshold of intracranial reinforcement. *Psychopharmacologia* **30**: 283–290, 1973.
3. Crow, T. J. Enhancement by cocaine of intra-cranial self-stimulation in the rat. *Life Sci.* **9**: 375–381, 1970.
4. Engen, T. Psychophysics. In: *Experimental Psychology*, edited by J. W. Kling and L. A. Riggs. New York: Holt Rinehart and Winston, 1971, pp. 11–46.
5. Esposito, R. U. and C. Kornetsky. Morphine lowering of self-stimulation thresholds: lack of tolerance with long term administration. *Science* **195**: 189–191, 1977.
6. Jaffe, J. H. Drug addiction and drug abuse. In: *The Pharmacological Basis of Therapeutics*, edited by L. S. Goodman and A. Gilman. New York: Macmillan, 1975, pp. 284–324.
7. Leith, N. J. and R. H. Barrett. Amphetamine and the reward system: evidence for tolerance and post-drug depression. *Psychopharmacologia* **46**: 19–25, 1976.
8. Liebman, J. M. and D. S. Segal. Lack of tolerance or sensitization to the effects of chronic d-amphetamine or substantia nigra self-stimulation. *Behav. Biol.* **16**: 211–220, 1975.
9. Marcus, R. and C. Kornetsky. Negative and positive intracranial reinforcement thresholds: effects of morphine. *Psychopharmacologia* **38**: 1–13, 1974.
10. Nelsen, J. M. and C. Kornetsky. Morphine-induced EEG changes in central motivational systems: evidence for single dose tolerance. *Fifth Int. Congr. Pharmac.*, 1976, p. 166.
11. Stein, L. Effects and interactions of impramine, chlorpromazine, reserpine, and amphetamine on self-stimulation: possible neurophysiological basis of depression. In: *Recent Advances in Biological Psychiatry*, edited by R. Heath. New York: Plenum Press, 1962, pp. 288–309.
12. Valenstein, E. Problems of measurement and interpretation with reinforcing brain stimuli. *Psychol. Rev.* **71**: 415–437, 1964.
13. Wauquier, A. and C. J. E. Niemegeers. Intracranial self-stimulation in rats as a function of various stimulus parameters V. influence of cocaine on medial forebrain bundle stimulation with monopolar electrodes. *Psychopharmacologia* **38**: 201–210, 1974.